UNIVERSITE BOURGOGNE FRANCHE COMTE

UMR A 102-02 Procédés Alimentaires et Microbiologiques, Université de Bourgogne /AgroSup-Dijon
(UNIVERSITY OF BURGUNDY, University of Burgundy – AgroSup Dijon, Department of Microbiology and Food Process)

SZKOŁA GŁÓWNA GOSPODARSTWA WIEJSKIEGO W WARSZAWIE

Wydział Nauk o Żywności,
Katedra Inżynierii Żywności i Organizacji Produkcji
(WARSAW UNIVERSITY OF LIFE SCIENCES, Faculty of Life Sciences, Department of Food Engineering and Process Management)

STUDY OF THE CHEMICAL, PHYSICAL AND FUNCTIONAL PROPERTIES OF EDIBLE STARCH-BASED FILMS

By **Ewelina BASIAK**

Cotutelle supervision by

Prof. Frédéric Debeaufort & Prof. Andrzej Lenart

Jury

Prof. Pascal Degraeve	Université Lyon I- IUT Bourg-En-Bresse	Reviewer,
Prof. Tomasz Jankowski	Poznań University of Life Sciences	Reviewer
Dr. hab. Sandra Domenek	AgrosParisTech – Massy	Examiner
Dr. hab. Hanna Kowalska	Warsaw University of Life Sciences	Examiner
Prof. Frédéric Debeaufort	University of Burgundy	Co-supervisor
Prof. Andrzej Lenart	Warsaw University of Life Sciences	Co-supervisor

2016, Dijon

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ÉTUDE DES CARACTERISTIQUES PHYSICO-CHIMIQUES ET FONCTIONNELLES D'ENROBAGES ET DE FILMS À BASE D'AMIDON

STUDY OF THE CHEMICAL, PHYSICAL AND FUNCTIONAL PROPERTIES OF EDIBLE STARCH-BASED FILMS

STUDIA NAD WŁAŚCIWOŚCIAMI CHEMICZNYMI, FIZYCZNYMI I FUNCJONALNYMI JADALNYCH FILMÓW SKROBIOWYCH

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Prof. Andrzej Lenart	Warsaw University of Life Sciences	Co-supervisor

June 6th, 2016, Dijon-France

Oświadczenie promotora pracy

Oświadczam, że niniejsza praca została przygotowana pod moim kierunkiem i stwierdzam, że spełnia ona warunki do przedstawienia jej w postępowaniu o nadanie tytułu zawodowego.

Data ………………………… Podpis promotora pracy ……………………………………

Oświadczenie autora pracy

Świadom odpowiedzialności prawnej oświadczam, że niniejsza praca dyplomowa została napisana przeze mnie samodzielnie i nie zawiera treści uzyskanych w sposób niezgodny z obowiązującymi przepisami.

Oświadczam również, że przedstawiona praca nie była wcześniej przedmiotem procedur związanych z uzyskaniem tytułu zawodowego w wyższej uczelni.

Oświadczam ponadto, że niniejsza wersja pracy jest identyczna z załączoną wersją elektroniczną.

Data ………………………. Podpis autora pracy …………………………………………

ACKNOWLEDGEMENT

This doctoral thesis was established on the basis of the Cotutelle agreement (of double PhD) between University of Burgundy and Warsaw University of Life Sciences. Laboratory tets were implemented in three countries: France (University of Burgundy, INRA, AgroSup Dijon), Germany (Leibniz Institute for Agricultural Engineering Potsdam-Bornim) and Poland (Warsaw University of Life Sciences). In each of them I spent around 1.5 year. Well, this period of time helped me to learn new methods, to get a new knowledge and the first of all to gain a new experience. I am very grateful for all what happened that time. I am very thankful for such unique, exceptional, experienced and helpful supervisors as Profesor Frédéric Debeaufort, Profesor Andrzej Lenart, Doctor Manfred Linke and Doctor Martin Geyer.

The greatest thankful I address to Mr. Frédéric DEBEAUFORT, Professor of I'IUT de Dijon-Auxerre, vice-president of University of Burgundy, supervisor of my thesis, my personal tutor. Contribution of his work exceeds the wildest expectations; his person is one of the highest authorities for me. Knowledge, dependability, availability, reliability, helpfulness, personal values, unconditional support – that are only some of the numerous attributes of Professor.

I wish to express the sincerest gratitude for Mr. Andrzej LENART, Professor of Warsaw University of Life Sciences, the right hand of President of University for economic affairs, the Head of Department of Food Engineering and Management of Produce. Being a student of Profesor is a great honor for me. Discipline, order, knowledge are just only some of the many positive attributes of Professor's character.

From whole my heart, from whole my soul I would like to thank Mr. Manfred LINKE, one of the greatest scientists, who I ever met. Dynamic, ready for new challenges, open minded, clever, helpful – it is just a drop in the sea with his many advantages. Collaboration with Doctor Linke in Leibniz Institute for Agricultural Engineering Potsdam-Bornim it was the real pleasure and glory.

I would like to express the deepest appreciation to Mr. Martin GEYER, the Head of Department of Horticultural Engineering of Leibniz Institute for Agricultural Engineering Potsdam-Bornim. Each conversation with Doctor Geyer resulted in increased interest for further action, lots of new ideas in my head. Doctor Geyer is always a favorably disposed to new challenges, person with specific aims and objectives, ingenious, ambitious, inventive, full of energy and passions.

Of course it is my pleasure either to acknowledge all my colegues, all employees of University of Burgundy, INRA, AgroSup Dijon, Leibniz Institute for Agricultural Engineering Potsdam-Bornim and Warsaw University of Life Sciences who were the instrumental to completion of my PhD research. It was a great pleasure to work with all of you.

I would like to thank my friends, who always supported me, and especially Ani Pinachyan.

These acknowledgements would not be complete without mentioning committees which gave me possibility to met interesting people, for instance in Business School of Alberta (the main price of Ministry of Science and Higher Education in Poland) or to accomplish own projects as these one from Warsaw University of Life Sciences or from Polish Federation of Food Producers.

My deepest appreciation belongs to my deceased Grandmother, Mother and my Aunt who I am very grateful for education, for all values, hope and the lack of temptation in me.

ABSTRACT

The amount of waste increased annually, mainly from plastic industry. Plastic materials were more produced during the only last ten years than during the last millennium. A potential solution of the ecological and economic problems can be biodegradable or edible films and coatings.

The goal of this thesis was to study edible films and coatings based on starch. Fifteen types of film-forming solutions were made: 3 types of starch, starch + different amounts of plasticizer, starch + proteins, starch + oil. For better understanding the interaction between film components, physical, chemical and functional tests were done. Finally, validation on real foods (plums) as coatings and films helped to improved edible barrier films for fruits and vegetables preservation.

Preliminary physico-chemical studies of corn, potato and wheat starch film properties allowed choosing the wheat starch-based films for further experiments. Then, a 50% amount of plasticizer related to dry biopolymer weight was selected aiming to obtain films being not too rigid, that did not break and without blooming. To prove the barrier moisture efficacy, rapeseed oil was added as multilayers films. Microstructure observations displayed that oil was dispersed as droplets instead of layer, thus emulsion-based films were obtained instead of multilayer starch-oil-starch films. Various ratios of starch/protein were assessed to improve functional properties of films. The more the protein content was, the better the barrier efficiency against water vapour, oxygen or aroma were. Indeed, higher protein content films were more dense and homogeneous. From these data obtained on films, and the better understanding how composition and structure affect film performances, several recipes were tested as coatings or films for wrapping fresh plums. Thermographic analysis was used to study the plums behavior during storage, and starch coating was efficient to delay fruit degradation.

Key words: starch, proteins, oil, biodegradable films, edible coatings, plums

STRESZCZENIE

Z każdym rokiem wzrasta liczba produkowanych odpadów, w szczególmości tych z plastiku. W ciągu pierwszych dziesięciu lat wyprodukowano więcej tworzyw sztucznych niż w przeciągu całego ubiegłego tysiąclecia. Rozwiązaniem tych ekologicznych i ekonomicznych problemów mogą okazać się filmy i powłoki do żywności.

Celem tej pracy były studia nad jadalnymi filmami i powłokami na bazie skrobi. Piętnaście rodzajów roztworów powłokotwórczych zostało wytworzonych: z 3 typów skrobi, skrobia + różne stężenia plastyfikatora, skrobia + białka, skrobia + olej. W celu lepszego zrozumienia interakcji pomiędzy komponentami filmu właściwości fizyczne, chemiczne i funkcjonalne zostały zmierzone. W ostatnim etapie walidacja na prawdziwej żywności (powlekanie i pakowanie śliwek) pomogła w udowodnieniu istnienia właściwości barierowych owoców i warzyw podczas przechowywania.

Próbne testy fizyko-chemiczne skrobi kukurydzianej, ziemniaczanej i pszenicznej pomogły w wyborze skrobi otrzymywanej z pszenicy do dalszych badań. Następnie wybrano zawartość plastyfikatora. 50% glicerolu względem suchej masy substancji powłokotwórczej nie powodowało twardości i pękania filmów ani też tzw. efektu kwitnienia (intensywnie żółty/ pomarańczowy kolor filmów). W celu poprawy właściwości barierowych olej rzepakowy został dodany. Zdjęcia mikroskopowe obrazują zawieszone krople oleju w matrycy jako emulsja zamiast dodatkowej warstwy, której oczekiwano. Do skrobi zostały dodane również białka serwatkowe w kilku proporcjach. Im więcej białek jest w stosunku procentowym skrobia/proteiny tym lepsze są właściwości barierowe dla pary wodnej, tlenu i aromatów. Dodatkowo filmy zawierające więcej protein w stosunku procentowym są bardziej gęste i jednolite. Uzyskane informacje pozwoliły na lepsze zrozumienie wpływu kompozycji i struktury filmów i powłok na pakowanie świeżych śliwek. Analiza z użyciem kamery termowizyjnej pozwoliła na ocenę zmian w owocach podczas przechowywania, zaś powłoka skrobiowa efektywnie opóźniała procesy degradacyjne w owocach.

Słowa kluczowe: skrobia, białka, olej, biodegradowalne filmy, jadalne powłoki, śliwki

RESUME

La quantité de déchets augmente depuis plusieurs décénies, issus principalement de l'industrie du plastique. La production cumulée de matières plastiques au cours des dix dernières années est supérieure que pendant le dernier millénaire. Une des solutions pour réduire les impacts écologiques et économiques est de dévelopement de films et enrobages biodégradables et/ou comestibles.

L'objectif de cette thèse est d'étudier des films et enrobages comestibles à base d'amidon. Quinze types de solutions filmogènes ont été formulés: 3 types d’amidons, amidon + protéines, amidon + huile de colza, amidon + plastifiant. Afin de mieux comprendre le rôle des constituants et les interactions mises en jeu, les propriétés physico-chimiques et structurales des films ont été réalisées. Enfin, les films présentant les meilleurs compromis ont été appliqués sur des prunes.

L’étude physico-chimique des films à base d’amidon de maïs, de pommes de terre et de de blé ont permis de retenir l’amidon de blé pour les études suivantes. Une quantité de plastifiant de 50% (par rapport à la masse sèche de biopolymère) a été sélectionnée car elle permet d'obtenir des films souples et resistants sans blanchiment. Afin d'améliorer l'efficacité barrière à l'humidité, de l'huile de colza a été ajoutée par laminage sur le film d’amidon. Les observations de la microstructure montrent une dispersion de gouttelettes d’huile dans la matrice à la place d’une structure multicouche amidon-huile-amidon. Diverses proportions amidon/protéine ont été testées pour améliorer les propriétés fonctionnelles des films. Plus la teneur en protéines est élevée, meilleure est l'efficacité barrière à la vapeur d'eau, à l'oxygène ou aux arômes. En effet, les films sont plus denses et homogènes en présence des protéines. A partir de la meilleure compréhension du rôle de la composition et de la structure sur les performances des films, plusieurs formulations ont été testées comme enrobage ou film sur des prunes fraîches. L’analyse thermographique a été utilisée pour étudier le comportement des prunes pendant le stockage, l’enrobage/film composé de l’amidon enrobage s’est avéré efficace pour retarder la dégradation des fruits.

Mots clés: amidon, protéine, huile, films biodégradables, enrobage comestible, prunes

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EWELINA BASIAK CURRICULUM VITAE

PROFESSIONAL EXPERIENCE:

2003 - 2011 **” Foundation for culture, recreation and family assistance ATN” (surplus voluntary labour)**
(„Fundacja kultury i wypoczynku, oraz pomocy rodzinie ATN”)
Voluntary work (English translation, camp organization, acquiring donors, work - office administration).

2006 - 2007 **Nestlé Waters (full - time work)**
Assistant in the department of quality and laboratory
Responsibilities: microbiological and physicochemical research, analyses, quality control, et cetera.

2005 - 2008 **Advertisement agency Adeo B. (surplus labor)**
Designing of advertisements, leaflets and posters, et cetera

EDUCATION:

2014 - present **Leibniz Institute for Agricultural Engineering, Potsdam, Germany**

2012– present **University of Burgundy/AgroSup Dijon France (double PhD study)**
Food processes and microbiology

2011– present **Warsaw University of Life Sciences (double PhD study)**
Food processing, specialization: food technology and production management

2010 - 2011 **University of Warsaw (Master study) (grade: excellent)**
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2004 – 2011 **Warsaw University of Life Sciences (Bachelor's and Master studies) (both grades: excellent)**
Food processing, specialization: food technology and production management

2009 - 2010 **Russian State Agrarian University – Moscow Timiryazev Agricultural Academy (grade: excellent)**

2008 - 2009 **Warsaw University of Life Sciences (Postgraduate study) (grade: excellent)**
Management and production control

2003 - 2005 **English Language College (grade: excellent)**

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biological-chemical class

LANGUAGES:

English	advanced C1
Russian	advanced C2
French	basic (during the training) A2
German	intermediate (during the training) B1

SKILLS:

driving license cat. B
proficient computer literacy (MS Office, Internet, graphic programs)
internal auditor of quality management system ISO 9001 and ISO 22000

INTERESTS:

Learning foreign languages, travels with backpack (these oriental and these short ones), sport, especially swimming, sailing, skiing, taekwon-do, shooting, climbing, ballet (here only as the spectator), haute couture, etymology, macroeconomics.

LIST OF PUBLICATIONS:

Basiak E., Galus S., Lenart A. (2012). Filmy i powłoki skrobiowe do żywności. *Przemysł Spożywczy, 66*, 28-30 (English title: Starch films and coatings for food, *Food Industry*). Ministry of Science and High Education: 6 points

Basiak E., Lenart A. (2012). Skrobia jako składnik powłok jadalnych. *Postępy Techniki Przetwórstwa Spożywczego, 22, 2,* 84-88 (English title: Starch like component of edible coatings, *Development of Techniques and Food Industry*). Ministry of Science and Higher Education: 5 points

Basiak E., Lenart A. (2013). Powłoki skrobiowe stosowane w opakowalnictwie żywności. *Żywność, Nauka, Technologia, Jakość, 20, 1,* 21-31 (English title: Starch coatings used in food packaging, *Food, Science, Technology, Quality*). IF = 0.31; Ministry of Science and Higher Education: 15 points

Basiak E., Lenart A. (2013). Wpływ oleju rzepakowego na właściwości optyczne filmów skrobiowych. *Postępy Techniki Przetwórstwa Spożywczego, 23/42, 1,* 23-26 (English title: Influence of rapeseed oil on optical properties of starch films, *Development of Techniques and Food Industry*). Ministry of Science and Higher Education: 5 points

Basiak E., Galus S., Lenart A. (2015). Characterisation of composite edible films based on wheat starch and whey-protein isolate. *International Journal of Food Science and Technology, 50,* 372-380. IF = 1.65; Ministry of Science and Higher Education: 25 points

Basiak E., Lenart A., Debeaufort F. (2015). Effect of oil lamination and starch content on structural and functional properties of starch-based films. *Food Chemistry,195, 56-63.* IF = 3.90; Ministry of Science and Higher Education: 40 points

Basiak E. (2015). Food industry: use of plastics of twenty-first century, in Goyal M.R., Meghwal M. (eds.), *Emerging Technologies and Innovations in Food Engineering*, Apple Academic Press, Canada. 1008, P9. Ministry of Science and Higher Education: 5 points

Basiak E., Lenart A., Debeaufort F. (2015). Carbohydrate/protein ratio affects microstructure, surface and transport properties of wheat starch – whey protein blend edible films. In *Journal* *of the Science of Food and Agriculture,* doi: 10.1002/jsfa.7807, IF= 2.08. Ministry of Science and Higher Education: 35 points

Basiak E., Lenart A., Debeaufort F. (2015). Role of starch nature on the physico-chemical properties of edible films. Submitted to *Journal of Food Science*, December 2015.

Basiak E., Lenart A., Debeaufort F. (2015). Glycerol and water contents affect the structural and functional properties of starch-based edible films. Submitted to *Food Science and Technology International,* November 2015.

Basiak E., Linke M., Geyer M., Debeaufort F., Lenart A. (2015). Relevance of interactions between starch-based coatings and plum fruit surfaces: a physical-chemical analysis. Submitted to *Journal of Food Engineering*, July 2016.

Basiak E., Linke M., Debeaufort F., Lenart A., Geyer M. (2015). Dynamic behaviour of coated plums during storage. Submitted to *Food Hydrocolloids*, July 2016.

Basiak E., Linke M., Debeaufort F., Lenart A., Geyer M. (2015). Impact of biodegradable coating materials on plums quality and physiology. Submitted to *Postharvest Biology and Technology,* July 2016.

LIST OF ORAL PRESENTATIONS AT THE CONFERENCES:

Basiak E.: "Physical and chemical properties of pectine coatings" – Conference in Russian State Agricultural University (Moscow Timiryazev Agricultural Academy), 16-18 March 2010, Moscow, Russia.

Basiak E., Lenart A.: "Sorption and mechanical properties of starch-whey protein films" - XVII Session of the Food Sciences Committee, 10-11 May 2012, Cracow, Poland.

Basiak E., Lenart A.: "Physical properties of starch-protein films" - XVIII Session of the Food Sciences Committee, 14-16 May 2013, Poznań, Poland.

Basiak E., Debeaufort F., Lenart A.: "Contact angle and microscopy of starch-protein films" - 12 Workshops of Young Scientific BioPhys, 21-23 May 2013, Lublin, Poland.

Basiak E., Debeaufort F., Lenart A.: “Physical and chemical properties of whey protein films” - Summer School on Industrial Biotechnology: Stability, Folding, and Misfolding of Recombinant Proteins, 6-10 October 2014, Milan, Italy.

Basiak E., Debeaufort F., Linke M., Lenart A.: “Impact of coating and wrapping with starch-based films on quality of plums” - Innovations in Food Packaging, Shelf Life and Food Safety, 15-17 September 2015, Stadthalle Erding, Munich, Germany.

LIST OF POSTERS AT THE CONFERENCES:

Basiak E., Sitkiewicz I.: ”Rheological parameters and stickiness of selected coatings-forming solutions” - FoodBalt-2012, 17-18 May 2012, Kaunas, Lithuania.

Basiak E., Lenart A.: ”Selected properties of starch-whey protein films” – III Symposium of Food Engineering, 26-27 June 2012, Warsaw, Poland.

Basiak E., Debeaufort F., Lenart A.: ”Goniometry and barrier properties of selected aroma compounds of starch-whey protein films” – Session of Committee the Food Sciences, 1-3 July, Cracow, Poland.

Basiak E., Debeaufort F., Lenart A.: “Physical properties of whey protein films”- 10 International Congress of Protein Stabilisation, 7-9 May 2014, Stressa, Italy.

Basiak E., Debeaufort F., Lenart A.: “Relationship between surface properties and interfacial of starch – oil laminate based films” – XIX Session of Committee the Food Sciences, 7-9 May 2014, Warsaw, Poland.

Basiak E., Debeaufort F., Lenart A., Voilley A.: “Water properties of starch based films” - VIII Eurofood Water Conference, 25-27 May 2014, Timişoara, Romania.

Basiak E., Debeaufort F., Lenart A.: “Relation between moisture and mechanical properties of starch – oil laminate films” - Forum of Young Researchers, 23-24 June 2014, Besançon, France.

Basiak E., Debeaufort F., Lenart A.: “Moisture transfers at interfaces of starch-based edible films” - 3rd International Meeting on Material/Bioproduct Interaction, 17-19 June 2015, Saragossa, Spain.

Basiak E., Debeaufort F., Linke M., Lenart A.: "Impact of coating or wrapping with starch-whey protein films on quality of plums" - 3rd International Meeting on Material/Bioproduct Interaction, 17-19 June 2015, Saragossa, Spain.

Basiak E., Debeaufort F., Linke M., Lenart A.: "Quality improvement an extension of the shelf life of Japanese plum (*Prunus salicina Angeleno*) by starch-based films and coatings" - 3rd International Meeting on Material/Bioproduct Interaction, 17-19 June 2015, Saragossa, Spain.

Basiak E., Debeaufort F., Linke M., Lenart A.: "Laminates or emulsions? Edible starch films with oil addition" - Innovations in Food Packaging, Shelf Life and Food Safety, 15-17 September 2015, Stadthalle Erding, Munich, Germany.

Basiak E., Debeaufort F., Linke M., Lenart A.: "Moisture transfers at interfaces of starch-whey protein edible films" Innovations in Food Packaging, Shelf Life and Food Safety, 15-17 September 2015, Stadthalle Erding, Munich, Germany.

COURSES ATTENDED AND INTERNSHIPS:

Russia, Russian State Agricultural University in Moscow (Moscow Timiryazev Agricultural Academy), 17 February – 31 August 2010.

France, Laberca, Nantes, Intensive course of chromatography (IPARAF Intensive Programme for Advanced Residue Analysis in Food), 26 March – 6 April 2012.

France, University of Burgundy/AgroSup Dijon, 28 January – 6 May 2013 and 15 August 2013 - 22 June 2014.

Germany, Humboldt University in Berlin, Career Day, co-organizer, 25-26 February 2014.

France, University of Burgundy/AgroSup Dijon, Intensive course on proteins (IPARAF Intensive Programme for Advanced Residue Analysis in Food), 23 June - 4 July 2014.

Italy, University of Biccocca, Summer School on Industrial Biotechnology: Stability, folding and misfolding of recombinant proteins, 6-10 October 2014.

Canada, University of Alberta, Business School, Edmonton, Training for coach competences: soft skills development and ability to cooperate with the business sector. The 1st prize won in the competition of Polish Ministry of Science and Higher Education. 8 – 29 November 2015.

Germany, Leibnitz Institute for Agricultural Engineering Potsdam-Bornim, training, 5 July 2014 - 15 July 2016.

GRANTS:

Grant for own project contains fruit and vegetable covering by starch-based coatings. **Warsaw University of Life Sciences**, May 2014.

Science grant of **Polish Federation of Food Producers** (grant w ramach programu grantów naukowych Polskiej Federacji Producentów Żywności Związku Pracodawców) – the 1st place, May 2015.

COURSES (TEACHING ACTIVITIES):

125 hours realized on Faculty of Food Sciences, Faculty of Civil and Environmental Engineering, Faculty of Science of Commodities.

- Technical drawing with the elements of machines theory
- Physical properties of food
- Modern technologies in food processing industry
- Theory of machines in food processing industry
- Ecology, energy and environmental protection management
- Textural properties of food products

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Meaning and unit
A	film area or fruit surface [m^2]
A_{600}	absorbation at wavelength 600nm [A mm^{-1}]
a_w	water activity
a	colour parameter/chromaticity coordinates: greenness – redness
b	colour parameter/chromaticity coordinates: blueness – yellowness
C	GAB parameter constants related to the sorption enthalpy of the first subsequent layers
C_{colour}	chroma parameter
C_0	initial concentration of diffusing substance [kg m^{-3}]
C_{max}	maximum concentration of diffusing substance [kg m^{-3}]
C_t	concentration at t time [kg m^{-3}]
C_∞	concentration at infinite time, *ie* at equilibrium time [kg m^{-3}]
CS	corn starch
D	diffusion coefficient [$m.s^{-2}$]
D_{app}	apparent diffusivity [$m.s^{-2}$]
DSC	differential scanning calorimetry [^{o}]
e	thickness [m]
E	elongation [%]
FM	fruit mass [g]
FTIR	Fourier transform infrared with attenuated total reflection
g	constant parameter of equation of absorbed moisture contentconstant parameter of
h	equation of absorbed moisture content
j	constant parameter of equation of absorbed moisture content
K	GAB parameter constants related to the sorption enthalpy of the subsequent layers
L	lightness
M	weight of plum [g]
MCF	mass of coated plum [g]
Me	mass of the coating material [g]

Mi	initial weight of plum [kg]
Mo	GAB parameter the water related to the monolayer [$g.g^{-1}$ dry basis]
M_0	amount of water vapour sorbed at zero time [g]
M_t	amount of water vapour sorbed at time t [g]
M_w	mass of water inside coating [g]
M_∞	maximum amount of water sorbed at equilibrium (theoretical infinite time) [g]
MRE	mean relative error [%]
m	mass of film forming solution [g]
n	number of iterations in Crank model equations
OP	oxygen permeability [$cm^3.m^{-1}s^{-1}Pa^{-1}$]
PS	potato starch
p	barometric pressure [bar]
RH	relative humidity [%]
R^2	determination coefficient
R_{CO2}	respiration rate of carbon dioxide [mL/kg h]
R_{O2}	respiration rate of oxygen [mL/kg h]
rB	boundary layer resistance in the water vapour pathway [$s\ cm^{-1}$]
rC	coating resistance in the water vapour pathway [$s\ cm^{-1}$]
RR	tissue resistance in the water vapour pathway [$s\ cm^{-1}$]
rT	respiration rate [mL/kg h]
S	wheat starch
Si	saturation index
SI	swelling index [%]
t	time [s]
t_i	initial tilme [s]
TA	titratable acidity
Tg	glass transition temperature [^{o}]
TGA	thermo-gravimetric analysis
TL	air temperature [^{o}]
TR_m	transpiration rate per unit of initial mass of the produce [$g\ kg^{-1}\ h^{-1}$]
TR_s	transpiration rate per unit surface area of the produce [$mg\ cm^{-2}\ h^{-1}$]
TS	tensile strength [MPa]
TSS	total soluble solids [%]
u	water content [$g_{water}\ g.^{-1}$ d.m]

Vc	volume of the coating onto fruit surface [cm^3]
V	volume of film-forming solution [cm^3]
V_f	empty volume inside the chamber [mL]
W	total weight of the product [kg]
Wa	work of adhesion [$mJ.m^{-2}$]
Wc	work of cohesion [$mJ.m^{-2}$]
Wi	initial weight [g]
Ws	spreading coefficient [$mJ.m^{-2}$]
Wsp	weight at sampling period [g]
WPI	whey protein isolate
WVP	water vapour permeability [$g.m^{-1}s^{-1}Pa^{-1}$]
W%	relative water content [%]
x	distance [m]
xL	volumic water contentof the air unaffected by the product [$mg\ m^{-3}$]
xP	volumic water content of air in the intercellular spaces in the center of the product [$mg\ m^{-3}$]
xPS	volumic water content of the air at the product surface [$mg\ m^{-3}$]
YM	Young modulus [MPa]
Y_{CO2}	concentration CO_2[%] at time τ (h)
Y^{t}_{CO2}	concentration CO_2 [%] at the initial time τi (h)
Y_{O2}	concentration O_2[%] at time τ (h)
Y^{t}_{O2}	concentration O_2 [%] at initial time τi (h)
γ_{C}	critical surface tension of film surface [$N.m^{-1}$]
γ_{LV}	surface tension at the liquid-vapour interface [$N.m^{-1}$]
γ_{SL}	surface tension at the solid-liquid interface [$N.m^{-1}$]
γ_{SV}	surface tension at the solid-vapour interface[$N.m^{-1}$]
	surface tension of the film surface [$N.m^{-1}$]
γ_{S}	dispersive component of the surface tension of liquid [$N.m^{-1}$]
γ^{D}_{L}	polar component of the surface tension of liquid [$N.m^{-1}$]
γ^{P}_{L}	dispersive component of the surface tension of film [$N.m^{-1}$]
γ^{D}_{S}	polar component of the surface tension of film [$N.m^{-1}$]
γ^{P}_{S}	total colour difference
ΔE	water vapour pressure differential between the two sides of the film [Pa]
	density of film-forming solution [$g\ cm^{-3}$}

Δp	contact angle [º]
ρ	films containing 3g of wheat starch in 100 mL of water with oil addition
Θ	films containing 5g of wheat starch in 100 mL of water with oil addition
3L	films containing 3g of wheat starch in 100 mL of water
5L	films containing 5g of wheat starch in 100 mL of water
3S	
5S	

INTRODUCTION

Since the dawn of history people have to be able to deal with the storage of food. However, methods such as freezing or drying were not possible for using in all places in the World. Carriage was also not so commode. Moreover, frequently some foods spoil quickly, resulting in numerous deaths occurred. Appearance of the first packaging was a milestone in the food storage. But the real revolution came in the early of the twentieth century. With the discovery of polyethylene the entire logistics chain production, transportation and storage of food gained. Plastic packaging were not only convenient, functional but either not expensive. So many advantages of them decided about replacing the other packaging material by plastic. Even so popular wood baskets used for consumers on bazars were replaced by this one made from plastic and by plastic bags. Annually one person in Europe consumes 450 plastic bags. 500 thousands ton is thus produced in the world. Moreover, every year people generate 250 kg of waste per person. Part of produced packages goes on fulfills, part of them goes to the water and kills marine animals or it causes their diseases. This has enough negative consequences: sick animals are eaten by others, and those by the people, either directly by the people (depending from the order in food chain) and this causes many disorders as increases of xenoestrogens (disorder of endocrine) or rise of food prices. However, production of packaging gives 80 million tons of plastic waste per year. Indeed, in the twenty-first century even lettuce, oranges, broccoli, biscuits or rolls are individually packaged in foils. With such their habits and consumption trends, serious consequences would occur due to the amount of wastes increase and because the area of earth still is finite and reducing. Furthermore oil-based packages need up to 1000 years for degradation/decomposition. According to data reported by UE in 2040, Earth will have 10 billion of people. It means the sources of petroleum will be exhausted and the Earth and its citizens will require maximum landfills area for food production. An excellent solution for these problems can be useing of biodegradable or edible packages. They can be produce from natural sources as plants and/ or animal products. One of the most abundantly polysaccharides is starch. It is a spare material of higher plants, available almost everywhere. Starch does not cause allergies; it is tasteless, odorless, is easy for printing and can play not only the function of packaging but also can be use as a tag. Moreover starch in form of edible coating can be modern and futuristic packaging. Proteins are hydrocolloids used for making bio-packages. They provide valuable nutrition and in combination with the starch can reduce high water vapour permeability. Either oil addition causes the significant reducing of high permeability properties, especially for water vapour, which is the main drawback of starch. According to Kyoto Protocol in 1997, using biodegradable materials has also two more impacts: the lower production of carbon dioxide and/or possibility to sell the higher limitation of this gas to the other countries. Biopolymers have influence on the environment and directly onto the food. Nowadays the speed of life is 2.5 times higher than several years ago; people thus expect convenient and minimally processed food. To reach these aims, coatings and films can be envisaged.

The range of application of biodegradable materials in the food industry is very wide. Films made from starch with glycerol addition should have not worse than plastic mechanical properties. The high mechanical properties must be maintened. Tensile strength of produced films should be aroud 3.5 MPa, which is the requirement for plastic materials. Then starch polymers can be easily used for carriage food stuff, as bags for shopping, treys for meat, fish, cheese, fruits etc. Below this value, starch-based films can be used as foils or coatings. Thus measuring and calculations of elongation, Young modulus, and tensile strength are mandatory. Wetability properties of starch films are the main element of hydrophilicity. They are directly related to adsorption of water, which in case of food product should be relatively low. Hence, moisture sorption isotherms, water vapour permeability, solubility in water, swelling index, kinetic, diffusion and related surface properties as contact angle, critical surface tension, work of adhesion, work of cohesion etc. should be measured either. Moisture exchange between product and ambient environment may cause modifications in food stuff and film properties.The knowledge of the moisture sorption isotherms of bio-based films and food water activity plays an important role for predicting quality changes and shelf-life. For that, moisture sorption equations (GAB, BET) were determined. Goniometry displays wettability of film surface with direct contact with liquids like water. Critical surface tension informs about hydrophilic or hydrophobic character and also influences film performances. Beyond moisture properties optical parameters also involved on functional qualities. Consumers buy with their eyes. Hence, measurements colour parameters play such an important role. It is not only encourageing the buying, but also its discouragement. Depending on ageing, sensory characterisics like colour aspects, odour may change. Physical ageing can cause also delamination, phase separation, migration of glycerol from biopolymer and lead to packaging with undesirable properties. It can negatively influences on the customers.

The choice of the adequate and optimized biodegradable packaging or coating can significantly reduce the amount of plastic products as bags. The next advantage of these materials is to prolongate the shelf-life of seasonal fruits and vegetables for instance.

Objectives of the work

The objective of the PhD work was to find appropriate material, concentration, amount of plasticizer of starch-based films and coatings for an application in the food industry (fruits and vegetables preservation). To reach this final target, the understanding of interactions between components, the physico-chemical properties of films, the interfacial properties of both films and coatings related to fruit skin, the structure of films, the mechanisms of mass transfers must be studied and understand.

Fifteen kinds of films were prepared, all based on starch, in combination or not with protein or lipids. Physical, chemical and functional properties, including geometrical, sorption, optical, mechanical, thermal, structural, wettability and surface characterisations were conducted. After that and from that, few recipes of films were selected to be tested on real food stuff (plums) as coatings and film wrappings. Physiological, chemical and physical tests were done on the plums according storage time. Development of new methods to assess the coating thickness, weight loss in fruits and vegetables, and related mathematical equations were set up.

This thesis is organized in 3 main sections divided in 6 chapters.

Chapter 1 (section 1) gives an overall and detailed survey of the literature on bio-based materials, in particular starch as novelty film-forming material. It is composed of 4 publications.

Materials and methods section is the compilation of materials and methods sections from all the publications constituting the results and discussion section and are presented in *chapter 2*.

The results and discussion section is composed of 9 publications accepted or submitted: the firsts dealing with the physico-chemical properties of films, and the last ones concerns the application on real food products.

The *chapter 3* contains the study of several starches, and at the end the selection of the most appropriate for combination with other film constituents and for the final application. Then, in the same chapter the content of glycerol effects were analyzed to find the most suitable concentration of plasticizer and polarity for good covering of plum surface are included. *Chapter 4* is focused on the mixture of whey protein and starch and their impact on structure, functional and surface properties of films. *Chapter 5* deals with the influence of oil incorporation on film physical-chemical properties and structure. *Chapter 6* is related to applications of coatings on plums or packaging (wrapping) of plums in films. Surface properties, physiology parameters, shelf-life prolongation of plums were analyzed as well as the bahaviour of coated of packaged fruits.

Finally, overall conclusions and perspectives are presented before the list of references.

SECTION ONE: STATE OF ARTS

1. Chapter 1: STATE OF ARTS

1.1. Starch films and coatings for food (Basiak E., Galus S., Lenart A. (2012). Filmy i powłoki skrobiowe do żywności. Przemysł Spożywczy, 66, 28-30. Starch films and coatings for food. Food Industry, 66, 28-30).

1.1.1. Abstract

Nowadays, when amount of people are increasing and natural resources are decreasing, edible and/or biodegradable films and coatings from starch became one of the solution in food packaging. Starch occupies a leading position in production of coatings and films because it has unique properties as global availability, attractive price, biodegradability. Also it has attractive sensory properties (tasteless, odorless), very good physical properties like barrier properties (impermeable for gases, and after added hydrophilic component also can be a barrier for water), optical (translucent or possibility to dye) and rheological (consistency, viscosity). Films and coatings of starch are usually made by casting. Starch polymers could be used without any restriction and interest of them on global market every year has increased. In this article starch raw materials formation of edible films or coatings and application in the food industry are characterized.

1.1.1. Abstrakt

W związku z rosnącą liczbą ludności i zmniejszającymi się zasobami naturalnymi na świecie jednym z rozwiązań w opakowalnictwie żywności są jadalne i biodegradowalne filmy oraz powłoki wykonane ze skrobi. Dzięki dużej dostępności, relatywnie niskiej cenie, skrobia zajmuje jedną z głównych pozycji w wytwarzaniu opakowań biodegradowalnych do żywności. Ma ona atrakcyjne właściwości sensoryczne (brak smaku, zapachu), bardzo dobre właściwości fizyczne, tj. barierowość (niska przepuszczalność gazów, a po dodaniu składników hydrofobowych także i wody), optyczne (przezroczystość lub możliwość nadawania pożądanej barwy) i reologiczne. Filmy i powłoki skrobiowe są najczęściej wytwarzane techniką wylewania. Biopolimery skrobiowe mogą być stosowane praktycznie bez żadnych ograniczeń, a zainteresowanie nimi na rynkach światowych z roku na rok jest coraz większe. W artykule scharakteryzowano surowce skrobiowe, tworzenie jadalnych filmów i powłok oraz ich zastosowanie w przemyśle spożywczym.

1.1.2. Wstęp

Jedną ze składowych ceny produktu jest koszt wytworzenia, magazynowania i recyklingu opakowania. W celu zmniejszenia kosztów i obniżenia masy produkowanych odpadów, coraz częściej stosuje się jadalne i biodegradowalne powłoki do żywności. Są one wykonane z różnych rodzajów skrobi, mają wiele zalet, jak dostępność, niska cena (wielokrotnie tańsze niż z polietylenu), dobre właściwości mechaniczne, sorpcyjne, optyczne, a w połączeniu z woskami i tłuszczami - także dobre właściwości barierowe. Dobór rodzaju skrobi zależy od właściwości surowca bądź produktu, na który nanoszona jest powłoka. Zastosowania jadalnych powłok skrobiowych z każdym rokiem jest coraz większe (Ghanbarzadeh, Almasi & Entezami,2010; Jiménez i in., 2012).

Rosnący popyt na żywność o wysokich cechach jakościowych, bezpieczną i taką, którą można szybko przygotować oraz o długim terminie przydatności do spożycia powoduje dynamiczny rozwój nowych technologii jej wytwarzania (Kokoszka & Lenart, 2007). W procesie tym nie bez znaczenia jest opakowanie. Jedną z najważniejszych funkcji, jakie pełni, jest ochrona przed niepożądanym działaniem środowiska zewnętrznego (pary wodnej, tlenu, światła, promieni UV), aromatów, barwników, ochrona surowca lub produktu przed migracją globalną bądź migracją tylko niektórych substancji (Mieliwodzka, 2007; Mieliwodzka, 2006). Oprócz tego współczesne opakowanie pełni wiele innych funkcji (m.in. marketingowo-promocyjną, dystrybucyjno-logistyczną, ekonomiczną, ekologiczną). Coraz częściej koszt opakowania jest znaczący w porównaniu z kosztami zapakowanych produktów (Ziemnicka-Niewczas, 2007). Z każdym rokiem produkuje się coraz więcej opakowań. Rozwiązaniem problemu nadmiernej liczby opakowań i odpadów stała się produkcja jadalnych i biodegradowalnych filmów i powłok. Są to cienkie warstwy materiału formowane na produkcie (powłoki) lub poza nim (filmy). Mają wiele zalet, takich jak funkcja ochronna, wysokie bezpieczeństwo i jakość, a przede wszystkim redukcja masy wytwarzanych opakowań i odpadów opakowaniowych (Chen, Kuo & Lai, 2009).

Jadalne filmy i powłoki mogą być produkowane z białek, węglowodanów, tłuszczów i z ich połączeń. Najczęściej stosowanymi biopolimerami węglowodanowymi do wytwarzania filmów i powłok jest skrobia, alginiany, celuloza, pektyny i żywice roślinne (Kokoszka & Lenart, 2007). Szczególnym zainteresowaniem wśród producentów żywności cieszy się skrobia. Jest łatwa w przetwarzaniu i użyciu, biodegradowalna, dostępna i tania, ma korzystne właściwości fizyczne i sensoryczne. Te właściwości skrobi, które zdają się być wadami, po niewielkiej modyfikacji zamieniają się w zalety, np. właściwości barierowe. Już niewielki dodatek tłuszczów do skrobi zapobiega przepuszczalności wody - filmy i powłoki skrobiowe zmieniają swoją naturę z hydrofilowej na hydrofobową (Chen, Kuo & Lai, 2009; Jiménez, 2012; Yan i in., 2012).

1.1.3. Surowce skrobiowe

Właściwości skrobi zależą m.in. od stosunku dwóch polimerów: amylozy i amylopektyny, z których składa się cząsteczka skrobi. Amyloza jest polimerem nierozgałęzionym, zbudowanym jedynie z łańcuchów liniowych połączonych wiązaniami α-1,4-glikozydowymi. Amylopektyna różni się od amylozy tym, że dominują w jej rozgałęzionej strukturze wiązania α-1,6-glikozydowe, połączenia liniowe α-1,4 są zaś rzadkie. Stosunek amylozy do amylopektyny jest zróżnicowany w zależności od gatunku botanicznego (Singh, Belton & Georget, 2009; Yuryev, Tomasik & Bertoft, 2007).

Skrobia jest głównym materiałem zapasowym roślin wyższych dostępnym niemalże we wszystkich strefach klimatycznych. Pozyskuje się ją najczęściej z bulw (np. ziemniaka, manioka jadalnego), korzeni (np. batata), kłączy (np. tara, pochrzyna), łodyg (np. sagowców), owoców (np. chlebowca, banana, kasztana) i ziaren (np. kukurydzy, pszenicy) (Torres i in., 2011). Oprócz skrobi wyizolowanej w postaci naturalnej wyróżnia się skrobię modyfikowaną i natywną. Skrobię modyfikowaną otrzymuje się najczęściej w wyniku modyfikacji chemicznej, enzymatycznej lub fizycznej. Obecnie otrzymuje się również skrobię o zmodyfikowanych właściwościach - przez zmianę układu genetycznego roślin produkujących skrobię. W wyniku oczyszczenia skrobi naturalnych usuwa się część białek oraz tłuszczów i otrzymuje się skrobię natywną. Źródłami skrobi natywnej i modyfikowanej są - tak jak w przypadku skrobi naturalnej - surowce roślinne (Liu, 2005). W przemyśle spożywczym najczęściej stosuje się skrobię kukurydzianą, ziemniaczaną i pszenną. Rodzaj surowca ma wpływ na skład, wielkość i kształt ziaren skrobi, co przekłada się na zastosowanie biopolimeru w przemyśle (Torres i in., 2011).

1.1.4. Tworzenie

Filmy i powłoki skrobiowe utworzone jedynie z biopolimeru oraz wody są kruche i łamliwe. W celu poprawienia ich elastyczności i grubości, do struktury skrobiowej jest dodawany plastyfikator, tj. alkohol polihydroksylowy (jeden lub więcej) oraz woda. Niekiedy wprowadza się dodatek surfaktantów lub innych biopolimerów - tłuszczów i białek. Dodatek tych substancji sprawia, że otrzymane filmy i powłoki mają ciągłą strukturę o pożądanych właściwościach mechanicznych, sorpcyjnych, barierowych, optycznych i sensorycznych (Jiménez i in., 2012; Yan i in., 2012).

Istnieje kilka sposobów, za pomocą których można utworzyć filmy i powłoki skrobiowe. Do najpopularniejszych metod należy wylewanie roztworu powłokotwórczego (Kokoszka & Lenart, 2007). W tym procesie, w wyniku fizykochemicznego oddziaływania międzycząsteczkowego tworzy się i stabilizuje ciągła struktura powłoki. Makrocząsteczki w roztworze powłokotwórczym zostają rozpuszczone w rozpuszczalniku, takim jak woda, etanol czy kwas octowy oraz połączone z ewentualnymi dodatkami. Następnie roztwór powłokotwórczy jest wylewany w postaci cienkiej warstwy, suszony i zdejmowany z powierzchni (Cagri, Ustunol & Ryser, 2004).

Müller i wsp. (2009) wytwarzali filmy z trzyprocentowego wodnego roztworu skrobi z manioka jadalnego z dodatkiem glicerolu jako plastyfikatora (0,30 g/g skrobi), włókien celulozy (od 0 do 0,5 g celulozy) i gumy guar (0,01 g/g skrobi). Roztwory wylewano na płaskie powierzchnie i suszono w temp. 40°C przez 16 h w komorze z cyrkulacją powietrza. Zawartość wody w filmach wynosiła 0,098-0,120 g wody/g suchej substancji. Powłoki były kondycjonowane w temp. 25°C, w higrostatach o wilgotności 58 i 75%. Muscat i wsp. (2012) przygotowywali powłoki przez mieszanie roztworu skrobi kukurydzianej: 20 ml skrobi o niskiej zawartości amylozy i 10 ml roztworu o wysokiej zawartości. Roztwory wylewano na plastikowe płytki o średnicy 90 mm. Filmy suszono przez 12 h w temp. 25 ±1°C w komorze suszarniczej z cyrkulacją powietrza. Tak wytworzone powłoki przed analizą były przechowywane przez 48 h w temp. 25 ±1°C i wilgotności względnej 52,9%.

Innymi metodami, za pomocą których można uzyskać powłoki skrobiowe, są: ekstruzja, powlekanie, wtryskiwanie, wydmuchiwanie i termiczne formowanie (Fu i in., 2011).

1.1.5. Zastosowanie

W związku z rosnącym zainteresowaniem żywnością jak najmniej przetworzoną, o wysokiej jakości i świeżą, jadalne i biodegradowalne filmy oraz powłoki znalazły zastosowanie w różnych gałęziach przemysłu spożywczego, m.in. w cukiernictwie, piekarnictwie, w przemyśle mięsnym i owocowo-warzywnym. Dzięki badaniom naukowym okazuje się, że różne kombinacje filmów i powłok, sposoby wytwarzania i nanoszenia, dodatki aromatów, barwników, antyoksydantów, tłuszczów itp. mogą znacznie rozszerzyć możliwości ich użycia i zastosowania w przemyśle spożywczym (Liu, 2005).

Filmy z wysokoamylozowych skrobi grubości 0,0015-0,05 mm były stosowane do pokrywania produktów piekarniczych w celu przedłużenia ich świeżości, do oddzielnego pakowania składników dań garmażeryjnych przed ich uwodnieniem, a także do przechowywania mrożonego drobiu, ryb i mięsa (Tederko, 1995).

Ribeiro i wsp. (2007) wykorzystywali powłoki skrobiowe o zawartości ok. 30% amylozy, sorbitolu, glicerolu, κ-karaginianu i surfaktanta Tween 80 do pokrywania truskawek. W wyniku dodania chlorku wapnia i chitozanu po sześciu dniach przechowywania barwa truskawek nie zmieniła się. Utrata jędrności i masy owoców była minimalna. Także dodatek jednoprocentowego di-uwodnionego chlorku wapnia do powłok spowodował zmniejszenie szybkości wzrostu mikroorganizmów na owocach. Minimalny wskaźnik wzrostu mikroorganizmów uzyskano dla truskawek powleczonych chitozanem i chlorkiem wapnia.

Hydrolizaty skrobi (dekstryny) mają gorsze właściwości barierowe dla pary wodnej niż skrobia, ale są lepszą barierą dla tlenu. Stosowano je do pokrywania takich produktów, jak orzechy i migdały, aby zachować dobre cechy organoleptyczne i odpowiednią teksturę podczas przechowywania (Tederko, 1995).

Ogonek i Lenart (2002) określili wpływ powłok z niskometylowanej pektyny i skrobi ziemniaczanej na proces odwadniania osmotycznego. Stwierdzili oni zależność ubytków wody i przyrostu masy suchej substancji od rodzaju powłoki. Ogólnie wartości tych wskaźników były niższe w truskawkach powleczonych niż w tych bez powłoki. Najlepsze właściwości barierowe wykazywała powłoka ze skrobi.

1.1.6. Summary (short translation of the polish publication)

One of the components of the product price is the cost of producing, storing and recycling. In order to reduce of costs and weight of trash, edible and biodegradable coatings are used. Made from different types of starch, biomaterials have many advantages such as availability, low price (many times cheaper than polyethylene), good mechanical properties, sorption, optical, and combined with waxes and fats – and also good barrier properties. The choice of starch depends on the raw material or product on which the coating is applied. Every year the demand on edible materials is increasing, because demand on food with high quality, minimally processed and safe increasing either. Rapid development of new technologies for its production causes growing up value of the packages. Nowadays, packaging should discharge a lot of expectations, so finally belching on the price out. It happens increasingly often the cost of the package is higher than that packed in product. Production of thin material layers onto a product (coating), and beyond it, seems to be an excellent solution of economy and environmental problems.

Edible films and coatings can be produced from proteins, carbohydrates, fats, and their combinations. The most commonly used carbohydrate biopolymers for the production of films and coatings are starch, alginates, cellulose, pectin and resins (Kokoszka & Lenart, 2007). Due to many advantages, starch is appreciated by food producers. Properties of this polysaccharide depend on amylose/amylopectine ratio, and thus the species of botanical plants from which it is sourced (Singh, Belton & Georget, 2009; Yuryev, Tomasik & Bertoft, 2007). Starch is the main spare material of higher plants, available in almost all climates. Starch can be used as modified or native forms. A modified starch is obtained usually by chemical, enzymatic or physical modification. Purification of the natural starch caused removing some proteins and fats to obtain a native starch (Liu, 2005). In the food industry the most commonly used are corn, potato and wheat starches. Type of raw materials has an influence on the composition, size and shape of the starch granules, which translates into the use of biopolymer in the industry (Torres et al., 2011).

Films and coatings made only from starch and water are brittle. In order to improve the flexibility and thickness, plasticizer is added to film-forming solution. Sometimes surfactants or other biopolymers - fats and proteins are incorporated too. The addition of these substances allows to form a continuous structure with desirable mechanical, sorption, barrier, optical and sensory properties (Jiménez, et al., 2012; Yan et al., 2012). Films and coatings are produced by several ways. The most popular is a casting method (Cagri, Ustunol & Ryser, 2004). The other methods belong to extrusion, coating, injection molding, blow molding and heat molding (Fu et al., 2011).

Due to the growing interest of minimally processed, high quality and fresh food, edible and biodegradable films and coatings can be applied in various sectors of the food industry, including: confectionery, bakery, meat industry, fruits and vegetables (Liu, 2005).

1.2. Starch like component of edible coatings (Basiak E., Lenart A. (2012). Skrobia jako składnik powłok jadalnych, Postępy Techniki Przetwórstwa Spożywczego, 22, 2, 84-88. Starch like component of edible coatings. Development of Technic and Food Industry, 22, 2, 84-88).

1.2.1. Abstract

Polyethylene and its copolymers were discovered at the end of nineteenth century. Several decades later, when they were used for food packaging, they became world widely known. The edible films and food coatings made from starch, show potential to become as popular as copolymers. This is due to many advantages they possess. The coatings formed from starch are tasteless, odorless and they do not cause allergy. Moreover they are cheap to manufacture, comprehensively available and biodegradable. This all means that they can be used for extended spectrum of food coatings. Starch coatings and films can be used for example as raw materials. It results in chemical, physical or genetic modifications of starch and easiness to their retrieve. There are lots of possibilities that are helpful in obtaining edible and/or biodegradable packaging from starch. One of most popular techniques of acquiring is casting. Application on an output consists of spraying, immersing or coating one or several layers. Waxes, flavours, colours, which include starch, are usually more attractive for customers as well as more environmentally friendly.

1.2.1. Abstrakt

Odkryty pod koniec XIX wieku polietylen i jego kopolimery kilkadziesiąt lat później, jako opakowania do żywności, odniosły globalny sukces. Podobnie zapowiada się z jadalnymi filmami i powłokami do żywności ze skrobi. Wynika to z wielu zalet, które posiadają. Powłoki utworzone ze skrobi są bez smaku i bez zapachu, niealergenne, tanie, szeroko dostępne i biodegradowalne, a to wszystko przekłada się na szerokie spektrum w ich wykorzystaniu do powlekania żywności. Powłoki i filmy skrobiowe stosuje się m.in. do produktów nieprzetworzonych. Wynika to z możliwości chemicznej, fizycznej bądź genetycznej modyfikacji skrobi i łatwości w jej otrzymaniu. Istnieje wiele metod, za pomocą których można uzyskać jadalne i/lub biodegradowalne opakowania ze skrobi. Do najpopularniejszych należy technika wylewania. Aplikacja na produkt odbywa się poprzez zanurzenie, spryskanie lub nanoszenie w postaci jednej lub kilku warstw. Dodatek do struktury filmów i powłok substancji takich jak: woski, aromaty i barwniki powoduje, że produkty spożywcze są atrakcyjne dla konsumenta i dla środowiska naturalnego.

1.2.2. Wstęp

Jak szacuje Unia Europejska liczba ludności na Świecie do roku 2040 wzrośnie do 10 miliardów, to jest w porównaniu z rokiem 1990 dwukrotnie. W związku z tym zapotrzebowanie na energię i surowce organiczne także wzrośnie (nawet trzykrotnie). W takiej sytuacji popyt na materiały roślinne będzie większy niż podaż, gdyż liczba terenów rolnych jest ograniczona, a dodatkowo ich ilość z każdym rokiem maleje. Podobna sytuacja ma miejsce w przypadku wyczerpywalnych surowców kopalnianych. Podczas ich pozyskiwania dochodzi do negatywnych skutków ekologicznych, co odbija się na cenie ropy naftowej, gazu i węgla. Aby zapobiec tym narastającym problemom gospodarczym coraz częściej stosuje się szeroko dostępne, relatywnie niedrogie, biodegradowalne i odnawialne surowce polisacharydowe takie, jak skrobia. Dzięki swoim unikalnym właściwościom jest ona najczęściej wykorzystywanym polisacharydem na kuli ziemskiej oraz pretendentem na materiał przyszłościowy XXI wieku (Tomasik, 2000).

1.2.3. Charakterystyka skrobi

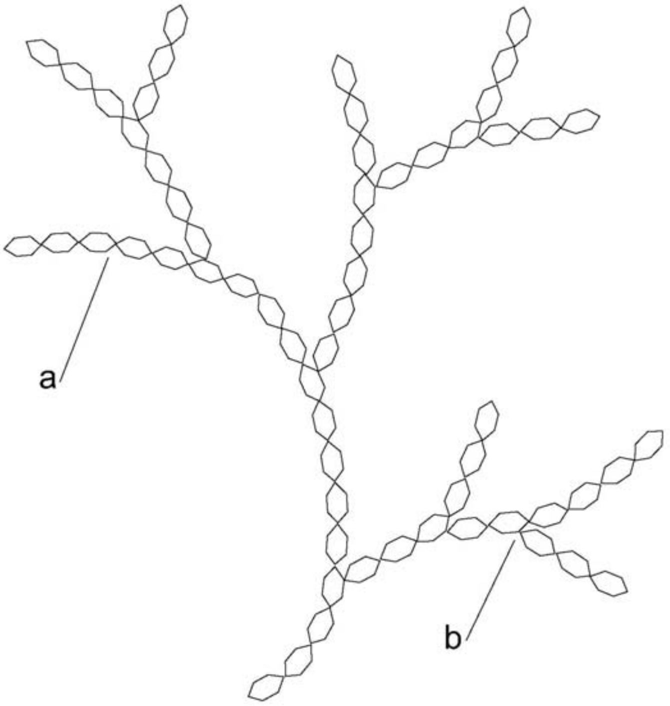
Skrobia jest najbardziej dostępnym surowcem roślinnym (tabela 1.1) i pełni w roślinach funkcję materiału zapasowego. Jest najczęściej używanym hydrokoloidem, ponieważ ma niską cenę, wiele korzystnych właściwości funkcjonalnych, może być w formie zwykłej, modyfikowanej lub natywnej.

W tabeli 1.1 przedstawiono przykładowe surowce skrobiowe i ich występowanie na świecie.

Tabela 1.1 Występowanie skrobi różnego pochodzenia (Tomasik, 2000). Table 1.1 Presence of starch from various origins

Surowiec skrobiowy	Występowanie
jęczmień, kukurydza, pszenica, ziemniaki	Ameryka Północna
banany, kukurydza	Ameryka Południowa
banany, sago, tapiok	Afryka
jęczmień, owies, pszenica, ziemniaki, żyto	Europa
ryż, kukurydza	Azja
kukurydza, palma	Australia

Zboża, nasiona warzyw, bulwy i owoce zawierają w suchej masie od 30 do 85% skrobi (Baldwin, Hagenmaier & Bai, 2012; Embuscado & Huber, 2009). Np. ziarno ryżu w swym składzie zawiera 1-3% tłuszczu, 8% białek i 80% skrobi (Lin i in., 2011). Skrobia to połączenie dwóch frakcji polisacharydowych: amylozy i amylopektyny (rys. 1). Obie frakcje zawierają cząsteczki glukozy, ale innego rozmiaru i kształtu. Amyloza jest frakcją mniejszą i jest definiowana jako linearna molekuła jednostek (1→4)-α-D-glukopiranozy, której stopień polimeryzacji jest poniżej 500. Zawiera w swym składzie niewiele rozgałęzionych łańcuchów (1→6)-α. Amylopektyna jest zaś cięższą, rozgałęzioną makromolekułą z wiązaniami (1→6)-α i (1→4)-α-D-glukozy. Zawartość amylozy w skrobiach jest rzędu 18-30%, z wyjątkiem skrobi z kukurydzy woskowej, która składa się w pełni z amylopektyny (Baldwin, Hagenmaier & Bai, 2012; Hoover, Hughes & Liu, 2010). Filmy i powłoki mogą być utworzone z każdego rodzaju skrobi zawierającej amylozę, gdyż linearna budowa ma większy wpływ na tworzenie połączeń między molekułami niż rozgałęziona struktura (Embuscado & Huber, 2009).



Rysunek 1.1 Struktura skrobi: a) amyloza, b) amylopektyna

Figure 1.1 Structure of starch: a) amylose, b) amylopectin

W zależności od rozmiaru, ziarna skrobi mogą być pogrupowane w 4 klasy wielkości:

- wielkie – ponad 25 µm np. skrobia ziemniaczana (41,5 µm) i pszenna (20,2 µm),
- średnie – od 10 do 25 µm np. skrobia z tapioki (15,6 µm), kukurydzy (13,8 µm),
- małe – od 5 do 10 µm np. skrobia z owsa, gryki, prosa ryżowego,
- bardzo małe – do 5 µm np. skrobia z amarantusa (3,0 µm), komosy ryżowej.

Oprócz tej klasyfikacji morfologicznej ziarna skrobi można uszeregować pod względem formy. Mogą mieć kształt owalny (eliptyczny), prostokątny, sferalny, eliptyczny, poligonalny, nieregularny, bądź kształt soczewki (Hoover, Hughes & Liu, 2010; Molenda i in., 2006).

Jak wykazał Molenda i wsp. (2006) cechy morfologiczne decydują o właściwościach mechanicznych polisacharydów. Zbadano pięć rodzajów skrobi: ziemniaczaną, pszenną, kukurydzianą, tapiokową i amarantusową. Wszystkie skrobie różniły się wielkością i kształtem cząstek. Wykazano, że wraz ze wzrostem wymiarów ziaren zwiększa się ściśliwość i sypkość materiałów. Skrobie o największych cząstkach (ziemniaczana i pszenna) wykazywały oscylacje przebiegów naprężenia od odkształcenia. Skrobia tapiokowa i kukurydziana posiadały ziarna o podobnej wielkości, stąd ich ściśliwość i krzywe naprężenia były do siebie bardzo zbliżone.

Najważniejszym źródłem skrobi na świecie jest kukurydza i jej udział stanowi 83% w całkowitej produkcji. Największym eksporterem, wytwarzającym ponad 60% skrobi są Stany Zjednoczone. Drugim, co do wielkości surowcem, z którego pozyskuje się skrobię jest pszenica. Mimo niewielkiego udziału w produkcji światowej (poniżej 7%), jest bardzo cenionym źródłem skrobi w Europie. Jest to spowodowane sprzyjającymi warunkami w uprawie. Kolejne miejsce zajmuje skrobia ziemniaczana – 6% i tapiokowa 4% (Liu, 2005; Molenda i in., 2006).

Ze względu na szereg zalet, które posiada skrobia (szeroko dostępna, odnawialna, będąca w stanie tworzyć ciągłą matrycę itd.), polimer ten jest jednym z najczęściej wykorzystywanych surowców do wytwarzania biodegradowalnych opakowań w postaci filmów i powłok. Oprócz tego charakteryzuje się dobrymi właściwościami geometrycznymi i optycznymi. Powłoki i filmy ze skrobi są łatwo formowane, o pożądanej grubości, zazwyczaj bezbarwne lub mlecznobiałe (np. ze skrobi ryżowej). Dzięki temu można je łatwo barwić lub zadrukowywać.

Transparentność błon może być wykorzystana również do uwydatniania świeżości owoców i warzyw. Pokrywanie jabłek filmami ze skrobi z dodatkiem wosków sprawia, że owoce są błyszczące i przyciągają uwagę konsumentów. Powłoki skrobiowe mają również dobre właściwości mechaniczne – dzięki dodatkowi plastyfikatora nie są kruche, nie łamią się i nie pękają. Do wad tego polisacharydu należy silny charakter hydrofilowy i słabe właściwości sorpcyjne (przede wszystkim duża przepuszczalność dla pary wodnej), tendencja do retrogradacji i słaba rozpuszczalność w wodzie. Dlatego skrobia jest modyfikowana (najczęściej metodami chemicznymi, fizycznymi, enzymatycznymi lub ich kombinacjami) bądź wytwarzana w postaci natywnej (Ghanbarzdeh, Almasi & Entezami, 2011; Guilbert, Gontard & Gorris, 1996; Jayakody i in., 2009; Lin i in., 2011; Singh, Kaur & McCarthy, 2007). Także modyfikacja genetyczna pozwala na uzyskanie produktu (bądź produktów) o zmienionych właściwościach lub produktu dopasowanego do specyficznych celów (Berski i in., 2011). Wśród właściwości fizykochemicznych najczęściej modyfikowana jest rozpuszczalność proszków skrobiowych w wodzie, temperatura żelowania, pęcznienie, właściwości lepkoplastyczne oraz temperatura zamrażania i rozmrażania skrobi (Nwokocha i in., 2009).

1.2.4. Modyfikacja chemiczna

W celu optymalnego wykorzystania skrobie są modyfikowane chemicznie. Uwydatnia to ich cechy lub daje możliwość na stworzenie produktów skrobiowych, które mają unikalne właściwości przydatne do różnego zastosowania. Najczęściej występujące kierunki modyfikacji chemicznej skrobi to:

- Stabilizowanie skrobi – proces polegający na wprowadzeniu do struktury skrobi podstawników tj. grupy estrowe (Krysińska, Gałkowska D & Fortuna, 2008; Pal, Singhal & Kulkarni, 2002). W rezultacie otrzymane skrobie są znacznie odporniejsze na retrogradację i synerezę podczas chłodzenia, zamrażania i rozmrażania kleików, mają obniżoną temperaturę kleikowania i zwiększoną lepkość kleików. Ponadto charakteryzują się ich dużą przejrzystością.
- Sieciowanie skrobi, czyli wprowadzenie do struktury chemicznej skrobi dodatkowych wiązań z innych reagentów. Ma ono na celu usztywnienie struktury polisacharydu. Wiele typów skrobi modyfikowanych jest produkowanych za pomocą sieciowania przy użyciu tlenku fosforu w celu zapobieżenia fragmentacji cząsteczek skrobi. Powstałe wiązania chemiczne wzmacniają cząsteczki budujące strukturę skrobi

natywnej, które z kolei zapobiegają przedwczesnemu pęcznieniu i niepożądanemu zniszczeniu granulek.

- Substytuowanie skrobi – istnieje kilka pozytywnych atrybutów wspomagających modyfikację substytutów. Pierwszym jest wprowadzenie grup funkcjonalnych. Dzięki nim wzrasta podobieństwo skrobi dla wody. Te rezultaty są widoczne przy mniejszej temperaturze żelowania skrobi i lepszym nawodnieniu. Kolejną zaletą jest fakt, że produkt żelowania skrobi jest znacznie mniej twardy, ale jednocześnie mocny i wydaje się bardziej przejrzystym. Hydroksypropylowane skrobie zazwyczaj dają filmy, które są czystsze i bardziej elastyczne w porównaniu do filmów ze skrobi natywnej.
- Utlenianie skrobi – proces prowadzący do poprawy właściwości adhezyjnych w powłokach nakładanych na materiały o luźnej konsystencji jak np. rzadkie ciasta. Głęboko mrożone powłoki do żywności jak np. powłoki do owoców morza, mięsa, ryb czy warzyw często kontaktują się ze skrobią utlenioną. Często też frytki są pokrywane cienką warstwą w celu poprawy jakości poprzez utrzymanie chrupkości po smażeniu. Idealne płynne roztwory powłokotwórcze posiadają dużą siłę adhezji w stosunku do powierzchni żywności podczas mrożenia i w trakcie zamrażania.
- Hydroliza kwasowa to inny typ modyfikacji chemicznej skrobi, który znalazł zastosowanie w produktach skrobiowych. Poprzez hydrolizę kwasową otrzymuje się dekstryny i maltodekstryny. Te pierwsze są często używane w celu stworzenia powłok w różnych wariantach, zaczynając od produktów farmaceutycznych poprzez słodycze i jadalne „spoiwa". Tradycyjnie w procesie wytwarzania dekstryn stosowane jest suszenie. Dekstryny mogą być rozpuszczane w zimnej wodzie i mieć dobre zdolności do formowania filmów o wysokiej sile adhezji. Znalazły one zastosowanie w cukiernictwie. Dekstryny z tapioki i kukurydzy są używane jako glazura do czekolady. Także maltodekstryny są produkowane poprzez depolimeryzację skrobi. W przeciwieństwie do dekstryn maltodekstryny są wytwarzane na mokro. Maltodekstryny są rozpuszczane w wodzie i mają dobre właściwości do formowania filmów. Tworzą powłoki o dobrych właściwościach barierowych dla tlenu (Baldwin, Hagenmaier & Bai, 2012; Embuscado & Huber, 2009).

1.2.5. Otrzymywanie skrobi

Skrobia jest najważniejszym materiałem zapasowym występującym w różnych organach roślin. Szczególnie bogate w skrobię są bulwy ziemniaka, kolby kukurydzy i ziarna zbóż. Niezależnie od gatunku rośliny i rodzaju skrobi, do izolacji tego polisacharydu stosuje się metody kombinowane, to jest mechaniczne w połączeniu z chemicznymi (Araujo-Farro i in., 2010; Lin i in., 2011).

Lin i wsp. (2011) otrzymywali skrobię z ryżu siewnego indyjskiego i japońskiego. Ziarna mielono, oczyszczano i zanurzano w wodzie w temperaturze pokojowej na 12 h, a następnie suszono. Skrobię ekstrahowano także z mąki ryżowej poprzez zanurzenie na pół doby w roztworze 0,2% zasady sodowej. Obie skrobie połączono, mieszając przez 5 minut w temperaturze pokojowej. Następnie mieszaninę przemyto kilkakrotnie w wodzie destylowanej do osiągnięcia pH 6-7 i suszono w suszarce próżniowej w temperaturze 45°C przez 48 godzin – do osiągnięcia wilgotności 13%.

Lin i wsp. (2011) pozyskiwali skrobię z ryżu indyjskiego i japońskiego także drugim sposobem. 40 gramów skrobi było moczone w fosforanie sodowym. Roztwór podgrzano do 50°C i ogrzewano przez 30 minut cały czas mieszając. Uzyskaną mieszaninę o pH 6,0 filtrowano. Wyizolowaną czystą skrobię suszono w temperaturze 45°C przez 48 h w suszarce próżniowej. Uzyskano susz o wilgotności 5-10%.

Podobną metodę stosował Yuan i wsp. (2007). Bulwy *D. nipponica* Makino (pochrzyn) były rozcinane w celu wyodrębnienia pewnych niekorzystnych fragmentów i myte w wodzie. Następnie cięto je na plasterki o grubości 3 i 5 mm i suszono do uzyskania stałej masy. Małe plasterki inkubowano w 0,2% roztworze zasady sodowej (pH 10-11) przez 24 h, po czym filtrowano przez membranę 150 μm. Dodawano 95% etanolu. Następnie frakcje skrobi były przemywane wodą destylowaną – do czasu uzyskania neutralnego odczynu. Otrzymaną skrobię suszono w 50°C do momentu, w którym została uzyskana stała masa.

Kong, Bao i Corke (2010) ekstrahowali skrobię z ziaren amarantusa. Nasiona suszono w suszarce w temperaturze 40°C przez 48 h a następnie mielono je na mąkę. Próbka mąki była zanurzana w 0,25% wodnym roztworze zasady sodowej i przechowywana przez 24 godziny w temperaturze 5°C. Kolejno zawiesinę mieszano przez 3 min., po czym filtrowano ją przez sita: 60 (250 μm), 100 (149 μm), 270 (53 μm) i 400 (37 μm). Zawiesinę przemywano podwójnie dejonizowaną wodą do momentu, kiedy skrobia nie została wypłukana. W ciągu 25 min. odwirowano 3 kg przesączu. Usunięto białka. Warstwę skrobi zmieszano ponownie z wodą podwójnie dejonizowaną i postępowano jak wcześniej. Procedurę powtórzono trzykrotnie. Wyekstrahowaną skrobię suszono w suszarce o temperaturze 40^{o}C, po czym została zmielona i przesiana na sitach 70 (212 µm).

1.2.6. Otrzymywanie powłok skrobiowych

Jadalne filmy i powłoki mogą być tworzone według następujących mechanizmów:

- prosta koacerwacja - rozpuszczający się w wodzie hydrokoloid jest wytrącany lub po odparowaniu rozpuszczalnika (suszenie) następuje zamiana fazy, w której się znajduje. Do mieszaniny powłokotwórczej jest dodawany nieelektrolityczny roztwór tj. np. etanol.
- kompleksowa koacerwacja – dwa rozpuszczalne w wodzie hydrokoloidy, o przeciwnych ładunkach elektrycznych, mieszają się powodując w ten sposób interakcje i wytrącenie się kompleksu polimerowego.
- żelowanie lub termiczna koagulacja – podgrzewanie makromolekuły prowadzi do jej denaturacji.

Filmy mogą być również otrzymywane standardowymi technikami tj. ekstruzja, wylewanie, termiczne formowanie. Filmy są najczęściej tworzone przez suszenie roztworu powłokotwórczego w suszarkach bębnowych, termoformowanie lub ekstruzja na gorąco (Guilbert, Gontard & Gorris, 1996).

Ghanbarzadeh, Almasi i Entezami (2011) wytwarzali powłoki ze skrobi kukurydzianej (zawartość wilgotności 12%). Rozpuszczano 5 gramów skrobi w 100 ml wody destylowanej. Dodawano 2 ml glicerolu i 0, 5, 10, 15 lub 20% kwasu cytrynowego względem zawartości skrobi. Wszystko ze sobą mieszano w temperaturze pokojowej przez 5 min. Następnie mieszaninę ogrzewano w łaźni wodnej w temperaturze 90^{o}C przez 30 min. z wytrząsaniem o amplitudzie 500 rpm. Tak otrzymany roztwór powłokotwórczy ochładzano i wylewano po 50 ml na płytki Petriego o średnicy 75 mm. Otrzymano błony o grubości 0,08 ± 0,01 mm, które następnie suszono w temperaturze 60^{o}C do odparowania rozpuszczalnika.

Torres i wsp. (2011) otrzymywali powłoki z 12 rodzajów skrobi natywnej pochodzącej z roślin andyjskich. Były to: biała marchew, ciecierzyca, banan, maniok jadalny, szczawik bulwiasty i kilka rodzajów ziemniaków, wśród nich m.in. słodki ziemniak i ziemniak złoty.

Powłoki skrobiowe były otrzymywane metodą wylewania. Skrobię mieszano z wodą destylowaną (5% skrobi względem wody). Część roztworu hydrolizowano rozcieńczonym kwasem solnym – do uzyskania pH 2,0. Dodano glicerol w proporcji 2 : 5 (glicerol : skrobia). Mieszaninę skrobi homogenizowano i mieszano przez 15 min. w temperaturze 95°C. Następnie roztwór neutralizowano rozcieńczonym wodorotlenkiem sodu (0,1 N) - do chwili zatrzymania procesu hydrolizy. Roztwór powłokotwórczy skrobi wylano na płytki Petriego i umieszczono w suszarce. Suszono w temperaturze 40°C przez 16 h do uzyskania powłok o grubości 200 μm.

Również Singh, Belton i Georget (2009) wytwarzali powłoki poprzez wylewanie. Źródłem skrobi była fasola. 5% skrobi mieszano z wodą destylowaną, dodawano 30% glicerolu i przez 5 min. mieszano przy pomocy mieszadła magnetycznego, a następnie podgrzewano w temperaturze 90°C przez 10 min. ciągle mieszając z częstotliwością 300 rpm. Roztwór ochłodzono do temperatury 50°C. Odmierzano po 6,2 gramów mieszaniny i wylewano na plastikowe płytki Petriego o średnicy 8,5 cm. Suszono w temperaturze 40°C przez 24 h. Otrzymane filmy przechowywano w higrostatach z tlenkiem fosforu (V).

Metodę wylewania zastosowali także Wu i wsp. (2009). Do skrobi ziemniaczanej (o 17% zawartości amylozy) dodawali agar i wodę destylowaną. Zawartość skrobi wynosiła: 3,3; 3,2; 3,0; 2,8; 2,7; 2,5; 2,3 i 0%. Zawartość agaru była odwrotnie proporcjonalna do skrobi: 0; 2,3; 2,5; 2,7; 2,8; 3,0; 3,2; 3,3%. Do wszystkich próbek dodawano 240 g. wody destylowanej i po 2 g. glicerolu. Mieszano i ogrzewano w temperaturze 95 ± 5°C przez 30 min. Otrzymano jednolite, homogeniczne roztwory błonotwórcze. Następnie zostały one odgazowane w próżni w celu usunięcia rozpuszczonego w nich powietrza i wylane na akrylowe płytki o wymiarach 18 x 27 cm. Roztwory suszono w temperaturze 50°C przez 10 h.

1.2.7. Zastosowanie

Powłoki mogą być aplikowane na powierzchnię produktu dowolną metodą, np. poprzez zanurzenie (najczęściej do żywności, która wymaga kilku warstw powłokotwórczych – laminatów), spryskanie (zazwyczaj w celu rozdzielenia dwóch warstw) i polewanie produktu. Dla produktów mięsnych jest używana metoda zalewania za pomocą specjalnej maszyny funkcjonującej jak wodospad (Embuscado & Huber, 2009).

Filmy są wytwarzane poza produktem i dopiero później na niego nanoszone. Mogą być aplikowane w postaci laminatów skrobiowych z dodatkiem innych składników tj. tłuszcz. Wadami podwójnych filmów skrobiowych jest skłonność wraz z upływem czasu do delaminacji, tworzenie się dziur, pęknięć i szczelin, oraz słabe siły kohezji. Filmy w takiej postaci są często kruche i niepraktyczne w zastosowaniu do wielu składników żywności (Liu, 2005).

Sukcesywny rozwój jadalnych filmów i powłok jest oparty na specyficznych właściwościach funkcjonalnych polimerów. Dodatek komponentów wchodzących w ich skład tj. plastyfikatory, stabilizatory, tłuszcze, białka w optymalnej koncentracji i we właściwej kolejności ma wpływ na ich właściwe zastosowanie (Liu, 2005).

Skrobia i jej pochodne są wykorzystywane w przemyśle spożywczym między innymi jako zagęstniki, zmiękczacze, stabilizatory, substancje żelujące, dietetyczne, zamienniki tłuszczu i środki modyfikujące strukturę w procesie technologicznym (Lin i in., 2011). Skrobia jest także dodawana m.in. do zup, sosów (również mięsnych), płatków śniadaniowych, mufinów, ciasteczek, przekąsek, makaronów, dresingów, kremów i produktów mlecznych (Kong, Bao & Corke, 2009; Thirathumthavorn & Charoendrin, 2006).

Przemysł spożywczy wymaga użycia wielu materiałów opakowaniowych i nawet niewielka redukcja ich ilości ma istotne znaczenie w ograniczeniu masy i objętości wytwarzanych odpadów (Liu, 2005). Jednym z najważniejszych polisacharydów używanych do wytwarzania biodegradowalnych i/lub jadalnych filmów zastępujących plastikowe opakowania do żywności jest właśnie skrobia (Ghanbarzdeh, Almasi & Entezami, 2011).

Filmy i powłoki skrobiowe cieszą się zainteresowaniem wśród konsumentów od wielu lat i są przez nich bardziej akceptowane niż pochodne ropy naftowej - polimerowe tworzywa sztuczne. Jadalne i/lub biodegradowalne filmy i powłoki mogą mieć bardzo szerokie zastosowanie w przemyśle spożywczym. Mogą zapobiec nadmiernemu używaniu opakowań i/lub pełnić funkcję izolującą między dwiema lub więcej warstwami produktu.

Pokrywanie mięsa powłokami skrobiowymi ma na celu ochronę przed utratą wilgoci, poprawą właściwości organoleptycznych, zapewnia stabilność mikrobiologiczną, chroni przed autooksydacją tłuszczów (Baldwin, Hagenmaier & Bai, 2012; Cutter & Sumner, 2002;

Embuscado & Huber, 2009; Sikora i in., 2007). Powłoki na bazie skrobi można stosować również do proszków, nasion i innych produktów żywnościowych. Nadają się one także do mikrokapsułkowania aromatów i powlekania produktów świeżych i minimalnie przetworzonych, a w szczególności do owoców i warzyw. Oprócz tego mogą poprawiać połysk wielu owoców np. jabłek, cytryn, pomarańczy, owoców mango itp., a także uwydatniać cechy sensoryczne i teksturalne (Guilbert, Gontard & Gorris, 1996; Petersen i in., 2011).

1.2.8. Summary (short translation of the polish publication)

The European Union estimates the world's population in 2040 will increase up to 10 billion, ie twice more than in 1990. Accordingly, the demand for energy and organic materials will also increase (up to three times). In this situation, the demand on plant material will be greater than the supply, as the number of farmlands is limited, and additionally their number is decreasing every year. The situation is similar in case of the fossil fuels.

Thier extraction induces negative environmental effects, which is reflected in the price of oil, gas and coal, and subsequent wastes. To prevent these growing economic problems widely available, relatively inexpensive, biodegradable and renewable polysaccharide raw materials, like starches, are envisaged as substitute of petrol-based plastics. This biopolymer has unique properties and it is the most commonly used polysaccharide on Earth and contender for material of the twenty-first century (Tomasik, 2000).

The food industry requires the use of many packaging materials and even a small reduction in their number is significant in reducing the weight and volume of waste which are produced (Liu, 2005). One of the major polysaccharide used in the manufacture of biodegradable and/or edible films to replace plastic food packaging is the starch (Ghanbarzdeh, Almasi & Entezami, 2011). Starch is the most available plant material. You can find it on 6 continents. Cereals, seeds of vegetables, tubers and fruits contain in a dry matter from 30 to 85% of starch (Baldwin, Hagenmaier & Bai, 2012; Embuscado & Huber, 2009). For example rice grain contains 1-3% of fat, 8% of protein and 80% of starch (Lin et al., 2011). Starch is a polysaccharide combination of two fractions: amylose and amylopectin. Both fractions contain molecules of glucose, but with different size and shape. Amylose is a smaller fraction and is defined as a linear molecule of the units (1 → 4) -α-D-glucopyranose, the degree of polymerization is below 500. It includes a few branched chain (1 → 6) -α. Amylopectin is the heavier, branched macromolecules with bonds (1 → 6) -α and (1 → 4) -α-D-glucose. The amylose content of starches is in the range 18-30%, except of waxy maize starch, which contains only amylopectin (Baldwin, Hagenmaier & Bai, 2012; Hoover, Hughes & Liu, 2010). Films and coatings can be formed from any type of starch containing amylose, because a linear structure has a greater influence on the formation of connections between molecules than the branched structure (Embuscado & Huber, 2009).

Depending on the size, the starch granules can be grouped into four size classes:

- huge - more than 25 microns, for example potato starch (41.5 microns) and wheat starch (20.2 microns),
- medium - from 10 to 25 microns, for example tapioca starch (15.6 microns), corn starch (13.8 microns),
- small - from 5 to 10 microns, for example oat, buckwheat, millet, rice starches,
- very small - up to 5 microns for example amaranth starch (3.0 microns), quinoa starch.

Besides, the morphological classification of the starch granules can be ranked by the mold. They can be oval (elliptical), square, elliptical, polygonal, irregular, or lens shape (Hoover, Hughes & Liu, 2010; Molenda et al., 2006). According to Molenda et al. (2006), these parameters influence the mechanical properties. It has been shown the grain size increases the compressibility and flowability of the materials. Tapioca and corn starch grains have similar size hence their compressibility and stress curves are very similar to each other.

The most important source of starch in the world is corn with 83% of total production. The biggest exporter, which produced more than 60% starch are the United States. The second largest raw material is wheat. Despite the small share of world production (below 7%), it is a very valued source of starch in Europe. This is due to the favorable conditions to grow. Next places belong to potato starch (6%), and tapioca (4%) (Liu, 2005; Molenda et al., 2006). Due to the many advantages of starch, the polymer is one of the most commonly used raw materials for the production of biodegradable packaging in the form of films and coatings. In addition it has good geometrical and optical properties. The coatings and films of starch are easily formed, having the optimal thickness, usually are transparent or milky as rice starch. This can be easily dyed or printed. Transparency of membranes can be also used to emphasize the freshness of fruits and vegetables. Starch coatings with wax addition make fruits shinier and concentrate the attention of consumers. Starch coatings have good mechanical properties too. Addition of plasticizer causes they are not fragile, they do not break and do not crack.

Strong hydrophilic nature and strong sorption (particularly high permeability to water vapor), the tendency to retrogradation and poor solubility in water are disadvantages of this polymer.

Therefore, starch is modified (usually by chemical, physical, enzymatic, or combinations of these methods), or produced in the native form (Ghanbarzdeh, Almasi & Entezami, 2011, Guilbert, Gontard & Gorris, 1996; Jayakody et al., 2009; Lin et al., 2011; Singh Kaur & McCarthy, 2007). By genetic modification starch helps for creating products tailored to specific purposes (Berski et al., 2011). So, the physicochemical properties of films are most often modified by the solubility of the powders of starch in water, the gelling temperature, swelling properties and the temperature of freezing and thawing starch (Nwokocha et al., 2009).

For optimal uses and applications, starches are thus often chemically modified. These highlight the features and give the ability to create starchy products, which have unique properties for various useful applications. The most common directions of chemical modification of starch include: thermal stabilization, cross-linking, substitution of some chemical groups, substitution of some branches in amylopectine, oxidation, acid hydrolysis…

Regardless of the plant species and the type of starch, the combined methods, i.e. mechanical combination with chemical are used for the isolation of the polysaccharide (Araujo-Farro, et al., 2010; Lin et al., 2011). Edible films and coatings can be made according to the following mechanisms: simple coacervation, complex coacervation, thermal gelation or coagulation. Films could also be prepared by standard techniques, i.e. extrusion, casting, thermoforming (Guilbert, Gontard & Gorris, 1996). Coatings can be applied onto the surface of products by many methods, for example by dipping (usually food that requires several layers of film-forming - laminates), spraying (usually to separate the two layers) and pouring. For meat products, flooding method is used with a special machine functioning like a waterfall (Embuscado & Huber, 2009). The films are produced independently to the food product and only then applied onto it. They can be multilayer systems with other ingredients, i.e. fat. The disadvantages of bilayer or multilayer films composed of starch-based layers is a tendency to delamination with time, to formation of holes, cracks and crevices, due to weak cohesive forces. Such films are often fragile or brittle and thus impractical for use with many food ingredients (Liu, 2005).

The successive development of edible films and coatings is based on the specific functional properties of biopolymers. The components included in the film composition, i.e. plasticizers, stabilizers, fats, protein, in the optimal concentration and in the correct sequence, have an impact on their proper use (Liu, 2005). Starch and its derivatives are used in the food industry, as thickeners, plasticizers, stabilizers, gelling agents, dietary fat substitutes and structure modifying agents (Lin et al., 2011). Starch is also added for soups, sauces (including meat), cereals, muffins, cookies, snacks, pasta, dressings, creams and dairy products (Kong, Bao & Cork, 2009; Thirathumthavorn & Charoendrin, 2006). Films and coatings made from starch are appreciated and used by consumers and they are more accepted than petroleum derivatives - polymer plastics. Edible and/ or biodegradable films and coatings can be widely used in the food industry. They can prevent excessive use of packaging and/or can act as isolation between two or more layers of the product. Covering meat with starch coatings intends to protect against moisture loss, improved organoleptic properties, provides microbiological stability, protects against autoxidation of fats (Baldwin, Hagenmaier & Bai, 2012; Cutter & Sumner, 2002; Embuscado & Huber, 2009; Sikora et al., 2007). Starch-based coatings can be applied onto powders, seeds and other granulated or small-size food products. They are also suitable for microencapsulation of flavors, coating of fresh product and minimally processed foods, especially fruit and vegetables. In addition, they can improve the shininess of fruits, for instance apples, lemons, oranges, mangoes, etc., as well as the enhancement of sensory and textural characteristics (Guilbert, Gontard & Gorris, 1996; Petersen et al., 2011).

1.3. Starch coatings used in food packaging and plasticizers (Basiak E., Lenart A. (2013). Powłoki skrobiowe stosowane w opakowalnictwie żywności, Żywność, Nauka, Technologia, Jakość, 20, 1, 21-31. Starch coatings used in food packaging and plasticizers. Food, Science, Technology, Quality, 20, 1, 21-31).

1.3.1. Abstract

Edible starch coatings are meeting with growing demand both by the producers and consumers for the cheap, save and biodegradable alternative for artificial coatings. Starch coatings have many advantages, e.g.: biodegradability, edibility, wide availability, low price and high quality. Edible starch coatings have good mechanical, optical, sorption properties and combined with others components (for example fats) starch coatings gain good barrier properties. They are very useful as a part of multilayer food products where they can serve as a barrier between layers. Additionally they can be used to encapsulate food ingredients making them easier to dose and mix. Most often starch films are made by casting. In combination with plasticizers and/or other components starch films can be used to form continuous structures, which could directly coat raw materials and food products.

1.3.1. Abstrakt

Coraz większym zainteresowaniem wśród producentów żywności bezpiecznej i o małym stopniu przetworzenia, cieszą się jadalne powłoki skrobiowe. Mają one wiele zalet tj. biodegradowalność, szeroką dostępność, niską cenę i wysoką jakość. Charakteryzują się dobrymi właściwościami mechanicznymi, optycznymi, sorpcyjnymi, a w połączeniu z innymi składnikami (takimi jak np. tłuszcze) także właściwościami barierowymi. Są szczególnie przydatne do wprowadzenia substancji dodatkowych do żywności z możliwością ich kontrolowanego uwalniania w czasie spożywania potraw, jak i ich przygotowywania. Powłoki i filmy skrobiowe są wytwarzane najczęściej techniką wylewania. W połączeniu z plastyfikatorem i/lub innymi składnikami tworzą ciągłe struktury nadające się do bezpośredniego powlekania surowców i produktów spożywczych.

1.3.2. Wstęp

Od momentu, w którym na rynku produktów spożywczych pojawiły się syntetyczne polimery dominują opakowania z tworzyw sztucznych. Mają one szereg zalet tj. łatwość dostępu surowca, niską cenę, dogodność w transporcie, dużą i wybiórczą barierowość dla gazów, aromatów i pary wodnej. Jednak jednocześnie są wyjątkowo niekorzystne dla środowiska naturalnego. Ich rozkład trwa setki lat. Opakowania biodegradowalne wymagają zazwyczaj specjalnych warunków do kompostowania a ich cena jest wyższa niż syntetycznych. Innym sposobem zagospodarowania i zmniejszenia masy produkowanych odpadów mogą stać się powłoki jadalne.

1.3.3. Właściwości powłok jadalnych

Żywność surowa, o wysokiej jakości i dużym bezpieczeństwie, oraz produkty jak najmniej przetworzone są przedmiotem wzrastającego zainteresowania konsumentów. Przy ich wprowadzaniu na rynek istotne znaczenie odgrywa również ochrona środowiska, tzn. dąży się do wytwarzania jak najmniejszej ilości opakowań, które po jednokrotnym użyciu staną się odpadami (Alves i in., 2007; Wu i in., 2009).

Jednym z rozwiązań tego problemu są powłoki nakładane bezpośrednio na produkt i filmy otrzymywane poza nim. Wytwarzane z syntetycznych polimerów, takich jak np.: polietylen niskiej gęstości, chlorek poliwinylu, polipropylen mają wiele pozytywnych cech, chroniąc produkty przed mechanicznymi uszkodzeniami i ograniczają kontakt żywności ze środowiskiem. Pomimo tych zalet mają istotną, ograniczającą ich stosowanie wadę – wysokie koszty utylizacji (Viña i in., 2007).

Alternatywą dla tego typu opakowań okazały się jadalne i/lub biodegradowalne powłoki. Są one przyjazne dla środowiska, mogą promować jakość i dostępność minimalnie przetworzonych produktów. Są wytwarzane z węglowodanów, białek i tłuszczów, lub też mogą być kombinacją białek z węglowodanami i węglowodanów z tłuszczami (Alves i in., 2007; Chen, Kuo & Lai, 2009). Źródłami polisacharydów są np. korzenie warzyw i ziarna zbóż oraz pektyny, alginiany i celuloza wyizolowane z komórek roślin oraz karageny z wodorostów morskich. Białkami wykorzystywanymi do produkcji powłok są m.in. białka serwatkowe i sojowe oraz gluten pszenny, zaś tłuszczami estry, mono-, di- i trójglicerydy, woski pszczele i żywice (Kokoszka & Lenart, 2007; Kowalczyk & Pikula, 2010; Talja i in., 2007).

Główną funkcją powłok jadalnych jest zapobieganie wymianie pary wodnej i innych substancji tj. tlenu, ditlenku węgla i związków aromatycznych pomiędzy produktem a otoczeniem albo pomiędzy różnymi warstwami w materiale. Oprócz właściwości barierowych dla wielu składników żywności powłoki mogą spowalniać absorpcję pary wodnej w produktach o małej zawartości wody np. w krakersach (Talja i in., 2008). Wpływają na przenoszenie substancji przeciwdrobnoustrojowych, składników aromatu, barwników albo na polepszenie właściwości mechanicznych żywności czy produktów farmaceutycznych. Dodatkowo mogą być używane jako aktywne składniki materiałów opakowaniowych (Singh, Belton & Georget, 2009; Talja i in., 2008). Powinny charakteryzować się dobrymi właściwościami wiążącymi, adhezyjnymi i plastycznymi. Jadalne powłoki białkowe i polisacharydowe cechują się większą siłą rozciągania i lepszą selektywnością dla gazów niż tłuszcze, ale mniejszą barierowością wody, ponieważ są hydrofilowe. Mniejsza przepuszczalność pary wodnej w materiałach hydrofobowych tj. w tłuszczach powoduje, że są one dodawane do powłok hydrofilowych poprzez laminowanie, czyli pokrycie cienką warstwą lub poprzez wytworzenie emulsji (Chen, Kuo & Lai, 2009; Viña i in., 2007).

1.3.4. Charakterystyka powłok skrobiowych

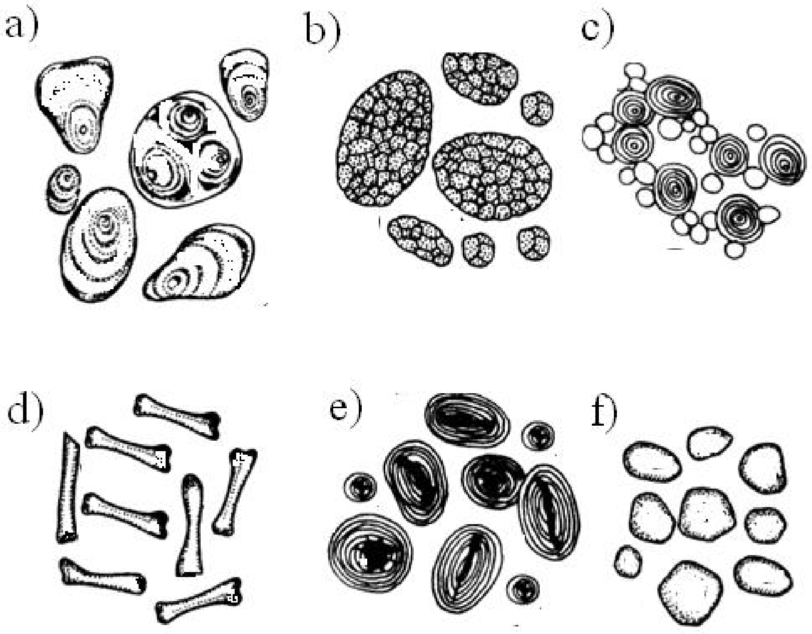
Skrobia jest jednym z najważniejszych naturalnych biopolimerów w technologii żywności, w szczególności w projektowaniu właściwości reologicznych żywności (Alves i in., 2007; Reis i in., 2008). Sikora i in. (2007) przeprowadzili badania reologiczne deserowego sosu truskawkowego, w skład którego wchodził 2% dodatek skrobi ziemniaczanej, kukurydzianej lub owsianej i 0,24% gumy ksantanowej. Wykazano, że rodzaj zagęstnika ma bardzo duży wpływ na cechy produktu końcowego i zależy on od użytego materiału biologicznego.

Skrobia może być jednym z najważniejszych składników materiałów opakowaniowych. Ma szereg pozytywnych cech m.in. relatywnie niską cenę (znacznie tańsza od polietylenu), dostępność i biodegradowalność. Ponadto jest jadalna i łatwa w obróbce technologicznej (Ghanbarzadeh, Almasi & Entezami, 2010; Jiménez i in., 2012; Rodríguez i in., 2006; Shen i in., 2010; Torres i wsp., 2011; Yan i in., 2012).

Skrobia jako węglowodan zapasowy stanowi główny zasób energii w roślinach wyższych (nawet około 90% w suchej masie) (Embuscado & Huber, 2009). Jest najczęściej akumulowana w bulwach, nasionach i korzeniach (Araujo-Farro i in., 2010; Torres i in., 2011). Największym źródłem skrobi dla przemysłu są kolejno: kukurydza, pszenica, maniok jadalny, ziemniak i całkiem współcześnie ponownie odkryte amarant i komosa ryżowa

(Araujo-Farro i in., 2010; Li i in., 2008). Ważną rolę odgrywa skrobia natywna, jak i modyfikowana (Liu, 2005; Torres i in., 2011).

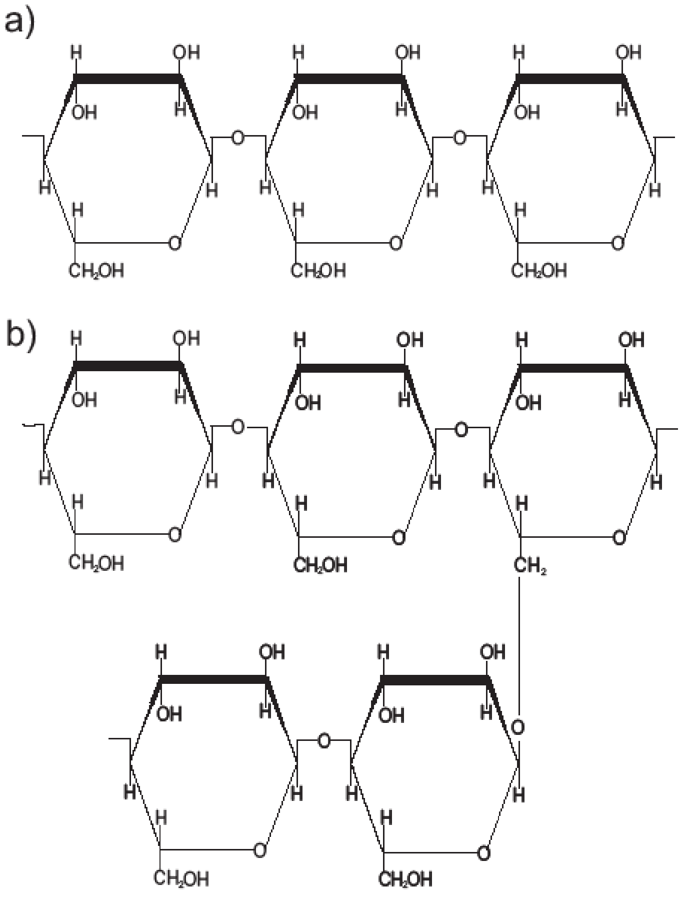
W zależności od rodzaju surowca ziarna skrobi różnią się od siebie składem, rozmiarem i kształtem (rysunek 1.2). Cechy te przekładają się na funkcjonalność, właściwości barierowe, mechaniczne i sorpcyjne. Wymienione właściwości sorpcyjne skrobi wpływają na skład powłok, proces ich formowania, metody nakładania na produkt i zastosowanie (Li i in., 2008; Mali i in., 2006; Rodríguez i in., 2006).



Rysunek 1.2 Kształt ziaren skrobi z: a) ziemniaka b) ryżu c) pszenicy d) wilczomleczu e) tapioki f) kukurydzy

Figure 1.2 Shape of starch grains obtained from: a) potato b) rice c) wheat d) euphorbia e) tapioca f) maize starches

Cząsteczka skrobi zbudowana jest z dwóch rodzajów polimerów D-glukozy (rysunek 1.3): amylozy (20 - 30%) i amylopektyny (70-80%). Amyloza jest polimerem składającym się z łańcuchów liniowych, połączonych wiązaniami α-1,4 glikozydowymi. Natomiast w amylopektynie większość stanowią wiązania α-1,6 glikozydowe zapewniające rozgałęzienie łańcucha liniowego, z częścią wiązań liniowych α-1,4 glikozydowych. Skrobia pochodząca z różnych źródeł ma różny stosunek amylozy do amylopektyny i przez to różne właściwości (Liu, 2005; Mali i in., 2006; Singh, Belton & Georget, 2009; Staroszczyk i in., 2007; Torres i in., 2011; Yuryev, Tomasik & Bertoft, 2007). Przewaga amylozy w skrobi powoduje, że filmy z niej otrzymane są bardziej wytrzymałe, zaś rozgałęziona struktura amylopektyny generalnie prowadzi do uzyskania powłok o zróżnicowanych właściwościach mechanicznych tj. o mniejszej sile rozciągania w porównaniu do tych, które mają w swym składzie większą zawartość amylozy (Torres i in., 2011). W celu uzyskania powłok z wysokim dodatkiem amylozy stosuje się metody alternatywne, np. modyfikację genetyczną. Są one niestety bardzo kosztowne i nie są dozwolone we wszystkich krajach. Stąd inną, bardziej pospolitą techniką do separacji amylozy jest jej selektywne ługowanie. Polimery te ekstrahuje się ze skrobi poprzez ługowanie w gorącej wodzie (50-70ºC) (Alves i in., 2007; Torres i in., 2011). Po ochłodzeniu ze skrobi uzyskuje się cenne w przemyśle spożywczym żele (Talia i in., 2007). W czasie żelowania amyloza i amylopektyna ulegają wewnątrz- i międzycząsteczkowemu sieciowaniu. W rezultacie dochodzi do produkcji makrocząsteczkowych sieci (Liu, 2005; Zavareze i in., 2012).



Rysunek 1.3 Rodzaje polimerów D-glukozy w skrobi: a) amyloza, b) amylopektyna (Mitrus, Wojnowicz & Mościcki, 2009)

Figure 1.3 Types of D-glucose polymers in starch a) amylose b) amylopectin

Możliwości wykorzystania jadalnych powłok na bazie skrobi są bardzo duże. Wynika to z wcześniej wymienionych zalet tego surowca, ale przede wszystkim z dobrej barierowości dla gazów. Najważniejszym ograniczeniem w użyciu skrobi jest wysoka przepuszczalność pary wodnej, która dodatkowo negatywnie wpływa na jej właściwości mechaniczne (Rodríguez i in., 2006). Pod wpływem wysokiej wilgotności względnej otoczenia powłoki mogą się fałdować (zwijać w rulon) lub wyginać. Właściwości mechaniczne filmów skrobiowych są determinowane przez jednokierunkową siłę rozciągania. Eksperymentalna krzywa naprężenia jest używana do obliczania wytrzymałości na rozciąganie powłok, odkształceń i wydłużenia prowadzących do zerwania lub pęknięcia. Właściwości te są zależne od długości łańcuchów polimerowych, interakcji łańcuchów i grubości powłok (Müller, Laurindo & Yamashita, 2009). Badana jest również barierowość powłok dla drobnoustrojów. Mimo, że drobnoustroje chorobotwórcze tj. *Escherichia coli* czy *Staphylococcus aureus* powinny być kontrolowane w przemyśle spożywczym, badania ich obecności w powłokach wykonuje się bardzo rzadko (Shen i in., 2010).

Właściwości funkcjonalne, mechaniczne i barierowe powłok spożywczych nie powinny być modyfikowane przez substancje chemiczne. Dodatki tego typu są niepożądane przez konsumentów, ponieważ powłoki i filmy są spożywane razem z produktem i muszą spełniać wszystkie wymogi dotyczące produkcji żywności bezpiecznej dla zdrowia. Dlatego preferuje się znacznie bardziej złożone postępowanie, jak np. modyfikacja właściwości skrobi. Powłoki wytwarzane z tego typu skrobi mogą mieć bardzo zróżnicowane właściwości użytkowe. Skrobia utleniona jest szeroko wykorzystywana w przemyśle, gdyż zapewnia uzyskanie bardzo dobrych zdolności powłokotwórczych (Zavareze i in., 2012). Natomiast roztwór powłokotwórczy ze skrobi natywnej z tapioki wymaga zawsze intensywnej homogenizacji przed utworzeniem powłoki (Chang, Karim & Seow, 2006).

1.3.5. Plastyfikatory i dodatki strukturalne w powłokach skrobiowych

Właściwości organoleptyczne, odżywcze, użytkowe, w tym i mechaniczne powłok jadalnych mogą być modyfikowane poprzez dodatek różnych substancji – tzw. plastyfikatorów (Rodríguez i in., 2006). Włączenie do struktury tych substancji jest niezbędne w celu uzyskania elastycznych powłok (Yan i in., 2012). Filmy składające się wyłącznie z samej skrobi mają dużą energię kohezji. Plastyfikatory redukują tę siłę poprzez zmniejszenie międzycząsteczkowych wiązań wodorowych występujących pomiędzy łańcuchami polimerów (Jiménez i in., 2012). Lourdin i wsp. (1995) porównywali mechaniczne właściwości filmów skrobiowych z i bez dodatku plastyfikatora. Powłoki z samej skrobi charakteryzowały się siłą rozciągania w granicach 45 MPa, a z glicerolem około 10 MPa. Dodatek plastyfikatora powoduje wzrost przestrzeni międzycząsteczkowych, a tym samym wpływa na zwiększenie ruchliwości cząsteczek, prowadzącej do obniżenia stopnia krystalizacji, zmiany modułu Younga i siły rozciągania (Mali i in., 2006; Muscat, Adhikari & Chaudhary, 2012; Rodríguez i in., 2006; Talja i in., 2008). Smits i wsp. (2003) wykazali, że tendencja do krystalizacji skrobi ziemniaczanej może być znacznie zredukowana, kiedy plastyfikator będzie w kontakcie z większą liczbą grup OH podczas żelowania.

Oprócz poprawy elastyczności i siły rozciągania plastyfikatory sprawiają, że powłoki nie są twarde i kruche oraz nie pękają (Jiménez i in., 2012). Zmniejszając ich sztywność zwiększają rozciągliwość polimerów poprzez zmniejszenie sił międzycząsteczkowych oddziałujących między sąsiadującymi łańcuchami (Rodríguez i in., 2006; Talja i in., 2008). Poprawiają ich właściwości strukturalne, ale wpływają też często negatywnie na właściwości barierowe (Rodríguez i in., 2006; Talja i in., 2007).

W przypadku dodawania do roztworu powłokotwórczego skrobi tylko jednego plastyfikatora w dużej ilości może następować rozdzielenie faz i krystalizacja plastyfikatora (Talja i in., 2008). Natomiast, kiedy jest obecny więcej niż jeden plastyfikator (oprócz wody) dochodzi do oddziaływań typu plastyfikator – plastyfikator. Ta interakcja prowadzi do uwydatnienia właściwości obu dodatków (Muscat, Adhikari & Chaudhary, 2012). Stosowane plastyfikatory muszą być kompatybilne z głównymi składnikami, to jest z polimerami powłokotwórczymi (Talja i in., 2008). Dlatego też najpopularniejszymi plastyfikatorami dodawanymi do powłok skrobiowych są alkohole polihydroksylowe tj. glicerol, sorbitol, ksylitol i mannitol (Wu i in., 2009). Nie powodują one sieciowania filmów podczas magazynowania i stanowią barierę dla różnego rodzaju gazów (Mali i in., 2006). Oprócz tego wpływają na właściwości powłok tj. sorpcję wody, migrację pary wodnej i właściwości mechaniczne (Talja i in., 2007).

Alkohole polihydroksylowe mają tendencję do silnej adsorpcji wody, która zależy od masy cząsteczkowej i liczby grup hydroksylowych (Muscat, Adhikari & Chaudhary, 2012). Jednym z najczęściej używanych plastyfikatorów wśród alkoholi polihydroksylowych, jest glicerol. Uwydatnia on właściwości mechaniczne, ponieważ ma naturę hydrofilową (Wu i in., 2009). Jego dodatek w odpowiedniej ilości może wpływać na interakcje pomiędzy łańcuchami innych polimerów poprzez zmianę wartości i sił wiązań wodorowych (Araujo-Farro i in., 2010). Powoduje także, że otrzymywane powłoki są przezroczyste, cienkie, elastyczne i mają jednolitą formę (Araujo-Farro i in., 2010; Wu i in., 2009). Jest rozpuszczalny w wodzie, polarny, nielotny, daje się łatwo zmieszać ze skrobią. Jego zawartość wpływa także na takie właściwości powłok skrobiowych, jak sorpcja i przepuszczalność pary wodnej oraz na temperaturę przemiany szklistej (Yan i in., 2012).

Adhikari, Chaudhary i Clerfeuille (2010) badali właściwości powłok ze skrobi kukurydzianej o małej zawartości amylozy z dodatkiem glicerolu (3 grupy hydroksylowe) i ksylitolu (5 grup hydroksylowych) w stosunku do skrobi 1:1. Wykazali, że ksylitol w porównaniu do glicerolu jest bardziej efektywnym plastyfikatorem, ma większą masę cząsteczkową i tendencję do tworzenia silniejszych wiązań wodorowych z cząsteczkami skrobi. Ksylitol jako plastyfikator powodował większą migrację wilgoci i miał lepsze właściwości dyfuzyjne. Jednocześnie Muscat, Adhikari i Chaudhary (2012) wykazali, że jego dodatek w ilości poniżej 15% powodował kruchość i łamliwość filmów. W tych powłokach, w których dodatek ksylitolu był na poziomie powyżej 30% po kondycjonowaniu do wilgotności względnej 52,9% dochodziło do rozdzielenia faz. Optymalnym rozwiązaniem okazało się połączenie glicerolu z ksylitolem. Filmy z udziałem obu plastyfikatorów miały mniejszą zawartość wilgoci niż te, które zawierały tylko jeden alkohol polihydroksylowy (Muscat, Adhikari & Chaudhary, 2012).

Woda również pełni funkcję plastyfikatora w powłokach polisacharydowych. Jej zawartość ma istotne znaczenie w kształtowaniu właściwości powłok i filmów skrobiowych (Torres i in., 2011; Zavareze i in., 2012). Gdy zawartość wody w filmach rośnie, wzrasta znacząco aktywność wody i analogicznie, gdy zawartość wody zmniejsza się, istotnie maleje aktywność wody (Talja i in., 2007). Najlepszym narzędziem do kontroli zawartości wody w powłokach polisacharydowych jest przebieg izoterm sorpcji pary wodnej (Godbillot i in., 2006). Woda jest także dobrym rozpuszczalnikiem innych substancji takich jak: glukozy, sacharozy, ksylozy, sorbitolu i glicerolu (Chang, Karim & Seow, 2006).

Wiedza z zakresu plastyfikatorów, a w szczególności alkoholi polihydroksylowych jest niezbędna do jak najefektywniejszego przygotowania powłok i filmów skrobiowych o pożądanych cechach jak np. o małej bądź dużej zawartości amylozy (Muscat, Adhikari & Chaudhary, 2012). Talja i wsp. (2007) zauważyli, że zwiększenie zawartości amylozy w skrobi kukurydzianej powoduje wzrost stopnia krystalizacji składników filmów. Dodatek plastyfikatora niweluje te niepożądane zmiany. Jednak ilość plastyfikatora musi być kontrolowana. Wysoka zawartość alkoholi polihydroksylowych powoduje rozdzielenie faz, a powierzchnia filmu jest lepka (dodatek glicerolu) i dochodzi do krystalizacji części składników (ksylitol i sorbitol). Do filmów z natywnej skrobi ziemniaczanej Talja i wsp. (2007) dodawali taką samą ilość glicerolu, ksylitolu i sorbitolu. Każdy alkohol inaczej wpływał na właściwości powłok, z uwagi na inną masę cząsteczkową. Na przykład przepuszczalność pary wodnej malała ze wzrostem masy cząsteczkowej plastyfikatora.

Kwasy tłuszczowe są dodawane do powłok skrobiowych w celu obniżenia przepuszczalności pary wodnej. Jej zawartość jest zazwyczaj duża w powłokach i filmach polisacharydowych, ponieważ są one silnie hydrofilne. W najnowszych pracach badawczych Galus i Lenart (2010) wykazali, że dodatek kwasów tłuszczowych do powłok i filmów oddziałuje na ich właściwości i wpływa na zdolność krystalizacji podczas formowania powłok i ich późniejszego przechowywania. Jiménez i wsp. (2012), badając powłoki i filmy na bazie skrobi kukurydzianej zauważyli, że kwasy tłuszczowe (palmitynowy i stearynowy) w kontakcie z glicerolem i przy zawartości wody poniżej 10% zmniejszają temperaturę przemiany szklistej i żelowania powłok skrobiowych.

Środki powierzchniowo - czynne jako substancje, które mają jednocześnie charakter hydrofilowy i hydrofobowy mogą być włączane w strukturę filmów skrobiowych w celu zredukowania napięcia powierzchniowego roztworów powłokotwórczych, polepszenia zwilżalności i zmiany siły adhezji (Chen i in., 2009; Rodríguez i in., 2006). Villalobos i wsp. (2006) wykazali, że dodatek środków powierzchniowo - czynnych (Span 60 i estru sacharozy P1570) do powłoki istotnie wpływa na równowagową zawartość wody i zwiększa barierowość dla wody w filmach z hydroksypropylometylocelulozy. Dla kontrastu Rodríguez i wsp. (2006) zaobserwowali, że powłoki ze skrobi ziemniaczanej z dodatkiem takich substancji jak Tween 20, Span 80 i lecytyna nie wykazują istotnego wzrostu barierowości dla wody, ale mają zróżnicowane właściwości mechaniczne.

1.3.6. Wytwarzanie powłok skrobiowych

Podczas wytwarzania powłok skrobiowych dąży się do uzyskania materiałów homogenicznych, cienkich i wytrzymałych (Fu i in., 2011). Filmy produkowane z jednego rodzaju skrobi mają zróżnicowane właściwości. W celu poprawy niezadowalających cech stosuje się mieszaniny kilku biopolimerów. Takie powłoki składają się z 2 – 3 rodzajów skrobi i są przygotowywane za pomocą różnych metod (Ghanbarzadeh, Almasi & Entezami, 2010).

Powłoki są wytwarzane następującymi sposobami: wylewanie, ekstruzja, termiczne formowanie, wtryskiwanie, powlekanie i wydmuchiwanie. Najpopularniejszą i najczęściej stosowaną z tych metod jest wylewanie, czyli tzw. metoda na mokro. Technika ta jest preferowana z tego względu, że powłoki można łatwo nakładać bezpośrednio na produkty spożywcze (Fu i in. 2011).

Jednym z najważniejszych parametrów przy wytwarzaniu powłok skrobiowych za pomocą metody na mokro są warunki suszenia. Mają one istotny wpływ na właściwości mechaniczne i barierowe, decydują o produkcie końcowym, np. jego homogeniczności itp. Podczas doboru urządzenia suszarniczego i warunków procesu ważne jest, by otrzymane produkty nie były zbyt wilgotne lub przesuszone (.Araujo-Farro i in., 2010).

Rodríguez i wsp. (2006) przygotowywali wodny roztwór skrobi ziemniaczanej i podgrzewali go w łaźni wodnej w temperaturze 70^{o}C przez 30 min. Następnie dodawali 20% glicerolu względem pierwotnej masy skrobi i 0,5 lub 5% środka powierzchniowo - czynnego Tween 20 lub Merc 80, także względem masy skrobi. Roztwory homogenizowano przez 10 min. przy prędkości 19000 rpm i 5 minut przy 22000 rpm. Wylewano 50 g tak przygotowywanego roztworu na płytkę Petriego o średnicy 147 mm i suszono w komorach klimatycznych (temperatura 60^{o}C, wilgotność względna 60%) przez około 24 h. Następnie zdejmowano powłoki z płytek i mierzono m.in. ich grubość, napięcie powierzchniowe, przepuszczalność pary wodnej i wytrzymałość na zerwanie.

Dias i wsp. (2009) uzyskiwali jadalne powłoki skrobiowe również techniką wylewania. Wodny roztwór zawierał 5% skrobi ryżowej i był mieszany przez 15 min., przy prędkości 4000 rpm. Jako plastyfikator był dodawany glicerol i sorbitol w ilości 0,20 lub 0,30 g/g skrobi. Całość podgrzewano w łaźni wodnej w temperaturze 85^{o}C przez 1 h. Następnie mieszaninę, po wylaniu na płytki, suszono w 30^{o}C w suszarce z cyrkulacją powietrza. Po upływie 14 h powłoki były zdejmowane z płytek i poddawane analizom. Wyznaczano izotermy sorpcji pary wodnej, współczynniki dyfuzji i rozpuszczalności oraz grubość i gęstość.

Wytwarzanie powłok skrobiowych metodą ekstruzji jest rozwijane od wielu lat. Na proces ten mają istotny wpływ: zawartość wody, temperatura, prędkość obrotów ślimaka w ekstruderze i prędkość podawania surowca. Ekstruzja z zastosowaniem surowców skrobiowych jest bardziej złożona niż z użyciem innych polimerów. Li i wsp. (2011) przygotowywali filmy skrobiowe przy użyciu matrycy do wytwarzania powłok. W ekstruderze znajdowało się 8 stref kontroli temperatury. Od pierwszej do szóstej strefy temperatura wzrastała od 60 do 115-180°C. W kolejnych strefach malała do 90-100°C. Prędkość obrotów ślimaka w urządzeniu wynosiła 30-120 rpm, a wydajność 1,2-2,4 kg/h. Po procesie ekstruzji filmy były kondycjonowane przez tydzień w eksykatorach z chlorkiem sodu o wilgotności względnej 75% w temperaturze pokojowej. Końcowa zawartość wody, po suszeniu w suszarce próżniowej w temperaturze 120°C przez 24 h, wynosiła 17 ± 0,5%.

Chen i wsp. (2008) próbowali zmieszać skrobię z polimerami syntetycznymi. Do natywnej skrobi wyizolowanej z grochu dodawali syntetyczny polimer (polialkohol winylowy) PVA. Jednak nie uzyskano zadawalających właściwości powłok. Filmy wykazywały zwiększoną przepuszczalność dla światła, słabsze właściwości mechaniczne, większą przepuszczalność dla pary wodnej, a ich wytworzenie było droższe niż filmów ze skrobi modyfikowanej z grochu czy z natywnej skrobi z grochu z nanokryształami.

1.3.7. Zastosowanie powłok skrobiowych

Powłoki ze skrobi mają szereg zalet: niski koszt, są łatwe w otrzymywaniu i zastosowaniu, chronią nie tylko żywność, ale i środowisko przed odpadami z tworzyw sztucznych. W przechowalnictwie owoców i warzyw są wykorzystywane do kontroli wymiany gazów między surowcem, a otoczeniem. Dzięki nim dochodzi do modyfikacji składu atmosfery gazowej będącej w kontakcie z powierzchnią surowców i mniejszych ubytków wody, powodując spowolnienie procesów metabolicznych i wydłużenie trwałości przechowywanych surowców (Kowalczyk & Pikula, 2010; Talja i in., 2007).

W przemyśle mięsnym powłoki i filmy są najczęściej wykorzystywane jako osłonki do kiełbas. Mogą stanowić również składnik mieszaniny peklującej, lub glazurę nanoszoną na produkty mięsne i rybne. Także owoce morza takie jak krewetki i ośmiornice są pokrywane powłokami – zarówno w formie półproduktu, jak i produktu końcowego. Innym zastosowaniem jadalnych powłok i filmów okazało się sushi z ryżu i ryb. Pokrywa się je filmami z wodorostów morskich (nori). Również papier ryżowy jest coraz częściej stosowany do pakowania żywności, nie tylko w Azji, ale i na świecie. Powłoki i filmy ze skrobi są pozbawione smaku, zapachu i barwy. Stąd dzięki tym pozytywnym cechom mogą być aplikowane m.in. na słodycze i leki (Dias i in., 2010).

Powłoki ze skrobi dają możliwości pakowania produktów spożywczych, o różnym stopniu przetworzenia. Mogą służyć jako torebki lub saszetki do pakowania suchych składników np. do napojów w proszku. Mogą być też używane do kapsułkowania składników w celu ułatwienia ich dozowania i mieszania (Abdorreza, Cheng & Karim, 2011). Sothornvit i Pitak (2007) wykazali, że cukier może być przechowywany nie tylko w papierowych czy polietylenowych torebkach, ale także w opakowaniach z filmów ze skrobi bananowej. Wprowadzenie do powłok skrobiowych barwników, witamin, przeciwutleniaczy, substancji przeciwdrobnoustrojowych, enzymów itp. daje duże możliwości użycia powłok i filmów jadalnych w technologii żywności (Kowalczyk & Pikula, 2010).

Jadalne filmy i powłoki ze skrobi są coraz częściej wykorzystywane w przemyśle spożywczym i farmaceutycznym. Zainteresowanie nimi z każdym rokiem rośnie. Prowadzone są coraz liczniejsze badania naukowe nad poprawieniem ich negatywnych cech i zwiększeniem możliwości w jeszcze szerszym użyciu. Obecnie dąży się do zmniejszenia masy powłok, oraz do zastosowania ich do produktów niszowych. Planuje się również zastąpienie etykiet i folii polietylenowych powłokami skrobiowymi. Drukowanie lub grawerowanie informacji o produkcie i producencie może następować bezpośrednio na powłoce. Jednocześnie przeprowadza się badania nad polepszeniem właściwości barierowych powłok, precyzyjnym dozowaniu substancji aktywnych oraz wytworzeniem powłok i filmów z nanoemulsji skrobiowych i wielowarstwowych laminatów (Dias i in., 2010).

1.3.8. Podsumowanie

Opakowania w przemyśle spożywczym odgrywają istotną rolę z następujących względów: stanowią barierę dyfuzyjną dla pary wodnej, aromatów i gazów, chronią przed drobnoustrojami i uszkodzeniami mechanicznymi, są łatwe i wygodne, zarówno w transporcie, jak i użyciu przez konsumentów. Konkurencją dla opakowań syntetycznych stały się jadalne powłoki wytwarzane z biopolimerów, głównie z polisacharydów takich jak np. skrobia. Mogą być one spożywane bezpośrednio przez konsumentów, mogą być w nie pakowane różne produkty, bądź mogą stanowić składnik produktów wielowarstwowych. W większości przypadków bardzo szybko rozpuszczają się w wodzie lub w jamie ustnej. Takie rozwiązanie znacznie redukuje masę zużytych opakowań i czas ich rozkładu w warunkach naturalnych.

Powłoki i filmy skrobiowe stanowią nie tylko opakowanie dla produktu. Mogą być dodatkiem funkcjonalnym, stanowić warstwę ochronną, lub rozdzielającą produkty o różnym pH i o różnej aktywności wody. Ze względu na wiele zalet wynikających z ich struktury, powłoki i filmy skrobiowe do żywności można łatwo barwić, wzbogacać w aromaty, enzymy, witaminy i substancje chroniące przez rozwojem drobnoustrojów.

1.3.9. Summary (short translation of polish publication)

When on the food market, plastic synthetic polymers had appeared dominating the packaging. Materials made from crude oil have plenty advantages, i.e. they are easily available, they have quite low price, they are convenience in transportation, they have a large and selective barrier for gases, aromaand water vapor. However, they are also very unfavorable for the environment. The decomposition takes hundreds of years.

The main function of edible coatings is prevention of water and other substance exchanges, i.e. oxygen, carbon dioxide and aromas between the product and the environment or between various layers of material. Additionally coatings can slow down the absorption of water vapor in products with low water content, for instance in crackers (Talja et al., 2008). Moreover coatings affect on the transfer of antimicrobial substances, aroma components, pigments or to improve the mechanical properties of food or pharmaceutical products. They can also be used as active components of packaging materials (Singh, Belton & Georget, 2009; Talja et al., 2008). Coatings should be characterized by good bonding, adhesive and plastic properties. Edible protein and polysaccharide coatings are characterized by a higher tensile strength and better selectivity for gas than fats, but the lower barrier for water vapour, as well as they hydrophilic. Lower water vapor permeability of hydrophobic materials (fats) is considered to be mixed to hydrophilic coatings by lamination, or by coating techniques as a thin layer, or by forming an emulsion (Chen, Kuo & Lai, 2009; Viña et al., 2007).

Amylose/ amylopectin ratio plays significant role on physical and chemical parameters of starch films. The predominance of amylose in starch makes films more resistant, while the branched structure of amylopectin generally results to obtaining coatings with different mechanical properties, i.e. with lower tensile strength than in those with higher amylose content (Torres al., 2011). In order to obtain coatings with a high amylose content alternative methods are used for example as genetic modifications. This method is very expensive and is not allowed in all countries. Thus, another more common technique of amylose separation is its selective leaching. These polymers are extracted from the starch by leaching in hot water (50-70ºC) (Alves et al., 2007; Torres et al., 2011). After cooling, valuable starch gels are obtained (Talja, et al., 2007). During the gelling process, amylose and amylopectin are involved in intra- and intermolecular crosslinking. Consequently, macromolecular networks are produced (Liu, 2005; Zavareze et al., 2012).

The possibilities of using of edible starch-based coatings are very large. This is due to the plenty advantages of this material, but above all are good barrier for gases. The most important limitation in the use of starch is its high vapour permeability which further adversely affects mechanical properties (Rodriguez et al., 2006). Thus, high relative humidity can cause folding, rolling and benching of biopolymers. The mechanical properties of starch films are determined by one direction tensile strength. Experimental stress curve is used to calculate the tensile strength of coatings, deformation and elongation leading to breaking or cracking. These properties are dependent on the length of the polymer chains, interacting chains and thicknesses of coatings (Müller, Laurindo & Yamashita, 2009). Barrier properties against microorganisms are also studied. Although the pathogenic microorganisms, i.e. *Escherichia coli* or *Staphylococcus aureus* should be controlled in the food industry, testing their presence in the coatings is carried out very rarely (Shen et al., 2010).

The functional mechanical and barrier properties of films and coatings should not be modified by chemical substances. This type of additives is undesirable for consumers since the coatings and films are consumed with the product and must meet all requirements for safe food production. Thus processes as modification of the starch are preferred (Zavareze et al., 2012).

Organoleptic, nutritional and functional, chemical and physical properties of edible coatings and films can be modified by the addition of various substances - plasticizers (Rodríguez et al., 2006). The inclusion in the structure of these compounds is necessary to obtain a flexible coating (Yan et al., 2012). Films consisting only of the same starch have a high cohesive energy. Plasticizers reduce this force by reducing the intermolecular hydrogen bonds occuring between the polymer chains (Jiménez, et al., 2012). Lourdin et al. (1995) compared the mechanical properties of the starch films with and without the addition of plasticizer. The coatings made from a starch without plasticizer characterized by a tensile strength in the range of 45 MPa, and with glycerol addition about 10 MPa. The incorporation of a plasticizer causes an increase of intermolecular spaces and thereby increases the mobility of molecules, leading to a reduction in the degree of crystallization, changes in Young's modulus and tensile strength (Mali et al., 2006; Muscat, Adhikari & Chaudhary, 2012; Rodriguez et al., 2006; Talja et al., 2008). Smits et al. (2003) proved that the tendency to crystallize of potato starch can be significantly reduced when the plasticizer will be in contact with a larger number of OH groups during the gelling.

Moreover plasticizers make the coatings flexible, not brittle and more resistance for breaking (Jiménez, et al., 2012). Reducing their rigidity increases the extensibility of polymers by reducing intermolecular forces interacting between adjacent chains (Rodríguez et al., 2006; talje et al., 2008). They improve their structural characteristics, but also often have a negative influence on the barrier properties (Rodriguez et al., 2006; Talja al., 2007).

When only one plasticizer in a large amount is added to starch film-forming solution, a phase separation and crystallization may follow (Talja et al., 2008). In contrast, when there is more than one plasticizer (other than water), they induce a plasticizer – plasticizer interaction. This interaction leads to enhancement properties of both additives (Muscat, Adhikari & Chaudhary, 2012). Plasticizers used must be compatible with the main film-forming substances (Talja et al., 2008). Therefore, the most popular plasticizers added to the starch coatings are polyols, i.e. glycerol, sorbitol, xylitol and mannitol (Wu et al., 2009). They do not cause crosslinking of films during storage and form a barrier for different types of gases (Mali et al., 2006). In addition, they influence the film properties, i.e. water sorption, migration of water vapor and mechanical properties (Talja et al., 2007).

Polyols have a strong tendency to water adsorption, which depends on the molecular weight and the number of hydroxyl groups (Muscat, Adhikari & Chaudhary, 2012). One of the most commonly used plasticizers of polyols is glycerol. It highlights the mechanical properties, because it has a hydrophilic nature (Wu et al., 2009). The additive in sufficient quantities may affect the interactions between the chains of other polymers by changing the value and strength of hydrogen bonds (Araujo-Farro, et al., 2010). Also it makes the coatings transparent, thin and flexible (Araujo-Farro and al., 2010; Wu et al., 2009). It is soluble in water, non-volatile polar solvents, and can be easily mixed with the starch. The starch

content also affects the coating properties of starch such as sorption and water vapor permeability and the glass transition temperature (Yan et al., 2012).
In the polysaccharide materials water also acts as a plasticizer. Water content is important in shaping the properties of starch coatings and films (Torres et al., 2011; Zavareze et al., 2012). When the water content of the films increases, the activity of the water significantly increases and vice-versa (Talja et al., 2007). The best opportunity of water content controlling is isotherm of water vapour sorption (Godbillot et al., 2006). Water is also a good solvent of other components such as glucose, sucrose, xylose, sorbitol and glycerol (Chang, Karim & Seow, 2006). Knowledge of plasticizers, particularly polyols is essential to maximize the preparation of coatings and films of starch with the eligible characteristics, such as a low or high amylose (Muscat, Adhikari & Chaudhary, 2012).

For the coatings and films preparation, it is seeked to obtain homogeneous materials, thin and durable (Fu et al., 2011). Films produced from one type of starch have different properties. In order to improve the unsatisfactory characteristics mixtures of several biopolymers are used. Such coatings consist of 2 - 3 kinds of starch and are prepared by different methods (Ghanbarzadeh, Almasi & Entezami, 2010). Drying conditions influence the homogeneity of starch films affecting the mechanical and barrier properties. Then, the selection of drying equipment and process conditions are parameters to be controlled to obtain biomaterials that can not be too wet or desiccated (Araujo-Farro et al., 2010).

In fruits and vegetables preservation starch polymers are used to control the gas exchange between the raw material and the environment. They modify the composition of the gaseous atmosphere in contact with the surface of the material and lower water losses, causing lower metabolic processes and extend the durability of the stored raw material (Kowalczyk & Pikul, 2010; Talja al., 2007).

In the meat industry, coatings and films are the mostly used as a casing for sausages. They can also be a component of the mixture of curing or glaze applied on meat and fish. Moreover seafood such as shrimp and octopus are coated. Edible coatings and films are used as well for sushi with rice and fish. They are covered with films from seaweed (nori). Rice paper is also increasingly used for food packaging, not only in Asia but in the world. Coatings and films made from starch are tasteless and colourless. Thus, due to these positive characteristics, they can be applied on sweets and medicines (Dias et al., 2010). The starch polymers are capable to packaging food products with varying degrees of processing level. They can be used as a bag or pouch for packaging of dry ingredients, e.g. beverage powders, they can also be used to encapsulate components in order to facilitate their dosing and mixing (Abdorreza, Cheng & Karim, 2011). Sothornvit and Pitak (2007) have shown that the sugar can be stored not only in paper or polyethylene bags, but also in packages made from the banana starch films. Incorporation to the starch matrix inks, vitamins, antioxidants, antimicrobials, enzymes, etc. gives great possibilities for the use of edible coatings and films in food technology in many branches (Kowalczyk & Pikul, 2010).

1.4. Food industry: use of plastics of twenty-first century. Basiak E. (2015) in Goyal M.R., Meghwal M. (eds.), *Emerging Technologies and Innovations in Food Engineering*, Apple Academic Press, Canada. 1008, P9.

1.4.1. Abstract

Every year the amount of people increasing, the gross domestic product is increasing and the consumption is increasing. Only during the first decade of twenty-first century more petroleum based polymers were produced than during the whole of the last century. Average, 80 million tons of plastic waste is generated annually, including approximately 500 billion un-biodegradable bags are used worldwide. Virtually every piece of plastic material that was ever made still exists in some shape or form (with the exception of the small amount that has been incinerated). From the second hand the amount of landfills increases because the decomposition process takes even 1000 years and the Earth area still is the same. Moreover price of plastic is required by several countries belong to OPEC. The brilliant denouement of monopoly for fossil fuels and reducing mass of thousand year biodegradable trash can be organic plant and animals sources, as polysaccharides, proteins and fats. They are most suitable, abundant, renewable and low-cost materials. They are able to replace plastic packaging as biodegradable and/or edible coatings and films. Is estimated, that even 1/3 of plastic waste can be replace by biopolymers.

1.4.2. Food packages: early nineteenth century

The Industrial Revolution which began in the eighteenth century in England and Scotland was the transition to the process of technological, economic, social and cultural rights and it was associated with the transition from an economy based on agriculture and manufacturing industry or craft to relying mainly on mechanical factory production at large scale (industrial). The major reason of the Industrial Revolution was the rise of population. It has led to an increase in the number of inhabitants, the result of which also grew market needs. They could not satisfy them manufactory which of craft production differed only in the organization of the production process. Second, in order of importance, the cause of the industrial revolution was the agrarian revolution, which led to the transformation of traditional feudal agriculture in modern agriculture. Manner of food production changed and demand on it increased. In order to adapt to market needs in nineteenth century appeared packaging for food (Berlanstein, 1992; Griffin, 2010). At the beginning the main role of packages was protection of food products and the ability to convenient transportation - counted so almost exclusively due to practical and functional. However, technological developments made it appear never forms of packaging, and thus the same packaging acquired additional significance. Producers were able to lock products in attractive forms, which contributed to increasing consumer interest in product and sales growth. It is also conditioned the growth of interest in the visual side of the packaging by manufacturers, who saw sales potential in packaging, and later also consumers, who began to differentiate the package among themselves - over time began to play the role of colour, typography and the general nature of the appearance and layout of the package. The excellent example can be a bottle of whiskey. In 1985 Jack Daniel trying to differentiate their product from the common then round bottles of this drink, put on a rectangular bottle, which further emphasized the character of this filtered through charcoal whiskey. This led to other manufacturers slowly started harder to see in the package a new feature - namely product differentiation. That made packaging history began to acquire momentum. A bottle of whiskey signed with the name Daniel, quite different from previously known bottles of alcohol, has given a new identity and brands it effectively manifested (http://blog.michalgosk.com/opakowania/historia-opakowan-czesc-1/, 2015). 30 years later, it is in 1915 year Coca-cola company also provided the patented design of a battle. This event was scheduled between the growing interest in packaging and their potential sales. The appearance of polymer materials completely revolutionized the market of the twentieth century. Many advantages such as low density, corrosion resistance and ease of processing (low costs of running large series of finished products compared to other groups of materials) made from polymers have dominated the market for packaging of the last century. Moreover economic boom, the appearance of television led to dynamic growth in the packaging market. The main driving force behind this situation was the emergence of self-service stores, which appeared in the 30s of the twentieth century in the United States and in Europe a little bit later - just after the end of World War II. Self-service stores revolutionized the current nature of the purchases. Customers could choose by themselves and they had time for that. Packaging design has become so popular and profitable that the resulting separate fields of study, dealing only packaging design (http://blog.michalgosk.com/opakowania/historia-opakowan-czesc-2/, 2015). Packaging became an integral part of the product is not packaged in. Compared to the end of the twentieth / beginning of twenty-first centuries people used to buy in the bazaars. Today, even the salad and rolls are sold in the plastic foil. Many times the packaging value exceeds the price of the product. Consumers buy by eyes. With each year increasing the amount of packaging particular those made of plastic. The process of decomposition takes hundreds of years, sometimes can occur even thousand. The amount of plastic thrown away annually can circle the Earth four times. For instance one American citizen throws away 84 kilograms of plastic. Furthermore every year approximately 500 billion bags made from plastic are used worldwide. Thus, society uses more than one million bags every minute. 50% of synthetic polymers is used only one time. The number of landfills increasing, the Earth area decreases due to the growing number of society. The amount of waste generated also increases. Only five percent of produced plastic is recovered. What's more, the production of synthetic materials use around eight percent of the world's oil production, so accounting amount of produced plastic for several decades crude oil deposits will be completely exhausted. Part of the waste goes to the reservoirs disrupting natural ecosystems (wastes are eaten by marine animals, marine transport causes environmental disasters, etc.). In oceans break down into such little forms that for instance pieces of plastic from one litre bottle are able to end up on every kilometre of beach throughout the whole world. Billions of kilograms of synthetic materials can be found in rotating convergences in the oceans representing approximately 40 percent of the world's ocean surfaces. 80 percent of pollutants flowing into the ocean off the land. Plastic accounts for about 90 percent of all rubbish floating on the surface of the ocean, with 46.000 pieces of plastic per square kilometre. The largest ocean garbage site in the world (the Great Pacific Garbage Patch) is located near the cost of California. This is a floating mass of synthetic polymers twice the size of Texas. Every year one thousands of marine mammals and one million of sea birds are killed by plastic occurred in the oceans. Numerous studies confirmed the plastic is presented in bodies of all sea turtle species, many kinds of fish, 44 percent of all seabird species and 22 percent of cetaceans. Moreover, parts of these compounds, which have been found, have influence on hormonal system of people (xenoestrogens), and the same potential human health effects. Chemicals present in plastic may be absorbed by the human body even in 93 percent. The next disadvantage of plastic material can be the other environmental problem of twenty-first century, it is dioxide emission. According to words of United States Secretary of State John Kerry (during Expo 2015) comparing the waves of immigrants to emission of greenhouse gas it is nothing. Participate to Kyoto Protocol 141 developed countries confirmed how serious problem can it be for the Earth and societies. One of the main reasons of dioxide production is production of plastic. Only in this century was produced more plastic than during the whole last one. That is why it is estimated that the total dioxide emissions from the base year increased approximately 49% (Environmental Health News).

1.4.3. Food packaging in twenty-first century

In connection with numerous disadvantages of plastic producers and scientists have begun to search for packaging material, which would be able to replace it. “Plastic” of twenty-first century should fulfil diverse functions. One of the most important is technological function – a package protects against deterioration and undesirable from outside, protects against microbiological, chemical and physical damages and helps with food trade, the same protected against scattering and spilling. The package is the carrier of information. All informations about product, contain, nutrition, mass, volume, quantity of pieces, method of using, date of shelf-life, brand, producer and others are placed there. In the free market economy period marketing function seems to be quite important either. Colour, design, form of package, information and nutrition claims or healthy information can influence on increasing product demand. Moreover package must be safety (regulated in legislations). In this respect the basic act in Europe is the European Union Regulation 1935/2004 of the European Parliament and European Council of 27 October 2004 for materials and articles intended to come into contact with foodstuffs. These materials under normal or reasonably foreseeable conditions of using can not constitute a danger for human life, cause unacceptable changes in the composition of the food as well as the deterioration of its organoleptic characteristics. These risks could be the result of migration packaging ingredients to food in quantity exceeding the allowable amounts (Polish Committee for Standardization).

The perfect solution of the problem of plastic packaging seems to be biodegradable and/or edible films and coatings for food. Edible coatings are a thin layer of edible material applied directly on food product and meantime films are produced separately from the foodstuff – as solid sheets and then they are used as a foil, trays etc. Bio-packaging can protect the food stuff from mechanical damage, chemical, physical, and microbiological activities. Such systems can be a carrier of nutraceuticals, antioxidants, antimicrobial substances, flavorings and colour agents. They are able to improve several parameters and properties in food products as mechanical integrity, appearance quality and others (Tavassoli-Kafrani, Shekarchizadeh, and Masoudpour-Behabadi; 2016). Biopolymers are usually edible, but if customer does not want to eat food with package can wash it and the coating will solve into water, or consumer can throw it away (Debeaufort, Martin-Polo and Voilley, 1996). Process of degradation takes usually few days - few months, it means a few hundred - few thousand times shorter than in case of plastics. Production of films and coatings is safety for environment. Any greenhouse gas does not arise in the process. According to Kyoto Protocol from 1997 it means, production of biopolymers is not only environmentally safety and friendly. You can also have profits by selling surpluses of these gases. Both films and coatings can occur as a single layers, bi-layers, multilayers or emulsions (Debeaufort and Voilley, 1995). The source materials of biopolymers are plants (spare plants 'materials, waxes, fats), shellfish (chitin armors) and animals' products (milk protein). Generally following classification is used (according to their structural component): saccharides, lipids, proteins and composites.

The most popular polysaccharides are: alginate, carrageenan, chitosan, cellulose with its derivatives, pectin, pullulan and gellan gums and starch (Han and Gennadios, 2005). Polysaccharides' films and coatings have good barrier properties to gases as oxygen, carbon dioxide. Laufer et al. (2013) worked with multilayered thin films. Bio, totally renewable materials were produced from following polysaccharides: chitosan, carrageenan and montmorillonite clay. Polymers characterized low oxygen permeability. After addition of one more chitosan layer (the 4th layer) the oxygen permeability was reduced of two orders of magnitude under the same conditions. Authors applied these coatings onto banana. Banana kept in 22ºC and 55% of relative humidity without any coating exhibit visible changes after 9 days. In the meantime on banana coated by three-layers' coatings were not observed so many changes, thus the lowest amendments could be observed on four-layer coated fruit. After 13 days the skin of uncoated banana was completely dark. Fruit coated by three-layers were brightness, fruit with four coating layer reminded three layers coated fruit after 9 days. So, multilayer coatings significantly are able to reduce physiological processes as respiration and transpiration and the same inhibit the fruit aging. Moreover, the post-harvest loses can be reduced. So, this kind of package, which has high gas barrier and optical transparency, may be promising replacement for foils used for food packaging. Anyway, weak properties for water vapour permeability are attributed to these systems. After lipid addition to their matrix this disadvantage can be significantly reduced. Basiak et al. (2015a) added rapeseed oil to wheat starch films. Water vapour permeability (in 75% of relative humidity) was reduced several times, from 8.77 x 10^{-10} $gm^{-1}s^{-1}Pa^{-1}$ to 3.55 x 10-10 $gm^{-1}s^{-1}Pa^{-1}$ in the case of 5% starch films (amount of starch powder in solution) and from 4.55 x 10^{-10} $gm^{-1}s^{-1}Pa^{-1}$ to 0.25 x 10^{-10} $gm^{-1}s^{-1}Pa^{-1}$ in the case of 3% starch films. Moreover these polymers have excellent mechanical properties, especially tensile strength and elongation at break (Tavassoli-Kafrani, Shekarchizadeh and Masoudpour-Behabadi, 2016). Most of polysaccharide materials is transparent, enhance, they can be easily coloured and printed (Guerrero et al., 2013).

Polysaccharide materials have particular application in fruits and vegetables industry. In couple works was confirmed positively influence of coatings on transpiration and respiration resistances and other parameters as firmness, colour, pH, sugar/acid ratio, even on antioxidant and antimicrobial properties. Arancibia et al. (2014) produced polysaccharide films (made from agar and alginate) with cinnamon essential oil as bi-layer packaging and they used them for microbial growth inhibition in chilled shrimps. The agar and alginate combination allowed reducing meaningly the quantity of microorganisms as pathogenic bacteria *Listeria monocytogenes*, in peeled shrimps during the chilled storage. Besides, bi-layer films have strong antioxidant properties and they are able to prolong the shelf-life of food products. However, any negative impact on the organoleptic properties was observed. The other antimicrobial effect was indicated in the work of Maftoonazad et al. (2007). Emulsions made from pectin and coated onto avocado fruit inhibited growing of *Lasiodiplodia theobromae*. Additional boundary layer caused decreasing of physical and physiological changes as colour and texture (caused by the ageing of the fruit). Tavassoli-Kafrani et al. (2016) noted the carrageenan and alginate biomaterials have good mechanical and barrier properties.

However caseins, collagen, corn zein, egg white protein, gelatin, keratin, myofibrillar protein, quinoa protein, soy protein, wheat gluten, whey protein are common as protein structural materials. Films produced from proteins are flexible, usually transparent with superb aroma-, oxygen-, and oil-barrier properties at low relative humidity (Janjarasskul and Krochta, 2010). Thus to keep good barrier properties in high relative humidity addition of the second film-forming substance, as fat for instance, to protein system is necessary. Janjarasskul et al. (2010) added candelilla wax to whey protein matrix and observed the potential to reduce the water vapour permeability and oxygen permeability. Authors also admitted the making technique plays significant role. Comparing to extruded sheets the solution cast matrix were more effective barriers against water vapour transmission. Moreover the extruded sheets subsequently compressed to biofilms, at all incorporated candelilla wax. Besides, incorporation of agar to soy protein isolate matrix produces changes on the surface, such as the orientation of the polar groups and hydrogen bonding interactions between polysaccharide and protein isolate. Films obtained from agar and soy protein isolate have good functional properties due to the formation of a compact and reinforced three dimensional network.

Lipids as animal and plant waxes (beeswax, candellila wax, carnauba wax, shellac, sugar cane wax), fatty acids (oleic acid, stearic acid), vegetable oils (corn oil, mineral oil, nut oil, olive oil, rapeseed oil, sunflower oil) are usually used as a monolayer or one of the layers in coatings. Actually, already in twelve century in China was recorded using lipids in oranges and lemons for preventing moisture loss (Allen et al., 1963). Liquid lipids can not be used as self-standing films. Separately they do not form films, but with other film-forming material (as a layer or as emulsions 'component) they prove sorption properties.

Polyols as glycerol, sorbitol, monoglycerides, oligosaccharides, polyethylene glycol, glucose, water, lipids are commonly used plasticizers. They are added to film-forming solution for facilitate processing and/or to increase films flexibility and elasticity (Cerqueira et al., 2012). Jost and Langowski (2015) tested several plasticizers as glycerol, propylene glycol, triethyl citrate, polyethylene glycol castor oil and epoxidised soybean oil in content 5-20 wt.%. They noted when the amount of plasticizer increasing crystallinity increasing too, melting temperature decreasing, Young's Modulus decreasing as well, and tensile strength and elongation at break increasing. Thus the oxygen and water vapour permeability is grown up with growing plasticizers content but to a different extent. Similar conclusion suggested Hanani et al. (2013) who measured effect of plasticizer content on the functional properties of extruded gelatin-based composite films. Moreover, authors admitted the solubility in water increases with increasing glycerol content. As well, amount of plasticizer influences on barrier properties.

SECTION TWO: MATERIALS AND METHODS

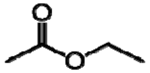
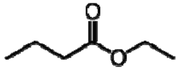
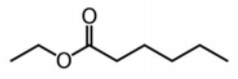
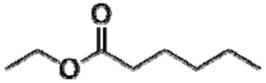
Chapter 2: MATERIALS AND METHODS

2.1. Materials

Wheat (25% of amylose), corn (27% of amylose) and potato (20% of amylose) starches were supplied by Hortimex (Konin, Poland), whey protein isolate (WPI, ~90% protein) BiPRO was obtained from Davisco Foods International Inc. (Le Sueur, MN., USA), rapeseed oils from Bouton d'Or (Tourcoing, France) and Kruszwica (Kujawski, Poland), anhydrous glycerol (99.9% of purity) from Sigma-Aldrich (Germany) and Chempur (Poland) and ten saturated salt solutions (all Prolabo, Fontenay-sous-Bois, France) were used for fixing a wide range of relative humidities (RH) at 25°C: calcium chloride (~3% RH), lithium chloride (11%), potassium acetate (22%), magnesium chloride (33%), potassium carbonate (43%), magnesium nitrate (53%), sodium nitrite (65%), sodium chloride (75%), ammonium sulphate (81%) and ammonium dihydrogenophosphate (93%). Four ethyl esters (Aldrich Chemical Company and Saint-Quentin Fallavier, France) have been chosen as model of aroma compounds (Fenaroli, 1975) and allowing studying a wide range of volatility and solubility. Ethyl acetate (99.5% purity), ethyl butyrate (99%), ethyl hexanoate (98%), ethyl octanoate (98%), all from Aldrich Chemical Company (Saint-Quentin Fallavier, France) have been selected. N-hexane (98% purity, Sigma-Aldrich, Germany) was used as solvent for the ethyl ester extraction from films after sorption.

The physicochemical characteristics of the volatile compounds are given in table 2.1. Log P is the mass partition coefficient of the aroma compound between water and octanol. It represents the polarity/hydrophobicity of the aroma compound. When log P is higher than 2, it is considered as hydrophobic. Then, ethyl acetate and ethyl butyrate can be considered as hydrophilic compounds, whereas ethyl hexanoate and ethyl octanoate have hydrophobic characters.

Table 2.1 Physico-chemical characteristics of aroma compounds used in this study.

Aroma compound formula	Chemical structure	Log P at 25°C	Molecular weight (Da)	Vapour pressure at 25°C (mmHg)	Solubility in water at 20°C (g/mL)
Ethyl acetate $C_4H_8O_2$		0.71	88.11	111.7±0.1	8.3/100
Ethyl butyrate $C_6H_{12}O_2$		1.77	116.16	13.9±0.2	Soluble in 150 parts
Ethyl hexanate $C_8H_{16}O_2$		2.83	144.21	1.7±0.3	Insoluble
Ethyl octanoate $C_{10}H_{20}O_2$		3.90	172.26	0.2±0.4	Insoluble

2.2. Film preparation

2.2.1. Preliminary experiments

3 varieties of starch were chosen: corn, potato and starch. Film-forming solutions were formulated with 2, 2.5, 3, 3.5, 5, 6, 7, 8 and 10 % content of starch in solutions. The same preliminary tests were done for whey protein isolate. Film forming solutions were prepared with the same concentration of proteins as for starch. 3 and 5% solutions were chosen in case of starch and 5% in case of whey proteins. In the next step plasticizer content was selected. Films with 0, 10, 20, 30, 40, 50, 60, 70 and 80 % of glycerol (based on the weight of film-forming powder) were made.

2.2.2. Starches films preparation

Film-forming suspensions of wheat, corn and potato starches were prepared by gelatinisation of 5 g of wheat, corn and potato starch powders in 100 mL distilled water (each separately). The suspensions were heated in a water bath at 85°C for 30 minutes under a 700 rpm stirring to obtain complete gelatinization of starch. Then, film-forming suspensions were cooled down to 40°C. The concentration of plasticizer was chosen based according to preliminary experiment, so the plasticizer was added at a weight ratio of 0.3:1 glycerol : starch under stirring at 150 rpm. A defined volume of film-forming suspension was poured into a Petri dish to obtain a constant film thickness whatever the composition. Films were dried at 25°C and 30% relative humidity (RH) for 48 hours in a climatic chamber (KBF 240, Binder, Germany). Dry films were peeled off and stored at 53±1% RH and 25±1°C in desiccators containing saturated magnesium nitrate for at least 7 days prior to any testing.

2.2.3. Starch film with different glycerol content preparation

Wheat starch film-forming suspensions were prepared by gelatinisation of 5 g of wheat starch powder in 100 mL distilled water. The suspensions were heated in a water bath at 85°C for 30 minutes under a 700 rpm stirring to obtain complete gelatinization of starch. Then, film-forming suspensions were cooled down to 40°C. The plasticizer was added at a weight ratio of 0.5:1 and 0.3:1 glycerol: starch it is 33% and 25% weight/ total dry matter respectively, under stirring at 150 rpm. The concentration of plasticizer was chosen based according to preliminary experiment, i.e., minimum concentration of 30% (weight/ total starch weight) is required in aim the film is ductile and does not brittle and maximum 50% because for higher glycerol content, the films become soggy and white (over-plasticized). A defined volume of film-forming suspension was poured into a Petri dish to obtain a constant film thickness whatever the composition. Films were dried at 25°C and 30% relative humidity (RH) for 48 hours in a climatic chamber (KBF 240, Germany). Dry films were peeled off and stored at 53±1% RH and 25±1°C in desiccators containing saturated magnesium nitrate for at least 7 days prior to any testing. Films without glycerol were very brittle and cannot be analyzed in this work because they broke during all the tests.

2.2.4. Starch-oil film preparation

Wheat starch film-forming suspensions were prepared by dispering of 3g or 5g of wheat starch powder in 100 mL distilled water. The suspensions were heated in a water bath at 85°C for 30 minutes under a 700 rpm stirring to obtain complete dispersion and gelatinization of starch. Film-forming suspensions were cooled down to 40ºC. Then the plasticizer was added at a weight ratio of 0.5:1 glycerol:starch, i.e. 33% to the total dry weight. Some preliminary trials have been done, using various content of glycerol. Thus, 50% (w/w d.m.) of plasticizer contain was considered as the best compromise to get elastic, not breaking, colourless materials. A fixed volume of film-forming suspension (30 mL) was poured into a Petri dish (ϕ = 13.8 cm^2). Then the dry matter per surface unit, and thus the final thickness varied according the film-forming suspension composition. Films were dried in the same conditions as non lipid films. After drying, a layer of rapeseed oil (3g) was deposited by brushing. The layer of rapeseed oil was spread and kept in previous aforementioned conditions for 24 hours. After this time, a third layer of the starch film-forming suspension was cast and dried for 48 hours in same conditions. Dry films were peeled off and stored at 53±1% RH and 25±1°C in desiccators containing saturated magnesium nitrate for at least 7 days prior to testing.

Films were coded as 3S, 3L and 5S, 5L, respectively according their starch content in film forming suspension (3 or 5g of starch in 100 mL of water) and oil layer addition (L) or not (S). To summarize, the dry compositions of films: 3S and 5S have a 1:0.5 starch:glycerol ratio, 3L has a 1:0.5:1 starch: glycerol : oil ratio and the 5L has a 1:0.5:0.6 starch:glycerol:oil ratio.

2.2.5. Starch film preparation with different amount of oil

Wheat starch film-forming suspensions were prepared by dispersing 5 g of wheat starch powder (Hortimex, Poland) in 100 mL distilled water. The suspensions were heated in a water bath at 85°C for 30 minutes under a 100 rpm stirring and shaking to obtain complete dispersion andgelatinization of starch. Then film-forming suspensions were cooled down to 40°C. The glycerol was added (Chempur, Poland) at a weight ratio of 0.5:1 and 0.3:1 glycerol:starch. Then, rapeseed oil (Kujawski, Poland) at 0, 1, 2 and 3% (to 100g film-forming suspension weight) was added. The mixtures were homogenized with an Ultra Turrax homogenizer (T25 IKA, Germany) at 24 000 rpm for 2 minutes. Emulsions of fixed volume were poured into a Petri dish and then dried at 25° C for 48 h. Dry films were peeled off and stored at 53±1% RH and 25±1°C in desiccators containing saturated magnesium nitrate for at least 7 days prior to any testing.

2.2.6. Starch-whey proteins film preparation

Film-forming aqueous suspensions were prepared by mixing by hand and then casting wheat starch and whey protein isolate in the following proportions: 100-0%, 80-20%, 75-25%, 60-40%, 50-50%, 40-60%, 25-75%, 20-80% and 0-100 %. Glycerol was used as a plasticizer at 50% w/w of biopolymer dry weight (i.e. 33% of total dry basis). It was optimal amount because films of starch, whey protein and their mixtures, which containing less glycerol were more brittle, broke and cracked. Films which contain more than 50% were sticky. Wheat starch film-forming suspensions were prepared by dissolving of 5 g of wheat starch powder in 100 mL distilled water. Whey protein film-forming suspensions were also prepared by 100 mL dissolving of 5 g of WPI in distilled water. The suspensions were separately heated in a water bath under a 700 rpm stirring at 85°C for 30 minutes to denature the whey protein and to obtain complete gelatinization of starch. Then, film forming suspensions were cooled down to 40°C. The plasticizer was added. 30mL of each film-forming suspension was poured onto a Petri plate to obtain a constant film thickness of about 80 µm. Films were dried at 25°C and 30% relative humidity (RH) for 48 hours. Dry films were peeled off and stored at 53±1% RH and 25±1°C in desiccators containing saturated magnesium nitrate for 7 days prior to testing.

2.3. Coatings preparation

Film-forming aqueous suspensions were prepared by mixing by blender (Bosch ErgoMixx, Germany) wheat starch and whey protein isolate in the following proportions: 100-0% and 20-80%. Procedure of film-forming suspensions of starch-whey proteins is similar as for films up to the casting step. Instead of casting on Petri dishes fruit were immersed in film-forming suspension for 20 seconds, removed and dried 2 hours in room conditions on threads tied to steams.

2.4. Plum preparation

Different varieties of European plums are available altogether only 8 weeks during the year. It is not sufficient time to perform tests using thermographic camera for this experiment (without earlier trials the needs time amounts is 12 weeks). For that two varieties of Japanese plums were used: "Lady red" and "Angeleno" originally from South Africa, where plums are harvested relatively unripe and ripen slowly whilst in transit for up to 42 days in the cold chain to the European markets. After that both of them were provided from wholesale fruit and vegetable market before the commercial ripening stage and transported immediately to the laboratory. South Africa has a large sector of Japanese plum - 35 plum cultivars are produced there, that is why it was not also possible to get plum of one variety (Louw and Theron, 2012). Plums were kept in cooling room in temperature 5°C and ~35% of relative humidity (RH).

For the second experiment 600 plum of Prunus domestica variety Jojo were harvested in ripening time from 4 trees. Plums were picked up in Garden of Institute for Agricultural Engineering Potsdam-Bornim ATB (Germany) and they were directly transported to laboratory. Fruit were put in a storage chamber (3.5°C, 35% RH) and were kept there one day. After that fruit were divided onto five parts: fresh, coated in starch solution, coated in starch-whey protein solution (80/20), wrapped in starch films and wrapped in starch-whey protein films (80/20). In each of groups were 120 plum (picture 2.1). Next these plums were put onto plastic trays. So, onto each of trays were 6 plums. It means 20 trays with the same treated fruit were prepared for 10 days of measurements. Every week 4 trays of each sort were taken out from the cooling room. One part was used for measurements on the same day and the second part was kept few days in room conditions (for getting the same conditions as are in grocery). After that tests were done. Experiments were conducted 28 days.

Picture 2.1 Coated and wrapped *Prunus domestica* L. cv. Jojo plums

2.5 Composition of film-forming solution (100g) used for both coatings or film making

Table 2.2 Film content per 100g of film-forming solution.

Film code	Wheat starch (S) (g)	Corn starch (CS) (g)	Potato starch (PS) (g)	Whey protein isolate (WP) (g)	Rapeseed oil (O) (g)	Glycerol (g)	Water (g)
3S 30G	3					1.5	97
5S 30G	5					1.5	95
5S 50G	5					2.5	95
5CS 30G		5				1.5	95
5PS 30G			5			1.5	95
80S 20WP	4			1		2.5	95
75S 25WP	3.75			1.25		2.5	95
60S 40WP	2.4			1.6		2.5	95
50S 50WP	2.5			2.5		2.5	95
40S 60WP	1.6			2.4		2.5	95
25S 75WP	1.25			3.75		2.5	95
20S 80WP	1			4		2.5	95
WP				5		2.5	95
3L	3				3	1.5	97
5L	5				5	2.5	95

The film codes are built on the basis on the following examples:

- 3S 30G means 3% starch in solution with 30% of glycerol (% to dry matter of starch)
- 80S 20WP means 80% starch and 20% whey protein (% of dry weight of biopolymers)
- 3L means 3% starch based films containing oil as laminates.

2.6. Transport and solubility properties

2.6.1. Water content

The water content was measured by determination of the weight loss of the film after drying at 105°C for 24 hours and is expressed as gram of water per gram of dry matter. All samples were performed in triplicate.

2.6.2. Swelling index

The swelling index was measured in aim to assess the impact of immersion in water on films. Films' samples were cut into 2x2 cm pieces and weighed. They were then immersed in distilled water (25°C) for 2 minutes. Wet samples were wiped with filter paper to remove excess liquid and weighed. The amount of adsorbed water was calculated in percentages. The measurements were done in triplicate for each type of film from 5 films samples, and the average was taken as the final result.

2.6.3. Solubility in water

The water solubility was determined according to Gontard et al. (1994) method. Films were cut into 2x2 cm pieces dried at 105°C for 24 hours and weighted. Films were individually placed in 50 mL beakers filled with 20 mL of distilled water, capped and stored at 25±1°C for 24 hours. Film pieces were then taken out and dried at 105°C for 24 hours to determine the final weight of dry matter. These steps were repeated five times. Loss of total soluble matter was calculated from the initial and final dry weight of films.

2.6.4. Moisture sorption isotherms

The sorption isotherm of films was determined at 25°C. Samples of films were cut into small pieces (2x2 cm) and weighed to the nearest 0.0001 g into pre-weighed vials. Films were stored up to equilibrium in desiccators, each containing a different saturated salt solution which fixed the relative humidity (RH) at 25°C. A wide range of RH was selected: calcium chloride (~3%), lithium chloride (11%), potassium acetate (22%), magnesium chloride (33%), potassium carbonate (43%), magnesium nitrate (53%), sodium nitrite (65%), sodium chloride (75%), ammonium sulphate (81%) and ammonium dihydrogenphosphate (93% RH). Film samples were weighted periodically up to equilibrium reached. Water content was checked up to 9 months. The final (equilibrium) water content was checked by drying the films at 105°C for 24h. The amount of water absorbed is expressed as gram of water per gram of dry matter. Measurements were done in triplicate for each film recipe.

Sorption isotherms of water vapour were fitted with the Guggenheim-Anderson-deBoer GAB model (equation 1), for water activities up to 0.85:

$$m = \frac{m_0 \cdot C \cdot K \cdot a_w}{(1 - K \cdot a_w) \cdot (1 - K \cdot a_w + C \cdot K \cdot a_w)} \tag{1}$$

where: m is the water content at equilibrium, m_0 is the water of water related to the monolayer, a_w is the water activity of the sample, C and K are constants related to the sorption enthalpy of the first and of subsequent layers, respectively. The GAB model was fit to experimental values using the Table-Curve 5.1 software (Sigmaplot, United States of America).

2.6.5. Diffusivity of water

From the kinetics of water sorption, the diffusivity of water in films was calculated according the Crank's solution of the 2nd Fick law. Indeed, the diffusivity was obtained from the kinetics, assuming the following hypothesis. When the apparent diffusivity D_{app} is constant and independent of the concentration and time, the transfer rate through a sheet of thickness e (m) immersed in an atmosphere of infinite volume with a constant concentration of diffusing substance (C_{eq}, kg m^{-3}) and with an infinite mass convection coefficient can be expressed by eq. (2) (Dury-Brun et al., 2008)

$$\frac{\partial C}{\partial t} = D\,\frac{\partial^2 C}{\partial x^2} \tag{2}$$

Taking into account the following initial and boundary conditions for sorption experiment (eqs. (3)-(5), eq. (6) were obtained by integrating eq. (2) for a sheet of e thickness) (Crank, 1975)

At $t = 0$, $C_0 = 0 =$ for $0 \geq x \geq e$; (3)

At $t \geq 0$, $C_t = C_{max}$ for $x = e$; (4)

And at $t=\infty$, $C_\infty = C_{max}$, for $0 \geq x \geq e$

$$\frac{\partial C}{\partial x} = 0;\ x = 0;\ t \geq 0 \tag{5}$$

$$\frac{C_t}{C_{max}} = \frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2+1)^2\pi^2} \exp\left[-\frac{(2n+1)^2\pi^2}{2e^2}\,Dt\right] \tag{6}$$

where: t is the time (s), M_t the amount of water vapour sorbed by the sheet at time t, and M_∞, the maximum amount of water sorbet at equilibrium (theoretical infinite time), C_o the initial concentration at zero time, C_t,the concentration at time t, C_{max}, the maximum concentration of sorbed vapour at infinite time also noticed C_∞. Water apparent diffusivity within the films was estimated by fitting eq. (5) to the experimental release kinetic data using the NLIN procedure of SAS after a pre-estimation of D using Excel. The equation was fit with n = 5.

2.6.6. Water vapour sorption kinetic

Measurement of water vapour sorption kinetics was conducted in at least three repetitions for each type of film under conditions of constant temperature of 25°C and relative humidity of 75.3%. The samples (2x2 cm) were removed from the desiccator and weighed periodically for 158 hours (until the samples reached a constant mass). The experimental absorbed moisture content values were averaged and fitted by a mathematical exponential equation (Kowalska et al., 2006) as follows:

$$u = h + g(1 - \exp^{(-jt)}) \tag{7}$$

where: u – water content (g water g d.m.$^{-1}$), h, g, j - constant parameters of equation, t – time (h).

The adequacy of the fitted exponential equation was evaluated by the determination coefficient (R^2) and mean relative error (MRE) using the following equations:

$$R^2 = \frac{\sum_{i=1}^{n}(u_p - \bar{u}_e)^2}{\sum_{i=1}^{n}(u_e - \bar{u}_e)^2} \tag{8}$$

$$MRE = \frac{100}{n} \cdot \sum \left| \frac{u_e - u_p}{u_e} \right| \tag{9}$$

where: n - number of observations; u_p - predicted water content (g water g d.m.$^{-1}$); $\bar{u}_e$ - average water content (g water g d.m.$^{-1}$); u_e - experimental water content (g water g d.m.$^{-1}$).

2.6.7. Aroma compound sorption

Ethyl acetate (99.5% purity), ethyl butyrate (99%), ethyl hexanoate (98%), ethyl octanoate (98%) have been selected to mimic fruit flavour compounds. In order to assess the aroma compound retention by the films or their affinity for the aroma compounds, films were exposed to atmospheres being saturated with the vapour of the aroma compounds. Pieces of dry film were placed in a glass vials along with respected aroma compound. All vials were put in sealed jar containing pure aroma compounds. The jars containing vials of films and the aroma were kept for a minimum of 3 weeks at 25°C. After equilibrium was reached, n-hexane was added in vials for extraction and stored for 24 hours at room temperature at a ratio of 500 mg of film in 4 mL solvent. The yield of extraction was previously set up in the lab (41) and ranged between 95-97% (Descours et al., 2013). The volatile flavour compounds were quantified by injection of the n-hexane extract into a gas chromatograph. The amount of aroma extracted from the films was calculated from an external calibration curve.

The chromatography analyses was done using a Shimadzu GC 2014 gas chromatograph, equipped with a flame ionization detector (GC-FID) and 30 m length DB-Wax column (J&W) with 0.53 mm i.d. and 1.0 µm film thickness and nitrogen as carrier gas (60 kPa). Hydrogen and air were used as ignition gases. The oven temperature program was set at 210ºC, isothermal. The injector and detector temperatures were at 240ºC, isotherm. For each sample minimum three repetitions have been done.

2.7. Structure and optic characterizations

2.7.1. Electron microscopy observations

Film microstructure was observed using an environmental scanning electron microscope (ESEM, Philips XL 30 ESEM, Japan). A 0.5 x 1.0 cm film was fixed on the support using double side adhesive, at an angle of 90º to the surface which allowed observation of the cross-section of the film. All the film samples were cut with a new razor blade to prevent as much as possible any morphological damage. Films were focused up to x15000, and magnifications ranging from x800 to x8000 where selected, with an intensity of 8kV and absolute pressure of 230 Pa in presence of water (RH~30% at 5ºC). No special preparation such as palladium or gold coating was necessary for ESEM observations.

2.7.2. Polarizing microscopy observations

Film microstructure was observed using the polarizing microscope (Olympus BX51, Japan) and additional light 1146 (light-x-cite, series 120Q Exfo). Uncoated plum skin, skin of plum directly coated and skin of plum coated and dried were cut with a new razor blade to prevent as much as possible any morphological damage in 0.5x0.5 cm and placed on a slide. Samples were focused up to x15000, and magnifications ranging from x100 to x4000 where selected, with an intensity of 8kV and absolute pressure of 230 Pa in presence of water (RH~30% at 5ºC). No special preparation such as palladium or gold coating was necessary for microscopic observations.

2.7.3. Light microscopy observations

Film structure was observed using the light microscope (Vision Engineering DX21, United Kingdom) with coupled camera (Nikon Coolpix 990, Japan). Uncoated plum skin, skin of plum directly coated and skin of plum coated and dried were focused up to x400, and magnifications ranging from x10 to x40 were selected. No special preparation was necessary for microscopic observations.

2.7.4. Thermographic camera observations

2.7.4.1. Temperature measurements

One raw plum was placed in a special thin basket made from wire. The tip of wire was fastened to the balance type CPA 224S-OCE (Sartorius, Germany). Thermographic camera supported 3D scan system ScanBook (Scanbull, Germany) was switched on. Pictures were taken every 2 minutes (100 pictures). After 3 hours and 20 minutes camera took photographs every 40 minutes (20 pictures). Temperature and relative humidity were measured every 2 seconds by logger type Almemo 2290-8 (Ahlborn Meß, Germany) connected with sensor type Almemo Heyco (Ahlborn Meß, Germany). The next day, at the same hour when the first picture was taken plum was dipped for 60 seconds in film forming solution and directly handled on the previous place. Camera, logger and sensor were turned on with the same settings as the first day. The third and fourth day plum was dipped for 60 seconds in film-forming solution and again directly handled on the same place, exactly the same as of the second and the third time, respectively.

2.7.4.2. Transpiration model for uncoated and coated fruit

This model is dedicated for calculating mass loss and surface temperature of food product items. It bases on only simple to measure parameters of environment (air temperature, relative air humidity, barometric pressure) and food product (mass loss, surface temperature). The core of the model is Fick's law of diffusion in terms of resistances, where water (water vapour) moves from locations with high concentrations to others with lower concentrations depending on involved resistances along the water vapour pathway. According the equations (10) and (11) the transpiration rate (TR) is equal to the ratio of differences in concentration to the sum of resistances:

$$TR = (xP-xL)/(rB+rT) \tag{10}$$

$$TR = (xP-xL)/(rB+rT+rC) \quad (11)$$

where: TR (mg $cm^{-2}h^{-1}$) is the area normalized transpiration rate, xP (mg m^{-3}) is the volume normalized water content of air in the intercellular spaces in the centre of the fruit, xL (mg m^{-3}) is the volumic water content of the air unaffected by the product, rB (s cm^{-1}) is the boundary layer resistance in the water vapour pathway, rT (s cm^{-1}) is the tissue resistance in the water vapour pathway, rC (s cm^{-1}) is the resistance of the coating in the water vapour pathway.

It is common to use the partial difference of water vapour pressure instead of the volume related water content difference. By simple conversion, it can also be demonstrated how water potential difference can be used as a driving force for the transpiration in order to explain events occurring in the food product itself and on the border between the fruit and its environment. Analogous to equation (10), equation (12) is valid:

$$TR = (xPS-xL)/rB \quad (12)$$

where: xPS (mg m^{-3}) is the volumic water content of the air at the product surface.

The area normalized transpiration rate can also be determined by equation:

$$TR = (FM1-FM2)/(A * t) \quad (13)$$

where: FM1 (mg) is the fresh matter before climatic treatment weighing by means of sensitive balance, FM2 (mg) is the fresh matter after climatic treatment weighing by means of sensitive balance, A (cm^2) is the fruit surface approximation equation, t (h) is duration of climatic treatment. All parameters were measured.

The boundary layer resistance (rB) for the water vapour diffusion between product and its environment under natural convection is only dependent on dimension, shape and surface structure of the product (and known temperature dependent on air properties). It can be determined from preliminary experiments if equations (10) and (12) are equated and the boundary layer resistance is solved for:

$$rB = ((xPS-xL) *A*t))/(FM1-FM2) \quad (14)$$

variants for the determination of the boundary layer resistance exist:

- wetted surface, eq.(10) with rT = 0 (no water comes out direct from inside of the product)
- water and product must be in thermal equilibrium at the starting point (xPS = f (T))
- drying up of the wetted surface can be observed from weight loss curve (characteristic edge)
- under normal room conditions and unrestricted free convection the surface is completely dried in around 12 minutes for coatings it needs longer time
- forced air, with velocity > $2ms^{-1}$, eq. (10) with rB = 0 (surface is in direct contact with ambient air)
- direct determination of rT under forced convection
- rB (free convection) is calculated from the difference of rLges (sum of resistance from layers) under free and forced convection.

[1] Environmental conditions

[1.1] Air temperature TL (in sufficient distance to the food product)

- measuring value (recorded over time in relatively short time intervals (some minutes, some hours)

- air temperature can change during the experiment

[1.2] Relative humidity RH (in sufficient distance to the food product)

- measuring value (recorded over time in relatively short time intervals (some minutes, some hours)

- relative humidity can change during the experiment

- alternatively air humidity measuring values from other measuring principles (psychrometer, dew point meter)

[1.3] Barometric pressure, p (bar)

- measuring value (recorded over time (some minutes, some hours))

- barometric pressure can change during the experiment

- if not measured a good approximation is 100 kPa

[1.4] Boundary layer resistance, rB

- characterises the flow conditions against and around the food product

- depends on main dimensions, shape, surface structure (and properties of the air)

- normally determined in preliminary (short) experiments (some minutes)

- constant during the operational time

[2] Food product properties

[2.1] Initial fresh matter, FM0

[2.2] Surface area, A

- normally an approximation equation, A = f(FM0), sometimes combined with main dimensions

- for some food product items an approximation exclusively from main dimensions (as sphere or cylinder) is common

[2.3] Food product (surface) temperature, TP (TPS)

- initially food product and environment should be at equilibrium

Model psychrometric calculations:

- for both outside air (in sufficient distance to the food product surface) and air in intercellular spaces of the food product

- assumption is that inside air (in intercellular spaces) is saturated, valid as long as the food product is visible fresh

- ambient air is measured (temperature, relative humidity, barometric pressure)

- partial pressure of water vapour at saturation depending on temperature

- partial pressure of water vapour
- absolute air humidity related to mass
- density of wet air
- absolute air humidity related to volume
- difference in absolute air humidity (related to volume) between outside air and air in intercellular spaces.

Calculation of the transpiration rate:

Calculation of the transpiration rate according to eq. (10)

determination of mass loss per unit time

Overall model output parameters:

[1] Weight loss per unit time

- it is assumed that the whole weight loss is caused by water loss
- weight loss due to respiration is neglected as long as operational time is not too long

[2] Food product surface temperature

- changing with time
- as mean value

[3] Food product surface relative humidity (plus partial pressure of water vapour, absolute humidity, total water potential at food product surface)

- changing with time
- as mean value

2.7.5. Fourier transform infrared with attenuated total reflection (FTIR-ATR)

The Fourier Transform Infrared spectra from each film were obtained using a spectrometer (Brucker, IFS 28) using Attenuated Total Reflectance (ATR) using ZnSe crystal. All the spectra had an average of 64 scans at a resolution of 4 cm^{-1}, from 650 to 4000 cm^{-1} and determined at 25°C on films equilibrated at 53% RH. This analysis aimed at determining the modifications at the molecular scale at the surface induced by the glycerol addition.

2.8.Geometrical properties

2.8.1. Film thickness

Film thickness was measured with a PosiTector 6000 (DeFelsko, Ogdensburg, NY, USA) digital micrometer to the nearest 1 µm in 0-100 µm range and to the nearest 5µm in the 100-1000µm. Prior to film thickness measurements, the electronic gauge was calibrated at 74 and 139 µm using standards to be close to thicknesses of samples. Thickness of each film was measured in five places, one in the centre part of the film and four around its perimeter, and an average value was used in the calculations. At least 10 replications of each formulation have been made.

2.8.2. Coating thickness

Changing of coating thickness (when not all amount of water evaporates) and constant thickness (after water evaporation) were assessed according to the following equations:

$$A = 0.809*MF + 19.405 \tag{15}$$

$$Me = MCF - MF \tag{16}$$

$$Mw = Me - 0.05 * Me \tag{17}$$

$$W\% = \frac{Mw}{Me*100} \tag{18}$$

$$\rho = \frac{W\%}{100} *\rho w + \left(\frac{100-W\%}{100}\right)* \rho w \tag{19}$$

$$Vc = \left(\frac{Me}{1000}\right)/ \rho \tag{20}$$

$$u = \frac{Mw}{0.06}* Me \tag{21}$$

$$e = 1000*\left(\frac{Vc}{A}\right)*10 \tag{22}$$

where: where: V – volume of film-forming suspension (cm^3), Δm - mass of film forming suspension (g), ρ – density of film-forming suspension ($g\ cm^{-3}$), e – mean thickness of coating layer onto a fruit (cm), FM – fruit mass (g), A – fruit surface area (cm^2), Me – mass of the coating material (g), MCF – mass of coated plum (g), Mw – mass of water inside coating (g), W% - relative water content (%),Vc – volume of the coating onto fruit surface (cm^3), u – water content ($g_{dry\ mass}/g_{water}$). The relevant mass of film forming suspension Δm was determined from the weight variation of plums after coating process (dipping).The relevant mass of film forming suspension Δm was determined from the weight variation of plums after coating process (dipping).

2.9. Surface properties

2.9.1. Contact angle determination

Contact angle (*θ*) is the angle described as a relationship between the surface tensions between the three phases: liquid phase L, solid phase S, or vapour phase V according Young equation (Young, 1805).

The contact angle was measured with sessile drop method: a droplet (about 1.5 µL) of a test liquid was placed on a horizontal film surface. Measurements were done using a DGD-DX goniometer equipped with the DIGIDROP image analysis software (GBX, Romans-sur-Isere, France), according to Karbowiak, Debeaufort and Voilley (2006) methodology. The contact angle was measured on both sides of the drop and averaged. The contact angle and drop volume measurements were carried out over 120 seconds. The effect of evaporation was assessed on an aluminum foil considered as an impermeable reference surface and subtracted from sample. Then the rate of evaporation was considered in the study of the kinetic of wetting and absorption. Measurements for all samples were done on the side of the films exposed to air during drying in aim to prevent the support (Petri dish) effect. Measurements were done in minimum 10 replications on each film recipe.

2.9.2. Films critical surface tension

The surface tension of the liquid tested (γ_L) was measured by the sessile drop method and Laplace-Young approximation (Song & Springer, 1996a,b). The estimation of the critical surface tension (γ_C) of the starch-based films was obtained from the Zisman method (Zisman,

1964) as used by Basiak, Lenart and Debeaufort (2015b). The critical surface tension (γ_C) value of film is obtained from the extrapolation the linear regression of the cos θ of various liquids according their surface tension (γ_L). Extrapolation at cos θ = 1 yields the value of the critical surface tension of the film. Cyclopentanol, diiodomethane, ethylene glycol, glycol, methyl benzoate, n-octane, polyethylene glycol, tetradecane, water and 1-bromonaphtalane were selected as the liquids for which the surface tension properties, dispersive and polar components are known and which are also used for determination of the surface tension and its components. Ten repetitions at least were done on each film formulation.

2.9.3. Surface tension

The surface tension (γ_S) and its dispersive (γ_S^D) and polar (γ_S^P) components were calculated by the Owens-Wendt method (Owens and Wendt, 1969). Equations (23) and (24) show this method:

$$\gamma_S = \gamma_S^P + \gamma_S^D \tag{23}$$

$$\gamma_L = (1 + \cos\theta) = 2\left(\sqrt{\gamma_S^P \gamma_L^P} + \sqrt{\gamma_S^D \gamma_L^D}\right) \tag{24}$$

In eq. (23) appears two unknown γ_S^P and γ_S^D, so it is insufficient to determine the γ_S of a polymer. Thus, the contact angle has to be measured using at least two liquids, knowing their respective surface tensions and their dispersive (γ_S^D) and polar (γ_S^P) components. That yields to the two equations in the form of eq. (24). Water and diiodomethane were used for the determination of surface tensions components. Measurements were performed at least 10 times.

2.9.4. Adhesion, cohesion, spreading coefficient

Work of adhesion (per unit area, W_a), work of cohesion (per unit area, W_c) and spreading coefficient (for a liquid over a solid, W_s) were calculated using Dupre (1869) equations on the basis of water and diiodomethane:

$$W_a = W_a^P + W_a^D \leftrightarrow 2\left(\sqrt{\gamma_S^P \gamma_L^P} + \sqrt{\gamma_S^D \gamma_L^D}\right) \qquad \gamma_L = (1 + \cos\theta) \tag{25}$$

$$W_c = 2\,\gamma_{LV} \tag{26}$$

$$W_s = W_a - W_c = \gamma_{SV} - \gamma_{LV} - \gamma_{LS} \tag{27}$$

2.10. Barrier properties

2.10.1. Water vapour permeability (WVP)

Water vapour permeability of films was measured gravimetrically according to Debeaufort et al. (1993) who adapted the ASTM E96-80 (1980) standard method to hydrophilic edible films and coatings. Film samples were placed between two rubber rings on the top of glass cells containing silica gel, sodium chloride or distilled water allowing obtaining internal relative humidity (RH) of the permeation cells at ~3%, 75 %, and 100%, respectively. The permeation cells were then placed in a climatic ventilated chamber (KBF 240, Binder, Germany) maintained at a RH of 30% and temperature of 25°C and the weight was recorded daily for at least 10 days. Water vapour permeability was calculated using the following equation:

$$\mathrm{WVP} = \frac{\Delta m \cdot e}{A \cdot \Delta t \cdot \Delta p} \tag{28}$$

where: $\Delta m/\Delta t$ - weight of moisture loss per unit of time (g s^{-1}), A - film area exposed to moisture transfer ($8.04\ 10^{-4}$ m^2), e - film thickness (m), Δp - water vapour pressure differential between the two sides of the film (Pa). Measurements were performed at least three times for each RH differential tested.

2.10.2. Oxygen permeability (OP)

Oxygen permeability was measured using the manometric method according the ISO 15105-1 standard using the Brügger equipment, Type GDP-C (Brügger Feinmechanik GmBH, Germany). The tests chambers of the permeation cell were first degassed under vacuum, then the upper side was swept by an humidified oxygen flow at a rate of about 80 mL min^{-1} at atmospheric pressure. The increase in pressure in the downside chamber during the test period was assessed and displayed by an external computer. Data were recorded and permeance was calculated by GDP-C software (with temperature compensation). The sample temperature (25°C) was adjusted using external thermostat (HAAKE F3 with Werbath K). The desired RH was regulated in an external saturation system (53% and 75% RH), so humidified oxygen gas circulated in the permeation cell.

2.11. Mechanical properties

Tensile strength (TS), elongation at break (E) and Young modulus (YM) of the films were measured using a TA-XT2i Texture Analyser (Stable Microsystems, United Kingdom) according to the ASTM D882-95 method (ASTM 1995). 10x2.5 cm specimens were cut by scissors. Self-tightening roller grips were used to perform tensile tests. The initial distance between the grips and the initial velocity were adjusted to 50 mm and 1mm s^{-1}. Mechanical properties were replicated 15 times at least and calculated using the average thickness of each film sample.

Firmness of fruit was measured using texturometer TA.XT plus (Stable Microsystems, United Kingdom) equipped with a special 4 mm tip for this study. Fruit flesh firmness was analysed by means of puncture tests. Skin of plum was removed as thin as it was possible (with a new sharp razor blade) and flesh was located on the texturometer table (diameter of flesh was around 15-30mm). The penetration depth was 1mm and the penetration speed rate was 2mm s^{-1}. Measurements were done in 15th repetitions.

2.12. Colour parameters of films and coated plums

Colour of films was determined using a colorimeter (Minolta, Model CR-300, Japan) using the CIE LAB colour parameters: L, from black (0) to white (100); a, from green (-) to red (+); and b, from blue (-) to yellow (+) (Gennadios et al., 1996). Colour of films was expressed as the total colour difference (ΔE), saturation index (Si) and colour chroma (Ccolour) according to the following equations (Sobral, dos Santos and Garcia, 2005):

$$\Delta E = \sqrt{(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2} \tag{29}$$

$$C_{colour} = \sqrt{(a - a^*)^2 + (b - b^*)^2} \tag{30}$$

$$Si = \sqrt{(a^2 + b^2} \tag{31}$$

where: L*, a*, b* and C_{colour} are the colour parameters of a white standard support used as the film background (L*=96.74, a*=0.09, b*=2.20).

6 replicates of the colour parameters of plum skin coated and not coated were determined on each fruit samples (measured in 3 places of each fruit sample surface), using the Minolta colorimeter CM-2600d model (Minolta Camera Co., Japan).

Opacity was measured using spectofotomether UV/VIS Helios Gamma (Thermo Electron Corporation, Germany). Films were cut into rectangles 1x4 cm^2. The absorbance was measured at visible light wavelength of 600 nm. 10 repetitions for each type of films were done. The opacity was calculated according the equation (32) proposed by Han and Floros (1997):

$$O = \frac{A_{600}}{e} \tag{32}$$

where: O – opacity (A mm^{-1}) A_{600} – absorbation at wavelenght of 600 nm; e – thickness (m).

2.13. Thermo-gravimetric analysis (TGA)

Dried films were scanned using a thermogravimetric analyser (TGA-7, Perkin Elmer, Norwalk, CT, USA) from 40 to 800°C at a rate of 10°C/min. Nitrogen was used as the purge gas at a flow rate of 20 mL/min and also prevent oxidative degradation. Measurements were done in 3 replications.

2.14. Physiological properties

2.14.1. Transpiration rate

Plums were storaged in 3.5°C in ~35 and 80% relative humidity. Fruit were taken out from the cooling room and replaced to the other room with following conditions: temperature 22°C and ~35% of RH. After temperature equilibria (fruit and environment), the transpiration rate was measured. Plum were weighted on balance CPA 1003S (Sartorius, Germany), then surface temperature was measured by microscanner D-series (Exergen, Germany) in 5 points. Plum were placed on a mesh, logger Almemo 2290-8 (Ahlborn Meß, Germany) with sensor Almemo Heyco (Ahlborn Meß, Germany) was switched on. After 1.5 hour logger with sensor was switched off, weight and temperature were measured once again. Calculations were done by using special software set up for this measurement. From the changes of plum weights over time and expressed as weight variation (g) per initial weight (kg) and unit time (day), the transpiration rate (TR) was calculated according to equations (33) and (34):

$$TR_m = \frac{Mt - M}{t x \left(\frac{M}{1000}\right)} \tag{33}$$

$$TR_s = \frac{1000 x (Mt - M)}{t x A} \tag{34}$$

where: TRs is the transpiration rate per unit surface area of the product in mg cm^{-2} h^{-1} and TR is the transpiration rate per unit of initial mass of the produce in g kg^{-1} h^{-1}, Mi is the initial weight (g), M is the weight of plum (g) at time t (h) and A the surface area of the plum (cm^2).

2.14.2. Respiration rate

After the transpiration test measurement the plums were weighted CPA 1003S (Sartorius, Germany) and placed into respirator's chambers at the same distance on the wire mesh. Chambers were tightly closed and software for respiration (Atmung, Germany) was switched on to monitor the gas composition in the chamber. After reaching the appropriate difference in the gas levels chambers were opened and temperature and weight were measured once again. Then, calculations were done by using special software set up for this measurement.

The rates of carbon dioxide production (R_{CO2}) and oxygen consumption (R_{O2}) were determinated by fitting experimental data of Y_{O2} and Y_{CO2}, according to the method used by Caleb et al. (2012) (equations 35, 36):

$$Y_{O2} = Y^{i}_{O2} - \frac{R_{O_2}M}{V_f}(t - ti)\ x\ 100 \tag{35}$$

$$Y_{CO2} = Y^{i}_{CO2} - \frac{R_{CO_2}M}{V_f}(t - ti)\ x\ 100 \tag{36}$$

where Y^{i}_{O2} is the oxygen concentration (%) at initial time t_i (h) and Y_{O2} at time t (h), Y^{i}_{CO2} is the CO_2 concentration (%) at the initial time t_i (h) and Y_{CO2} at time t (h), R_{O2} and R_{CO2} are RR in mL kg^{-1} h^{-1} and Mis the total weight of the product (kg). V_f is the empty volume inside the chamber (mL). The volume occupied by the fruit was calculated from the fruit mass.

2.14.3. Mass loss

To characterise water losses in plum tissue mass changes in time were calculated. On the beginning of experiment, before and after every measurement fruit were weighted on balance CPA 1003S (Sartorius, Germany). Following equation was used:

$$weight\ loss\ (\%) - \frac{(Wi - Wsp)}{Wi} * 100 \tag{37}$$

where: Wi - initial weight (g); Ws - weight at sampling period (g).

2.14.4. Titratable acidity and total soluble solid

Using garlic press equipment, the juice was squeezed from plums. Juice samples were centrifuged and devided onto two parts. One part was used for the total soluble solid content (TSS) determination. Using a digital refractometer, the TSS was measured and expressed as a percentage (%) of dry content. The second part of juice was used for titratable acidity (TA) measurement by automatic titrator (compact titrosampler rondo tower, Metter Toledo, Switzerland) with 0.1 mol L^{-1} NaOH to an end-point of pH 8.2. Titratable acidity (TA) was expressed as a gram of of citric acid per litre of plum juice (g L^{-1}). TSS:TA ratio was calculated and expressed as %. 3 replications of each sample were made.

2.15. Statistical analysis

Statistical analysis was performed with the program Statgraphics Plus, version 5.0 (Manugistics Corp., Rockville, MD, U.S.A.). The analysis of variance (ANOVA) and Fisher's LSD multiple comparisons were performed to detect significant differences in properties of films. The significance level used was 0.001.

SECTION THREE: RESULTS AND DISCUSSION

Chapter 3: Starch source and plasticizer content (glycerol) to cover plums, which constitute the end-product application of starch-based wrappings/coatings in order to extend their shelf-life.

3.1. Role of starch nature on the physico-chemical properties of edible films (Basiak E., Debeaufort F., Lenart A. (2016). Role of starch nature on the physico-chemical properties of edible films. Submitted in Journal of Food Science in January 2016).

3.1.1. Abstract and introduction

Food preservation is mostly related to packaging in oil-based plastics, inducing environmental troubles but this drawback could be limited by the use of edible or biodegradable films and coatings. Physical and chemical properties were assessed and display the role of the starch nature (wheat, corn or potato) and thus that of the amylose/amylopectin ratio which influences thickness, colour, moisture, wettability, thermal, surface and mechanical properties. Higher amylose content in films induces higher moisture sensitivity, and thus affects the mechanical and barrier properties.

The most suitable, abundant, renewable and low-cost material for producing edible coatings based on biopolymers is starch (Ghanbarzadeh and others 2011; Al-Hassan and Norziah 2012). Due to this fact, there is a growing need of understanding the interactions between the film structure, water molecules and other constituents used for edible film making. Starch is very well known carbohydrate polymer growing in mostly all the tempered zones (Bergo and others 2012). Corn, wheat, potato and rice take the world lead positions: 84%, 7%, 4and 1% respectively. Tapioca, yam, oat, sweet potato, barley, rye, taro, millet, amaranth grain, quinoa, favas, lentils, mung beans, peas, chickpeas, palms, chestnuts, acorns, arrowroot, arracacha, bananas, breadfruit, buckwheat, canna, colacasia, katakuri, kudzu, malanga, oca, polynesian arrowroot, sago, sorghum, water chestnuts are the other starch sources that could be envisaged for such applications (Araujo-Farro and others 2010; Piyada and others 2013; Prakash Maran and others 2013, Zavareze and others 2011). Amylose is a sparsely branched carbohydrate mainly based on α(1-4) bonds with a molecular weight of 105 to 106. The number of macromolecular configurations based on α(1-6) links is directly proportional to the amylose molecular weight (Phan and others, 2005). Amylopectin is a highly multiple-branched polymer with a high-molecular weight of 107 to 109. Depending on botanical resource starch granules vary in size from about 2 to 150 µm, the amylose content from 0% to about 75%, but typical is 20-25% (w/w) (Arvanitoyannis and Tserkezou 2009). The so-termed waxy starch contains low or no amylose, whereas high-amylose starch contains more than 50% of the linear polymer. Starches with various amylose: amylopectin ratio have different chemical, physical and functional properties of edible films and coatings (Parker and Ring 2001; Xie and others 2012). Palviainen and others (2001) described better mechanical and gas barrier properties of starch with high amylose content such as from pea. Joshi and others (2013) noticed that compared to corn and potato starches, lentil starch (30% of amylose) possesses strong gel forming tendency at relatively low concentration. Whatever the botanical origin, starch exhibits several disadvantages such as strong hydrophilic character (water sensitivity), which make it unsatisfactory for some applications. The objective of this study was to investigate microstructural, optical, mechanical, barrier and thermal properties of 3 types of films made from corn, potato and wheat starch and containing 33% (to starch dry basis) glycerol. Besides, the main goal of this work was to display the difference in amylose content has significant influence on some properties.

3.1.2. Practical application

In case of fruit and vegetables, starch thin layers suit because they do not inhibit but they retard the physiological processes (respiration and transpiration). The films made from potato starch constitute a greater barrier for oxygen and water vapour. But their mechanical properties are weaker than that of wheat and corn starch films. Starch species with high amylose content have lower wettability properties, and better mechanical resistance, so they could be used for products with higher water activity such as cheese, fruits and vegetables etc.

3.1.3. Microstructure, thickness and optical parameters

The environmental scanning electron microscopy (ESEM) of starch matrix gives relevant information about the microstructure of the films. Depending on the nature of the components and the interactions expanded in the film-forming solutions, the hydrocolloids arrange themselves in different ways in the film matrices. That in turn, allows the data received for the mechanical, optical, barrier properties to be comprehended (Jiménez et al., 2012). Images of corn, potato and wheat starch film microstructure are presented on figure 3.1. Cross section of all starch films displays heterogeneities, looking like a network or fibres. However, according to the very homogeneous and smooth aspect of the surface, there is no porosity. This microstructure is maybe due to the incomplete dispersion/gelatinisation of starch granules linked by the solubilized-gelatinized fraction of starch. Bonilla et al. (2013), Mali and Grossmann (2002), Phan The and others (2009), Shi et al. (2013), Sun and others (2013) and several other authors also displayed similar results for films based on various starch types containing glycerol content from 10 to 50% to dry matter. As confirm by the cross-section pictures, corn starch films are the thickest (112 µm). The thinnest and the less heterogeneous films are those from potato starch (55µm). Differences appeared from amylose/amylopectin ratio, 27/73%, 25/75%, 20/80% for corn, wheat and potato starches, respectively. The less amylose is the thinner and the less heterogeneous the films seem to be. In this study, the film forming suspensions have always the same concentration for all type of starch and thus the film forming composition cannot explain thickness variations. The retraction of the starch gel during drying is favoured by the lower content of amylose. Amylopectin favour to obtain less heterogeneous and denser films and thus thinner films.

Film microstructure and thickness affect optical properties of the films, which are greatly influenced by the internal and surface heterogeneity of the matrix (Jiménez and others, 2013b). Wheat, corn and potato starch films are shinny and glossy at the support side (surface in contact to the Petri dish during film drying) and dull from the air side (surface exposed to air during drying) as also observed by Bonilla and others (2013). Depending on the starch origin films present opacity for corn and wheat starches, but transparent for the potato starch. The same results have been obtained by Gutiérrez and others (2014), Jiménez and others (2012), Sun and others (2013), Zavareze and others (2010). According to Mali and others (2004) colour parameters values are either dependent on the film thickness. The higher the thickness the more opaque the films appear. That explains probably why potato starch films exhibit transparency, whereas corn and starch films are more opalescent because they are thicker.

Colour parameters display also differences. In the present study the lightness parameter are very close to the white standard. Both *a* (redness) and *b* (blueness) parameters were similar for the corn and potato starch films. However, the wheat starch surfaces had greenness *a* parameter and yellowness *b*. Araujo-Farro and others (2010) reported the following values for quinoa starch systems were obtained: *L*=92.86, *a*=-0.87 and *b*=1.96, Muscat and others (2013) for high amylose content starch films: *L*=92.76, *a* =0.12 and *b* =1.40, Flores and others (2007) for tapioca starch films: *L*=85.42, *a*=-1.08 and *b*=5.02, Li and others (2015) for pea starch matrix: *L*=86.93, *a*=1.15 and *b*=2.10 and Zavareze and others (2011) for native potato: *L*=95.70, *a*=-5.14 and *b*=7.38. In general, the film optical values presented here have good transparency. In any case the values of *a* and *b* parameters are of a magnitude that does not interfere with the colour perception by consumer of coated food products with such type of film.

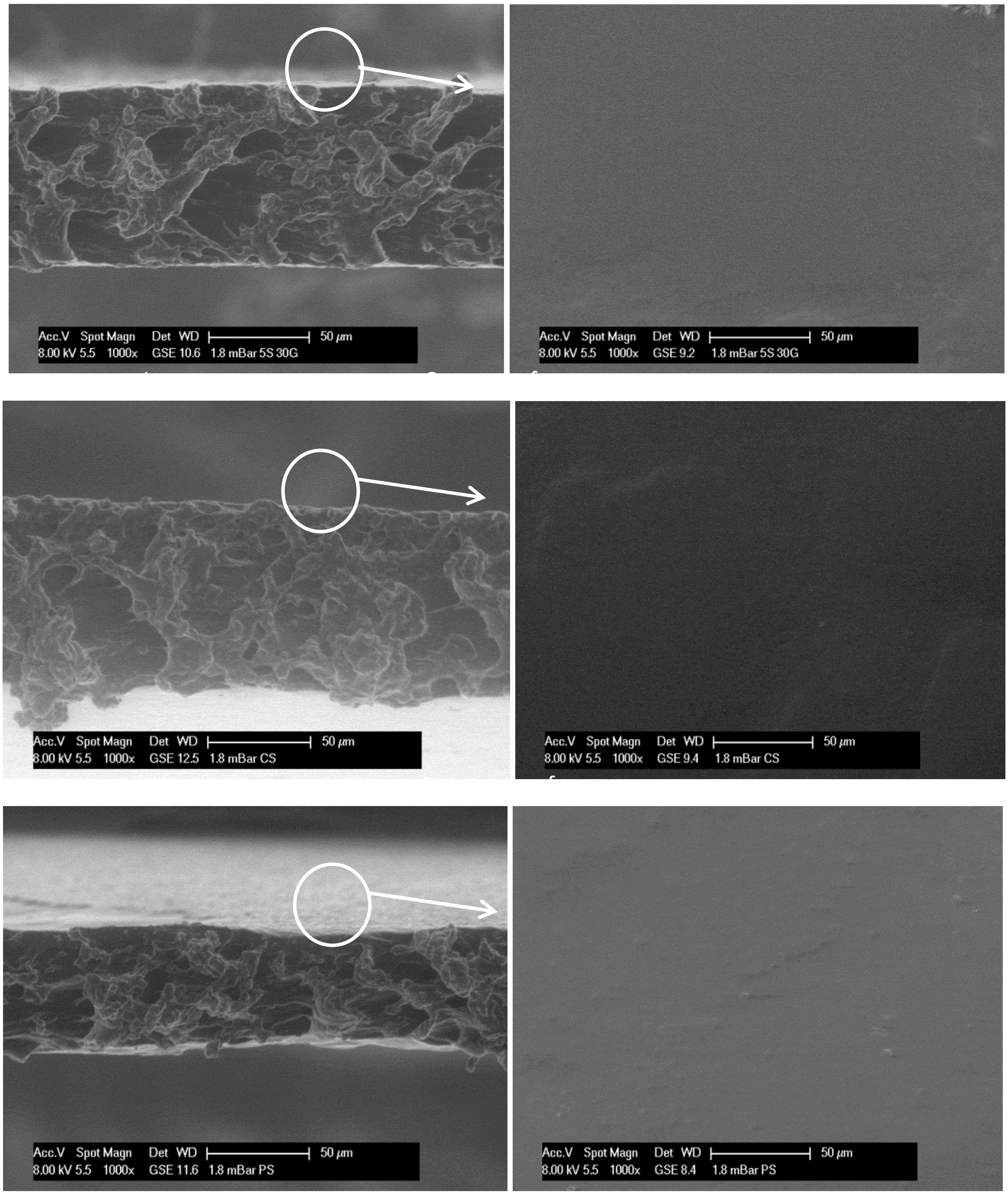


Figure 3.1 ESEM micrographs of the cross section and of the surface exposed to air during drying of wheat starch-based films, of corn starch-based films and of potato starch-based films (magnification x1000).

3.1.4. Surface properties

The effectiveness of edible films depends primarily on the control of the wettability properties, i.e. contact angle, surface energy, surface tension, works of adhesion and cohesion (Souza and others, 2010). Contact angle measurements provide a quantitative way to characterise the surface properties of starch films (He and others, 2013). Usually, contact angle is used as an indicator of the degree of surface hydrophobicity or hydrophilicity (Su and others, 2010). Time lapsed images made by goniometer show shape changes of the liquid drops deposited onto the film surface as a function of time. Contact angle was determined at equilibrium. Results for corn, potato and wheat starch matrix are presented in table 3.1. The highest values of the water contact angle were obtained for wheat starch films. The contact angle is higher than 90°, so the film surface has low wetting properties. It is noteworthy, the values of contact angle of edible films for water are very similar to values of synthetic polymers as polystyrene – 91°, nylon 6-6 – 70°, PTFE 88°, polycarbonate 70°, polyethylene 90° confirming the hydrophobicity. The contact angle for corn and potato starch films display more hydrophilic surfaces, which the works of cohesion, adhesion and spreading confirm they are more wettable. These data are in accordance with the study of He and others (2013) on potato starch films and with that of Białopiotrowicz (2003) for potato and corn starch films.

Table 3.1 Water content at 53% RH, swelling index, film solubility in water, GAB equation parameters (M_o, C, K), colour parameters (L, a, b, ΔE), surface tension (γ_S) and its dispersive (γ_S^D) and polar (γ_S^P) components, critical surface tension (γ_C), works of adhesion (W_A), work of cohesion (W_C) and work of spreading (W_S), tensile strength (TS), Young modulus (YM), elongation at break (E), water vapour and oxygen permeabilities of corn, potato and wheat starch films.

Parameters			**Starch films**		
			wheat	**corn**	**potato**
Thickness (µm)			74.1 ± 8.04 ^{a}	112.2 ± 11.69 ^{b}	55.4 ± 8.73 ^{a}
Hydration properties	Water content (g $_{water}$ g^{-1} $_{dm}$)		0.445 ± 0.060 ^{c}	0.367 ± 0.013 ^{b}	0.316 ± 0.003 ^{a}
	Swelling index (%)		38.99 ± 2.44 ^{a}	34.66 ± 1.34 ^{a}	69.90 ± 4.41 ^{b}
	Solubility in water (%)		30.16 ± 2.25 ^{b}	44.76 ± 0.31 ^{c}	14.52 ± 0.81 ^{a}
	GAB parameters	M_o (g $_{water}$ g^{-1} $_{dm}$)	0.2015 ± 0.0135 ^{c}	0.1437 ± 0.0311 ^{b}	0.0941 ± 0.0042 ^{a}
		C	264.22 ± 85.93 ^{b}	82.88 ± 37.81 ^{a}	41.25 ± 28.41 ^{a}
		K	0.968 ± 0.008 ^{a}	0.962 ± 0.028 ^{a}	0.965 ± 0.006 ^{a}
		R^2	0.988	0.774	0.995
Colour parameters		L	95.87 ± 0.51 ^{b}	92.35 ± 0.56 ^{a}	92.35 ± 0.87 ^{a}
		a	-0.15 ± 0.05 ^{a}	0.92 ± 0.09 ^{b}	1.32 ± 0.14 ^{c}
		b	2.93 ± 0.19 ^{b}	-1.95 ± 0.29 ^{a}	-1.51 ± 0.39 ^{a}
		ΔE	0.97 ± 0.49 ^{a}	6.13 ± 0.19 ^{b}	5.72 ± 0.33 ^{b}
Surface properties		θ_{water} (^{0})	103 ± 14 ^{c}	57 ± 10 ^{a}	75 ±7 ^{b}
		γ_S (mN.m^{-1})	56.22 ^{a}	64.94 ^{b}	71.07 ^{c}
		γ_S^D (mN.m^{-1})	38.01 ^{a}	42.30 ^{b}	42.95 ^{b}
		γ_S^P (mN.m^{-1})	18.21 ^{a}	22.64 ^{b}	28.12 ^{c}
		γ_C (mN.m^{-1})	36.0 ^{a} (R^2=0.87)	36.0 ^{a} (R^2=0.90)	36.0 ^{a} (R^2=0.86)
		W_A (mJ.m^{-2})	118.5 ^{a}	128.7 ^{b}	136.9 ^{c}
		W_C (mJ.m^{-2})	112.4 ^{a}	129.9 ^{b}	142.1 ^{c}
		W_S (mJ.m^{-2})	-6.05 ^{b}	-1.18 ^{a}	-5.24 ^{b}
Mechanical properties		TS (MPa)	3.29 ± 0.79 ^{a}	3.72 ± 0.81 ^{a}	26.26 ± 0.87 ^{b}
		YM (Mpa)	0.12 ± 0.05 ^{a}	0.10 ± 0.03 ^{a}	5.33 ± 0.40 ^{b}
		E (%)	15.21 ± 5.88^{b}	19.13 ± 4.53^{b}	5.67 ± 1.26^{a}

Barrier properties	Water vapour permeability (10^{-10} g m^{-1}s^{-1}Pa^{-1})	33-0% RH	0.52 ± 0.04^{b}	0.79 ± 1.04^{c}	0.01 ± 0.01^{a}
		75-30% RH	6.05 ± 0.62^{b}	8.72 ± 1.40^{c}	1.24 ± 0.34^{a}
		100-30% RH	5.48 ± 0.36^{a}	9.41 ± 1.29^{b}	5.04 ± 0.79^{a}
	Oxygen permeability (10^{-14} cm^{3} m^{-1}s^{-1}Pa^{-1})	53% RH	2.72 ± 0.68^{b}	3.66 ± 0.49^{b}	1.70 ± 0.20^{a}

Values having the same letter for a parameter are not significantly different at p level < 0.05

The critical surface tension (surface tension corresponding of zero energy for wetting) is the same whatever the starch nature (about 36 mN/m). Variety of starch completely does not influence on critical surface tension even when difference between polar and non-polar components in different starch specimens is significant. Indeed, the table 3.1 displays that the ratio between the polar and dispersive components of the surface tension increases from wheat starch to potato. This means the polar component is the more influencing on the surface tension of films. This suggest the higher the surface tension is, the more polar the surface film is. Usually, synthetic polymers have much greater dispersive component related to polar component whereas that obtained for starch films are only twice more dispersive (apolar). The surface tension of solids for water is 2-5 times higher for biopolymers than for synthetic materials (Subedi, 2011). It proves the biomaterials have more hydrophilic groups which could connect with the hydrophilicity surfaces.

3.1.5. Hydration properties

Water solubility is a key parameter for biodegradable materials made for water sensitive biopolymers. Potential applications of starch-based films may require water insolubility to enhance product integrity and water resistance for biodegradable packaging materials. However, the solubility in water or aqueous phases might be useful for coatings application or food encapsulation (Shen and others, 2010). As displayed in table 3.1, solubility in water is related to the amylose content. The higher the amylose content is, the greater the solubility index is. These data are in agreement with Ghanbarzadeh and others (2011) and Sun (2013). The solubility index is dependent on the starch origin and on the substitution groups if starch is modified (Ghanbarzadeh and others, 2011; Lin and others, 2011). Zavareze and others (2011) displayed a relationship between solubility and swelling parameters. Swelling influences the solubility and vice-versa. Indeed, when the structre is more dense or crystalline, the swelling is limited and thus the solubility delayed or reduced (Zavareze and others, 2011). This hypothesis has confirmation in present work. Indeed, the water solubility is reported in literature on the following level: 27.5% for cassava starch films (Chiumarelli and Hubinger, 2014), 20% for tapioca starch films (Kim and others, 2015), 38% for high amylose corn starch films (Bertuzzi and others, 2007), 27.26% for acetylated corn starch (López and others, 2008), 26.6% for sugarcane bagasse starch films (Slavutsky and Bertuzzi, 2014), 30% for wheat starch films (Farahnaky and others, 2012), Mehyar and Han (2004) found film solubility in water of 44.4% and 32.0% for rice and pea starch films, respectively. Table 3.1 shows a direct dependence of swelling index with the content of water as expected. As confirmed by the surface properties, wheat is more hydrophilic and absorbed more water than two others. Potato starch matrix had the lowest water concentration, the highest was observed for wheat starch, and is consistent with Talja and others (2008) results.

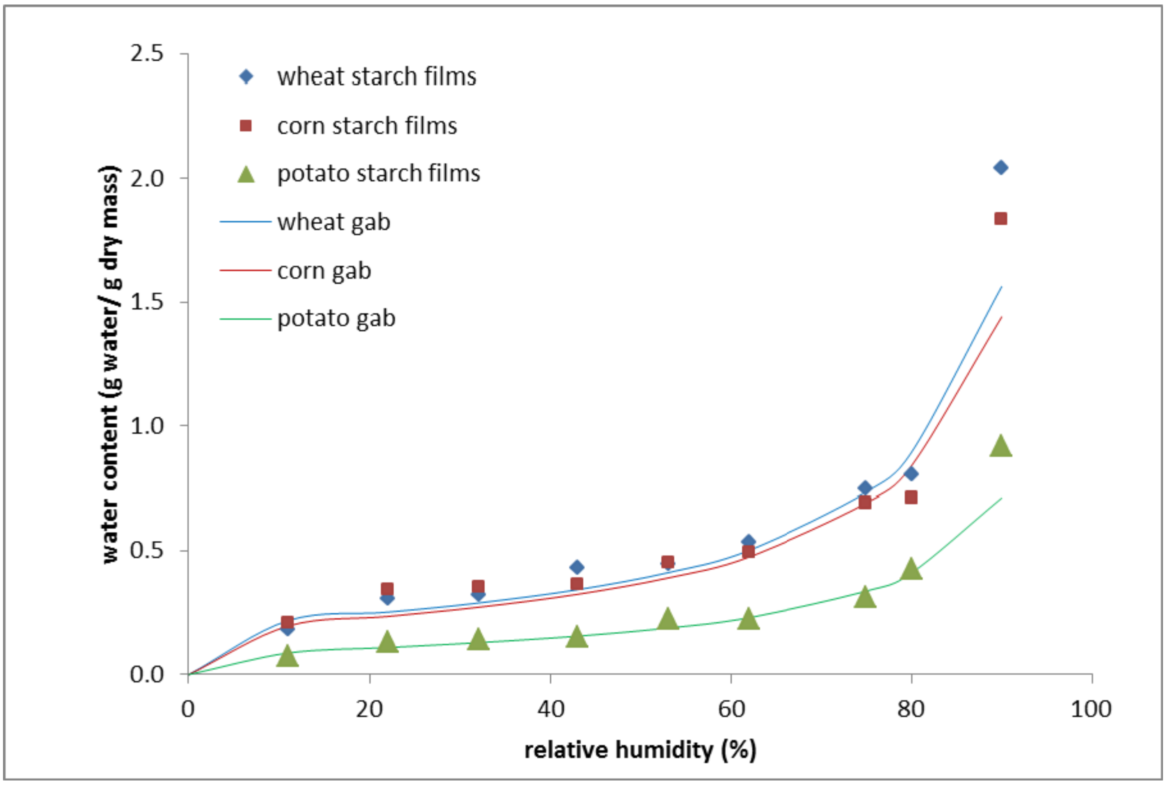
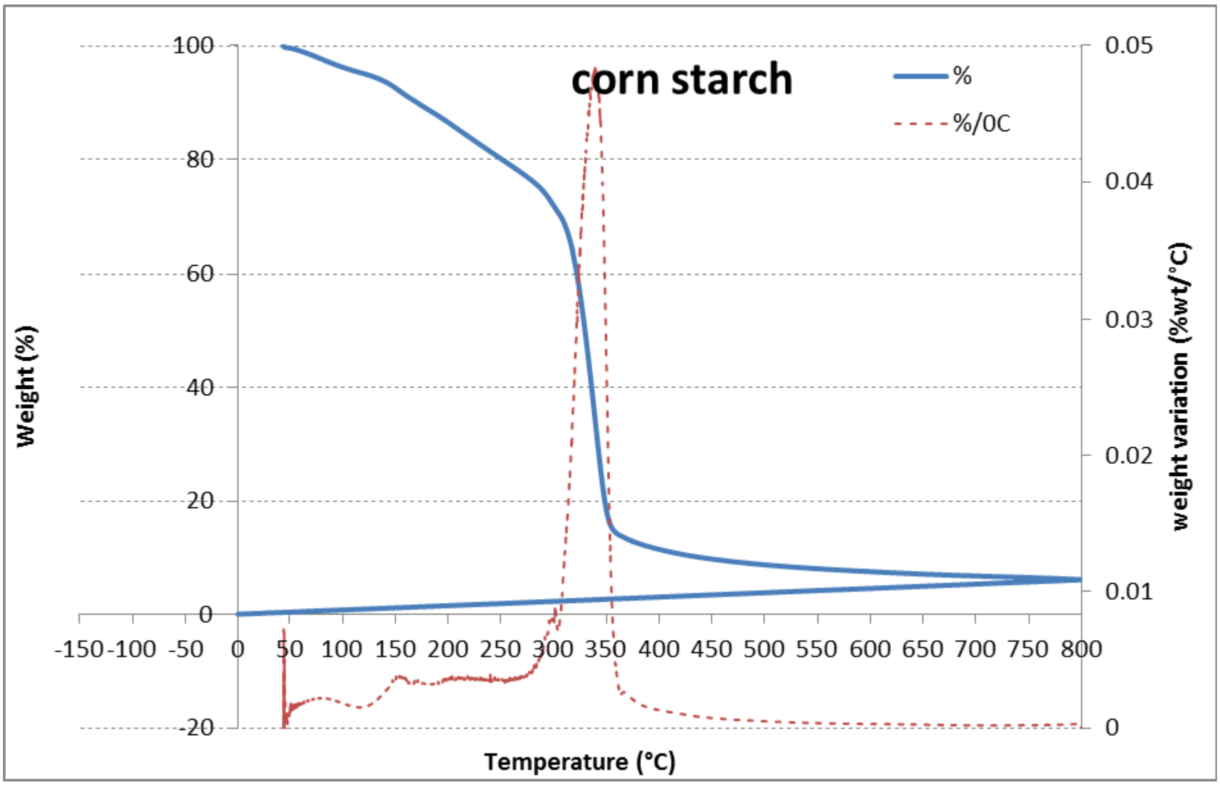


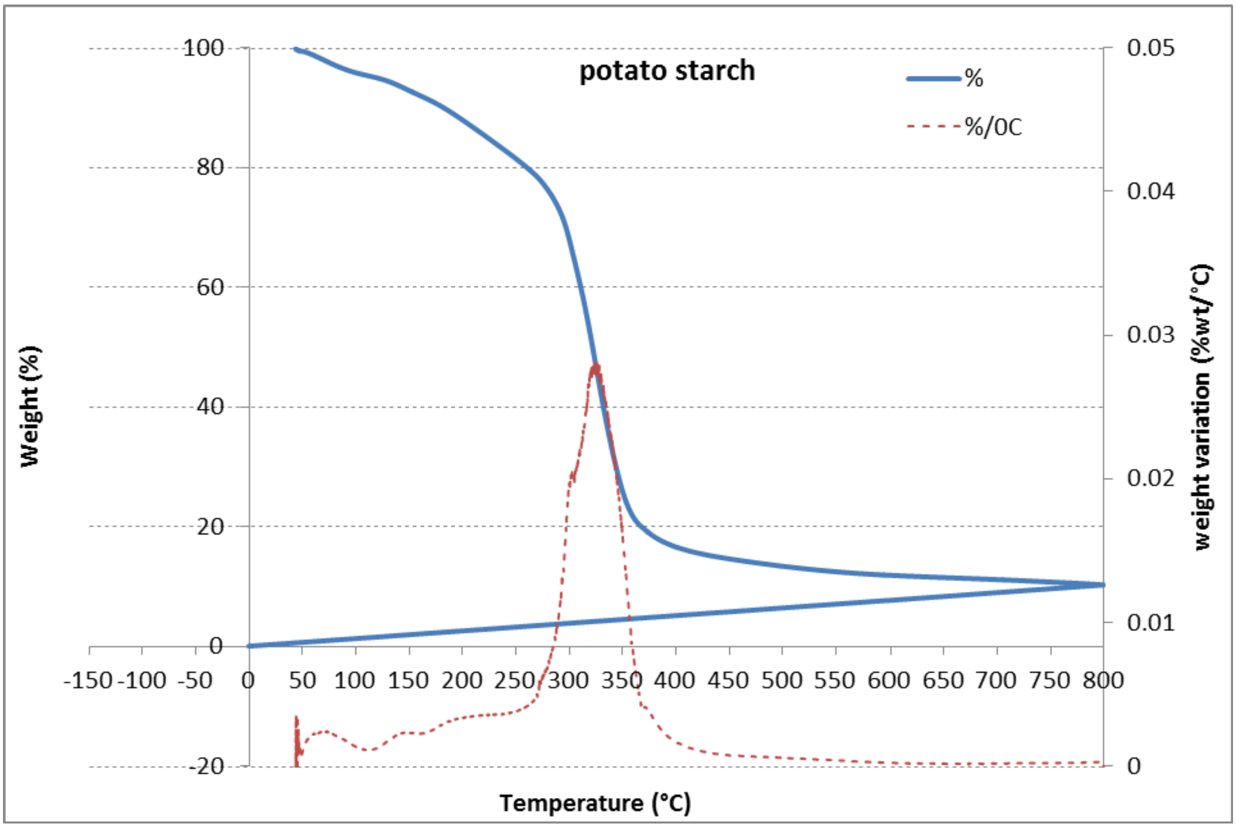
Figure 3.2 Moisture isotherm sorption of wheat starch (WS), corn starch (CS), potato starch (PS) at 25°C (symbols are experimental values, lines are from GAB model fitting).

Figure 3.2 gives the moisture sorption isotherm of films made of wheat, corn or potato starch at 25°C. All have the sigmoidal shape which is typical of dense and hydrophilic materials corresponding to the type II of the IUPAC classification (Suzuki, 1993). The potato starch films display the lowest affinity for moisture absorption. The GAB model fits well such isotherms. Two major coefficients i.e. monolayer water content (M_o) and parameter related with sorption enthalpy of the first layer (C) correlated with value of contact angle. The C parameter influences the sigmoidal shape of the isotherms particularly at low water activity range (Mali and others, 2005). The C values of starch films are high and there is no definite pattern these values. This may indicate that the moisture absorption of all studied matrix could occur in the upper layers more readily than in the monolayer (Tongdeesoontorn and others, 2009). Besides, sorption data corroborate the behaviour observed in contact angle of water measurements. Indeed, the water contact angle is the highest for potato starch and thereby M_o and C coefficients are the lowest. K parameter which is related with sorption enthalpy of the subsequent layers does not show significant differences between the starch sources. For all films the K value is close to 1 which confirms a predominancy of monolayer absorption as described by the BET model. Muscat and others (2013) described a similar dependence for high amylose starch surfaces. Furthermore, the GAB model provides a good fit to the experimental data for all samples, especially for potato starch with R^2 values > 0.99. Van den Berg (1984), McMinn and Magee (1999), Timmermann and others (2001) reported the GAB model adequately represented the sorption isotherms of potato and wheat starch. The values presented in this study for the GAB constants are in agreement also with the values reported by the other authors (Basiak and others, 2015e; Muscat and others, 2013; Talja and others, 2007; Viollaz and Rovedo, 1999).

3.1.6. Thermal and mechanical properties

To better understand the influence of starch origin on thermal properties, a thermogravimetry analysis was carried on. Figure 3.3 presents the weight change according the temperature and its derivative.


corn starch
%
%/0C
Weight (%)
100
80
60
40
20
0
-20
weight variation (%wt/°C)
0.05
0.04
0.03
0.02
0.01
0
-150 -100 -50 0 50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800
Temperature (°C)


potato starch
%
%/0C
Weight (%)
100
80
60
40
20
0
-20
weight variation (%wt/°C)
0.05
0.04
0.03
0.02
0.01
0
-150 -100 -50 0 50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800
Temperature (°C)

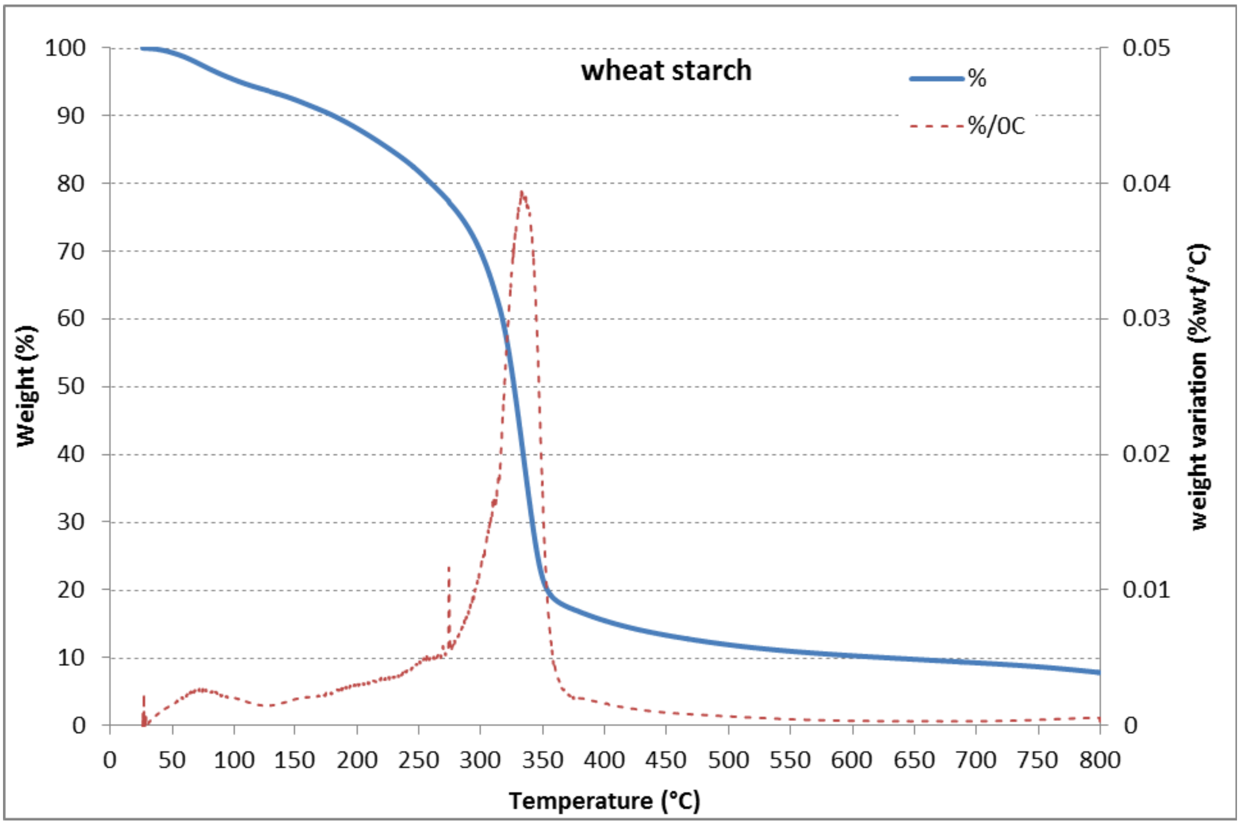


Figure 3.3 Thermogravimetry analysis of wheat starch, corn starch and potato starch based edible films equilibrated at 53% RH prior analysis.

The initial weight loss, starting at approximately 30°C for wheat starch films and 55°C for corn and potato starch films, is attributed to water evaporation. The weight loss in the second range of 120-270°C is related to a complex processes including the dehydration of the saccharide rings, depolymerisation (Mathew and Dufrene, 2002), and glycerol evaporation (boiling point = 182°C). The third zone ranging from 270 to 450°C corresponds to the degradation of starch. The total mass lost at 800°C is approximately 90-94%. Data obtained by de Silva and others (2012) for cassava starch confirmed the obtained results.

These results are also in agreement with the data reported by Piyada and others (2013) for rice starch systems. From both our results and those from literature, starch nature does not significantly influence the thermal properties (thermal resistance) of films.

Mechanical properties were measured on films equilibrated at 53% RH and are given in table 3.1. For both: wheat, corn and potato starches used, the recipe remained the same. However the final thickness as well as the water content of films varied and thus explain the difference in mechanical properties. Table 3.1 displays that wheat starch is more deformable (E) and less stiffness (TS and YM) than potato starch films. The higher thickness is related to the higher viscosity of the film-forming suspension obtained for the corn starch (results are not showed here; they are also confirmed in the literature). However, its water content is similar to that obtained for the potato starch based films for which the tensile strength is the higher. Mechanical resistance of film is thus more related to the thickness. The values obtained are similar to those of the literature. To compare with other starch origin (wheat, sago, potato, chickpea, banana), TS ranges from 2 to 10, YM from 5 to 106 MPa, and E from 14 to 45% according the authors (Farahnaky and others, 2013; Al-Hassan and Norziah, 2012; Muscat and others, 2012; Torres and others, 2011), but these values strongly depend on the plasticizer content, thickness, water content and additives. The obtained data are however opposite to the results got by several authors who displayed more mechanically resistant film for higher amylose content. Indeed, the amylose/amylopectin content causes significant changes in mechanical properties of starch plasticized films (Liu, 2005; Lourdin and others, 1995; Souza and others, 2010; Torres and others, 2011; Xie and others, 2014). Structure difference in amylose and amylopectine molecules induces different behaviours with regard to gelation, crystallinity and film forming capacity (Mali and others, 2006). Amylose content significantly favours TS and YM of films made from wheat, corn and potato starches (Chiumarelli and Hubinger, 2014; Li and others 2008, Mali and others, 2005, Zobel, 1988).

3.1.7. Water vapour and oxygen permeabilities

One of the purposes of edible films is to control moisture transfer between the food and the surrounding atmosphere. Therefore the water vapour permeability should often be as low as possible (Flores and others, 2007; Martins and others, 2012). As reported Alves and others (2007), Pushpadass and others (2008), Soliman and Fortuna (2014) and Souza and others (2010) water vapour permeability of edible films depends on several factors, such as the integrity of the film, the thickness, the glycerol content, the hydrophilic-hydrophobic ratio, the crystalline fraction, the polymeric chain mobility, and thus depends on the starch origin. Table 3.1 presents water vapour permeability measured at three relative humidity gradients: 33-0%, 75-100%, 100-30%. The potato starch films have the lowest permeability which is related to lower wettability and moisture absorption. It also corresponds to the lowest amylose content. Jiménez and others (2012) and Muscat and others (2012) confirmed the starch components, i.e. amylose and amylopectin have a significant influence on WVP. Moreover water vapour permeability is related to solubility in water as well as surface wettability and moisture absorption. Indeed, according to theory of transfers, permeability is directly dependent of both kinetics (diffusivity, mobility) and thermodynamics (affinity, sorption, solubility, partition). Increasing the RH gradient induces in all cases a rise of the WVP as expected but it occurs as a higher RH for the potato film in accordance with its isotherm. This increase of permeability with the RH was also demonstrated by Muscat and others (2012) for corn starch.

As observed for the WVP, the oxygen permeability is the lowest for the potato starch film (table 3.1). The same interpretations could be suggested. Indeed, the lower amylose content, lower moisture sorption (lower plasticization by water) can explain this data because potato starch films have a twice lower thickness. Thirathumthavorn and Charoenrein (2007) observed that tapioca starch films are excellent oxygen barriers at low to intermediate RH. At high RH oxygen permeability increased, due to the hydrophilic nature of starch films and plasticization by moisture. Liu (2005) confirms that gas permeability through starch films is connected with solubility in water. Starch films with higher amylose content are better oxygen barriers.

3.1.8. Conclusions

Amylose/ amylopectin ratio in starch films plays significant role on physical and chemical properties. This ratio strongly influences the microstructure, thus the viscosity of the film-forming suspension that affects the retraction of network during the film drying and then the final thickness. The film colour and transparency depend on the thickness. Indeed, potato starch films are transparency, while corn and wheat starch matrices are more opaque. The amylose/amylopectin ratio also influences the hydration characteristic. The lowest solubility in water, water vapour and oxygen permeabilities are observed for potato starch corresponding to the lowest amylose content. Surface and mechanical properties are also dependent. The lower the amylose content is, the greater are the barrier, surface resistance to moisture and mechanical properties are.

3.2. Influence of plasticizers (glycerol and water) on starch based films (Basiak E., Debeaufort F., Lenart A. 2015. Glycerol and water contents affect the structural and functional properties of starch-based edible films. Submitted in Food Science and Technology International, November 2015).

3.2.1. Abstract and introduction

The quite not expensive, widely available starch is able to replace plastics from petrol of even 1/3 for making biodegradable films and containers. Moreover, starch could be envisaged as edible coatings for food shelf life prolongation. Wheat starch films with two glycerol contents were formulated to mimic coatings. Their structural and functional properties were characterized. The transfer properties of starch films containing 33% of plasticizer such as the diffusivity, the oxygen permeability, water vapour permeability at 2 different humidity gradients, the polar component of the surface tension, the works of adhesion and cohesion, and the moisture sorption have lower values than films containing 50% of glycerol. Glycerol content does not play a significant role on colour or mechanical properties. This work shows that glycerol can strongly affects the functional properties of starch based coatings and films.

One of the major drawbacks to used packaging from petrol is their environmental impact and as well limited resources. One of the alternatives is the development of biodegradable films and coatings made from natural bio-sourced polymers. With the constant availability and low production costs, films made from starch could be an excellent solution of problem of packages for many kinds of food-stuff such as fresh fruits, but also for cosmetics or pharmaceuticals. Such packaging materials can be manufactured for minor investment (García et al., 2011).

Annually supply of starch is estimated to be billion tons in developed and developing countries (Kaur et al., 2005; Mali et al., 2006). The first position of producing is occupied by maize starch, on the second position ranks wheat starch and then is potato and rice starches. The top place holds wheat starch in European production (García et al., 2011). Its versatility in using has low price and it is available. Besides the starch is widely used as a thickener, texturizer, gelling agent, adhesive and moisture-retainer, it also has interesting film-forming ability and processability (Liang and Ludescher, 2015). Starch can be converted into a continuous polymeric entangled phase by mixing with enough water or plasticizer to increase flexibility by reduction in intramolecular hydrogen bonding along polymer chains thereby increasing the intermolecular spacing (Janjarasskul and Krochta, 2010, Lawton and Fanta, 1994). The plasticizing system can also be constituted of two or more components (Krogars et al., 2003). When several plasticizers (other than water) are presented in the system, strong plasticizer-plasticizer or plasticizer-water interactions are observed. These interactions can be cooperative and then enhance functional properties of biodegradable materials (Adhikari et al., 2010). Tensile strengths of starch films without added plasticizers are high, but films are brittle and exhibit little or any elongation (Talja et al., 2008). Thus, plasticizer is always required. While water is the most commonly used and effective plasticizer for starch, many other substances are used to plasticised this biopolymer. Generally there are hydrophilic, low molecular weight polyols (glycerol, sorbitol, xylitol, polyethylene glycols, etc.), compounds containing nitrogen (urea, ammonium derived chemicals, amines) and non-volatile acids such as citric or lactic or tartaric (Xie et al., 2014). Polyols tend to adsorb water which depends on their molecular weight and number of hydroxyl groups (Muscat et al., 2012). The most common used is glycerol which is considered as totally non-toxic and is, then, well indicated for use for the food industry (Pommet et al, 2003). It is compatible with amylose and could interfere with amylose chain packing (García, Martino and Zaritzky, 2000). Glycerol and other water-compatible diluents have plasticising rather than antiplasticizing effects on the mechanical properties of glassy composite systems; especially biopolymer based edible films (Debeaufort and Voilley, 1997). In several studies it has been reported that glycerol can exert various effects depending on its concentration. Heydari et al. (2013) studied wettability properties on corn starch films. Glycerol content less than 10% causes the brittle of films; content of plasticizer between 10-20% does not provide enough flexibility. The content of glycerol depends on the targeted applications, for instance the minimum amount of glycerol in order to acceptable mechanical resistance and heat sealability is 30% for sago starch films. Abdorreza et al. (2011) reported that glycerol at a content below 30% could decrease the ductility of sago starch films. When the amount of glycerol exceeded 50%, the plasticity increased largely as well as the permeabilities. The higher the glycerol content is, the higher is the ductility (Ollé Resaa et al., 2014), but lower the barrier and solubility efficacy.

Therefore, the objective of this work was to study the influence of plasticizer content on physical, chemical and functional, properties of films made from wheat starch, related to hydration behavior of the system, in aim to be used for fruit wrapping or coating.

3.2.2. Hydration properties

The moisture sorption characteristic of the films is important for predicting the stability of the films during storage, since the shelf life of the biodegradable packages in different storage conditions is related to their moisture uptake. The water sorption isotherms of films prepared with different plasticizer concentrations, i.e. 33 and 50% of glycerol, are shown in figure 3.4, while the GAB parameters (M_o, C, K) and correlation coefficients (R^2) are given in table 3.2.

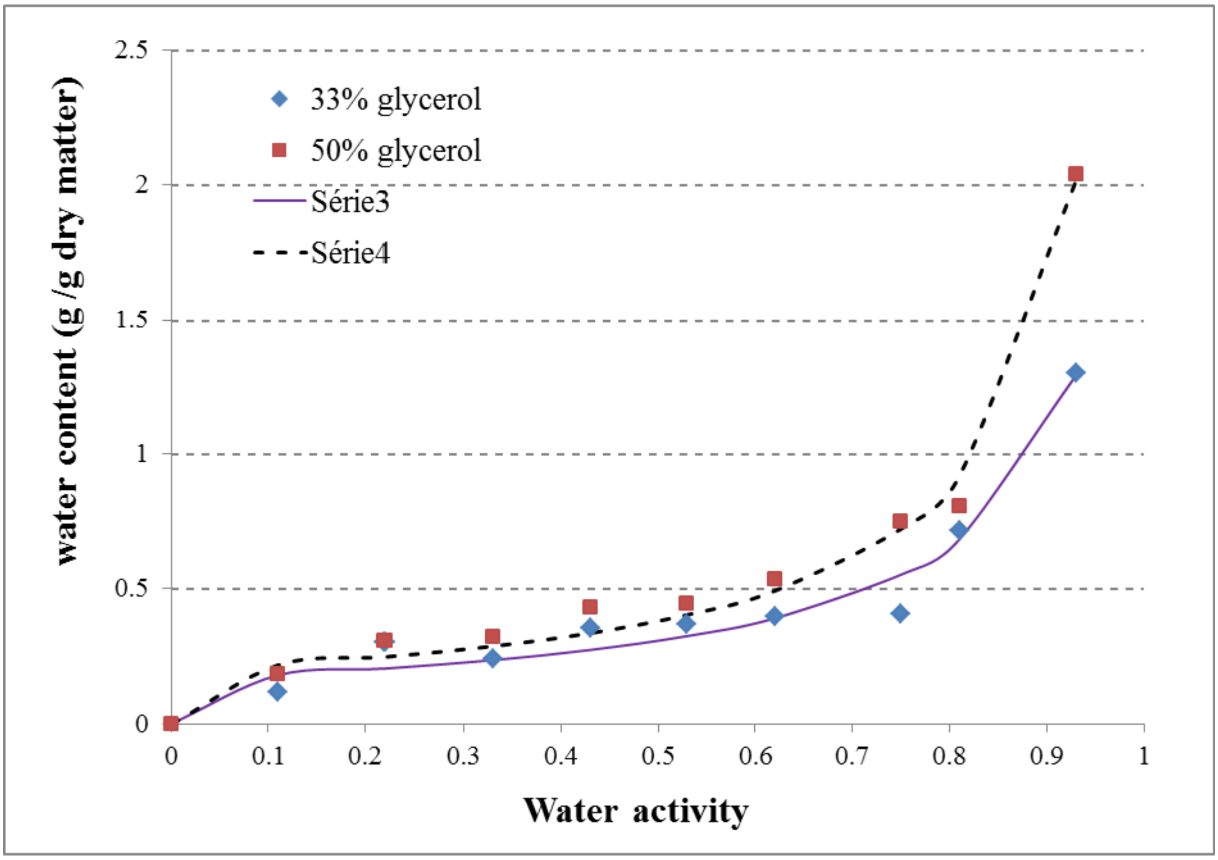


Figure 3.4 Moisture isotherm sorptions of starch containing either 33 or 50% (w/w) glycerol in 5% starch solutions (g water/g d.m.) at 25°C. Symbols are experimental values and lines values fitted by GAB model.

Table 3.2 Water content, swelling index, film solubility in water, parameters of the GAB equation (M_o, K, C), colour parameters (*L, a, b, ΔE*), surface tension (γ_S), dispersive (γ_S^D) and polar (γ_S^P) components of the surface tension, critical surface tension (γ_C), works of adhesion (W_A), of cohesion (W_C) and of spreading (W_S), tensile strength (TS), Yong modulus (YM), elongation at break (ε), water vapour and oxygen permeabilities of wheat starch films containing 33 or 50% glycerol.

Parameters			**Wheat starch films**	
			33% glycerol content	**50% glycerol content**
Thickness (µm)			64.1 ± 8.04^a	80.8 ± 12.59^a
Hydration characteristics	Water content (g $_{water}$ 100 g^{-1} $_{dm}$)		3.44 ± 0.50^a	3.72 ± 0.50^a
	Swelling index (%)		38.99 ± 2.44^a	39.20 ± 1.43^a
	Solubility in water (%)		30.16 ± 2.25^a	34.76 ± 2.18^a
	GAB parameters	M_o (g $_{water}$ 100 g^{-1} $_{dm}$)	16.4 ± 2.2^a	19.8 ± 1.6^b
		K	0.93 ± 0.02^a	0.97 ± 0.01^a
		C	$754 \pm 3\ 10^{5\ a}$	$105\ 10^9 \pm 10^{16\ a}$
		R^2	0.962	0.989
Colour parameters		*L*	95.87 ± 0.51^a	95.48 ± 0.39^a
		a	-0.15 ± 0.05^a	-0.24 ± 0.06^a
		b	2.93 ± 0.19^a	3.19 ± 0.29^a
		Δ*E*	0.97 ± 0.49^a	1.46 ± 0.43^a
Surface characteristics		γ_S (mN/m)	56.22^a	60.28^b
		γ_S^D (mN/m)	38.01^a	35.40^a
		γ_S^P (mN/m)	18.21^a	24.88^b
		γ_C (mN/m)	36.0^a (R^2=0.87)	36.0^a (R^2=0.88)
		W_A (mJ/m^2)	118.5^a	126.8^b
		W_C (mJ/m^2)	112.4^a	120.5^b
		W_S (mJ/m^2)	-6.05^a	-6.24^a
Mechanical properties		TS (MPa)	3.29 ± 0.79^a	2.10 ± 0.76^a
		YM (Mpa)	0.12 ± 0.05^a	0.10 ± 0.09^a
		E (%)	15.21 ± 5.88^a	18.08 ± 5.40^a

Transfer properties	Water diffusivity $(10^{-11}\ m^2s^{-1})$	at 75% RH	4.3 ± 0.9^{a}	7.0 ± 0.5^{b}
	Water vapour permeability $(10^{-10}\ gm^{-1}s^{-1}Pa^{-1})$	33-0% RH	0.52 ± 0.04^{a}	0.92 ± 0.06^{b}
		75-30% RH	6.05 ± 0.62^{c}	8.77 ± 0.59^{d}
		100-30% RH	5.48 ± 0.36^{c}	8.01 ± 0.15^{d}
	Oxygen permeability $(10\text{-}^{14}\ cm^3m^{-1}s^{-1}Pa^{-1})$	33% RH	3.58 ± 2.72^{a}	7.23 ± 1.00^{b}
		75%RH	4.30 ± 0.77^{a}	7.41 ± 1.39^{b}

Values having the same letter for a parameter are not significantly different at p level < 0.05

Below $a_w = 0.55$, the water is strongly involved in the structural organisation of a biopolymer network. Bound water is located in the immediate contact of the starch chains, has a reduced activity. Interactions occurring between starch and glycerol may promote adsorption of moisture as displayed on figure 3.4, due to changes of dimension in the area where the adhesion between interfaces (glycerol-starch) is weak, thus creating a step that simplifies the accumulation of water molecules (Wan et al., 2009). With the glycerol content increasing the moisture absorption increases.

In the range of $a_w = 0.60\text{-}0.75$ you are able to see the highest difference of water content between films with 33 and 50% of glycerol. Figure 3.4 shows the effect of glycerol incorporation to the starch matrix. Simultaneously, in experimental data the higher relative humidity is the stronger influence of plasticiser is. According to higher water activity, glycerol increases the water content in starch films matrix, however under these conditions hydrophilic polymeric chains swell, altering its structure.

The GAB model represented well the experimental data, as previously reported by other authors (Godbillot et al., 2006; Mali et al., 2005; Talia et al, 2007; Zhanga et al, 2011). Films with higher plasticiser content have higher value of the monolayer water content (M_o). These results may be related with high glycerol's hydroscopicity. Glycerol molecule presents upper water affinity, demonstrated by absorption isotherms reported in the literature (Leung, 1986). Kibar and Us (2013) reported that in starch films, plasticizers are generally more hygroscopic than starch. Indeed, Muscat et al. (2012) and Talja et al. (2008) showed that amount of sorbed water by starch films increases with the polyol content for potato starch, up to twice. Thus, the difference in the water adsorption capacity of starch films (when the amount of starch is constant) is mostly dependent on content of the plasticizers. Müller et al. (2008) also observed consistent effect for cassava starch films. The value for the parameters C and, relating to subsequent layer of moisture, decreased with the concentration of polyol. Moreover, the GAB model provided a good fit to the experimental data for all samples, with R^2 values > 0.98 (Teodoro et al., 2015). However, both samples (with 33 and 50% of glycerol) exhibited similar behaviour. The values presented in this study for the GAB constants are in agreement with the values reported in the literature (Dias et al., 2010; Mali et al., 2005; Müller et al., 2008).

Another important property of films for food packaging applications is them solubility in water. Some latent uses may require water insolubility to enhance product integrity and resistance of water (Farahnaky and Saberi, 2013). Difference in water solubility between the films is not significant (table 3.2). However, a slight increase of solubility is observed for the 50% glycerol content film. This is not proportional to the glycerol content which means the interaction between starch and glycerol rises with the glycerol content. Farahnaky and Saberi (2013) indicated that the addition of glycerol increased the water solubility of wheat starch films, from 11.7% to 27.7% when glycerol content roses from 30% to 50% w/w. In that case, it was supposed hydrogen bonds stabilizing the starch network were disrupted, reducing the cohesiveness of the starch matrix and then increasing its solubility in water. Ghanbarzadeh et al. (2011), Hu et al. (2009) and Laohakunjit and Noomhorm (2004) obtained similar dependences for corn, rice and potato starches films.

The swelling index and water content (table 3.2) are not significantly different between films with 33 and 50% of glycerol. In previous works Basiak et al. (2015a,b,c) displayed that only addition of protein isolate or rapeseed oil has significant influence on the swelling index and water content of wheat starch films.

3.2.3. Interactions involved in starch-glycerol films displayed by FTIR

The most important intermolecular interaction determining the properties of the starch films is the hydrogen bond. Changes in the hydrogen bond network due to changes in matrix composition alter this structure and in consequence modulate matrix network (Liang and Ludescher, 2015). The FTIR spectra of glycerol-starch films (33% and 50% plasticizer content) and that of pure glycerol are presented in figure 3.5 The broadband located at 3430 cm^{-1} (not showed onto the graph) has a very strong character and corresponds with vibrations models of intramolecular OH-groups from the absorbed water, from glycerol and from the starch polymer as also displayed by Jiménez et al. (2013). The peak located at 2928 cm^{-1} is weak and related with vibration of hydrogen bond. The absorption bands at 1655 cm^{-1} and 1373 cm^{-1} are also weak and related to CH and C=O groups. The broad bands located at 1165 cm^{-1} and 1084 cm^{-1} have strong character and assigned to δCH and δCOC. Peak with wavenumber 980 cm^{-1} has very strong character and it corresponds with vibrations δC-O (Zaleska et. al, 2001). Bands covers 1165–980 cm^{-1} are the typical region of saccharides. These bands turned out to be the most intensive absorbance in the IR-spectra (Souza and Andrade, 2002).

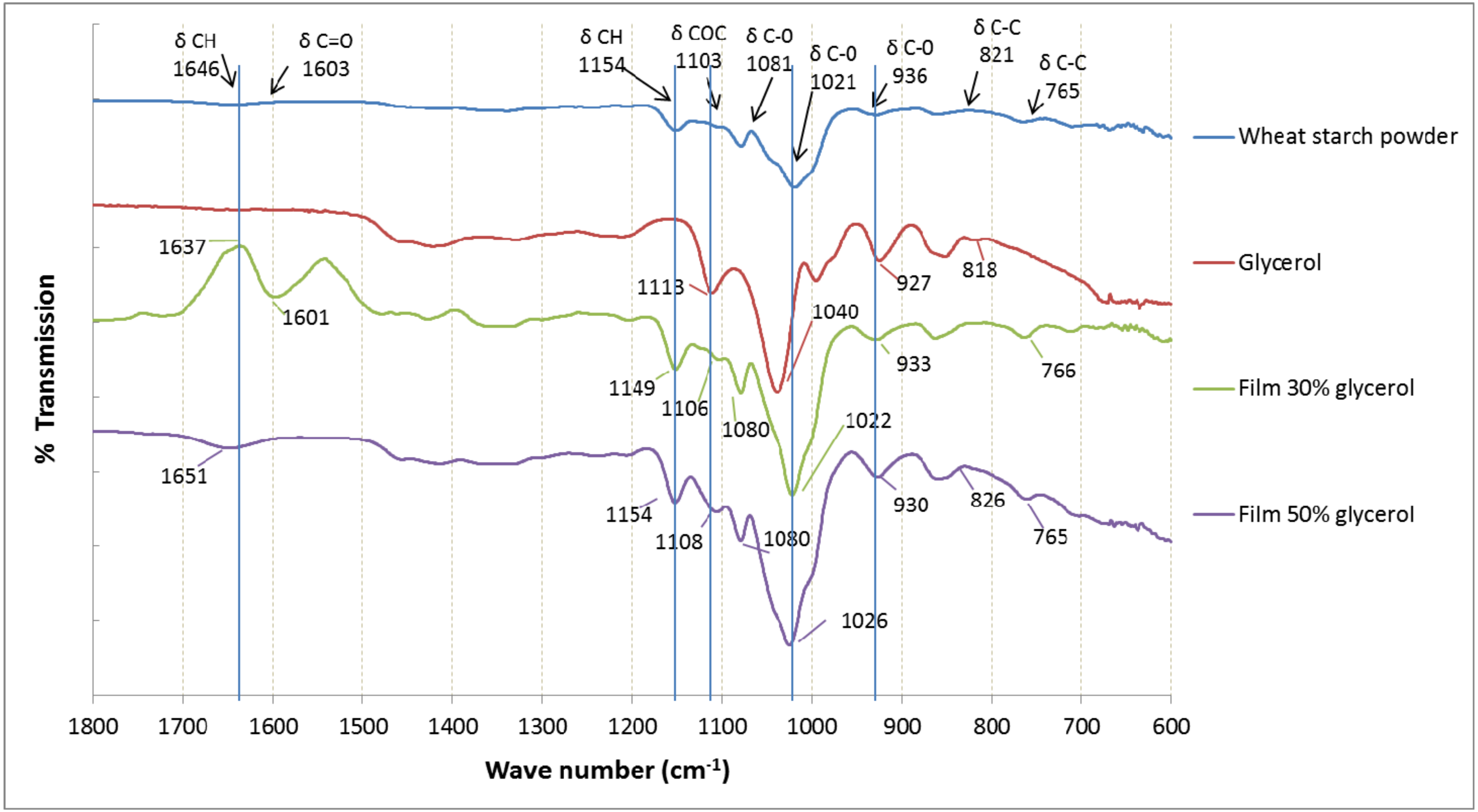


Figure 3.5 FTIR spectra for (a) wheat starch powder, (b) glycerol, (c) starch films with 33% of glycerol, (d) starch films with 50% of glycerol (blue vertical line corresponds to shift peaks).

The FTIR spectrum of pure glycerol shows five typical absorption bands located at 800 up to 1150 cm^{-1}, corresponding to the vibrations of C-C and C-O linkages. Three broad bands at 850, 925 and 995 cm^{-1} are correspond to the vibration of the skeleton C-C, the peak at 1045 cm^{-1} is associated to the stretching of the C-O linkage in C1 and C3 and the bond at 1117 cm^{-1} is corresponded to the stretching of C-O in C2 (Guerrero et al., 2010). The effect of glycerol and water can be analysed by comparing the spectra of wheat starch powder to the spectra of starch films with 33 and 50% of glycerol. As can be seen from figure 3.5, compared to the IR spectra of reference samples (wheat starch powder), characteristic peaks for saccharides are shifted. Peaks at 1014 cm^{-1} can be assigned to C-O stretching, are shifted to 1020 cm^{-1} (starch films with 33% of glycerol) and 1023 cm^{-1} (films with 50% of glycerol). Furthermore, the characteristic peaks in glycerol (2908, due to C-H vibrations), shifted to a higher wavenumber at 2926 cm^{-1} for films with 33% of glycerol and to 2932 cm^{-1} for the films with 50% of glycerol, and that at and 3320 cm^{-1} (affiliated to O-H bond) is shifted at 3338 cm^{-1} for both 33 and 50% of glycerol films. These shifts indicated that the addition of glycerol promoted the hydrogen bonding interactions among starch and glycerol. These results prove plasticisation effect of glycerol and they are expected considering the hydrophilic nature of glycerol and starch (Liu eta al., 2013).

3.2.4. Effect of plasticizer on the film appearance, colour, microstructure and thermal stability

The appearance of starch film which does not contain glycerol is completely different to the others. Indeed, glycerol free films are clear, transparent, though, brittle, rougher, rigid, unpeelable, and the texture on each of the two sides are different. The films containing 33% or 50% of glycerol are a little bit less transparent (opalescent), flexible and soft. Glycerol interacts with starch molecules, causes weakened of cohesive tension. In consequence structure of peeled films is more flexible. Moreover, no effect of blooming and blushing were observed. This occurs when the plasticizer concentration surpassed its capability limit in the polymer (overplasticization), causing phase segregation and physical exclusion of the plasticizer (Laohakunjit and Noomhorm, 2004; Suppakul et al., 2013).

Colour parameters: L, a, b and total colour difference ΔE of starch-glycerol films are presented in table 3.2 According to increase the L parameter biopolymers are more transparent. Values obtained for wheat starch films with 33 and 50% of glycerol have 95.87

and 95.48 values, respectively. Glycerol slightly decreases value of lightness parameter. Values of red-greenish and yellow-bluish rather increase with the plasticizer content. This means that films with higher polyol content are more coloured. Colour changes due to the incorporation of glycerol can be more fully described using other colour functions, such as total colour difference which increases. The same behaviour was observed by Farahnaky and Saberi (2013) or by Muscat et al. (2013).

Surface and cross-section images were investigated by environmental scanning electron microscopy (figure 3.6). Achieved photographs show microstructure of wheat starch-glycerol films. Micrographs, both for starch films with 33% and 50% of glycerol, presented smooth surfaces without pores nor cracks, and compact structures. The same matrix obtained Mali et al. (2002), who added 1.3% and 2% of glycerol (w/w d.b.) to yam starch films. Polyol addition to starch matrix changes the microstructural arrangement of starch chains that are less dense (Acosta et al., 2013). Farahnaky, Saberi and Majzoobi (2013) investigated the effect of glycerol (0, 20 and 50% of dry basis) in wheat starch films. Unplasticized films were rigid, fragile and brittle, they had cracks or pores that could possibly facilitate the transmission of the water vapour. Surface and cross-sections morphologies of plasticized films' were homogeneous, but crumpled at the same time. The more the plasticizer content in matrix was, the smoother and slender the films were (Jagadish and Raj, 2011). Liu et al. (2011) observed the same tendency for plasticizer concentration ranging between 50 and 70% (w/w) in maize starch films. However, the film surfaces of films with 70% (w/w) of polyol were less porous, with more concise networks. According to Xie et al. (2014), who also measured influence of glycerol concentration in maize films, a higher amount of glycerol could result in a more apparent granular morphology. The homogeneous structure of starch films is a good indicator of their structural integrity, and consequently good mechanical and physical properties are expected (Farahnaky et al., 2013).

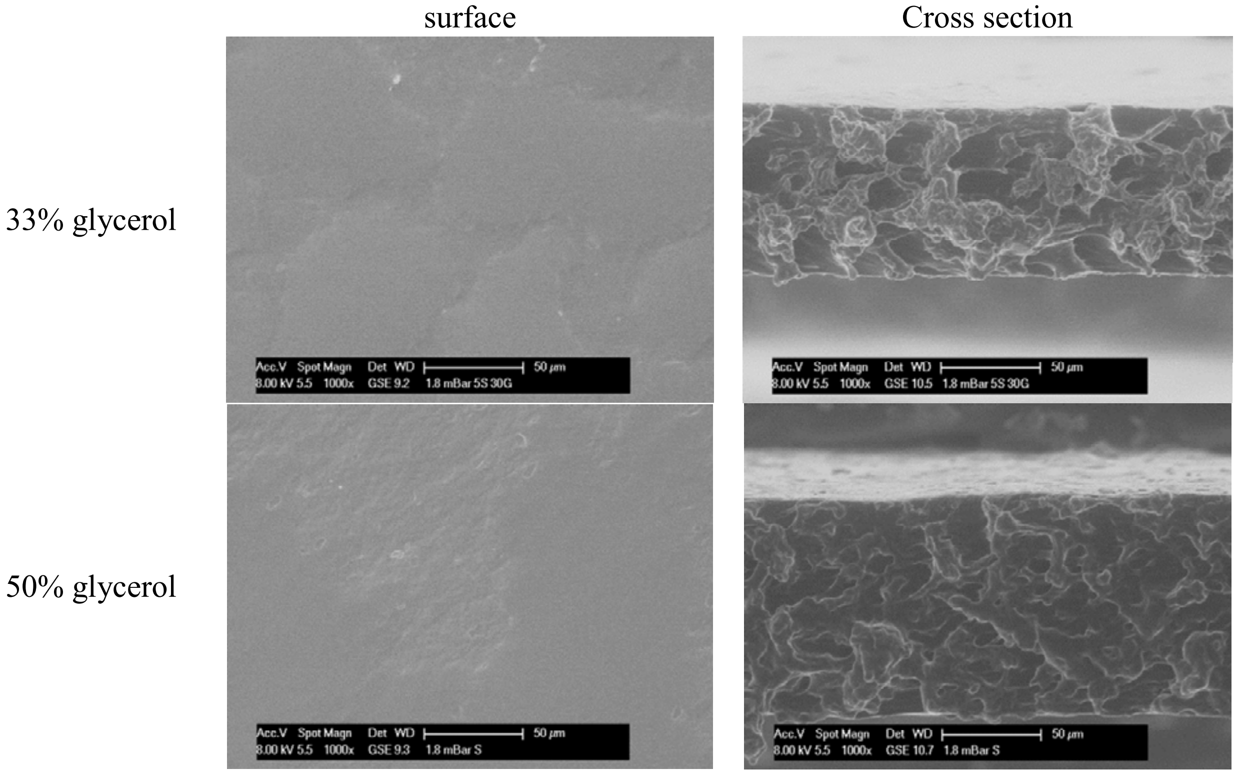


Figure 3.6 ESEM micrographs of surface exposed to air during drying and cross section of starch-based films containing 33% or 50% glycerol (w : w) at a magnification of x1000.

A well-understanding of how plasticizer affect thermal behavior of thermoplastic starch and to determine mass loss in starch matrices contain 33% and 50% of glycerol thermal gravimetric analysis (TGA) was conducted. Figure 3.7 presents the weight change and its derivative according temperature. Three stages of weight losses occur in the temperature range of 30 to 450°C. The first stage in the range of 30–120°C is attributed to water evaporation from the external surface as well as dehydration of the intern layer. In that stage, films loss 7.7% of their weight. It can be also related to the film water content as observed in the sorption isotherms for a 53% RH. The zone in the range of 120-260°C is related to the evaporation of glycerol (López et al., 2015). Incorporation of glycerol increases weight loss as the films with 50% of plasticizer display a loss of about 150% higher than films containing only 33% of polyols. The third zone ranges from 260 to 450°C and corresponds to the degradation of starch. The higher the glycerol content, the lower is the film thermal stability. The TGA results clearly indicate that thermal degradation of the starch films, are relevant features in order to convert this biopolymer into materials with useful and desirable properties, can be important for different packaging application.

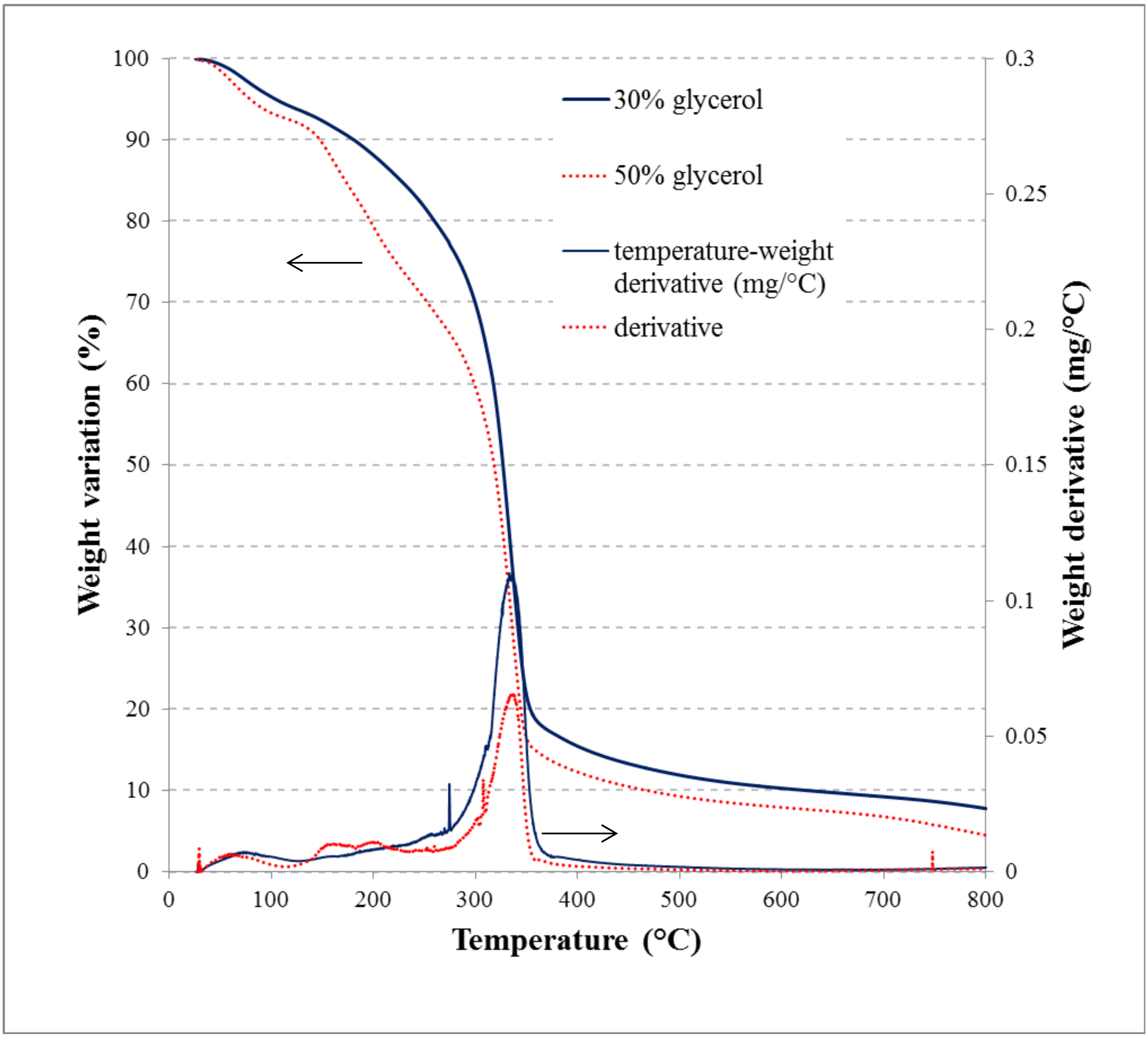


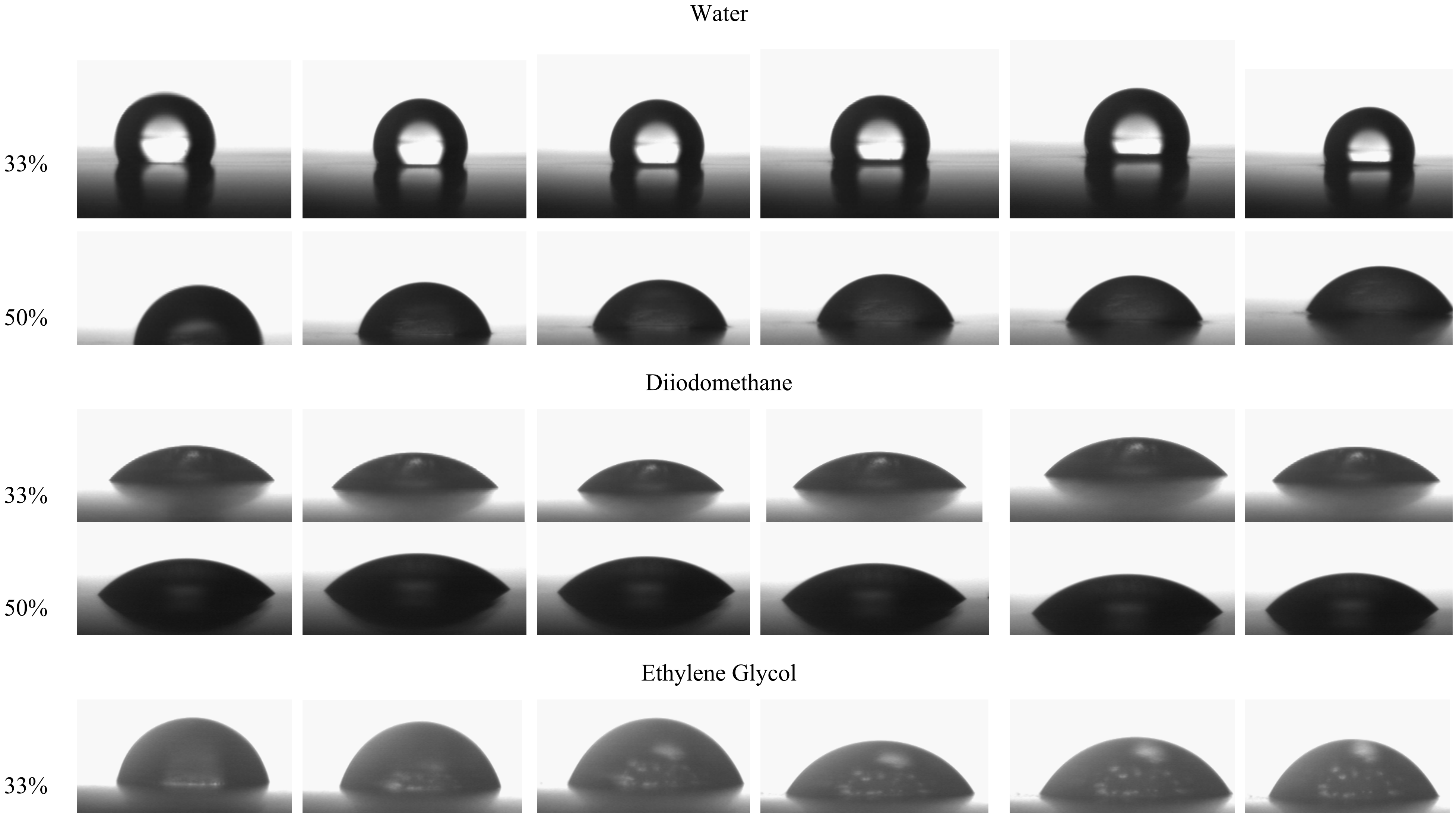
Figure 3.7 Weight loss vs temperature (and its derivative) of films containing either 33 or 50% of glycerol equilibrated at 53% RH prior thermogravimetry analysis.

3.2.5. Wettability properties

This experiment was designed to obtain information about the surface characteristics. Surface tension (γ_S) and its components (dispersive γ_S^D and polar γ_S^P), contact angles (*θ*) of various liquids, works of adhesion (W_A), cohesion (W_C) and spreading (W_S) are given in the table 3.2. The contact angle is a quantitative measure of the wetting of the solid by a liquid. The value of the contact angles corresponds to the value gained at zero time, i.e. it is the initial contact angle at first droplet contact. To assess the surface properties, the contact angle kinetics of 10 liquids deposit on the 33% and 50% glycerol film were determined. Figure 3.8 shows the droplets contact angle shape of 5 liquids (water, diiodomethane, ethylene glycol, glycerol, polyethylene glycol) at time: 0, 10, 30, 60, 90, 120 seconds. For five other liquids: cyclopenthanol, methyl benzoate, n-octane, tetradecane, 1-bromonaphtalane the spreading was instantaneous (less than 0.5 s) and then the contact angle value is considered as 0°. The shape of liquid droplets changed with the plasticizer content. In particular, water contact angles of films vary from 103° to 43° respectively for 33% and 50% of glycerol (w/w). Adding of glycerol reduces values of contact angle and causes faster the spreading. In the 33% of glycerol films, incomplete wetting of surface occurs (contact angle is higher than 90°).

However, it is noticeable that for diiodomethane and polyethylene glycol, contact angle is higher for films containing more plasticizer. When the shape of drop is more rounds this allows supposing that films have better barrier properties as absorption is unfavoured. Heydari et al. (2013) studying functional properties of corn starch films observed influence of the content of glycerol on contact angle. For films containing 25%, 30% and 35% of glycerol, the water contact angles were respectively 49°, 44° and 35°. The higher the glycerol content is, the lower the contact angle.

The surface tension and its dispersive and polar components are given in table 3.2. The surface tension of solid surfaces is the sum of polar and dispersive (apolar) components, indicating the nature of intermolecular interactions at the interface. Polar component is lower for the films with 33% of glycerol than for films with 50% plasticizer. The higher is the glycerol content, the greater the polarity of surface is, favouring hydrophilicity and then water spreading and absorption. This is confirmed by the works of adhesion and cohesion that increased whereas that of spreading decreases (less energy for spreading).


Water
33%
50%
Diiodomethane
33%
50%
Ethylene Glycol
33%

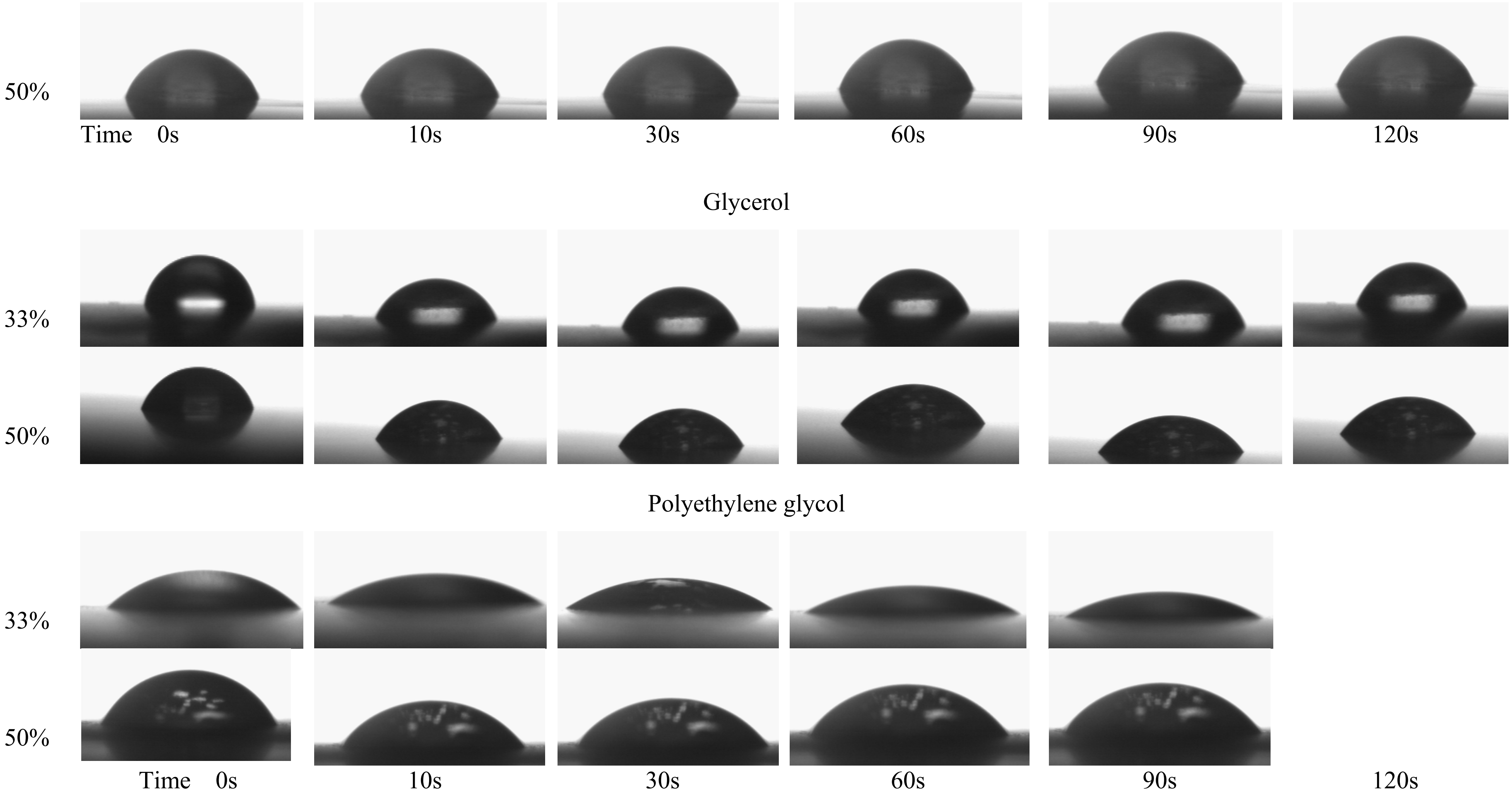


Figure 3.8 Behaviour of water, diiodomethane, ethylene glycol, glycerol and polyethylene glycol droplets on film surfaces as a function of time for starch films with 33 and 50% of glycerol.

Wheat starch films are hydrophilic as displayed from hydration properties; their surface has then strong affinity for moisture. It is confirmed by surface wettability and polarity. McHugh et al. (1993) stated that the equilibrium of water vapour partial pressure at the inner film surface increases with the content of polyols. Due to water content absorption depending on glycerol concentration, the morphology of films, as well as the swelling, affects significantly the surface properties (Laohakunjit and Noomhorm, 2004).

3.2.6. Influence of both glycerol and water on functional properties: mechanical and barrier efficiencies

The Young's Modulus (YM) is the staple test of the sample stiffness: the higher the stiffness of the material, the higher the modulus of the elasticity (Suppakul et al., 2013). The effect of the glycerol on the modulus of elasticity as well as on the tensile strength is not significant (table 3.2). However, the Young's modulus and the tensile strength at break tend to decreased with increasing plasticizer content, whereas the elongation increased. Talja et al. (2008) observed that the addition of glycerol+sorbitol or glycerol+xylitol reduces the Young's modulus by 14 times and a rise of 110% of the elongation. Mali et al. (2002, 2006) reported similar dependence for corn starch, cassava starch and yam starch films containing two different concentration of glycerol. On the contrary, Suppakul et al. (2013) did not displayed significant change in elongation at break, 5.20 and 5.29% respectively for cassava starch films containing 33 or 50% of sorbitol. Muscat et al. (2012) observed a stronger influence of glycerol on elongation than that of xylitol on corn starch-based films. According to conventional standards, packaging films should have tensile strength more than 3.5 MPa. So it is noteworthy that thus not significant difference in mechanical properties only the films containing 33% of glycerol satisfies the requirements and could be used as biodegradable packaging materials (Heydari et al., 2013; Kim et al., 1995).

When plasticizer is incorporated in a starch matrix, it reduced the intra-molecular affinity between the starch chains by forming hydrogen bonds between plasticizer and starch molecules, thus under tensile stress film matrix become less dense, movements of starch chains were facilitated and allows greater flexibility and pliability (Alves eta al., 2007; Heydari et al., 2013). The range of glycerol content tested does not allowed to modify significantly the mechanical properties. This means that 33% of glycerol is enough for gaining enough flexibility to film to be manipulate without risks of breaking or crack formation. This also means the higher concentration of glycerol (above 33%) is not significantly involved in the structure properties and then is only available for fixing or favouring moisture absorption. This probably may affects in a greater extend the molecular dynamics, reducing the local viscosity and then increasing the diffusivity of gases and of small solutes.

Nowadays, the main goal of packaged food is the prolongation of the food shelf-life and thereby minimizes the transfers between the food and the surrounding atmosphere (Gontard et al., 1992). That's why permeability determination is a key functional property for packaging materials. The water vapour permeability of all starch-based films increased with the RH gradient as well as with the glycerol content (table 3.2). Similar effect of the relative humidity gradient was reported by Chang et al. (2000) for tapioca starch films. Glycerol has the biggest effect on the water content of films at the stationary state of the permeation process which induced greater plasticization and thus favours the moisture transfer (Fu et al., 2011; Pushpadess et al., 2008). Farahnaky et al. (2013) reported that the WVP of the wheat starch films without plasticizer is higher than that of films containing 20 or 30% of glycerol. It is caused by microcracks in the unplasticized. In the films containing 50% glycerol, both gradients 75-30% and 100-30%RH exhibit the same WVP values. Indeed, the glycerol content (from 33 to 50%) induced a greater amount of water absorbed which acts as primary plasticizer (more efficient than the glycerol) and thus increases the permeation. These results have to be related to the diffusivity of water in films (table 3.2). Indeed, from kinetics of sorption at 75%RH, the diffusivity increases twice approximately from film containing 33% to film with 50% glycerol. Comparing our data to that of other authors, both wheat starch films with 33 and 50% of glycerol have lower diffusion coefficient. Slavutsky and Bertuzzi (2014) worked with corn starch with 20% glycerol content and they founded water diffusivity values 1000 times higher. Muscat et al. (2013) obtained values of diffusivity of about 10^{-13} m^2s^{-1} for high amylose starch films with very low glycerol content. Müller et al. (2008) also investigated glycerol influence on diffusivity of cassava starch films. Films containing 0.25 g/g of dry starch at 77%RH have an apparent moisture diffusivity of 1.64 x 10^{-11} m^2s^{-1} whereas those containing 0.30 g of plasticizer have a 1.10 x 10^{-11} m^2s^{-1}, and films with 0.35 g of glycerol addition a diffusivity value of 1.02 x 10^{-11} m^2s^{-1}.

García et al. (2011) observed a correlated dependence between glycerol content and water vapour and oxygen permeability. Indeed, the oxygen permeability of films twice increases when glycerol content rises from 33% to 50%, but no significant effect of the RH on the oxygen transfer have been displayed. The oxygen permeability mostly depends on the plasticizer ratio than on the relative humidity. Glycerol is more effective in increasing molecular mobility and in facilitating migration of either oxygen molecules or water vapour permeability (Suppakul et al., 2013).

3.2.7. Conclusions

The influence of glycerol content and moisture level on physical, chemical and functional properties of wheat starch films was studied. The main conclusions can be drawn as follows: films containing 33% of glycerol have lower moisture absorption capacity than films with higher glycerol content. When the concentration of plasticizers increased the tensile strength and the modulus of elasticity decreased, whereas the elongation at break increased as expected with higher amount of plasticizer. The lower the glycerol content, the best water vapour barrier properties (oxygen permeability, water diffusivity, water vapour permeability) are. Interactions between starch, glycerol and water occur between C-O and OH groups indicating mainly hydrogen bonds. Properties of starch-based films strongly depend on water glycerol contents, but this allows reducing surface water activity and transfers which are key parameters for fruit coating applications.

Chapter 4: Impact of protein addition in film-forming suspensions on mechanical, optical and gas barrier properties of films

4.1. Characterisation of composite edible films based on wheat starch and whey protein isolate (Basiak E., Galus S., Lenart A. 2015. Characterisation of composite edible films based on wheat starch and whey-protein isolate. International Journal of Food Science and Technology, 50, 372-380).

4.1.1. Abstract and introduction

Composite films prepared by casting wheat starch and whey-protein isolate at proportions of 100-0, 75-25, 50-50, 25-75, 0-100% were characterised. Combination of both substances gave continuous and homogeneous films. The more starch there is in a film, the more dull is the appearance. The highest water adsorption was observed for pure whey protein films and the lowest for pure wheat starch films with the final water content of 0.264 and 0.324 g water g d.m.$^{-1}$, respectively. An exponential equation well fitted the experimental data of water vapour kinetics ($R^2 \geq 0.99$). The highest values of thickness and elongation at break were observed for mixed films. With the increasing content of whey protein isolate, the values of the swelling index and tensile strength increased from 34.31 to 71.01 % and from 2.29 to 8.90 MPa, respectively. The values of water vapour permeability depended on humidity conditions and decreased slightly with the increasing content of whey protein isolate.

Every year the amount of produced trash increases, causing the landfills to increase. Aside from the fact that people throw away more things than they used to in the past, the main reason for continued growth of landfills is the fact that a large percentage of trash is made up of materials that are not biodegradable or take a long time to decompose (Żenkiewicz and Richert, 2008).

Since the majority of trash generated by households consists of materials used to package food and other necessities, it should be evident that whenever possible easily recyclable of biodegradable materials should be used instead of currently commonly used copolymers of ethylene, polypropylene and other materials that are difficult to utilise (Prakash et al., 2013). An excellent alternative to many of those materials would be to use edible and/or biodegradable coatings and films. Coatings are a kind of material that are deposited onto the surface of foods and films made apart from them. They can reduce packaging waste associated with processed foods. In addition edible films and coatings are environmentally friendly materials which can promote the quality and convenience of minimally processed foods. They can also be used to inhibit or regulate the migration of moisture, carbon dioxide, oxygen and other liquids. They are capable of providing many functional components including flavonoids, colours, vitamins, minerals, nutrition, antioxidants, antimicrobials and other preservatives. By affecting respiration they can modify metabolism of fruits and vegetable tissue (López et al., 2008; Martins et al., 2012).

One of the most abundantly occurring biopolymers in nature is starch. It is an inexpensive (much cheaper than polyethylene), widely available and biodegradable material. Furthermore it is edible, tasteless, colourless and easily used in treatment technology – it is easy to colour or add flavours and other components. Starch is the main resource of energy in higher plants (up to about 90% dry matter) (Schmidt et al., 2013). It is usually accumulated in tubers, seeds and roots (Araujo-Farro et al., 2010). The biggest sources of starch for the industry are corn, wheat, cassava, potato, and quite recently rediscovered amaranth and quinoa (Araujo-Farro et al., 2010). Native and modified starches play an important role (Liu, 2005).

Proteins are functional components used on a wide scale in the food industry. They can be used to produce a wide selection of food products (Schmidt et al., 2013; Talja et al., 2008). In recent years whey proteins are becoming increasingly popular. This is because they have high nutritional value and a useful configuration of molecules. They contain amino acids, essential for proper functioning of the human body, which it cannot produce itself. In addition, they are easily absorbed by humans. Whey proteins are obtained by separation of substances in the ultrafiltration process of protein substance and its drying. Whey proteins have high content of α-lactoglobulins. It is the reason that they have similar properties to human milk proteins (Gounga et al., 2007).

Many polysaccharide-based films and pure whey protein films are very brittle because they have a large cohesive energy. In order to overcome this they need the incorporation of plasticizers to reduce this force by reducing the intermolecular hydrogen bonding occurring between the polymer chains (Jiménez et al., 2012; Murillo-Martínez et al., 2011; Osés et al., 2009). The most popular plasticizers are polyhydroxy alcohols: glycerol, sorbitol, xylitol.

There are several ways to obtain coatings and films from starch and whey proteins. They include casting, injection, extrusion, thermo moulding, blowing and sheeting. During the manufacture of coatings and films the main aim is to obtain homogeneous material, thin and strong (Fu et al., 2011). Films made from one type of film-forming substance often have limited properties. In order to improve the process several mixtures of biopolymers are used. That is because films which consist of 2-3 types of substances have better properties than those made from a single type (Kurek, Galus & Debeaufort, 2014).

The aim of the study was to investigate physical properties of composite edible films based on wheat starch and whey protein isolate, including composition, thickness, swelling index, water solubility, colour, tensile strength, elongation at break, water vapour permeability, sorption properties and microstructure of composite films.

4.1.2. Thickness

Thickness, swelling index and water solubility of starch, starch-protein isolate and protein isolate films are shown in table 4.1. The thickness of all films ranged from 87 to 110 µm. For all films the same amount of film-forming suspension was casted. The films made from pure starch or protein isolate were the thinnest (pure wheat starch films and pure whey protein films) while the ones made from starch and protein isolate mixture were thicker than the ones made from a single ingredient. The films for which the starch and protein isolate proportions (75% starch and 25% protein isolate films and 25% starch and 75% protein isolate films) were 1:3 or 3:1 had a similar thickness, but they were thicker than pure ones. 50% of starch – 50% of protein isolate films with protein isolate and starch ratio 1:1 were the thickest from among all samples. Thickness was the lowest for pure starch films and pure protein isolate films, and the highest for films in a ratio 1:1. The thickness value for 75% starch and 25% protein isolate films was between the value for pure starch films and films in a ratio 1:1, for 25% starch and 25% protein isolate films was between the value for films in a ratio 1:1 and pure protein isolate films. It implies that addition of starch to protein isolate or protein isolate to starch in the same proportion causes an increase of films' thickness (Chang and Nickerson, 2013). Martins et al. (2012) cast 28 ml of film-forming suspension with κ-carrageenan and locust bean gum of 6 different concentrations into polystyrene Petri dishes and they also obtained results dependent on starch and κ-carrageenan/locust bean gum ratio from 39 to 61 µm. Similar results have been previously reported for potato starch films with other ratios of plasticisers (Rodríguez et al., 2006) and for wheat films, for which Roy et al. (2000) obtained results of 83±2 µm.

Table 4.1 Thickness, swelling index, water solubility of wheat starch/whey-protein isolate films (100S - 100% wheat starch ; 75S-25WP - 75% wheat starch + 25% whey protein; 50S-50WP - 50% wheat starch + 50% whey protein; 25S-75WP - 25% wheat starch + 75% whey protein; 100WP - 100% whey protein).

film indication	thickness (µm)	swelling index (%)	water solubility (%)
100S	87.4 ± 3.8^a	34.31 ± 2.7^a	8.37 ± 3.9^a
75S-25WP	95.2 ± 7.0^b	46.03 ± 0.7^b	18.32 ± 3.1^b
50S-50WP	109.3 ± 8.4^c	49.36 ± 1.6^c	10.53 ± 3.8^a
25S-75WP	98.9 ± 6.8^b	51.40 ± 2.6^c	8.68 ± 1.0^b
100WP	88.7 ± 5.8^a	72.01 ± 4.3^d	17.46 ± 1.7^a

$^{a\text{-}d}$ Values with different superscripts within columns are significantly different ($p<0.001$).

4.1.3. Swelling index

The results of film swelling research can be clearly seen in table 4.1. The results show that pure starch films have the lowest swelling index. It was also observed that addition of protein isolate to starch causes a great increase of the swelling index. The pure starch films have a swelling index of 34% while the pure protein isolate films have a swelling index of 72%, which is more than twice as high as for pure starch films. The addition of 25% whey protein isolate to wheat starch causes an increase of the swelling index to 46%. This means that the swelling index rose by 35% in comparison to the pure starch sample. In turn, a further increase of protein isolate content does not cause a significant increase of the swelling index. Increase of protein isolate to 50% and 75% increases the swelling index to 49.4% and 51.4%. This means that a further increase of protein isolate contents does not cause a meaningful increase of the swelling index. In conclusion, the addition of protein isolate to starch films results in creation of a product with an increased value of the swelling index. As in the case of water vapour permeability, more whey protein isolate in the wheat starch film structure entails an increase of water. This means that films made from protein isolate or with an admixture of protein isolate are distinguished by higher hygroscopic than pure starch ones or with a smaller amount of whey protein isolate.

4.1.4. Water solubility

Values of water solubility of different types of starch, starch-protein isolate and protein isolate films are shown in table 4.1. Both pure starch films and protein isolate films have similar values (respectively 13 and 16%).

Starch-protein isolate films have significantly different values depending on the proportions of starch and protein isolate. In the case of films with a starch and protein isolate ratio of 1:3 the value of water solubility decreases considerably in comparison to pure protein isolate or starch films. In films with a starch and protein isolate ratio of 3:1 the value of water solubility is the highest even if it is compared to the results of pure protein isolate or starch films. Schmidt et al. (2013) conducted research on films made of cassava starch without and with stearic acid (5, 10, 15, 20% g/100 g starch). They noted that water solubility increased with the addition of a higher concentration of stearic acid. Lower values for water solubility could be the result of greater interaction between components of these structures.

4.1.5. Colour

One of the most important parameters of food products, crucial for consumer's choice, is colour. Table 4.2 shows colour parameters for investigated films. According to the obtained results of lightness, three homogeneous groups were observed. The first group constitutes starch films (100S), starch-protein isolate films 1:1 (50S-50WP) and protein isolate films (100WP). Their values are of a similar level and their amounts are 95.62, 95.41 and 95.39. The value for the standard (white plate) is 96.74. This means that samples which are in the first homogeneous group are of high lightness. The second homogeneous group constitutes a sample with 75S-25WP. Its value is 94.23. The last homogeneous group consists of a sample with 25% wheat starch and 75% whey protein isolate. Also in this case the value is quite similar (94.78), which means that this kind of film is transparent, too.

Coefficient *a* has minus values for four types of films. These are samples which contain starch. Their values are between -0.10 and -0.81. This means that the colour of films changes to green. Parameter *a* for whey protein samples indicates a change of colour to red.

Coefficient *b* values for samples of pure starch films, 50% starch and 50% whey protein films and pure whey protein films are of a similar level and their amounts are 2.90, 3.62 and 3.92. Starch and protein isolate samples with a ratio of 3:1 (75S-25WP) and 1:3 (25S-75WP) have much higher values than the previous ones (pure starch films, films in a ratio of 1:1 and pure protein isolate films).

Values of total colour difference (ΔE) range from 0.81 to 13.27. Similarly to coefficient *b*, ΔE values show nearly the same trend for samples of pure starch films, films in a ratio of 1:1 and pure protein isolate films. Analogically, samples containing 75% starch and 25% protein isolate and 25% starch and 75% protein isolate are of much higher values than the pure ones (pure starch films and pure protein isolate films) and the one with a ratio of 1:1 (50S-50WP). Galus and Lenart (2012) obtained values of ΔE of alginate/pectin films between 1.90 to 5.49 and noted that ΔE increased significantly with the higher content of pectin.

Table 4.2 *L, a* and *b* colour values and total colour difference (ΔE) of wheat starch/whey proteins films (100S - 100% wheat starch ; 75S-25WP - 75% wheat starch + 25% whey protein; 50S-50WP - 50% wheat starch + 50% whey protein; 25S-75WP - 25% wheat starch + 75% whey protein; 100WP - 100% whey protein).

Film indication	*L*	*a*	*b*	ΔE
100S	95.62 ± 0.40^{a}	-0.20 ± 0.05^{a}	2.90 ± 0.16^{a}	0.81 ± 0.21^{a}
75S-25WP	94.23 ± 0.87^{b}	-0.57 ± 0.06^{b}	4.52 ± 0.85^{b}	13.27 ± 2.07^{d}
50S-50WP	95.41 ± 0.22^{a}	-0.10 ± 0.02^{c}	3.62 ± 0.22^{c}	$1.94 \pm 0.45^{a,b}$
25S-75WP	94.78 ± 0.86^{c}	-0.81 ± 0.14^{c}	6.89 ± 1.10^{d}	4.73 ± 2.14^{c}
100WP	95.39 ± 0.25^{a}	$+0.15 \pm 0.05^{d}$	3.92 ± 0.35^{c}	2.42 ± 0.81^{b}

$^{a-d}$ Values with different superscripts within columns are significantly different ($p<0.001$).

4.1.6. Water sorption kinetics

Fig. 4.1 shows water sorption kinetics of whey-protein isolate/wheat starch films at relative humidity of 75.3%. The highest water adsorption was observed for pure whey protein films and the lowest for pure wheat starch films. Final water content was 0.264 and 0.324 g water g d.m.$^{-1}$, in pure wheat starch and pure whey protein films respectively. Because of the hydrophilic nature of whey protein and wheat starch, high water vapour adsorption was observed for all investigated films. All of the analysed films reached their state of equilibrium within the 24 h time frame set for the tests. Water vapour sorption kinetic curves had a similar course for investigated films. Sorption is dependent on relative humidity of the environment. The higher the relative humidity of the environment the samples were stored in, the greater their ability to absorb water (Ciurzyńska et al., 2013). The highest changes of dry mass for all films were observed during the first 24 h.

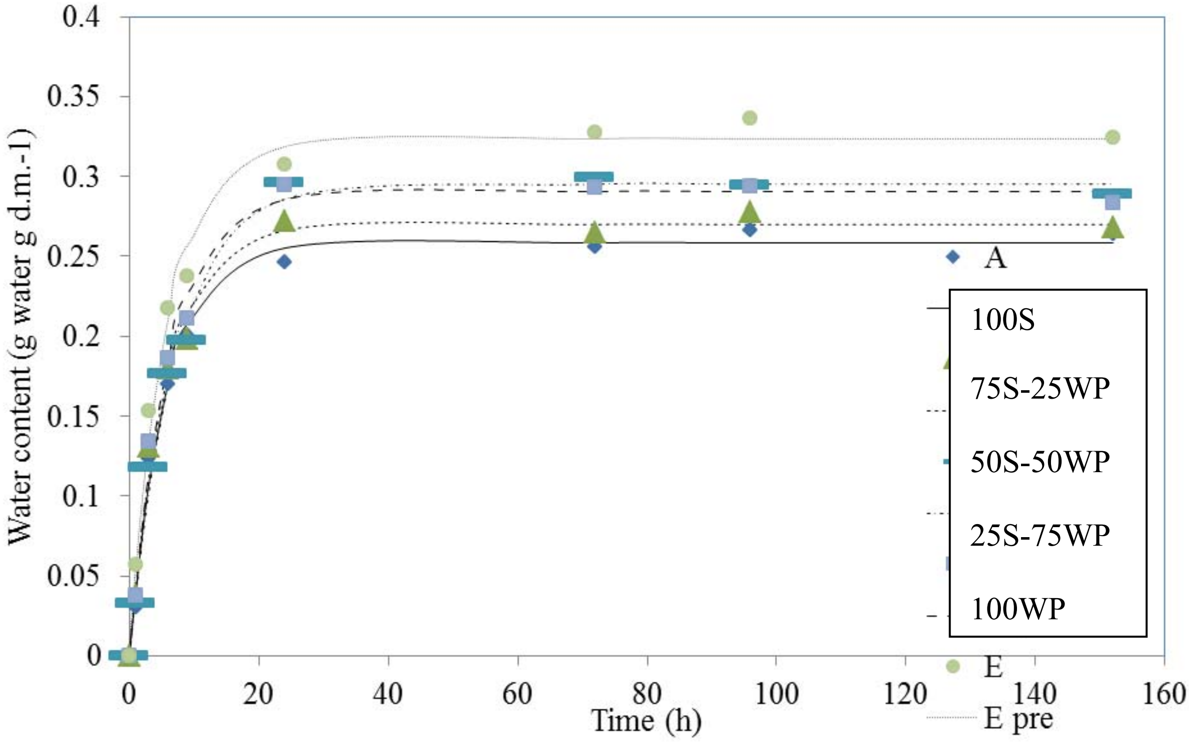


Figure 4.1 Water content as a function of adsorption time for wheat starch/whey-protein isolate films (100S - 100% wheat starch; 75S-25WP - 75% wheat starch + 25% whey protein; 50S-50WP - 50% wheat starch + 50% whey protein; 25S-75WP - 25% wheat starch + 75% whey protein; 100WP - 100% whey protein). Experimental points and fitted exponential equation (lines)).

Similar observations were obtained for other hydrocolloid films. Galus and Lenart (2013) observed an equilibrium state after 10 h of the kinetic process for pure pectin, pure alginate and composite pectin/alginate films. Mali et al. (2005) also observed more rapid moisture adsorption for cassava starch films at the initial stages of the storage at all relative humidity conditions (32, 58 and 90%). Moisture equilibrium time influenced by storage conditions was presented for soy protein films as well (Cho and Rhee, 2002).

Measured water sorption curve data were fitted to an exponential equation. The constants g, h and j, which were derived from the fit, are presented in table 4.3. The coefficients of determination are found to be very high in all cases ($R^2 > 0.99$) and values of mean relative error (MRE) were low, in the range of 3.578-6.863%. These two indicators proved a good fit to the experimental data. The highest sorption rate (j - parameter of eq. 1) was obtained for pure wheat starch films. The chosen equation was also used to describe the shape of water vapour sorption kinetics of soy protein/oxidized starch and soy protein/maltodextrin composite films (Galus et al. 2012a), pectin films (Galus et al.. 2012b) and also for food products, i.e. freeze-dried strawberries (Ciuszyńska and Lenart, 2010) or food powders (Kowalska et al., 2006). Based on the obtained results, the water vapour adsorption rate was the highest at the beginning of the process, when the initial water content in analyzed films was low.

Table 4.3 Exponential equation parameters fitted water vapour adsorption kinetics by of wheat starch/whey-protein isolate films (100S - 100% wheat starch ; 75S-25WP - 75% wheat starch + 25% whey protein; 50S-50WP - 50% wheat starch + 50% whey protein; 25S-75WP - 25% wheat starch + 75% whey protein; 100WP - 100% whey protein; R^2 – determination coefficient, MRE – mean relative error).

Sample	Equation parameters			R^2	MRE (%)
	g	h	j		
100S	0.259	1.007	0.182	0.993	6.863
75S-25WP	0.270	0.994	0.178	0.990	5.743
50S-50WP	0.296	0.998	0.144	0.993	6.088
25S-75WP	0.291	0.996	0.169	0.992	5.593
100WP	0.324	0.997	0.174	0.991	3.578

4.1.7. Water vapour permeability

Results of starch, protein and their components regarding water vapour permeability (WVP) are shown in table 4.4. Obtained values depend on relative humidity (RH). For films which were stored at 0-30% values are the lowest, for 30-75% values are higher, and the highest are for the ones stored at 30-100% (RH) which was also presented in literature (Olivas and Barbarosa-Canovas, 2008). You can also notice that when humidity decreases, water vapour permeability is lower. Starch films at relative humidity gradient (RHG) 0-30% have the lowest values. On the other hand, regardless of RH values of WVP are always of the same dependency. When the protein content increases, values of WVP rise too. For pure starch films values of WVP are at the lowest level. Increasing the addition of protein isolate causes growth of values. Jiménez et al. (2012) prepared films made of pure starch, pure hydroxypropylmethylcellulose (HPMC) and with their combinations with ratios of 3:1, 1:1 and 1:3. They reported that addition of HPMC to starch causes an increase of values of WVP. Values of pure starch films and films with HPMC are similar but not the same. Values increase with higher content of polysaccharides, which have hydrophilic character. Starch films have the lowest value of the swelling index. Values increase when the content of protein isolate rises. It means that more protein isolate causes higher levels of hydrophilicity. That is why adding protein isolate results in an increase of WVP values. Values of water vapour permeability (WVP) also depend on relative humidity. The lowest value is for samples with the lowest moisture content.

Table 4.4 Water vapor permeability (100S - 100% wheat starch; 75S-25WP - 75% wheat starch + 25% whey protein; 50S-50WP - 50% wheat starch + 50% whey protein; 25S-75WP - 25% wheat starch + 75% whey protein; 100WP - 100% whey protein).

Film	0 – 30 % (10^{-10} g m^{-1}s^{-1}Pa^{-1})	30 – 75% (10^{-10} g m^{-1}s^{-1}Pa^{-1})	30 – 100% (10^{-10} g m^{-1}s^{-1}Pa^{-1})
100S	3.94 ± 0.61^{a}	$6.11 \pm 0.21^{c,d}$	$7.70 \pm 0.03^{f,g}$
75S-25WP	$4.34 \pm 0.31^{a,b}$	$5.91 \pm 0.54^{c,d}$	$7.45 \pm 0.70^{e,f,g}$
50S-50WP	$4.28 \pm 0.44^{a,b}$	$6.61 \pm 0.88^{d,e,f}$	7.95 ± 0.33^{g}
25S-75WP	$4.60 \pm 0.75^{a,b}$	$6.52 \pm 0.42^{d,e,f}$	$6.88 \pm 0.40^{d,e,f,g}$
100WP	$5.24 \pm 0.26^{b,c}$	$6.37 \pm 0.57^{c,d,e}$	7.87 ± 0.54^{g}

$^{a-g}$ Values with different superscripts within columns are significantly different ($p<0.001$).

4.1.8. Mechanical properties

Table 4.5 shows values of mechanical properties: tensile strength and elongation.Tensile strength values range from 2.29 (pure starch films 100S) to 8.90 MPa (pure protein isolate films 100WP). Values for starch-protein isolate samples are between the lowest and the highest results. The lowest value was for pure starch films. When more protein isolate was added, values were higher. More protein isolate caused a higher value of tensile strength. Elongation results show that samples of pure starch films, 75% starch and 25% protein isolate films and pure protein isolate films, whose values vary from 10.22 to 16.17%, comprise one common homogeneous group, whereas samples of films of starch and protein isolate in a ratio of 1:1 and films of 25% starch and 75% protein isolate do not show any similar dependencies. Their values are a few times higher than the previous ones (pure starch films, 75% starch and 25% protein isolate films and pure protein isolate films), because the starch and whey protein content is the same so the amount of interactions beetwen them is higher than in the situation when one of components dominates in the ratio. Pure starch films are stiffer and more fragile than films with addition of protein isolate. Whey protein isolate films combined with wheat starch showed greater flexibility than pure films. In 1998 Rhim et al. reported on soy protein films, also prepared at a 5% protein concentration and with 50% glycerol (% plasticiser / 100% protein). Obtained values of tensile strength (TS) were ~6.3 MPa. In all formulations starch significantly reduced the TS. Cao et al. (2007) also reported that the tensile strength of soy protein and fish gelatin composite films increased when the content of gelatin was higher. Dong et al. (2006) made composite films of alginate and gelatin. The authors reported that TS increased when protein content increased. Also Chambi and Grosso (2006) found dependence between two film components. The higher the gelatin content, the higher was TS. The value of TS increased when the amount of protein increased in mixed films.

Table 4.5 Tensile strength and elongation at break of composite edible films based o wheat starch and whey protein isolate (100S - 100% wheat starch; 75S-25WP - 75% wheat starch + 25% whey protein; 50S-50WP - 50% wheat starch + 50% whey protein; 25S-75WP - 25% wheat starch + 75% whey protein; 100WP - 100% whey protein).

Film	Tensile strength (MPa)	Elongation at break (%)
100S	2.29 ± 0.33^{a}	10.22 ± 0.81^{a}
75S-25WP	3.90 ± 0.56^{b}	16.17 ± 1.11^{a}
50S-50WP	4.67 ± 0.19^{b}	76.26 ± 8.92^{b}
25S-75WP	5.10 ± 0.89^{b}	51.35 ± 4.83^{c}
100WP	8.90 ± 1.01^{c}	13.12 ± 1.42^{a}

$^{a-d}$ Values with different superscripts within columns are significantly different ($p<0.001$).

4.1.8. Microstructure

The structure of the film depends on chemical interactions of film hydrocolloids and on the drying method of film-forming solutions. Micrographs provide information about how the film's components are located and how they are arranged in a film matrix (Fabra et al., 2009).

Photographs taken with scanning electron microscopy (SEM) give a better insight into the homogeneity and the films' microscopic structure. In figures 4.2a-j scanning electron micrographs are presented (fig. 4.2a,c,e,g,i - surface and fig. 4.2b,d,f,h,j - cross-section).

Starch samples (pure starch films 100S) are dull, while those with protein isolate (pure whey protein isolate films 100WP) are shiny on the side facing the casting plate with the film but dull on its reverse side (fig. 4.2a, 4.2j). Sample, which contain 75% starch and 25% protein isolate are dull, while sample in a ratio of 1:1 are slightly shiny on the facing side of the plate whereas sample 25% starch and 75% protein isolate films are much shinier on this side and resembles sample of pure protein isolate films. The more protein is in the sample, the shinier is the facing side of the plate.

SEM photographs were taken at different magnifications and they show that starch samples (fig. 4.2a and 5.2b – pure starch films) are more granular than other ones. Sample 100WP of pure protein isolate films (fig. 4.2i and 4.2j) reveals the homogeneity of the structure. It is not possible to single out individual molecules or their complexes. When there is dominance or equilibrium of starch in the sample (75% starch and 25% protein isolate and 50% starch and 50% protein isolate), grains of it are visible (fig. 4.2c-f). In the sample with protein dominance over starch (25% starch and 75% protein isolate) the structure is quite homogeneous (fig. 4.2g and 4.2h). Jiménez et al. (2012) obtained very similar results. They added fatty acids (palmitic, stearic, oleic) to a film-forming solution of corn starch with glycerol (used as a plasticiser). Pure starch films were homogeneous but films with fatty acids had porous and/or heterogeneous structures. This fact implies that starch-protein isolate interactions occur in the film-forming dispersion. Murillo-Martínez et al. (2011) obtained protein and polysaccharide films which had pores and cracks in the surfaces. Galus et al. (2013) observed visually different internal arrangement as a function of composition in soy protein/oxidized starch composite films. While the control film containing only soy protein isolate and glycerol showed a rough and homogeneous microstructure, the films prepared with oxidized starch showed structural discontinuities due to the lack of miscibility of components. Hambleton et al. (2009) also obtained very similar results. They tested alginate films with and without fats (acetic acid ester of mono and diglycerides blended with 20% w/w beeswax). The second one had a more homogeneous structure than the pure ones.

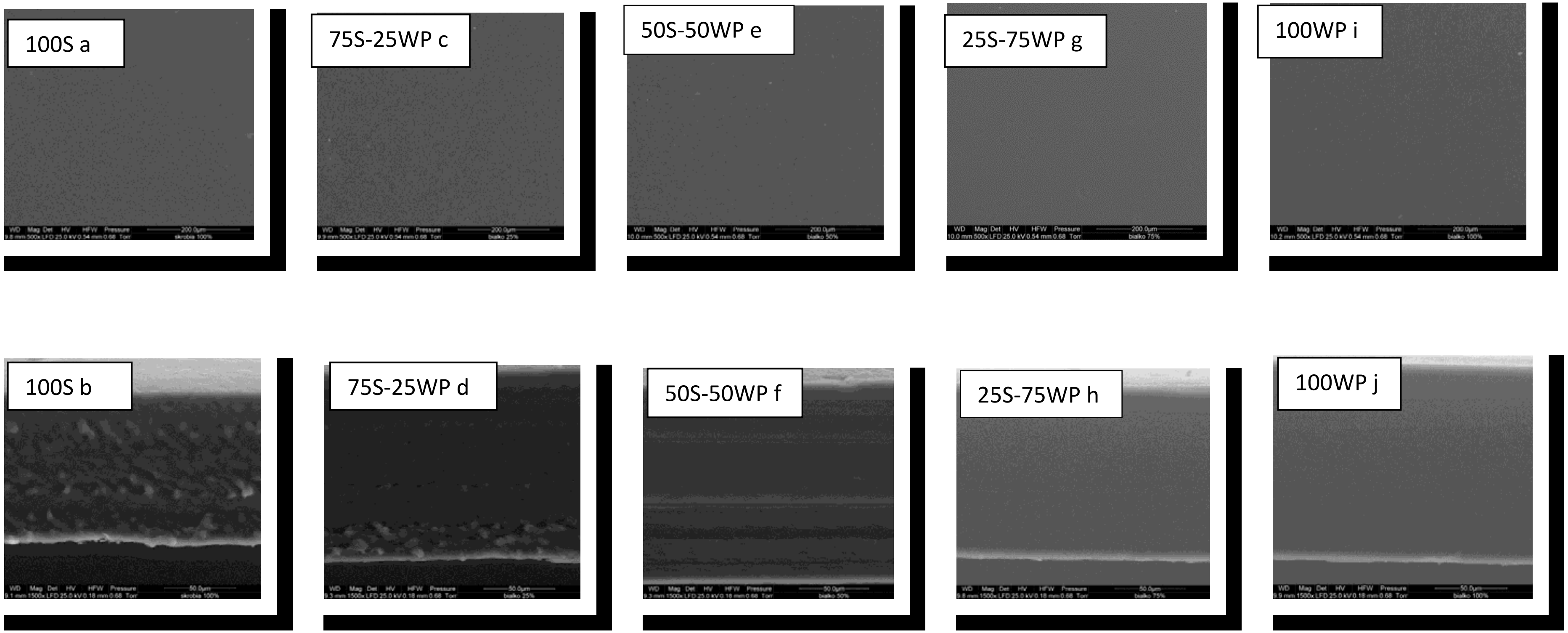


Figure 4.2 Scanning electron micrographs of view (a,c,e,g,i) and cross-section (b,d,f,h,j) of wheat starch/ whey protein isolate films (magnification 500x) - (100S - 100% wheat starch; 75S-25WP - 75% wheat starch + 25% whey protein; 50S-50WP - 50% wheat starch + 50% whey protein; 25S-75WP - 25% wheat starch + 75% whey protein; 100WP - 100% whey protein).

4.1.9. Conclusions

Wheat starch and whey protein isolate are excellent materials to make edible films. All investigated films, pure or composite, have a continuous and homogeneous structure. One difference between pure starch films and pure protein isolate films is surface area. The more protein there is in the sample, the shinier the facing side of the plate is. Film thickness depends on film-forming suspension compositions. Addition of protein isolate to starch films results in obtaining a product with an increased value of the swelling index. Water solubility is similar for pure films, and for mixtures this value decreases and depends on the ratio of substances. Colour of investigated films also depends on type and quantity of particular components. Sorption kinetics shows the hydrophilic character of both components. Values of water vapour permeability (WVP) depend on relative humidity and moisture. When the relative humidity (RH) increases, the value of WVP also rises. Moreover, adding more protein isolate causes growth of WVP values. Tensile strength grows with addition of protein isolate to the film-forming suspensions. Like other parameters and properties, the structure of edible films depends on their composition. Addition of protein isolate changes the visual appearance of films.

4.2. Starch-whey protein mixtures: effect of protein/polysaccharide ratio on film properties (Basiak E., Debeaufort F., Lenart A. (2016). Effects of carbohydrate/protein ratio effects on microstructure, barrier and sorption properties of wheat starch – whey protein blend edible films. Journal of the Science of Food and Agriculture, doi: 10.1002/jsfa.7807.In

4.2.1. Abstract and introduction

Starch and whey protein isolate have good film-forming properties and their mixtures were used for making edible films.Moisture sorption isotherms, water vapour permeability, sorption of aroma compounds, microstructure, water contact angle and surface properties were investigated. Water vapour permeability increased with both the humidity gradient and the starch content. For all samples, film hygroscopicity increased with starch content. Surface properties change according the starch: whey protein ratio and is mainly related to the polar component of the surface tension, as the dispersive component did not varied. With increasing protein content, the microstructure changed toward a more homogeneous. 80% starch - 20% whey protein films have more hydrophobic surfaces than the other films due to specific interactions.

Addition of proteins to starch matrix has strong influence on barrier properties for water vapour permeability and aroma compounds. Surface parameters as contact angle, work of adhesion, cohesion, spreading coefficient depend on starch-protein ratio in the film-forming suspension, hence knowing these parameters it is easier to shape the desirable Interest in edible films and coatings still increases to satisfy the rising demand for fresh and minimally processed or non-processed products. Since 20 years, a great amount of papers dealing with edible and bio-based films for packaging application has been published; displaying huge possibilities of applications for preservation of food and for reducing the use of conventional plastic materials (Debeaufort et al., 1998; Garcia et al., 2000; Guetller et al., 2013). Bio-based packaging materials could offer functional advantages, such as the modification of vegetable tissue metabolism that permits to control the respiration rate (Linke et al., 2013). Moreover, bio-based packaging materials can be used as carriers of antimicrobial and antifungal substances, antioxidants, and vitamins to be released to the specific food matrix according to deliberately induced doses. In addition they protect food products against the loss of valuable components, due to oxidation or to hydrolysis, by controlling permeation of gases and vapours. Coatings and films can contribute to the reduction of food and plastic packaging wastes by increasing

Cellulose, gums, starches and some proteins are the basic materials used for the production of edible films and coatings (Debeaufort et al., 1998; Chiumarelli et al., 2014). Films prepared from starch are tasteless and odorless, non-toxic, biodegradable, as well as colourless, or in the case of rice starch, films are more opaque. Therefore, starch materials can be applied to most of food products as coatings or wrapping. The main part of the wheat starch production are used for food as stabilizers, thickeners and gelling, as dietary substances, fat replacers and as substances that modify the structure of the product, but it is also used for making starch based edible films or coatings (Jagadish and Raj, 2011; Zullo and Iannacce, 2009). Whey protein (WP) was considered as a by-product of the cheese manufacture though it becames a key ingredient of the food industry. Whey protein can form transparent coatings and films which barrier properties to gases and flavours are of key interest but needs some additive for mechanical improvement (Anker et al., 2002; Baldwin et al., 2011; Soazo et al., 2011).

Compared to other protein films (wheat gluten, soy protein, zein and casein films), whey protein isolate films have poor moisture barrier properties (Yoshida and Antunes, 2004). Janjarasskul and others (2014) studied barrier and tensile properties of whey protein isolate–based films obtained from solution-castingand from compressed extruded sheets. Oxygen permeability is 10 times higher for extruded films than for casted films. Basiak, et al. (2015) also measured wettability properties of starch – whey protein films in various ratios. The higher the ratio of protein, the higher the water vapour permeability is. Values of swelling index and water solubility are higher for pure whey protein films than for films made from starch. Water content increases according to the protein content for a given relative humidity (Basiak et al., 2015). Guckian et al. (2006) concluded that films prepared from heated WPI solution have much higher mechanical properties (elongation, tensile strength and Young Modulus) than unheated solutions. Strong hydrogen bonding and disulphide bonds are ruptured during proteins heating. Thus the proteins denaturation induces more stretchable films. The degree of hydrolysis plays a very important role on the mechanical properties. Sothornvit and Krochta (2000) investigated the effect of whey protein hydrolysis and plasticizer content. They admitted the hydrolysed WPI required less glycerol to achieve the same mechanical properties than unhydrolysed WPI. This was attributed to the reduced chain length of hydrolysed proteins which had higher flexibility. Ozdemir and Floros (2008) observed that addition of preservatives like potassium sorbate in WPI films allows inhibiting both enzymes and microorganisms and can also improve the optical properties.

Films should also have appropriate mechanical and surface properties in order to satisfy an adequate adherence onto the food surface (Oh and Lunner, 1999; Ramirez et al., 2012). Parameters as: plasticity and elasticity, hardness, roughness, wettability, lipo-/hydrophilicity and/or hydro-/lipophobicity, solid-liquid properties also surface free energy, surface tension, and contact angle determine the film-food compatibility and performing application (Ganzevles et al., 2006). The most basic measure of wettability for a particular liquid/solid combination is the contact angle θ. The lower the contact angle is the more extensively is the liquid spreads on the solid. In applications of wetting, the aim is generally to engineer a desirable value or range of values for the contact angle able to favour adhesiveness, cohesiveness and spreading of the coating on a solid surface. The value of contact angle gives overall information about the surface hydrophobicity whereas the droplet volume and angle kinetics refer to spreading and wetting (Kalin and Poljanar, 2013). Ferreira et al. (2009) characterized chitosan–whey protein films. The water contact angle on the surface of this composite films at 25-50% protein slightly increased, compared with that of pure chitosan film, and then decreased for higher protein contents. All the blended films have a significantly different contact angle compared to pure starch films and pure whey protein films. The contact angle value measurement is also related to roughness of surface, its porosity or "surface texture". Thus, the angle measured on the film surface in contact to the support for the casting is mainly influenced by the smoothness/roughness of the support surface. On the contrary, the angle measured on the surface exposed to air during film drying is related to the natural self-organization of the biopolymer molecules exposed to air when film forming solution was dried (Phan The et al., 2009). Białopiotrowicz and Janczuk (2002) also displayed that the richer in protein content the surface is, the more hydrophobic the surface is, but this depends on the nature of the protein and its solubility in the solvent used for preparing the film-forming solution.

Edible films can be used as carriers of flavour compounds, either for flavouring food surface by coatings, or for providing essential oils having antimicrobial or antioxidant properties (Debeaufort et al., 2002). More and more edible films have been developed as active packaging by encapsulation/entrapment of active aroma compounds or plant extracts (essential oils). Whey protein as well as starch has been envisaged for flavour encapsulation.

They both allow to retain organic volatile compounds and to protect them against oxidation or temperature (Coupland et al., 2000; Fabra et al., 20012; Lubbers et al., 1998). Reducing the loss of organoleptic characteristics of food to levels below human sensory detection can be obtained by appropriate use of biodegradable or edible polymers with very low absorption or permeability to aroma compounds (Landois-Garza and Hotchkiss, 1987). Edible films having high barrier properties for aroma compounds (very low affinity or sorption), could be seeked.

Both pure biopolymers often couldn't form films with satisfactory properties (Debeaufort et al., 2002). Interactions between starch and whey proteins are anticipated to greatly affect the rheological properties of food systems, markedly changing the gel network structure and the rheological profile (Goel et al., 1999). Combination of two biomaterials (or more) together eliminates each drawback and facilitates to obtain the targeted properties. Polymer combinations can occur as various structure such as emulsions, dispersions or as laminates.

The objective of this work was to study the functional properties of edible films based on wheat starch and whey protein isolate. The ratio between starch and whey protein was focused and then microstructure, surface and transport (sorption and permeability) properties were assessed. Four ethyl esters (model aroma compound) having a wide range of hydrophobicity have been used to study the sorption behaviours of both polar and apolar volatile compounds. parameters of many food products.

4.2.2. Film microstructure

Differences in the morphology of the pure starch, mixture and whey protein isolate films were investigated by environmental scanning electron microscopy (ESEM). On the figure 4.3 are displayed the micrographs of films cross sections.

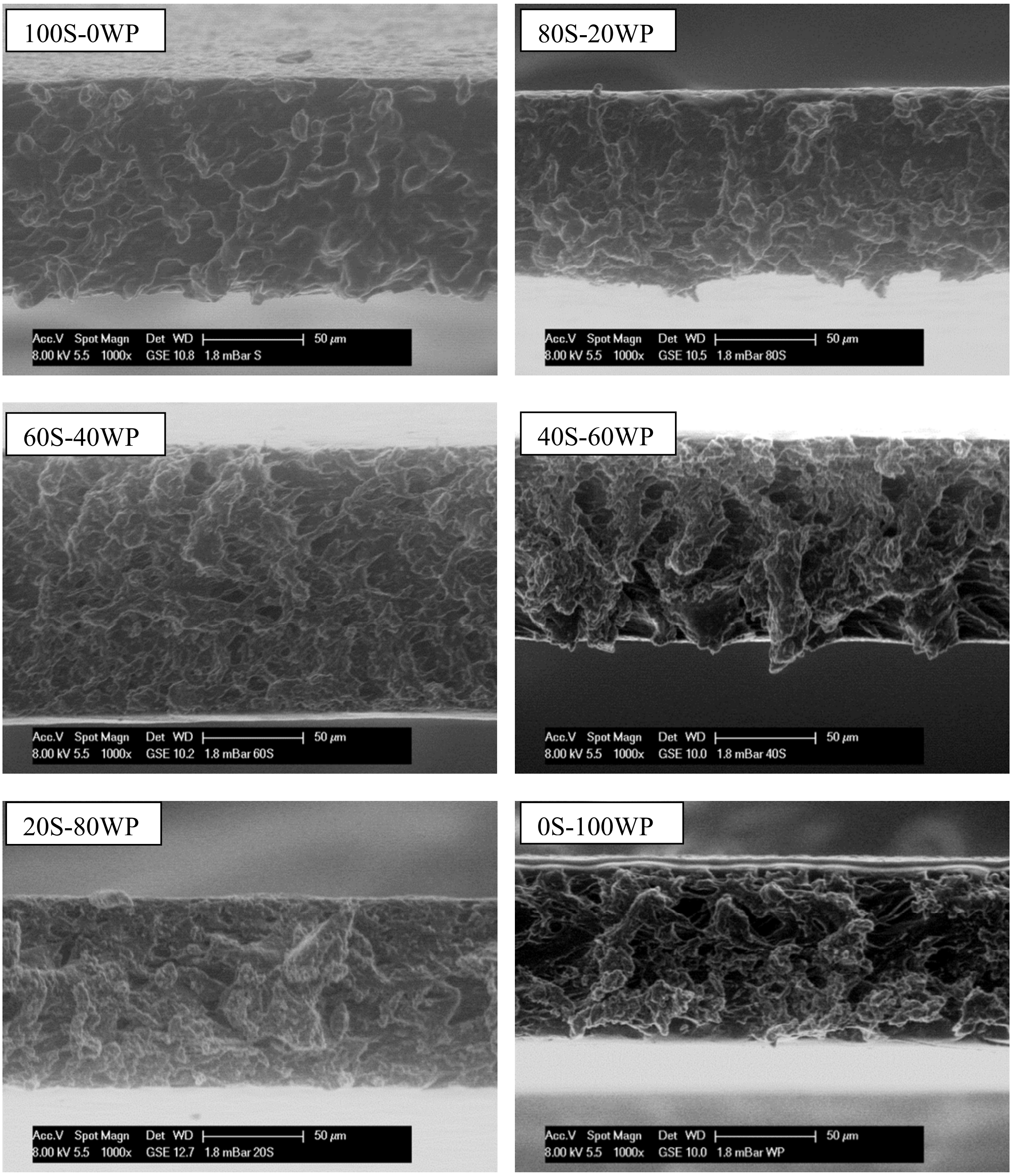


Figure 4.3 ESEM micrographs of cross section of films as a function of starch/whey protein ratio (Magnitude 1000x).

Preparing of starch, whey protein isolate (WPI) and their blends caused changes in the films structure and network mainly due to starch gelatinization and protein denaturation.

Morphology of all samples is significantly different. Structure of starch films (100S-0WP) seems much more heterogenous, fibrillar and less dense than structure of whey protein films (0S-100WP). The framework of blend films seems more complex (80S-20WP, 60S-40WP, 40S-60WP, 20S-80WP). The higher the protein content, the denser and more homogeneous films are. Sun et al. (2013) showed that starch/whey protein films are not completely homogenous but the homogeneity increases with the protein content. However, the difference in the colour of the images suggests a differentiation in density of packing the material in the films formed basing on the starch and whey proteins (Cieśla and Sartowska, 2015). Films containing more starch are thicker. Elsewhere, the glossy parameter depends on two factors: ratio of ingredients and film's side. Starch films are dull whereas, whey protein films are shiny. The more starch in the matrix the less glazy is the matrix. In all cases air side is matter than reverse side. Anyway, regardless of starch-whey protein ratio all films are smooth (smoothing shows the higher homogeneity of the films), without any cracks, holes and damage. Nor open pores are observed onto matrix' surfaces (are not showed in this paper). In previous work Basiak et al. (2015c) investigated wheat starch - whey protein isolate films in five different ratio ingredients. These authors noticed similar effects of view and cross section matrices. According to Fabra et al. (2009) and Jiménez et al. (2012) evaporation of solvent causes changes in component concentrations at the surface thus affects the inner structure of the film surface. These also affect the barrier, mechanical and optical properties.

4.2.3. Moisture sorption isotherm

Moisture sorption isotherms results are given in figure 4.4. The sorption isotherm curves of starch films (100S-0WP), of whey protein isolate films (0S-100WP) and their mixtures films showed a typical behaviour of water vapour sensitive hydrophilic biopolymers. Curves for all kinds of films did not show a significant difference of the shape. The isotherm displays typical shape for protein or polysaccharide edible film. Such non-linear water sorption isotherm is typical of hydrophilic biopolymers and is related to the swelling of the matrix at RH higher than 70%. Olivas and Barbosa-Canovas (2008) obtained similar dependence in whey protein films containing glycerol (50% of dry basis). Soazo and others (2011) reported that in WPI films, the moisture isotherm had sigmoidal shape that is typical of product rich in hydrophilic polymers. At $a_w > 0.58$, moisture content of films rapidly increased. Obtained values for films made from starch, whey proteins and their mixtures are weakly lower than observed by other researchers for same film compositions. This could be attributed to different contents of glycerol concentration and higher content of whey protein isolate. Mali et al. (2002) and Al-Hassan and Norziah (2012) observed in polysaccharides films relatively weak slopes at low water activity but a significant increase at a_w above 0.75. Starch structure consists of crystalline and amorphous parts. The starch adsorption isotherms are attributable to hydrogen bonding of water molecules to the available hydroxyl groups of the substrate, i.e. those in the amorphous parts and on the surfaces of the crystallites. The crystalline spheres typically exhibit resistance to solvent penetration, thus water affects the structure acting as a plasticizer of the amorphous surfaces. Plasticizing effect is low at low activity of water and the ability of the amorphous parts is restricted. At higher water activity, the sorbed moisture causes a subsequent swelling of the biopolymer, the degree of crystallinity goes down and increasing availability of the polar groups to the water molecules is observable. This explains why moisture content of films increased with the starch content (Al-Muhtaseb, McMinn & Magee, 2004).

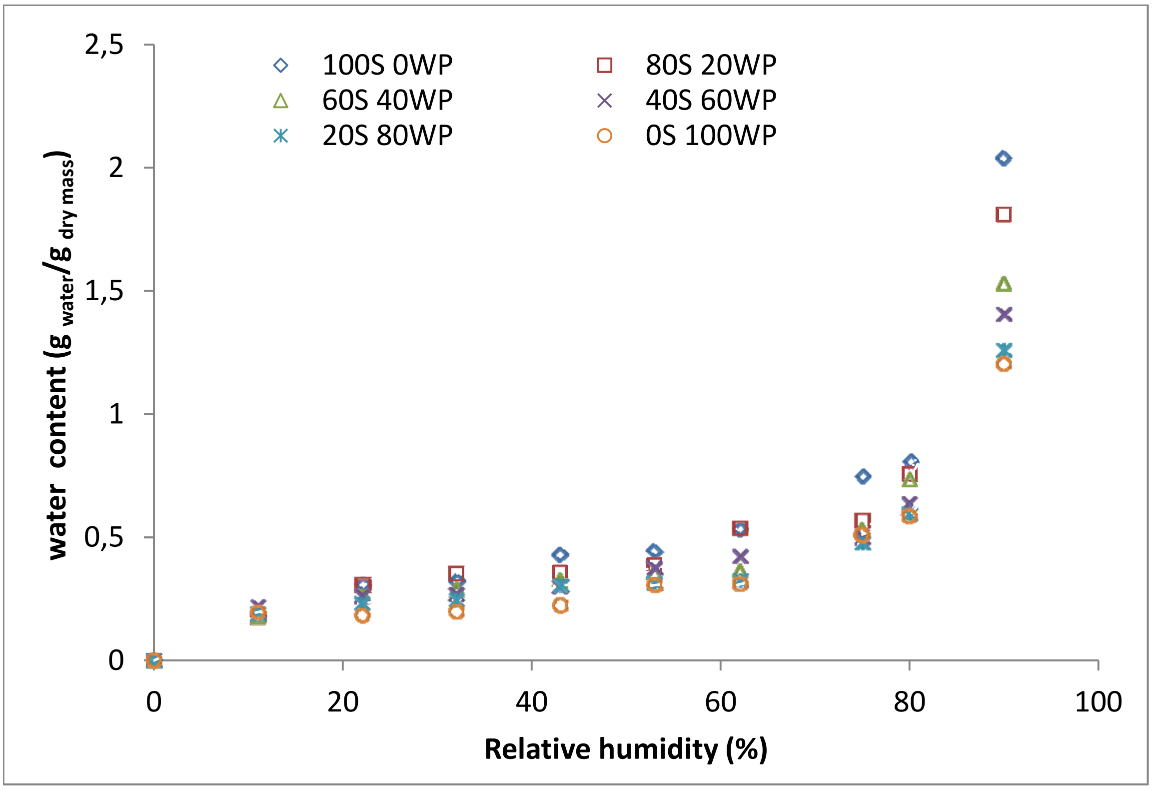


Figure.4.4 Moisture sorption isotherm of starch, starch- protein isolate and protein isolate films (g $_{water}$/g $_{d.m.}$).

4.2.4. Contact angles of water and other liquids

Surface wettability is assessed from the contact angle (θ) and the critical surface tension. These parameters are used to estimate the surface hydrophobicity of films. Table 4.6 gives the initial water contact angle of films (extrapolated at time = 0 s). The balance between adhesive and cohesive forces (between film area and drop of liquid) determines the contact angle. Thus, a water-wettable surface can exhibit its hydrophilic property. The value of contact angle for water is the lowest for starch films (43^{o}) and the highest for whey protein films (93^{o}). This is in agreement with the higher moisture affinity of moisture for starch as displayed on the sorption isotherms. In the end of experiments time (2 min), all films displayed a convex lens shape with a section less than the radius of curvature, because the contact angles are higher than 30^{o}. Only whey protein films can be classified to the group of incomplete wetting; it forms a section greater than the radius of curvature, corresponding to an angle higher than 90^{o}

(figure 4.5). The higher the protein content, the higher is the contact angle. Starch has more hydrophilic groups than whey protein. Increasing the amount of whey protein in films leads to increasing the hydrophobicity. Basiak et al. (2015c) observed that the addition of the whey protein increased contact angles. This appeared because starch has a higher hydrophilic character than proteins. Usually another substance added to starch matrix such as oil, proteins, chitosan caused increasing of surface hydrophobicity. Results are in good agreement with literature: Heydari, Alemzadeh, Vossoughi (2013), Jiménez et al. (2013) and Wiącek (2015) showed the effect of pure starch films on contact angles changed according the incorporation of hydrophobic or hydrophilic substances. The contact angle is directly related to the ratio of the both biopolymers. Shape of the curve (the angle versus the protein or starch content) is linear (R^2 = 0.93). In case of other liquids (cyclopentanol, diiodomethane, ethylene glycol, glycol, methyl benzoate, n-octane, polyethylene glycol, tetradecane, water and 1-bromonaphtalene), contact angles are very similar for all films. The contact angle at equilibrium for diiodomethane is 41-48^o, for ethylene glycol is 45-60^o, for glycerol is 59-68^o, but for cyclopenthanol, methyl benzoate, n-octane, tetradecane, 1-bromonaphtalene) and polyethylene glycol, contact angle was not measurable as these liquids spread immediately. These results can be related to the polar and dispersive components of the surface tension, detailed in following section.

The contact angle measurements deals with the attraction between the water molecules and on polymer surface (Heydari, Alemzadeh, and Vossoughi, 2013). The lower contact angle the stronger attraction value could be noted.

Table 4.6 Comparison of surface tension and the dispersive (γ_S^D) and polar (γ_S^P) components of starch, starch – protein isolate and protein isolate films, adhesion (W_A), cohesion (W_C) and spreading coefficient (W_S), contact angles (°) for water at 20°C. Measurements were done at air drying surface (air) and support drying surface (support).

Film sample	Contact angles (°)	Surface tension (mN/m)	γ_S^D (mN/m)	γ_S^P (mN/m)	Critical surface tension γ_c (mN/m)	W_A (mJ/m^2)	W_C (mJ/m^2)	W_S (mJ/m^2)
0S 100WP	93 ^{c}	63.70 ^{a}	38.45 ^{a}	25.25 ^{a}	35.95 (R^2=0.94)	113.74 ^{a}	129.67 ^{a}	-15.93 ^{a}
20S 80WP	89 ^{c}	71.58 ^{b}	39.11 ^{a}	32.47 ^{b}	33.01 (R^2=0.83)	133.98 ^{b}	139.79 ^{b}	-5.81 ^{b}
40S 60WP	86 ^{c}	70.21 ^{b}	37.99 ^{a}	32.22 ^{b}	33.85 (R^2=0.88)	131.66 ^{b}	138.63 ^{b}	-6.97 ^{b}
60S 40WP	65 ^{b}	73.28 ^{b}	37.99 ^{a}	35.29 ^{b}	31.98 (R^2=0.82)	139.22 ^{b}	142.41 ^{b}	-3.19 ^{b}
80S 20WP	61 ^{b}	60.88 ^{a}	38.01 ^{a}	22.87 ^{a}	33.64 (R^2=0.82)	106.12 ^{a}	125.86 ^{a}	-19.74 ^{a}
100S 0WP	43 ^{a}	60.28 ^{a}	35.40 ^{a}	24.88 ^{a}	34.31 (R^2=0.88)	108.08 ^{a}	126.84 ^{a}	-18.76 ^{a}

Values having same superscript in a column are not significantly different at p level = 0.05

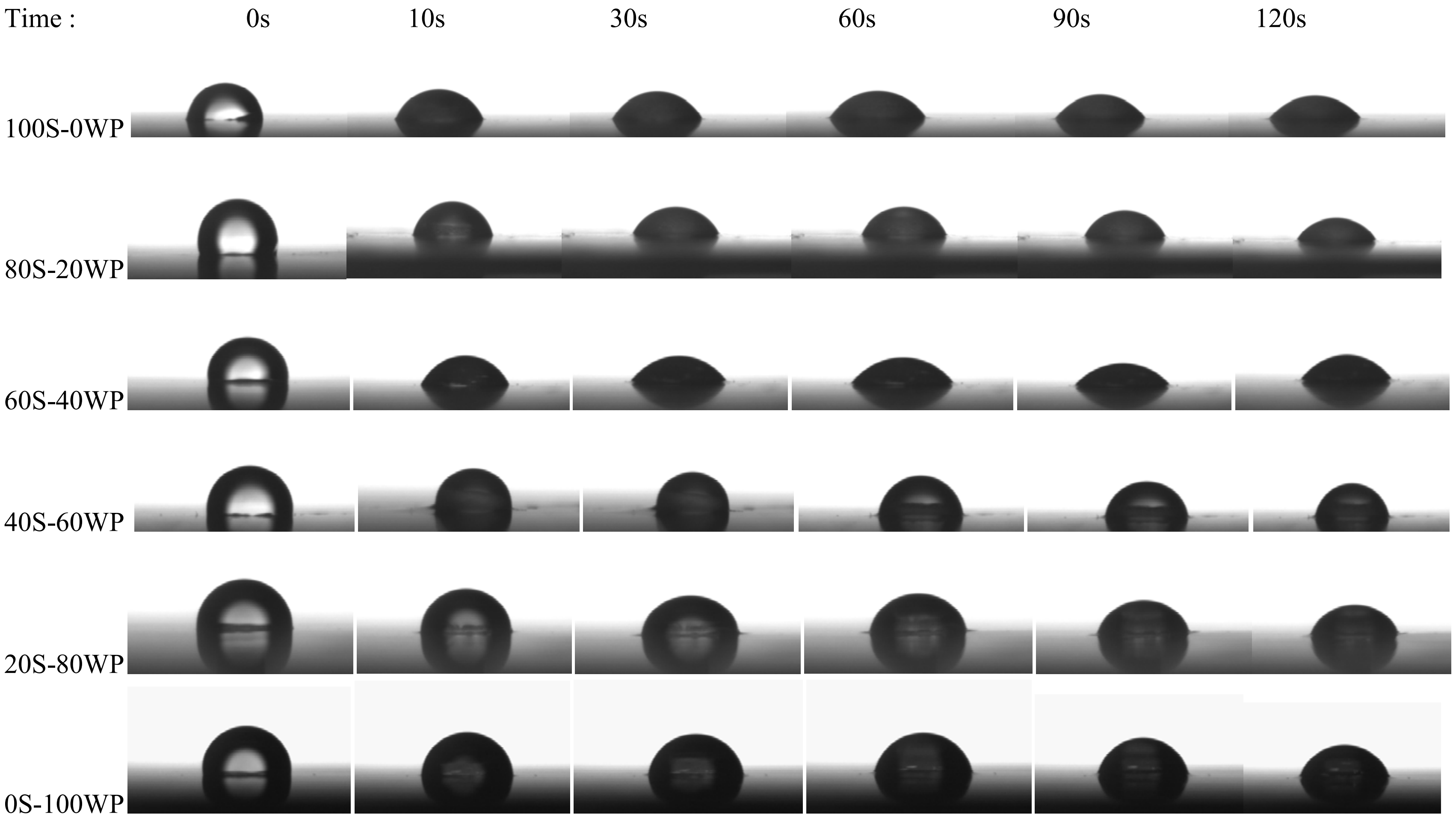


Figure. 4.5 Behaviour of water droplets on film surfaces as a function of time

4.2.5. Surface free energy and critical surface tension

The critical surface tension and the surface tension (or surface free energy) with its polar and dispersive components, the cohesion coefficient and the spreading coefficient, were determined for starch, whey protein and blend films (table 4.6). The surface tension of films was estimated from the contact angle measurements of the seven liquids, and then an estimation of the dispersive and polar surface energy components were obtained. Measurements on both film surfaces (air and support sides) did not give significant differences. The pure starch films, pure protein isolate films and films containing of 80% of starch and 20% of whey protein had the lowest value (about 60 mN m^{-1}), the value of the other blend films (40S-60WP, 60S-40WP and 20S-80WP) are of same value and the highest (more than 70 mN m^{-1}). This means they are more polar than other films. A slight difference in surface free energy between pure films and mixtures (except 80S-20WP films) is a result of a different molecular organisation in the network. This behaviour is explained by the polar component of the surface free energy. Indeed, all films, whatever their composition have same dispersive component. The γ^{D}S ranges from 35 to 39 and are not significantly different. On the contrary, the γ^{P}S value of starch films, whey protein films and 80S-20WP films have significantly reduced value, indicating that they are less polar. Critical surface tension is the highest for the starch films and for whey protein films. Blends of starch and whey protein at ratios 20: 80, 40: 60 and 80: 20% are very similar slightly above 33 mN m^{-1}. Finally, the 60: 40% starch – whey protein film displays the lower critical surface tension value. This critical surface tension value is obtained from linear extrapolation from contact angle of various liquids. However, it accuracy has to be related to the angle measured at a meta-stable equilibrium (the kinetic of wetting not considered). This value cannot easily been compared to surface surface tension value at the condition of angle measurement differs. However, these data seem to confirm that interactions between whey protein and starch induce a network with less hydrophilic groups at the film surface and then films having lower surface tension.

Dealing with the spreading coefficient (W_S) and the critical surface tension (γ_c), significant differences have been observed between all films (figures 4.6 a-b). Pure starch films, pure WPI films and films in ratio 80S-20WP have the highest Ws and γ_c. Values for mixtures films in ratio 60S-40WP, 40S-60WP and 20S-80WP are the lowest. In case of pure starch films, pure protein isolate films and mixture films of 80% of starch and 20% of protein isolate, the non-polar energy is predominant on polar energy. It means pure starch films, pure protein isolate films and their mixtures have more ability to participate in dispersive interactions.

Ghanbarzadeh et al. (2007) obtained similar results for polar part of zein films. Polar interactions were much stronger than apolar interactions. The disperse part was three times lower than polar part. They also admitted that critical surface tension values are mostly lower than the surface tension values of the same tested surfaces.

Interactions between WPI and polysaccharides depend on the colloidal systems and then affect final properties of systems (Ercelebi and Ibanoglu, 2007; Koupantsis and Kiosseoglou, 2009). If the interactions in solution at pH 7 between the whey protein molecules with those of the starch were hydrophobic and not electrostatic in origin (as charge is a non-charged polymer), the orientation of the hydrophobic groups of the adsorbed protein molecules might have prevented their interaction with the polysaccharide of the continuous phase (Cooper, Dubin, Kayitmazer and Turksen, 2005; Koupantsis and Kiosseoglou, 2009). Potato starch - WPI complexes in several ratios (from 1:0.5 to 1:5 starch – WPI) were studied by Zaleska et al. (2001). They displayed the composition of the product was dependent on the composition of solutions. The lower the starch content in the suspension, the slower is the rate. Between polysaccharides and proteins exists a numerous interactions. These authors also stated that water might take an active part in the construction of the network structure and then affects the final surface properties of films after cast solution have been dried.

Work of adhesion (W_A) and work of cohesion (W_C) fold on wettability (work of spreading W_S). Those parameters are opposite because the first one represents contraction, the second one the spreading of the liquid. Results for starch films, whey protein films and blend films are given in table 4.6. As in case of other surface parameters, spreading coefficient (W_S) and work of cohesion (Wc) behave differently for the pure starch films, pure whey protein isolate films and 80S-20WP films.

The highest the difference between those two parameters, the lower is the spreading coefficient. Work of spreading is an important process for coating applications to ensure the continuity of the layer and then its barrier efficiency. Liquids deposited on these films have a tendency to spread very easily. Guettler et al. (2013) studied soy materials and they obtained very similar values for work of adhesion: 104.3 mJ m^{-2} for soy protein isolate and 120.2 mJ m^{-2} for heated soy protein isolate. All protein materials displayed hydrophilic character and have relatively similar dispersive surface energy characteristic, which influence on parameters as adhesion work. The work of cohesion obtained was insignificantly lower: 88.2 (mJ m^{-2}) for soy protein isolate and 106.4 (mJ m^{-2}) for heat treated soy protein isolate. Values obtained by

Guettler et al. (2013) for soy protein films are similar to these obtained in this study for starch, whey protein and blend films.

4.2.6. Water vapour permeability

Water vapour permeability (WVP) is a measure of the amount of moisture passing through unit area of material per unit of time. For hydrophobic polymer films such as polyethylene or polypropylene, the WVP is proportionally constant whatever the water vapour pressure gradient applied across the films. Natural biopolymers used for making edible films are often hydrophilic. They contain polar groups which interact with permeating water molecules inducing plasticization during permeation. Indeed, water sorption occurring during permeation process increases the polymer free volume allowing the polymeric chain segments to raise their mobility due to swelling. Higher mobility leads to higher WVP (Gontard, Guilbert and Cuq, 1994; McHugh and Krochta, 1994).

WVP of films made from starch, WPI and their blends are given in table 4.7. The WVP depends on relative humidity gradient (RH) as expected. Indeed, the higher the gradient, the higher is the WVP. However, using different gradients allowed obtaining different average moisture contents in films at the stationary state of permeation, and then permitting to discriminate permeation behaviours according the film composition and plasticisation. As expected, the WVP obtained for the 33-0% RH differential were the lowest, for 75-30% values were higher and the highest were for the ones stored in 100-30% (RH). Regardless of RH, WVP values are always of the same dependency according the starch/protein ratio. When starch content increases, the WVP increases too. As WVP is the result of both sorption and diffusivity of moisture in solid matter, probable is that starch either increases the affinity of the film for water or induces a local viscosity decreases and then increases of the diffusivity. Sorption isotherms confirm that starch favours moisture absorption compared to whey protein which contributes to WVP increases with the starch content. So the increase of WVP observed for starch film is also attributed to the increases of moisture affinity. In that way, it is also suggested that less swelling occurs in presence of low content of starch.

The WVP could be related to the polar component of the surface tension, and then is directly related to the film surface polarity and work of spreading. At 30-75% RH differential and then higher, water content is high enough (according the moisture sorption isotherm) to induce water plasticization significantly.

Table 4.7 Water vapour permeability (10^{-10} $gm^{-1}s^{-1}Pa^{-1}$)) of films at 25°C at three relative humidity differentials

	33-0% RH	75-30% RH	100-30% RH
0S 100WP	3.94 ± 0.61 ^{a}	6.11 ± 0.21 ^{d,e}	7.45 ± 0.91 ^{g,h,i}
20S 80WP	3.97 ± 0.08 ^{a,b}	6.15 ± 0.39 ^{d,e,f}	7.11 ± 0.75 ^{e,f,g,h}
40S 60WP	4.27 ± 0.34 ^{a,b,c}	6.69 ± 0.93 ^{e,f,g,h}	7.08 ± 0.21 ^{e,f,g,h}
60S 40WP	4.86 ± 0.29 ^{a,b,c}	6.17 ± 0.70 ^{d,e,f}	6.58 ± 0.68 ^{e,f,g}
80S 20WP	4.99 ± 0.14 ^{b,c}	6.37 ± 1.03 ^{e,f}	7.16 ± 0.54 ^{f,g,h,i}
100S 0WP	5.24 ± 0.26 ^{c,d}	7.70 ± 0.85 ^{h,i}	7.87 ± 0.65 ^{i}

Value having the same letter are not significantly different at p level = 0.05

4.2.7. Aroma compound sorption

Vapour sorption coefficients of ethyl acetate, ethyl butyrate, ethyl hexanoate and ethyl octanoate in films are presented in table 4.8. The sorption coefficients were determined at equilibrium (after 21 days of storage). Ethyl acetate sorption seems not dependent on the starch content, and is slightly higher in whey protein film. Ethyl acetate is a polar substance which log P_{part} is the lowest (0.71) among aroma compounds studied (figure 4.7). This may explain its peculiar behaviour that seems not related to film composition. As it is highly soluble in glycerol, it is probable that sorption of ethyl acetate is dominated by its solubility in the glycerol. The ethyl butyrate has an average hydrophobicity, probably closer to that of protein which explains its sorption increases with whey protein content. It is probably also more sensitive to the polarity of the film as estimated from contact angle measurement. Increasing the hydrophobicity and decreasing the volatility of the aroma compound induce a reduced sorption as observed for ethyl hexanoate and ethyl octanoate. This means that whey protein films could be better barrier to more hydrophobic aroma compound of less volatile. However, the nature of the chemical group of the aroma compound could also play and important role on sorption and transport through protein film, through interaction with amino acids

Table 4.8 Concentration of aroma compounds (ethyl acetate, ethyl butyrate, ethyl hexanate, ethyl octanate) sorbed in films (mg aroma/g of film) according the ratio between starch and whey protein isolate.

Aroma compound	100S 0WP	80S 20WP	60S 40WP	40S 60WP	20S 80WP	0S 100WP
Ethyl acetate	5.07 ± 0.37 ^{h,i}	4.82 ± 0.24 ^{h,i}	4.80 ± 0.22 ^{h}	4.79 ± 0.17 ^{h}	4.73 ± 0.02 ^{h}	5.80 ± 0.24 ^{j}
Ethyl butyrate	2.11 ± 0.02 ^{f}	4.02 ± 0.07 ^{g}	5.19 ± 0.01 ^{i}	6.45 ± 0.05 ^{k}	7.09 ± 0.01 ^{l}	7.30 ± 0.02 ^{l}
Ethyl hexanate	1.15 ± 0.09 ^{c,d}	1.22 ± 0.07 ^{d}	1.75 ± 0.11 ^{e}	1.45 ± 0.11 ^{d,e}	1.28 ± 0.34 ^{d}	1.74 ± 0.17 ^{e}
Ethyl octanoate	1.32 ± 0.14 ^{d}	1.53 ± 0.01 ^{d,e}	1.52 ± 0.14 ^{d,e}	0.41 ± 0.03 ^{a}	0.81 ± 0.09 ^{b,c}	0.74 ± 0.10 ^{a,b}

Value having the same letter are not significantly different at p level = 0.05

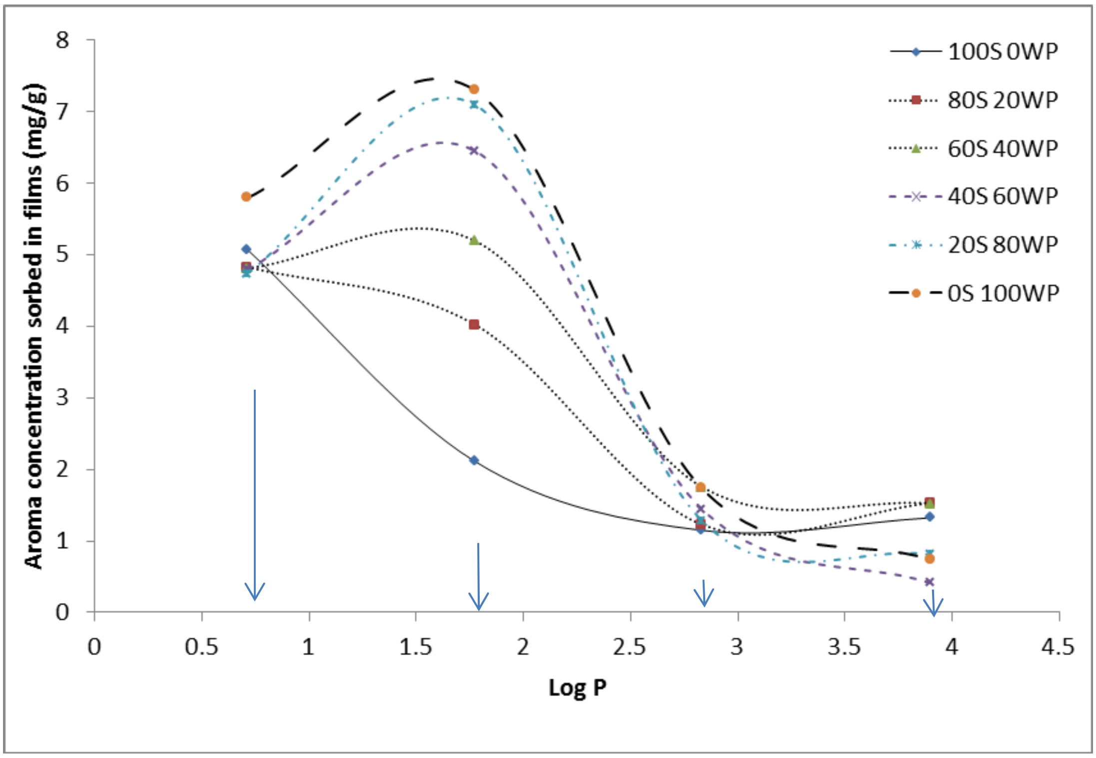


Figure 4.7 Relationship between Log P and concentration of aroma sorbed by starch-whey protein isolate films

Thanh et al. (1992) demonstrated that the caseinates substrates sorb higher quantities of volatile compounds that carbohydrates. Błaszczak et al. (2007) demonstrated the sorption capacity stronger depends on hydrophobicity and molecular structure of odorant volatiles rather than starch origin. In contrast, Boutboul et al. (2002) showed that interactions between aroma compounds and starch exist. Chalier et al. (2006) reported that the permeability of 2-heptanone was decreased by coatings made from casein or gluten.

Mauricio-Iglesias et al. (2011) investigated concentration of aroma compounds (ethyl butanoate and ethyl hexanoate) through polylactide films (PLA). The results obtained are on the level 0.3-0.6g of ethyl butanoate per kg of PLA films and 0.7-1.6g of ethyl hexanoate per kg of PLA film. Terta et al. (2006) used polysaccharide matrices for investigation of aroma compounds concentration (limonene and *trans*-2-hexanal). The data obtained show the aroma concentration sorbed in films is around 0.6-1.6g of aroma compounds per kg of biodegradable film.

As well as starch, starch-whey protein and whey protein films, PLA films and polysaccharide films have polar character and thus have less affinity for apolar volatile compounds like aromas. These films have amounts of aroma sorbed comparable with data obtained in this study. On the contrary, the quantity of aroma sorbed by plastic films like polyesters with high crystallinity or polyolefins (Mauricio-Iglesias et al., 2011 and Hirat and Ducruet, 2006) is at least two magnitudes higher.

4.2.8. Conclusions

The effect of transport properties at the starch-whey protein-based films was studied. The microstructure, surface and wettability properties of wheat starch films, whey protein films and their mixtures were investigated. Starch films are more heterogeneous and less dense than protein films. The less the starch content, the more homogenous and dense the films are. Starch films, whey protein films and 80S-20WP mixture films have higher dispersive component of the surface energy than other blends. Also surface tension, work of adhesion, work of cohesion and spreading coefficient are lower for mono-component and 80S-20WP films than for other mixtures. The contact angle and sorption of ethyl butyrate decrease with the starch concentration whereas WVP and the water content increased. Increasing the hydrophobicity and decreasing the volatility of the aroma compound induce a reduced sorption as observed for ethyl hexanoate and ethyl octanoate. This means that wheat starch films will be poorer barriers to less hydrophobic aroma compounds that are less hydrophobic.

Chapter 5: Effect of rapeseed oil addition (by a lamination process) on physico-chemical and mechanical properties of films

5.1. Influence of rapeseed oil on optical properties of starch films (Basiak E., Lenart A. (2013). Wpływ oleju rzepakowego na właściwości optyczne filmów skrobiowych, Postępy Techniki Przetwórstwa Spożywczego, 23/42, 1, 23-26. Influence of rapeseed oil on optical properties of starch films. Development of Technic and Food Industry23/42, 1, 23-26).

5.1.1. Abstract

The aim of this work was to analyse the effect of rapeseed oil addition on optical properties of wheat starch films. Films were prepared from 5% of starch film-forming water solutions and 50% of glycerol as plasticizer (w/w of starch) and rapeseed oil was added to the solutions at concentration of 0, 1, 2, 3%. Colour in the CIE *Lab* system was measured and colour discriminants were calculated: total colour difference, colour saturation and saturation index. Moreover the opacity at wavelength of 600 nm was calculated. Changing in colour parameters and discriminants was observed and decreased opacity of starch films with rapeseed oil addition.

5.1.1. Abstrakt

Celem pracy było wyjaśnienie wpływu dodatku oleju rzepakowego na właściwości optyczne filmów ze skrobi natywnej pszennej. Filmy utworzono z 5% wodnych roztworów powłokotwórczych skrobi natywnej pszennej z dodatkiem 50% glicerolu jako plastyfikatora względem masy skrobi oraz dodatkiem oleju rzepakowego w ilości 0, 1, 2, 3% (względem masy roztworu powłokotwórczego). Zmierzono barwę filmów w systemie CIE *Lab* z zastosowaniem wyróżników barwy takich jak bezwzględna różnica barwy, nasycenie barwy i indeks nasycenia. Policzono również nieprzezroczystość przy długości fali 600 nm. Zaobserwowano zmianę barwy oraz zwiększanie nieprzezroczystości filmów modyfikowanych dodatkiem oleju rzepakowego.

5.1.2. Wstęp

Zainteresowanie jadalnymi powłokami i filmami ciągle wzrasta, ponieważ rośnie popyt na żywność świeżą i minimalnie przetworzoną. Jednocześnie produkowane nowoczesnymi technikami filmy posiadają coraz więcej zastosowań. Mają wiele funkcjonalnych zalet jak modyfikacja metabolizmu tkanek warzyw prowadząca do zmian ich respiracji, są nośnikiem substancji przeciwmikrobiologicznych, antyoksydantów, witamin. Oprócz tego chronią produkty przed utratą cennych składników takich jak ww. przeciwutleniacze, mikro- i makroelementy, aromaty. Powłoki i filmy przyczyniają się do redukcji masy wytwarzanych opakowań z tworzyw sztucznych jak np. z politereftalanu etylenu i jego kopolimerów, polietylenu dużej i małej gęstości oraz polipropylenu. Marketing żywności stawia czoło wielu wyzwaniom, jak wytwarzanie produktów o wysokiej jakości, produktów odżywczych, trwałych i niedrogich. Jadalne filmy pomagają spełniać te oczekiwania.

Celuloza, gumy, skrobie i białka są podstawowymi materiałami wykorzystywanymi do produkcji jadalnych filmów i powłok. Filmy otrzymane ze skrobi są pozbawione smaku i zapachu, nietoksyczne, biologicznie degradowalne, jak również bezbarwne, lub jak w przypadku skrobi ryżowej mlecznobiałe (Flores i in., 2007; Guilbert, Gontard & Gorris, 1996; Souza i in., 2010). Pszenica jest drugim co do wielkości (7%) źródłem skrobi na świecie. W samej Europie wytwarza się rocznie 2,8 miliona ton. Sprzyjają temu korzystne warunki uprawy. Ziarna skrobi pszennej, zarówno zwykłej, jak i modyfikowanej, są średniej wielkości (10 – 25 μm) o kształcie owalnym (Molenda i in., 2006). W około 53% są wykorzystywane do produkcji żywności w postaci stabilizatorów, zagęstników, substancji dietetycznych i żelujących, zastępują tłuszcz i substancje modyfikujące strukturę w procesie technologicznym, a także są środkiem powłokotwórczym jadalnych filmów (Lin i in., 2011).

Ze względu na szeroką dostępność, niską cenę, duże możliwości w kształtowaniu cech fizyko-chemicznych i biologicznych filmy otrzymane ze skrobi są cenione przez producentów żywności i przez konsumentów. Filmy można łatwo barwić, zadrukowywać, dodawać do ich struktury substancje odżywcze i inne komponenty powłokotwórcze tj. białka i tłuszcze. Te ostatnie są w szczególności pożądane w celu zniwelowania głównej wady powłok skrobiowych, a mianowicie dużej przepuszczalności pary wodnej (Ghosh, Ziegler & Anantheswaran, 2005).

Związki lipidowe są substancjami o charakterze hydrofobowym. Stanowią barierę przeciwko wymianie wilgoci między produktem a otoczeniem i otoczeniem a produktem. Mogą być dodawane do struktury powłokotwórczej filmów i powłok, lub nanoszone w postaci oddzielnej warstwy.

Jednym z nieodłącznych wskaźników, który decyduje o wyborze produktu przez konsumenta jest barwa. Charakteryzuje ona świeżość surowca i jego przydatność do spożycia. Barwa środków spożywczych jest mierzona często za pomocą kolorymetru w systemie CIE *Lab*. O analizie kolorów produktów spożywczych decydują takie parametry, jak jasność *L, a* przejście barwy od zielonej do czerwonej, *b* przejście barwy niebieskiej do żółtej (Flores i in., 2007; Ghanbarzadeh , Almasi & Entezami, 2010; Viña i in., 2007; Zavareze i in., 2012). Charakteryzują one cechy barwy tj. indeks nasycenia, czyli nasycenie barwy produktu bez udziału wzorca (nasycenie barwy – intensywność barwy w porównaniu ze standardem) i bezwzględną różnicę w barwie – różnica między barwą materiału a barwą standardu. Różnica barwy jest wielkością wynikającą z 3 różnych komponentów: *ΔL, Δa i Δb* (Reis i in., 2008).

Również nieprzezroczystość jest jednym z wyróżników optycznych materiałów spożywczych. Charakteryzuje ona zdolność filmów jadalnych do wchłaniania i odbijania światła oraz decyduje o ich zastosowaniu jako materiałów opakowaniowych. Wartość nieprzezroczystości jest uzależniona od grubości (Flores i in., 2007; Reis i in., 2008).

Celem pracy była analiza barwy i nieprzepuszczalności filmów ze skrobi natywnej pszennej z dodatkiem oleju rzepakowego.

5.1.3. Wyniki i dyskusja

W pracy analizowano wpływ dodatku i ilości oleju rzepakowego na własciwości optyczne filmów skrobiowych (skład surowcowy podany w tabeli 5.1). Po suszeniu zaobserwowano pewne ilości tłuszczu pozostające na podłożu oraz zaobserwowano jego straty w wyniku przechowywania, ze względu na występowanie niewielkiej ilości oleju na powierzchni filmów. Wraz ze wzrastającym udziałem oleju (1-3%) w roztworach powłokotwórczych zaobserwowano większe ilości tłuszczu wodnego na powierzchni. Podobne zjawisko było prezentowane w pracach na temat modyfikacji składu surowcowego filmów białkowych dodatkiem emulsji tłuszczowych (Yang & Paulson, 2000). Zaobserwowano również, że wraz ze wzrostem zawartości oleju rzepakowego, zmienia się barwa filmów. Wszyskie filmy były mlecznobiałe, jednakże filmy kontrolne były matowe, a modyfikowane dodatkiem oleju połyskujące.

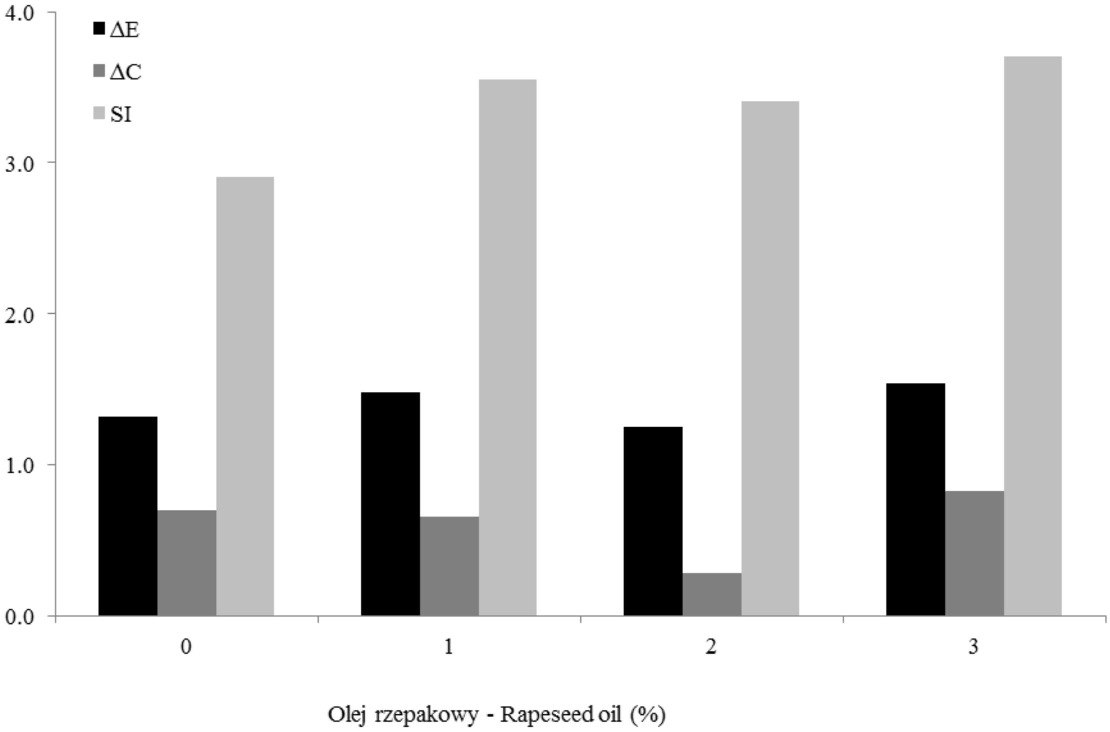
Tabela 5.1. Skład surowcowy wodnych roztworów powłokotwórczych z dodatkiem oleju rzepakowego i otrzymanych z nich filmów.Table 5.1. Composition of film-forming solutions in water with rapeseed oil and films obtained from them.

Roztwór powłokotwórczy Film-forming solution			
Olej rzepakowy Rapeseed oil (%)	Skrobia pszenna natywna Native wheat starch (g)	Glicerol Glycerol(g)	Masa roztworu końcowego Total mass (g)
0	5	2.5	100
1	5	2.5	100
2	5	2.5	100
3	5	2.5	100

Tabela 5.2. Parametry barwy filmów skrobiowych z dodatkiem oleju rzepakowego.
Table 5.2. Colour parameters of starch films with rapeseed oil.

Udział oleju rzepakowego w roztworze powłokotwórczym Participation in rapeseed oil in film forming solution (%)	*L*	*a*	*b*
0	95.62 ± 0.40^{a}	0.14 ± 0.07^{a,b}	2.9 ± 0.16^{a}
1	94.29 ±0.46^{a}	0.07 ± 0.05^{b}	3.55 ± 0.30^{a}
2	94.49 ± 0.35^{a}	0.00 ± 0.07^{b}	3.41 ± 0.33^{a,b}
3	94.32 ± 0.22^{a}	(-0.08) ± 0.04^{b}	3.7 ± 0.15^{b}

Analizując parametry barwy *L*, *a* i *b* filmów skrobiowych z dodatkiem oleju rzepakowego zaobserwowano obniżenie parametrów *L* i *a* oraz wzrost parametru *b* wraz ze zwiększającym się udziałem (1-3%) oleju rzepakowego w roztworze powłokotwórczym (tabela 5.2). Bezwzględna różnica barwy wskazuje, w jakim stopniu dany materiał różni się barwą od standardu. Nasycenie barwy charakteryzuje intensywność barwy w porównaniu ze standardem, a indeks nasycenia mówi o nasyceniu barwy materiału bez udziału wzorca (Galus & Lenart, 2012). W celu interpretacji otrzymanych wyróżników barwy posłużono się kryterium, które zostało opracowane przez Międzynarodową Komisję Oświetleniową. Wartości bezwzględnej różnicy barwy z zakresu 0-2 nie są rozpoznawalne przez człowieka, z zakresu 2-3.5 rozpoznawalne są przez obserwatora niedoświadczonego, zaś przy wartościach wyższych od 3.5 obserwuje się wyraźne różnice w odchyleniu barwy (Anonim, 1999). Wartości bezwzględnej różnicy barwy (Δ*E*) analizowanych filmów były na zbliżonym poziomie z zakresu 1.25-1.54 niezauważalne przez człowieka (rysunek 5.1). Podobne zależności zaobserwowano dla nasycenia barwy (C_{colour}), której wartości były z zakresu 0.28-0.83. Wartości indeksu nasycenia filmów tłuszczowo-skrobiowych były wyższe (3.41-3.70) w porównaniu z filmem kontrolnym (2.90). Yang i Paulson (2000) wykazali podobne zależności dla filmów z gumy gellan modyfikowanych dodatkiem związków lipidowych, jak również Monedero i in. (2009) dla filmów sojowych.



Rysunek 5.1. Bezwzględna różnica barwy (Δ*E*), nasycenie barwy (C_{colour}) i indeks nasycenia (*Si*) filmów skrobiowych z dodatkiem oleju rzepakowego.
Figure 5.1. Total colour difference (Δ*E*), colour chroma (C_{colour}) and saturation index (*Si*) of starch films with rapeseed oil.

Tabela 5.3. Grubość i nieprzezroczystość powłok skrobiowych z dodatkiem oleju rzepakowego.

Table 5.3. Thickness and opacity of starch films with rapeseed oil.

Olej rzepakowy Rapeseed oil (%)	Grubość Thickness (μm)	Nieprzezroczystość Opacity (A·mm^{-1})
0	86,4 ± 3,12^{a}	6,42 ± 1,33^{a}
1	50,2 ± 6,91^{b}	1,30 ± 0,09^{c}
2	46,1 ± 5,12^{b}	1,50 ± 0,24^{b,c}
3	43,4 ± 6,82^{b}	2,20 ± 0,19^{b}

Nieprzezroczystość powłok jadalnych jest ważnym czynnikiem decydującym o zastosowaniu materiałów jako opakowania do żywności. Niska bądź wysoka przezroczystość uzależniona jest od wymagań stawianych opakowaniom (Galus & Lenart, 2012). W tabeli 5.3 przedstawiono wartości nieprzezroczystości analizowanych filmów przy długości fali 600 nm. Zaobserwowano znaczne obniżenie nieprzezroczystości z 6.42 dla filmu kontrolnego do 1.30 ± 2.20 A·mm^{-1} dla filmów z najniższym dodatkiem oleju rzepakowego. Wraz ze zwiększającą się ilością oleju rzepakowego wartości nieprzezroczystości rosły z 1.30 do 2.20 A·mm^{-1}. Podobną metodę analizy nieprzezroczystości filmów jadalnych zaproponowali López, García i Zaritzky (2008) oraz Fu i in. (2011). Chen, Kuo i Lai (2009) badając filmy skrobiowe z tapioki z dodatkiem emulsji tłuszczowej i środków powierzchniowo-czynnych wykazali, że największą przezroczystością charakteryzują się filmy bez żadnego dodatku, a surfaktanty i tłuszcz zwiększają wartość nieprzezroczystości otrzymywanych filmów. Ponadto filmy z dodatkiem emulsji i środków powierzchniowo-czynnych były bardziej transparentne od tych, które zostały utworzone jedynie z dodatkiem emulsji tłuszczowej. Natomiast Wu i in. (2009) oraz Fu i in. (2011) zauważyli, że nieprzezroczystość filmów skrobiowych zależy od ich grubości. Zaobserwowano również, że roztwory powłokotwórcze zhomogenizowane są bardziej transparentne od tych, nie poddanych homogenizacji wysokociśnieniowej. Jiménez i in. (2012) badali właściwości optyczne skrobi kukurydzianej z dodatkiem kwasów tłuszczowych. Stwierdzono, że dodatek tłuszczu zwiększa nieprzezroczystość filmów ze skrobi. Kwasy tłuszczowe tj. palmitynowy, stearynowy lub oleinowy, dodane do hydrokoloidowej struktury filmu powodowały zmniejszenie współczynnika przezroczystości ze względu na różnice w załamaniu światła w fazie ciągłej i rozproszonej.

5.1.4. Wnioski

- Dodatek oleju rzepakowego do filmu skrobiowego powoduje obniżenie jasności powierzchni (parametr L) i parametru barwy a oraz podwyższenie parametru barwy b. Natomiast zmiana stężenia oleju w zakresie 1 - 3% w roztworze powłokotwórczym nie powoduje istotnych zmian tych parametrów.
- Dodatek oleju rzepakowego do filmu skrobiowego jak i zmiana jego stężenia w zakresie od 1 do 3% w roztworze powłokotwórczym, nie powoduje zmian bezwzględnej różnicy (□E) i nasycenia (C_{colour}) barwy.
- Dodatek oleju rzepakowego do filmu skrobiowego powoduje obniżenie stopnia nasycenia barwy (Si). Natomiast zmiana stężenia oleju w zakresie 1 - 3% w roztworze powłokotwórczym nie powoduje zmian.
- Filmy skrobiowe z dodatkiem oleju rzepakowego wykazują 3 - 4 krotnie niższą nieprzezroczystość w stosunku do filmów bez tłuszczu. Zwiększenie stężenia oleju w zakresie 1 - 3% w roztworze powłokotwórczym powoduje wzrost nieprzezroczystości.

5.1.5. Summary (short translation of the polish publication)

Increasing interest of edible coatings and films caused developing modern techniques for biodegradable material producing and using them for more and more applications. Biopolymers have many functional advantages as the modification of the metabolism of vegetable tissues, leading to changes in their respiration, are expressions of antimicrobial substances, antioxidants, vitamins. In addition films and coatings protect products against the valuable components loss, such as the above antioxidants, micro- and macronutrients, flavorings. Coatings and films contribute to reducing the weight of packaging made from plastics such as terephthalate and its copolymers, polyethylene of high and low density and polypropylene. Food marketing tries to meet of many challenges, such as the production of high quality nutritional products, durable and affordable. Edible films help to meet these expectations.

Cellulose, gums, starches and proteins are the basic materials used for the production of edible films and coatings. Films made from starch are tasteless and odorless, nontoxic, biodegradable, and colourless, or in the case of rice starch - milky white (Flores et al., 2007; Guilbert, Gontard & Gorris, 1996; Souza et al., 2010). Wheat is the second largest (7%) source of starch in the world. Only in Europe is produced 2.8 million tons annually. Encourage for that favorable growing conditions. Seeds of wheat starch, both native and modified, are of medium size (10 - 25 microns), oval (Molenda et al., 2006). In about 53% starch is used for the production of food in the form of stabilizers, thickeners and gelling agents dietetic substances, replace fat and substances that modify the structure in the process, and are also edible coatings and films (Lin et al., 2011).

Due to the wide availability, low price, great potential in the development of physico-chemical and biological properties starch films is appreciated by food manufacturers and consumers. Films can be easily coloured, printed, it is possible to add to their structure nutrients and other film-forming components i.e. proteins and fats. Especially these, the last one are particularly desirable in order to compensate the main drawbacks of starch polymers it is high water vapour permeability (Ghosh, Ziegler & Anantheswaran, 2005).

Lipids are substances with the strong hydrophobic character. They constitute a barrier against moisture vs. product, product vs. environment and environment and product exchange. Fats can be added to the structure of the film-forming suspensions and coatings, or applied as a separate layer.

One of the inherent indicators which determine the customer choice is colour. Colour reflects the freshness of the food product and the suitability for consumption. The colour of food is often measured by colorimeter in the CIE *Lab* system. About the colours analysis of the food products determine parameters as brightness *L*, *a* transition of colour from green to red, *b* transition blue to yellow (Flores et al., 2007; Ghanbarzadeh, Almasi & Entezami, 2010; Viña et al. , 2007; Zavareze et al., 2012). They characterized colour parameters i.e. the index of saturation, or chroma product without the intervention standard (saturation of colour - the colour intensity compares with the standard) and the total difference in colour - the difference between the colour of the material and colour standard. The difference is the size of the colour resulting from three different components: *L, a* and *b* (Reis et al., 2008).

Also the opacity is one of the differentiator of the optical properties of food stuff. Opacity describes the ability of edible films to absorption and reflection of the light and decides on their use as packaging materials. The opacity is also dependent on the thickness (Flores et al., 2007; Reis et al., 2008).

The aim of the study was to analyze the colour and impermeability of films made from the native wheat starch with the rapeseed oil addition.

The impact and the amount of rapeseed oil on optical properties of starch films were measured. After drying an amount of fat on the surface was observed. With increasing the oil content (1-3%), higher amount of oil was visible on the surface. A similar phenomenon was presented in the work of Yang & Paulson (2000) on the modification of the proteins with fat emulsions. It was also observed that higher contents of rapeseed oil changed the film colour. All films were milky white, but the control films were dull and modified with the addition of oil gliter.

Analyzing the *L, a* and *b* parameters of starch films with the rapeseed oil addition was observed to decrease parameters *L, a* and *b* according increasing oil concentration in the solution (1-3%) (table 5.1). For the interpretation of obtained colour data method created by the International Commission on Illumination was used. The values of total difference in colour from the range 0-2 are not recognizable by humans, in the range 2-3.5 are recognizable by the inexperienced observers, while at values higher than 3.5 is a clear difference in colour deviation (Anonymous, 1999).

The total colour difference (ΔE) values of analyzed films were on the similar level to the range of 1.25-1.54 it means invisible for the human eye (figure 5.1). Similar results were observed for the chroma (C_{colour}) - the values were between 0.28-0.83. Saturated index values for starch films with oil addition were higher (3.41-3.70) compared to the value of control film (2.90). Yang and Paulson (2000) showed similar relationships for gellan gum films modified with the addition of lipid compounds, as well as Monedero et al. (2009) for soy films.

The opacity of edible coatings is an important factor determining the use of materials as food packaging. Low or high transparency depends on the requirements for packaging (Galus & Lenart, 2012). Table 5.3 shows the opacity of films analyzed at a wavelength of 600 nm. There was a significant reduction in opacity from 6.42 for the control samples to 1.30 ± 2.20 A mm^{-1} for films with the lowest addition of rapeseed oil. According to increasing amount of rapeseed oil opacity grew from 1.30 to 2.20 A mm^{-1}. A similar method of opacity analysis of edible films presented López, García and Zaritzky (2008) and Fu et al. (2011). Moreover, films containing the emulsion and the surfactant were more transparent than those formed with the only addition of the lipid emulsion. In contrast, Wu et al. (2009) and Fu et al. (2011) observed that the opacity of starch films depends on their thickness. It was also observed that the homogenized film forming solutions are more transparent than those not undergo to high pressure homogenization. Jiménez et al. (2012) investigated the optical properties of corn starch with the addition of fatty acids. The addition of fat increases the opacity of the starch films. The fatty acids i.e. palmitic, stearic or oleic acid added to the hydrocolloid film structure caused a reduction of the coefficient of transparency due to differences in refraction of light in the continuous phase and dispersed.

Rapeseed oil of the starch films reduces the brightness (L) and the a value of colour parameter but increases b value. Anyway, changing the oil concentration in the range of 1 - 3% in a solution film forming does not cause significant changes in these parameters. Rapeseed oil addition to starch films as well as the change of the concentration range from 1 to 3% solution does not change the total difference (ΔE) and chroma (C_{colour}) colour. Rapeseed oil addition causes decreasing of the colour saturation index (Si). Thus, changing of the oil concentration in the range of 1 - 3% in the solution does not cause any changes. Starch films with rapeseed oil addition have 3 - 4 times lower opacity than films without fat. Increasing of the oil concentration in the range of 1 - 3% in the film coating solution increases the opacity.

5.2. Effect of oil lamination between plasticized starch layers on film properties (Basiak E., Debeaufort F., Lenart A. (2015). Effect of oil lamination and starch content on structural and functional properties of starch-based films. Food Chemistry, 195, 56-63.)

5.2.1. Abstract and introduction

To reduce the hygroscopic character of biodegradable starch-based films, rapeseed oil was incorporated by lamination (starch-oil-starch 3-layers technique). The lipid lamination followed by starch solution casting step induced an emulsion type structure of dried films. Composite films are more opalescent and glossier than fatty free starch films. For all the films, structure is heterogeneous in the cross-section only. Adding fat induced a twice decrease of the tensile strength. Thermal gravimetry analysis did not show differences between films with and without oil. Lipid reduced the moisture absorption particularly at higher RH as well as the surface swelling index, when water droplet contact occurred. Addition of lipids always decreases the contact angle for all liquid tested, except for water. Surface affinity of films for liquids less polar that water increased with rapeseed oil addition. The addition of rapeseed oil significantly reduces water vapour and oxygen permeability.

Most of papers dealing with multilayer films, since end of 80's, considered solid fats such as hydrogenated oils or waxes. However, solid fat layers are often very brittle, did not adhere to the polysaccharide or protein support layer and moreover their sensory characteristics are deleterious for the food onto which they will be applied as coatings. So liquid oil was selected to prevent these drawbacks even if we supposed the final structure probably differs to a real 3-layers system.

The first films and coatings appeared in China probably in twelfth or thirteenth centuries. Fresh food products were commonly dipped in hot waxes or soy milk skin (Yuba). Nowadays edible films and coatings are produced using a variety of methods and raw-materials: proteins, polysaccharides, lipids and even multi-components as composite structures. Despite of many advantages of each of constituents, carbohydrates and proteins have poor barrier properties. Exceptions are lipids, waxes and resins. Very often lipids are added to film-forming solutions or to films (Baldwin, Iagenmaier & Bai, 2012). Coated food products such as meat, cheeses, sweets, processed foods, the most common fresh fruits and vegetables have strong hydrophilic properties. To reduce moisture loss, control desiccation, and impart hydrophilicity, or to prevent loss of flavour, fatty acids and alcohols, acetylated glycerides, waxes, lacs and cocoa-based compounds and their derivatives, oils have been envisaged.

Fats in films might exist as a monolayer (pure fat films) or they can be added to other hydrocolloids and they can create structures in the form of emulsion, dispersion or bi/multilayers (Debeaufort, Quezada-Gallo & Voilley, 1998). Literature reviews on use of lipids, waxes (such as beeswax, carnauba, candelilla) or resins show that they are often used to coat fresh fruits and vegetables such as oranges, lemons, apples, bananas, plums, mushrooms (Bai, Baldwin & Hagenmaier, 2002; Hagenmaier & Shaw, 1991; Embuscado & Huber, 2009; Jongen, 2002; Rojas-Graü, Soliva-Fortuny & Martín-Belloso, 2009; Jiménez Fabra, Talens & Chiralt, 2012; Ramírez Gallegos, Ihl & Bifani, 2012; Jiménez Fabra, Talens & Chiralt,, 2013a,b,c; Linke & Gayer, 2013; Schmidt, Porto & Laurindo, 2013). Addition of lipids is one of two common ways (the second is cross-linking agents) to reduce the water vapour permeability. Water affinity often controls the water vapour permeability and is related to the hydrophilicity of the film (Wang Zhou, Wang, Zhang, Sun & Ma, 2014). Fatty coatings not only retard moisture loss, but also improve appearance by imparting shine to the surface, provide a carrier for fungicides or growth regulators, create a barrier for gas (oxygen, carbon dioxide, ethylene, …) exchange (Bai, Hagenmaier & Baldwin, 2003; Guilbert, 1986; Linke, Herppich & Geyer, 2010; Linke & Gayer, 2013). Indeed, Hall & Sorenson (2006) observed that lipid coatings preserve internal quality of fresh citrus, serve to prevent decay, improve appearance, control weight and carry postharvest fungicides. Rattanapnone, Plotto & Baldwin,. (2007) displayed that the colours of lychee was preserved on longer time when fruits were coated by films based on chitosan. Chiumarelli, Pereira, Ferrari, Sarantópoulos & Hubinger (2010), Rhim & Shellhammer (2005), Sothornvit & Krochta (2001) applied edible coatings and films based on starch and sunflower oil or stearic acidon fresh-cut products. Chiumarelli & Hubinger (2012) reported that cassava starch films with carnauba wax and stearic acid strongly influenced barrier and mechanical properties and then respiration and preservation of fresh cut apples, favouring their shelf-life..

The objective of this work is to study the functional properties of composite edible films obtained by laminations of wheat starch solution, rapeseed oil and again wheat starch solution as a 3-layer process. Two concentrations of starch in the film-forming solution were studied, and monolayer starch films were used as controls. Then, structural and physical-chemical characterization of the films has been conducted.

5.2.2. Structural properties of films

Several techniques have been used for characterizing the film structure such as the colour, the environmental scanning microscopy, the mechanical properties, the calorimetry analyses.

The film macroscopic appearance was assessed from colour measurements. The hue is one of the most important parameters, crucial for the consumer's choice. Table 5.4 presents the colour attributes as luminance (*L*), hue parameters (*a, b*) and colour difference obtained for pure starch and starch-oil films. Visually films with and without oil were from transparent to opalescent. One side of each of films was shiny (face in contact to Petri dish when cast), the other was dull. The gloss of films was greatly affected by the film composition. Indeed, films containing rapeseed oil were glossier than those of lipid-free films. This could indicate that oil migrated toward the surface during film preparation. However, gloss values of films containing rapeseed oil does not vary significantly with respect to films without oil. The lightness values of starch films (*L*) were insignificantly higher than films with rapeseed oil addition. Nevertheless all films were characterized by high brightness (94.69 – 96.20). Whatever the starch content or the presence of oil, there is no change in colour parameters. Some authors observed changes in colour parameter when oil was added in hydrocolloid-based films. Rao, Kanatt, Chawla and Scharma (2010) proved that blend films have higher ΔE than monolayer materials. Martins, Cerqueira and Vicente (2010) reported that the incorporation of α-tocopherol to chitosan based films influenced to decrease lightness (from 95.4 to 92.3) and increase greenness, *b* values were constant. But this behaviour is explained by the colour parameter of ingredients that are significantly different, whereas rapeseed oil and starch have very similar values.

Table 5.4 Physico-chemical characteristics of films according their composition (3S : 3% starch, 5S : 5% starch, 3L, 5L, 3and 5% starch respectively prepared as laminated with oil) : luminance (*L*), colour parameters (*a, b*), coulour difference (Δ*E*), thickness, tensile strengh (TS), Young modulus (YM), elongation (E), weight loss and residue after thermogravimetry, surface tension of liquids (γ_L), surface tension (γ_S)and the dispersive (γ_S^D) and polar (γ_S^P) components of film surfaces, adhesion (W_A), cohesion (W_C) and spreading coefficient (W_S) for water at 20°C, swelling index, water content, solubility in water, water vapour and oxygen permeabilities at 25°C

Film characteristics			Film composition			
			3S	5S	3L	5L
Colour parameter	*L*		96.20 ± 0.32 ^{b}	95.48 ± 0.39 ^{a,b}	94.73 ± 0.74^{a}	94.69 ± 0.31^{a}
	a		-0.32 ± 0.10 ^{b}	-0.24 ± 0.06 ^{a}	-0.39 ± 0.12 ^{b}	-0.38 ± 0.13 ^{b}
	b		3.72 ± 0.62 ^{a}	3.19 ± 0.29 ^{a}	3.20 ± 0.26 ^{a}	2.99 ± 0.21 ^{a}
	Δ*E*		1.51 ± 0.60 ^{a}	1.46 ± 0.43 ^{a}	2.13 ± 0.74 ^{a}	2.07 ± 0.31 ^{a}
Thickness (μm)			35.4 ± 5.47^{b}	80.8 ± 12.59^{c}	22.1 ± 5.40^{a}	27.7 ± 5.69^{a,b}
Mechanical properties	TS (MPa)		2.03 ± 0.79^{b}	2.10 ± 0.76^{b}	1.04 ± 0.45^{a}	0.92 ± 0.33^{a}
	YM (MPa)		0.08 ± 0.06^{a}	0.10 ± 0.09^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}
	E (%)		13.18 ± 4.49^{a}	14.16 ± 5.40^{a}	16.44 ± 6.72^{a}	18.29 ± 5.79^{a}
Thermal stability	Weight loss (%)	30-120°C	4.9 ^{a}	7.7 ^{b}	7.0 ^{b}	6.9 ^{b}
		120-260°C	21.9 ^{a}	23.7 ^{b}	47.2 ^{c}	25.5 ^{b}
		260-450°C	61.0 ^{a}	58.1 ^{a}	35.9 ^{b}	56.2 ^{a}
	Residue at 750°C (%)		8.1 ^{a}	4.5 ^{a}	6.4 ^{a}	7.6 ^{a}
Surface properties	Contact angles (°)	Polyethylene glycol (γ_L=43 mN/m)	42 ^{b}	41 ^{b}	34 ^{a}	31 ^{a}
		Ethylene glycol (γ_L=47.7 mN/m)	64 ^{c}	60 ^{c}	47 ^{a}	55 ^{b}
		Diiodomethane (γ_L=50.8 mN/m)	46 ^{b}	48 ^{b}	38 ^{a}	38 ^{a}
		Glycerol (γ_L=64 mN/m)	68 ^{a}	71 ^{a}	65 ^{a}	70 ^{a}

		Water (γ_L=72.8 mN/m)	46^{a}	43^{a}	67^{b}	77^{c}
	Surface tension (mN/m)	γ_S^D	36.5^{a}	35.4^{a}	40.6^{b}	40.6^{b}
		γ_S^P	18.7^{a}	24.9^{b}	23.1^{b}	15.5^{a}
		γ_S total	55.2^{a}	60.3^{b}	63.7^{b}	56.1^{a}
	W_A (mJ/m^2)		118.11^{a}	126.80^{b}	128.14^{b}	115.12^{a}
	W_C (mJ/m^2)		110.30^{a}	120.56^{b}	127.40^{b}	112.12^{a}
	W_S (mJ/m^2)		-7.81^{c}	-6.24^{c}	-0.74^{a}	-3.00^{b}
Transport and solubility properties	Swelling index (%)		44.76 ± 1.52^{c}	39.20 ± 1.43^{b}	$36.85 \pm 2.31^{a,b}$	34.91 ± 1.39^{a}
	Water content (g$_{water}$ g$^{-1}_{dm}$)		2.01 ± 0.09^{a}	3.24 ± 0.50^{c}	2.70 ± 0.10^{b}	$2.69 \pm 0.21^{b,c}$
	Solubility in water (%)		$14.49 \pm 4.84^{a,b}$	19.67 ± 0.17^{b}	7.83 ± 2.41^{a}	10.70 ± 0.72^{a}
	Water vapour permeability (10^{-10} gm^{-1}s^{-1}Pa^{-1})	33-0% RH	$0.56 \pm 0.06^{a,b}$	0.92 ± 0.06^{b}	0.28 ± 0.15^{a}	0.57 ± 0.46^{a}
		75-30% RH	4.55 ± 0.56^{d}	8.77 ± 0.59^{e}	0.25 ± 0.01^{a}	3.55 ± 2.80^{d}
		100-30% RH	3.98 ± 0.84^{d}	8.01 ± 0.15^{e}	0.92 ± 0.01^{c}	3.40 ± 0.31^{d}
	Oxygen permeability (10^{-14} cm^{3}m^{-1}s^{-1}Pa^{-1})	33% RH	7.55 ± 0.72^{d}	7.23 ± 1.00^{d}	0.14 ± 0.02^{a}	0.96 ± 0.02^{b}
		75% RH	6.63 ± 1.39^{d}	7.41 ± 1.39^{d}	1.00 ± 0.03^{b}	1.12 ± 0.05^{c}

The microstructure of the starch films with and without lipid was qualitatively observed using environmental scanning electron microscopy (SEM). Figure 5.2 gives the ESEM micrographs obtained for the cross-section and surface of the wheat starch-based films containing or not rapeseed oil. The surface of film without oil looks smooth, homogeneous without any defects up to size of 0.5 µm. However, their cross section pictures of starch films show the films are heterogeneous, with a seemingly alveolar organisation, but seem dens as no holes nor open pores are visible neither at the surface nor in cross section views. No starch granule remained which means a total gelatinization occurred during film making process. Garcia, Martino and Zaritzky (1999) observed quite similar structure for corn starch. However, Phan The, Debeaufort, Luu and Voilley (2008) observed much more homogeneous structure for cassava starch films but those films contained only 15% glycerol compared to ours. The starch concentration of the film-forming suspension did not affect the microstructure as same amount of dry matter per unit surface was poured in Petri dish when films were cast. Dealing with films containing rapeseed oil figure 5.2 displays large droplet of oil on film surface that indicate that the oil migrated toward the surface when the 3rd layer of starch solution was cast. Moreover, the cross section of starch-oil film does not present a 3-layers structure as expected. Indeed, the melting point of rapeseed oil was not high enough to provide that the layer solidified prior to the third starch layer to be dried. From cross section, oil seems to be spread close to the surface as large and very flat droplets. Phan The, Debeaufort, Luu and Voilley (2008) also displayed destructuration of the oil/starch emulsion during film casting and drying processes. On the contrary, Jiménez, Fabra, Talens and Chiralt (2013a) observed on SEM micrographs, that in corn starch films containing oleic acid, lipid droplets were suspended in the amylose and amylopectin network, whereas this was not observed for palmitic and stearic acids. This shows that lipid molecules are well integrated in the polymer structure and their aggregates were very small but dispersion and final structure of films depends on the nature of lipids. Hydrophobicity of oil as well as the difference of density between oil and film-forming aqueous suspension contribute to instability and then to film having heterogeneous structure with significant phase separation which tend to emulsion (Phan The, Debeaufort, Peroval, Despré, Courthaudon and Voilley, 2002).

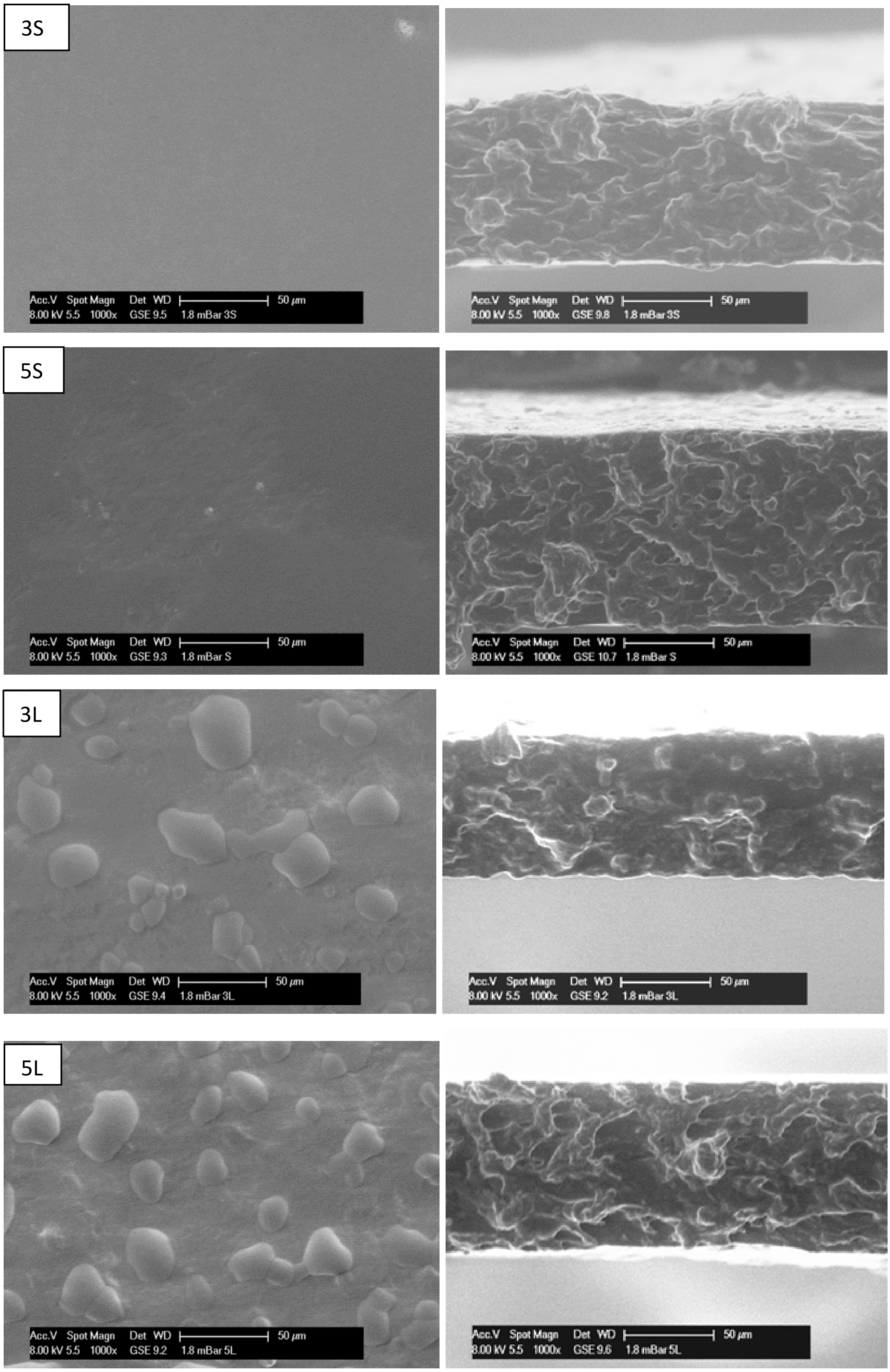
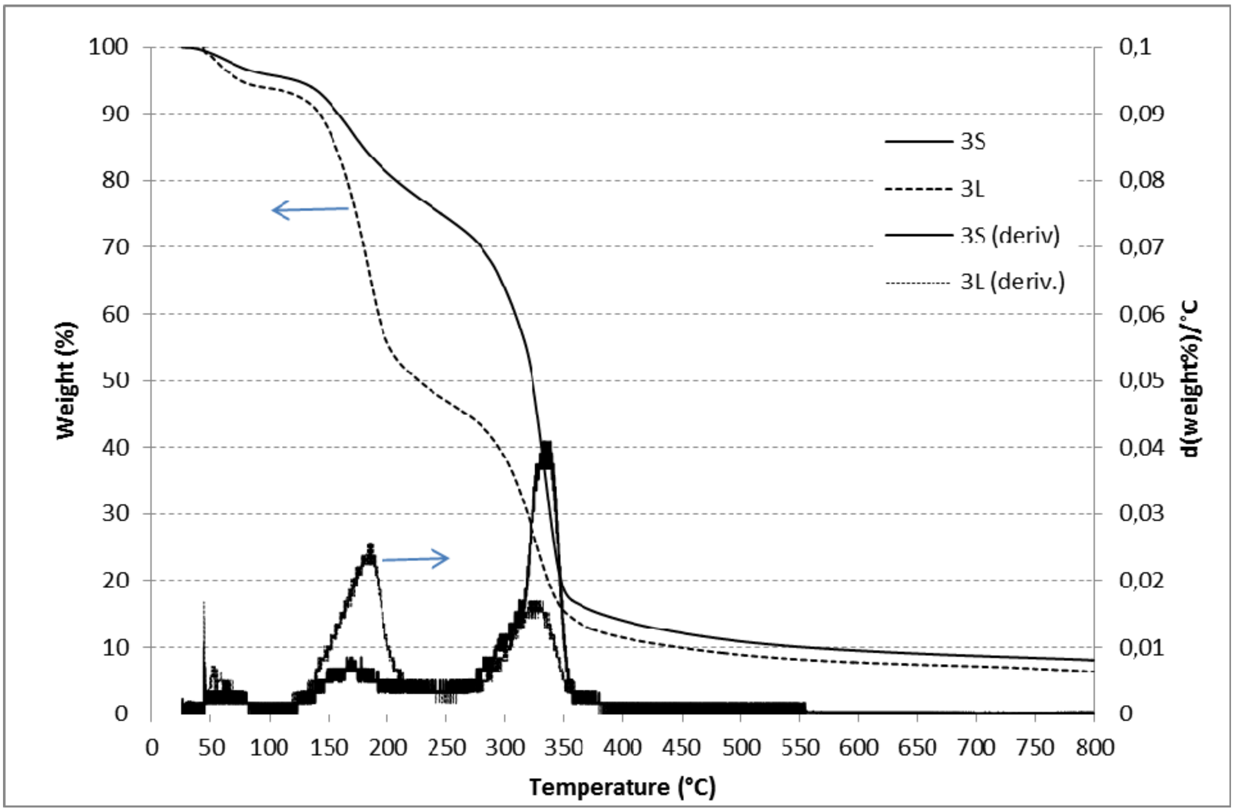


Figure 5.2 ESEM micrographs of surface exposed to air during drying (left) and cross section (right) of starch-based films (3S: 3% starch, 5S : 5% starch, 3L, 5L, 3and 5% starch respectively prepared as laminated with oil; magnification x1000).

Table 5.4 gives information about the thickness, tensile strength, Young modulus and elongation of film measured at 25°C and 50% RH. The lowest thickness values are observed for films with fat (3g) and with lower starch concentration (3S). The more starch is in film forming suspension the thicker films are. It is known that oils did not act as a plasticizer which often involves swelling, but as a lubricant which limits the swelling. Moreover, oils delay the water evaporation during drying which favour the organization of a more organized network and then favour a densification of the structure. In the case of 5% starch, the increase in thickness is more related to the starch content and higher viscosity which disturb the network organization and then the less structured is the network, the less density is. But at the end, the increase of thickness is mainly related to the amount of dry matter per unit area poured in Petri dish. However, García, Martino and Zaritzky (1999) also displayed that thickness increases with the amylose and the glycerol content in solution whereas the same dry matter per surface was cast.

The tensile strength of starch films without oil was the highest. Starch concentration in suspension did not influence on the tensile strength as this value is normalized by the thickness. This means nor the starch content, nor the thickness affected significantly the network. Adding fat induced a twice decrease of the tensile strength as observed by several authors for both polysaccharide and protein film containing oils as emulsions (Morillon, Debeaufort, Capelle and Voilley, 2002). While there is no significant differences between values of Young modulus whatever the film, the highest value are for films without fat and follows the trend observed for the tensile strength. On the contrary, even if not significant, elongation is slightly increased with oil addition as also observed by various authors for oils (Morillon, Debeaufort, Capelle and Voilley, 2002; Phan The, Debeaufort, Voilley and Luu, 2009). The opposite behaviour is usually observed for solid fat incorporation which induces strong decrease of both TS and E of biopolymer films having emulsion type structures (Janjarasskul, Rauch, McCarthy and Krochta, 2014). The behaviour of rapeseed oil-starch film as emulsion could be attributed to a lubrication or plasticization of the starch network by the rapeseed oil components. However, it is probable that only a lubrication process occurred as the E and then plasticity is not significantly increased as observed when plasticization occurs.


Weight (%)
d(weight%)/°C
Temperature (°C)
3S
3L
3S (deriv)
3L (deriv.)
0
10
20
30
40
50
60
70
80
90
100
0
0,01
0,02
0,03
0,04
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0,06
0,07
0,08
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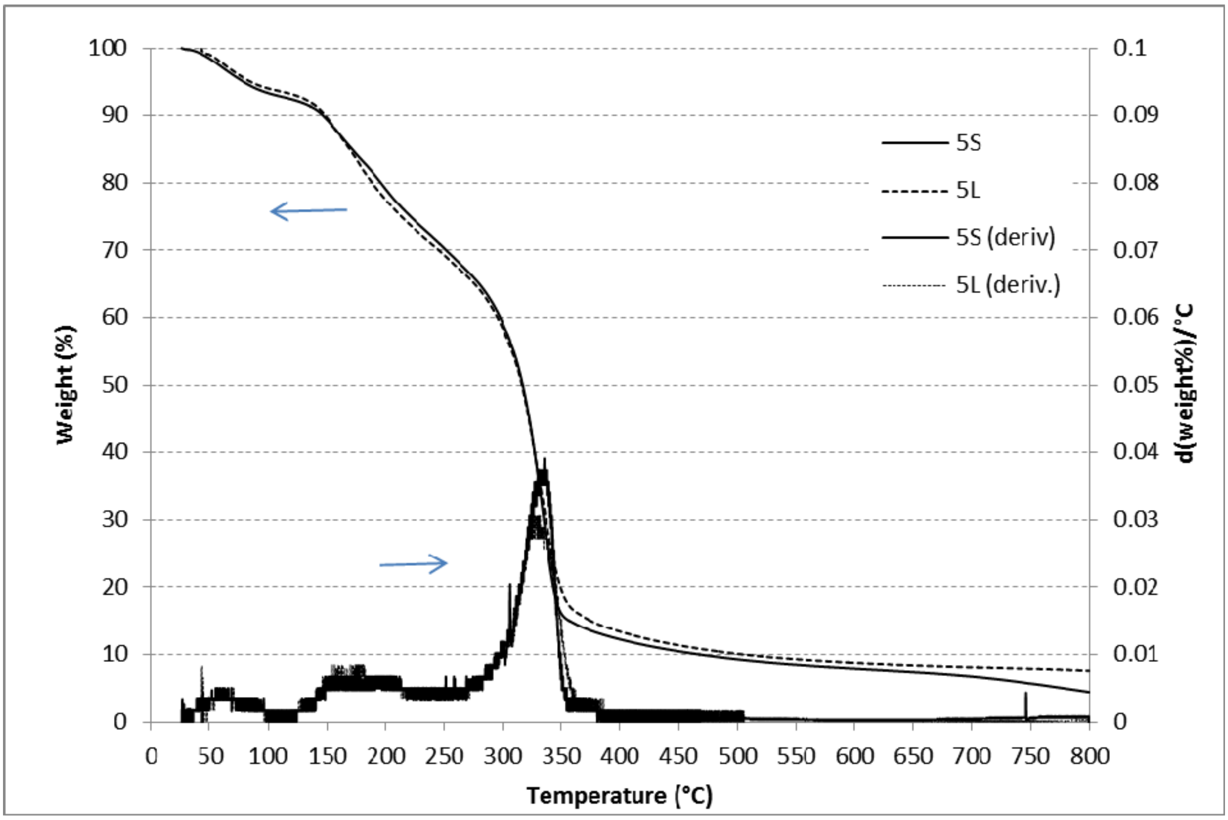

Weight (%)
d(weight%)/°C
Temperature (°C)
5S
5L
5S (deriv)
5L (deriv.)
0
10
20
30
40
50
60
70
80
90
100
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800

Figure 5.3 Thermal stability (weight loss versus temperature) of starch films and starch-oil films determined TGA at a rate of 10°C/min and under nitrogen to prevent oxidation (3S and 3L film on left, and 5S and 5L on right).

Thermogravimetry analysis on starch films and starch-rapeseed oil films are given in table 5.4 and figure 5.3. Three zones of thermal change of film properties (weight loss). The first one from ambient to about 120°C corresponds to the loss of water contained in the films. Without lipid, films loss about 5-8% which can be related to the film water content as observed from the sorption isotherm (figure 5.4). The second zone, between 120 and 260°C is related to evaporation of glycerol and of oil degradation for films containing oil. Indeed, the losses are greater in that range when oil was added for the 3L films. Lower starch content seems to decrease the thermal stability of film, containing or not oil. Then, it could be supposed that for 5% starch content, the higher concentration of starch in the film filming suspension induced an increase in the viscosity of the suspension and then of the thickness. The latter could explain a more organised network which tends to protect the films against thermal degradation. The last part of the curve (260-450°C) is related to starch degradation. These data are in agreement with Piyada, Waranyou and Thawien (2013). At 750°C, the residue obtained is not dependent of starch concentration in suspension, nor to the oil presence.

5.2.3. Film behaviours toward moisture and liquid water

The moisture sorption isotherm (figure 5.4) was used to describe the relationship between the moisture content and water activity. Except for the 5L (5% starch containing rapeseed oil) which the behaviour is unexpected, all films have a typical sigmoidal shape of starch based materials highly plasticized by glycerol (Mali, Grossmann, Garcia, Martino and Zaritzky, 2002). The higher the starch content, the higher the percent moisture sorption was observed, resulting in the enlargement of the hygroscopicity that highly increased for RH above 80%. Addition of lipid reduced the moisture absorption at higher RH as expected whereas is seems to slightly increases for lower RH values. The presence of rapeseed oil in the starch matrix did not significantly modify the sorption behavior despite their apolar character up to water activity 0.8. Comparing to lipid-free matrices, films with fat addition have lower water content. Lipids reduce the number of active sides where water molecules can interact over the film surface as expected and displayed by Slavutsky and Bertuzzi, (2015). However, in 3-80% of relative humidity, the presence of fatty acids enhanced the water sorption capacity of the starch matrix as displayed by Jimenez et al. (2013a), but no clear explanation were provided.

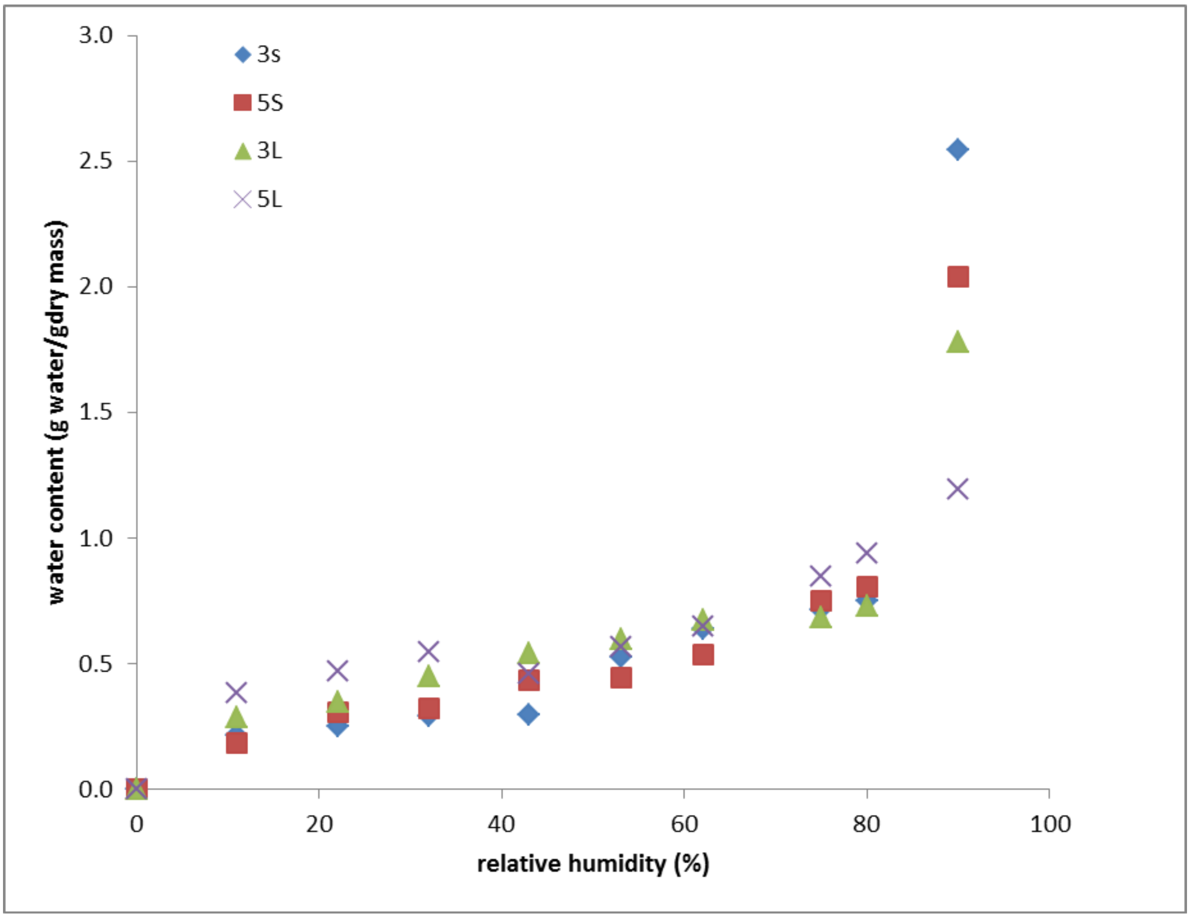


Figure 5.4 Moisture isotherm sorption of starch containing either 3 or 5% starch in solution (3 or 5) and from monolayer (S) or laminated with oil (L) at 25°C.

From table 5.4, it is noteworthy that the swelling index decreases according the starch concentration increase and the addition of rapeseed oil. This is coherent with the sorption isotherm as swelling index is measured after immersion and could then be related to saturation, i.e. 100% RH. Oil addition, due to hydrophobicity may protect the starch network against moisture absorption and then swelling. Moreover, from microscopy observation, oil is located at the surface which was probably more unfavourable for water penetration. This is confirmed by the solubility that is twice decreasing when oil was added. Of course rapeseed oil is not water soluble, so the decrease of the solubility is due to the oil fraction. Our results cannot display that interactions between fatty acids and amylose/amylopectin (if it is) is responsible of solubility reduction of starch complexes.

5.2.4. Surface properties of films

All the data related to surface properties, contact angles of various liquids, surface tension (γ_S) and its components (dispersive γ_S^D and polar γ_S^P), works of adhesion (W_A), cohesion (W_C) and spreading (W_S) are given in table 5.4 and figure 5.5. The contact angles given in the table 5.4 correspond to the value obtained at zero time, i.e. initial contact angle at first droplet contact. The contact angle represents equilibrium between surface tension of both air, liquid and film surface. The higher the contact angle is, the less the affinity for the surface. From table 5.4., it could be state that addition of lipids always decreases the contact angle for all liquids tested, except for water. For five other liquids: cyclopenthanol, methyl benzoate, n-octane, tetradecane, 1-bromonaphtalane the contact angle value is 0^o. Indeed, for these, just after the contact with the film surface, the drop of liquid undergoes spreading instantaneously (less than 0.5 sec). This indicates that surface affinity of films for liquids less polar that water increased when rapeseed oil was added. Behaviour of water droplets on the surface is clearly changed when films contain oil. However, it is noteworthy that the concentration of starch did not modify the initials contact angles. Indeed, contact angle and surface tension is more related to chemical composition of the surface that of the surface structure when film surface remained dense. Though initial contact angle is not affected by the starch concentration, it seems from figure 5.5 that the kinetic of wetting/absorption varies according both oil presence and starch concentration. The droplet of water spreads or was absorbed more rapidly that for 3L films. On the contrary, 3S spread faster that 5S. The shape of drop is more rounds for 3L, which let suppose that they have better barrier properties as absorption is delay. Kalin and Polajnar (2013) measured spreading parameters of five coatings with lubricating oils. Depending on the affinity of the oil to the surface droplets spread more or less and the shape of droplets were other. The more round is the drop on the surface the more adhesion forces dominate between molecules. Adding of polyol plasticizers reduces values of contact angle and can explain why values are much below to 90° (hydrophobic surface) as usually observed for most of fat products.

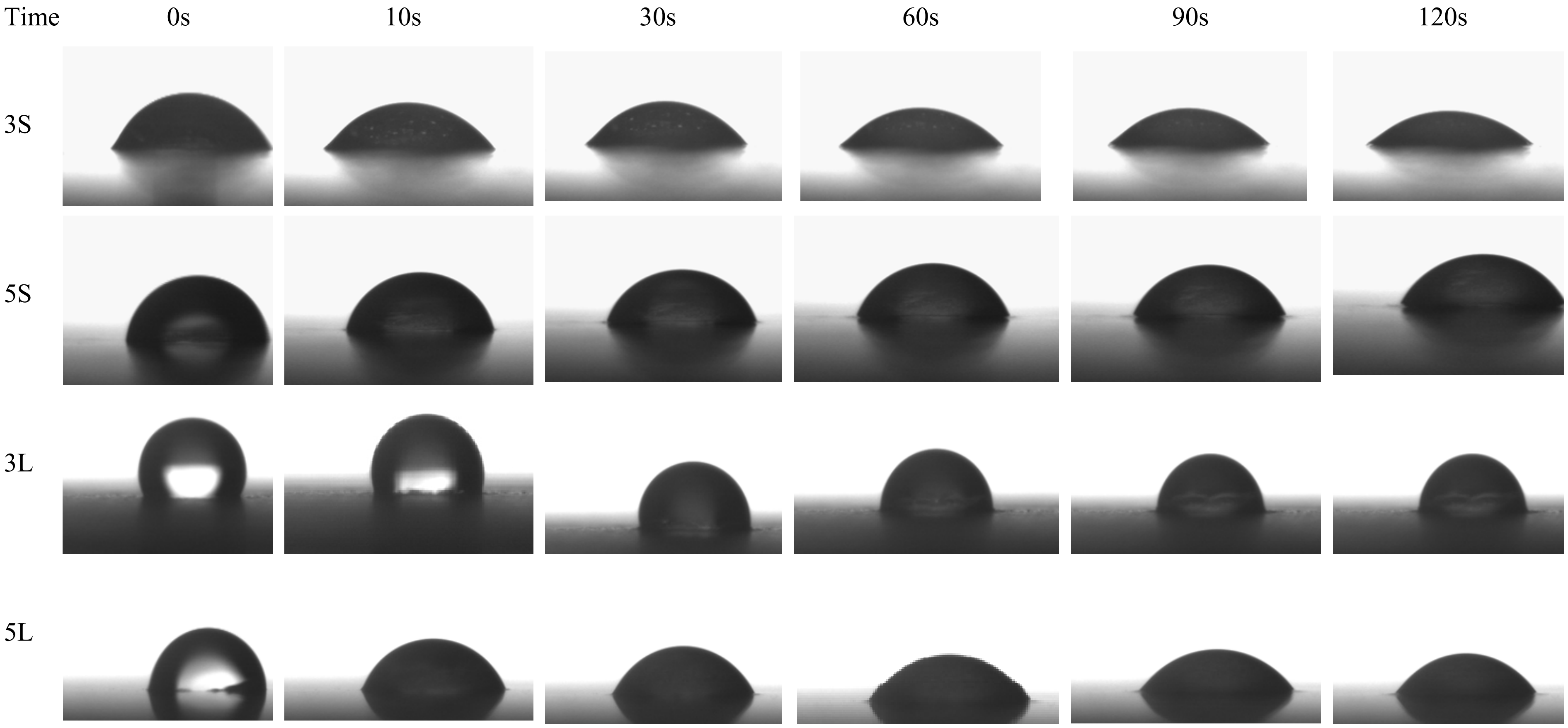


Figure 5.5 Behaviour of water droplets on film surfaces as a function of time (25ºC) – (3S: 3% starch, 5S: 5% starch, 3L, 5L, 3% and 5% starch respectively prepared as laminated with oil).

The surface energy of films was estimated from the contact angle measurements of ten liquids which allowed calculating the dispersive and polar surface energy components. The surface tension is relatively constant. There is neither effect of starch content nor of oil addition. However, it could be noted that the dispersive component of the surface tension seems related to the presence of oil, whereas the polar is not directly related to starch content nor to rapeseed oil. The critical surface tension gives a value of 36 mN/m for all films, value mainly due to the dispersive component of the surface tension contribution. No significant difference has been observed probably due to inaccuracy of the Zisman method. However, this value is quite far from the γ of water and indicates the surface of the starch films is not as polar as supposed.

Besides, wetting properties of films are characterised by spreading coefficient (W_S) which including work of adhesion (W_A) and work of cohesion (W_C). Adhesion forces connect phases of surface together while cohesion forces are a physical resistance and disconnect on parts (Chen, Feng, Gonzalez and Pugnaloni, 2008). Work of adhesion is not significantly higher than the work of cohesion. Both works are of the same order. Work of adhesion and work of cohesion are slightly higher for 5S and 3L than for 3S and 5L, and spreading coefficient does not show significant difference between 3S and 5S and 3L and 5L films. Moreover spreading coefficient is the highest for 3L laminates (-0.74 mN m^{-1}) and the lowest for 3S films (-7.81 mN m^{-1}).

5.2.5. Barrier properties of films

Water vapour permeability at 25°C for 3 different RH differentials as well as oxygen permeability at 33% and 75% are given in table 5.4. In all cases, the addition of rapeseed oil significantly reduces water vapour permeability. Oil addition reduces twice the WVP at 0-33% RH differential, up to 4 times for 100-30%. Drops of oil dispersed in starch network induce a kind or tortuosity. Then, because of their apolar character, they induce an increase in the preferential pathway length of water through starch network. Lipids addition as an emulsion structure, led to a significant decrease in the WVP. Our results are in agreement to those obtained by Jiménez, Fabra, Talens and Chiralt (2012), who observed that the addition of lipids to starch film matrix reduces WVP even by 36%.

The addition of lipids in starch matrix also reduces significantly the oxygen permeability by 7 times whatever the RH we tested. On the contrary, the incorporation of the oil in the starch-sodium caseinate films provoked a slighter increase in the oxygen permeability values due to the increase of oxygen solubility in the oil (Jiménez, Fabra, Talens and Chiralt, 2013). Same behavior was observed by García, Martino and Zaritzky (2000) for corn starch. This opposite behavior could be attributed to the lower content of plasticizer used. Indeed, oxygen solubility is very low in glycerol and probably reduces the oxygen permeability in our systems.

5.2.6. Conclusions

The incorporation of the lipid implied an increase in gloss, decrease transparency with respect to the lipid-free films. Also SEM photographs revealed films in cross-section view have heterogeneous character and confirm the 3 layers-structure get loss during film making when lipid added, driven an emulsion. The rapeseed oil is dispersed in the starch network. Fat addition into starch-glycerol films remarkably increased resistance to water vapour permeability. Addition of lipids also improved surface hydrophobicity, increasing the contact angle. However, increasing the starch content in solution slight modified the surface properties but not so much the barrier efficiency.

Chapter 6: Application of wraps/coatings for plums preservation

6.1. Relevance of interactions between starch-based coatings and plum fruit surfaces: a physical-chemical analysis (Basiak E., Geyer M., Linke M., Debeaufort F., Lenart A. (2016). Submitted to Journal of Food Engineering, July 2016).

6.1.1. Abstract and introduction

To extend the shelf life of the fruit, to improve appearance and to keep all nutrition properties of plums coatings based on edible wheat starch and on wheat starch-whey protein isolate (in ratio 80-20%) were used. Stand-alone films were also produced to assess some properties which helped to understand phenomena occurring at the surface of coated plums. The thickness of the starch and starch-whey protein films (80/20) was similar. Addition of rapeseed oil in starch decreased the film thickness from 80 to 28µm. When applied onto plums, both starch films and starch-whey protein (80/20) films had the same thickness. Coatings based on starch with oil and based on starch-whey protein isolate have better barrier properties than films without oil and then not coated plums. Oil addition increased twice the contact angle value and the dispersive component of the surface tension. Consequently, the works of adhesion and cohesion, the spreading coefficient, the absorption, the water content and the solubility in water decreased. New formed bonds or interactions established between starch, whey proteins, water, glycerol and oil have been displayed by FTIR analysis. The addition of oil to starch matrix or covering fruits by starch/starch whey-proteins strongly influence physical and chemical properties of coated layer. Colour parameters of coated fruits were improved. This work revealed interactions between coatings onto plum surface impacts the efficiency of coatings.

Biodegradable packages have significant impact on the environment which helps to reduce waste. Total biodegradability is the highest advantage of these materials. Films and coatings can replace the conventional packaging in many applications, for instance as disposable packaging materials, daily use bags, cups, plates, containers, egg boxes, lamination coatings etc. (Alves et al., 2007; Tharanathan, 2003; Tihminlioglu et al., 2010). Preservation, protection particularly from oxidative and microbial spoilage, extend shelf-life of many types foods and their raw materials, quite fast decomposition or easy separation from the surface makes biodegradable/edible films and coatings very attractive for both producers and customers (Garcia et al., 2010; Eum et al., 2009; Tharanatan, 2003).

Hydrocolloids and lipids are two types of biomolecules generally used for the preparation of biodegradable packages. Among all of them starch is one of the most promising materials. Starch has good film-forming and excellent gas barrier properties in dry conditions (Koch et al., 2010). To the other advantages belong: continuous matrix, structural complexity, functional diversity (Yan et al., 2012). Another type of hydrocolloids which is commonly used are proteins. In multilayer films, layers composed on the different types of polysaccharides, proteins or lipids they are able to improve the functional properties of biodegradable materials, allowing to reduce the drawback of each layer when they are used as monolayers (Ghanbarzadeh et al., 2011; Jiménez et al., 2013; Reinoso et al., 2008; Yan et al., 2012). The second coating material can occur also as food component. The first coatings used to reduce transpiration losses in lemon and in oranges contained from one kind of film-forming material, because the second material (wax) naturally occurs onto the fruit surface (Baldwin et al., 2012; Basiak et al., 2015a). In that case, additional single film-forming material can be one of laminate layers (Basiak et al., 2015a; Tharanathan, 2003). Compared to protein-based coatings, the barrier property of starch-based materials especially to water vapour is poorer. Starch exhibits a strong hydrophilic character whereas proteins have specific structures which confer a wider range of potential functional properties able to compensate starch drawbacks. Molecular weight of proteins is higher, so consequently they are generally insoluble in water and thereby suitable to form water vapour resistant coatings and films (Labuza and Altunakar, 2007; Tihminlioglu et al., 2010). However, it does not mean that bilayer or multicomponent films have always better properties than biopolymers alone. These properties depend on many factors such as uses, food water activity, fruit surface, barrier and mechanical properties etc. (Pérez-Gallardo et al., 2011).

Plums have natural wax onto the skin (Linke and Geyer, 2013). This thin layer of fat is permeable for water vapour and other gases. In consequence it is permeable for guard cells. The exchange of oxygen and carbon dioxide between the tissues of plums and the atmosphere takes place via pores, stomata, lenticels and cracks, so covering these structures also have influence on the further gas exchange. It explains why the storage time and shelf life are limited to few weeks (Banks et al., 1993). A simple technology to reduce physiological activities as mass loss, transpiration and respiration, is the application of edible coatings to fruit surface (Navarro-Tarazaga et al., 2011). The natural epicuticular wax layer on the plum surface (crystallized wax) also contributes to the fruit shelf life (Bickelmann, 2008). However, the film-forming solution coatings could influence this natural wax coating inducing changes in other properties such as colour, moisture properties etc. (Wisuthiphaet et al., 2014). Coatings influence the firmness, smoothness, brightness and darkness of blue colour and thus glossy, and resistance which convince the consumer to buy the plums in retails.

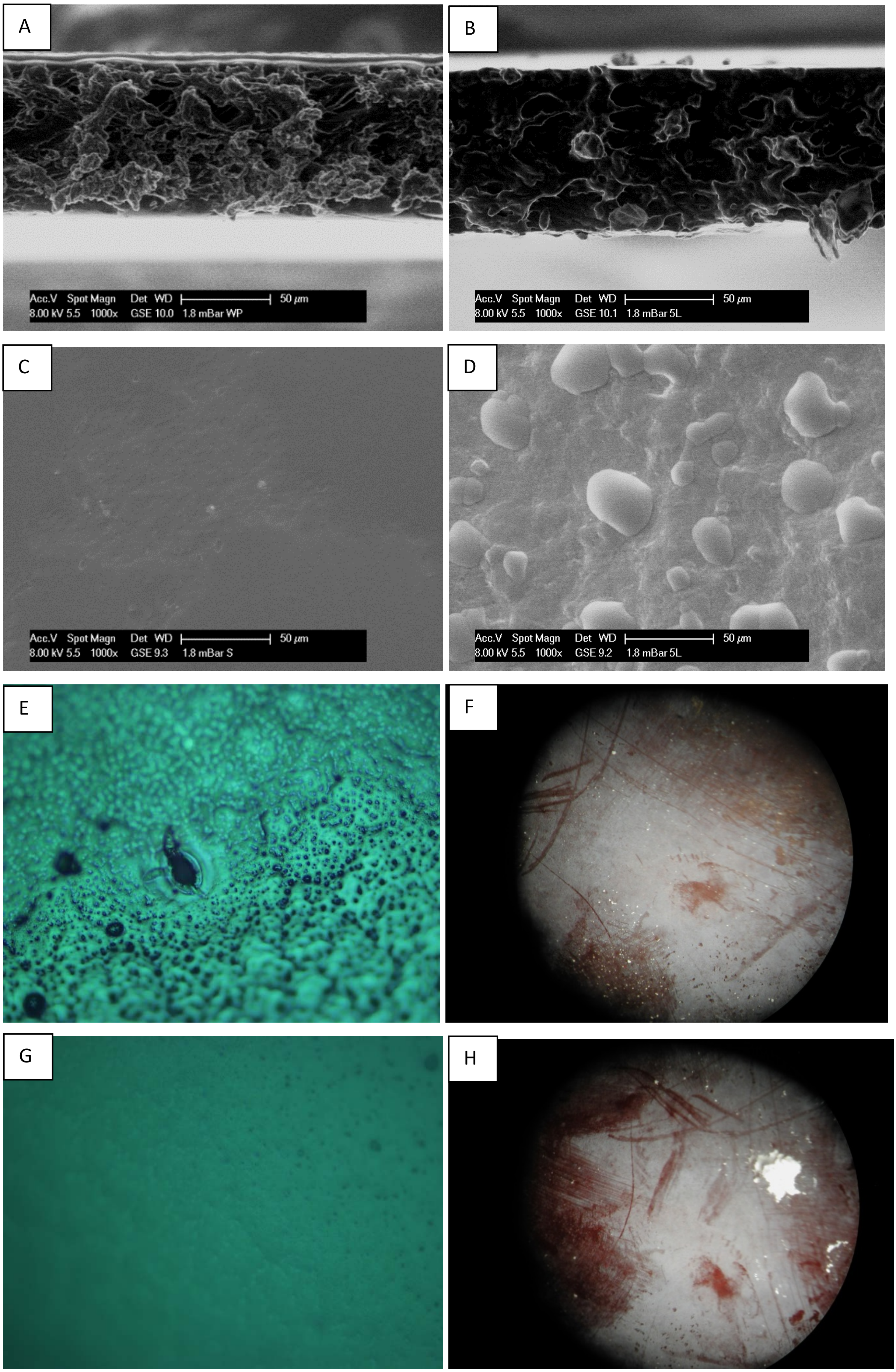
For this work, plums were chosen for their high nutrition value but also relatively short shelf life. Their skin can be consumed with the flesh, so plums are an excellent material for covering with edible coatings. The main objective of this study is a better understanding of the surface properties of the plum surface after coating. Stand-alone films were made as a model for understanding processes which are not possible to measure directly on the plum surface.

Table 6.1 Composition of film-forming suspension (100g) used for both plum coating and film making.

Film	Wheat starch (S) (g)	Whey protein isolate (WPI) (g)	Rapeseed oil (O) (g)	Glycerol (g)	Water (g)
Starch	5	0	0	2.5	95
starch-whey proteins (80/20)	4	1	0	2.5	95
Starch-oil-starch (3-layer)	5	0	3	2.5	95

6.1.2. Structural and visual properties of films and coatings applied onto plum surface

The surface and the cross-section pictures achieved from environmental scanning electron microscopy (ESEM), light, polarizing microscopies and thermographic photographs were presented in figure 6.1.


A
Acc.V Spot Magn Det WD 50 µm
8.00 kV 5.5 1000x GSE 10.0 1.8 mBar WP
B
Acc.V Spot Magn Det WD 50 µm
8.00 kV 5.5 1000x GSE 10.1 1.8 mBar 5L
C
Acc.V Spot Magn Det WD 50 µm
8.00 kV 5.5 1000x GSE 9.3 1.8 mBar S
D
Acc.V Spot Magn Det WD 50 µm
8.00 kV 5.5 1000x GSE 9.2 1.8 mBar 5L
E
F
G
H

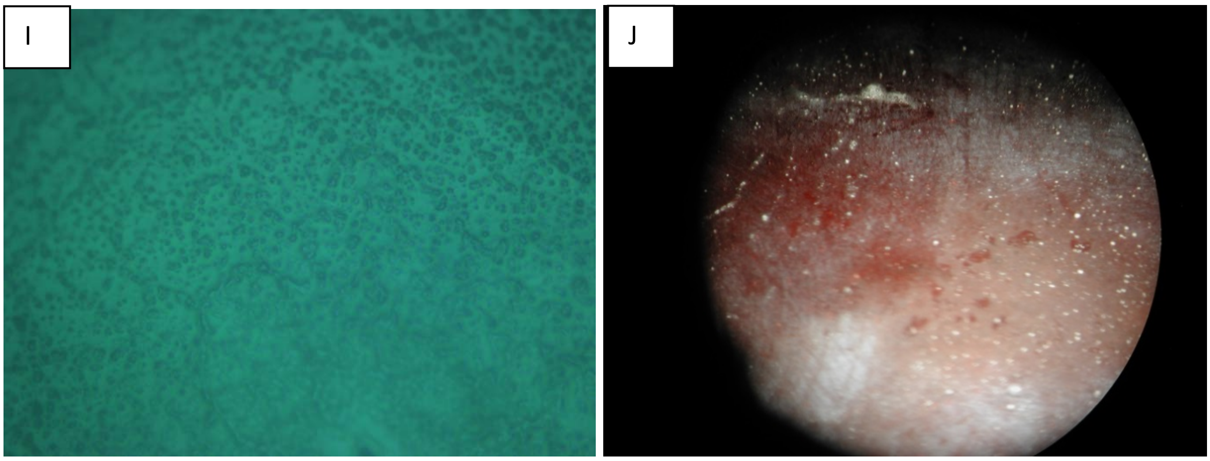


Figure 6.1 ESEM micrograph of a) cross section of starch-whey protein (80/20) films (magnification 1000x), b) cross section of starch-based films (magnification 1000x), c) surface exposed to air during drying of starch-based films (magnification 1000x), d) surface exposed to air during drying of starch-based films with rapeseed oil addition (magnification 1000x); Polarizing microscopy image of e) surface of raw plum (magnification 200x), g) plum surface after dipping in starch-based coating (magnification 200x); i) plum surface after 1 hour from dipping in starch-based coating (magnification 200x); and Light microscopy graph of f) surface of raw plum (magnification 20x), h) plum surface after dipping in starch-based coating (magnification 20x), j) plum surface after 1 hour from dipping in starch-based coating (magnification 20x).

View of wheat starch and wheat starch – whey protein (80/20) films revealed smooth, continuous and homogenous matrix without any cracks, holes, defects etc., but did not display any significant differences (one picture is presented fig. 6.1.c). In turn significant differences were observed for the cross-section micrographs (fig. 6.1.a,b). Wheat starch contains 75% of branched amylopectin chains, thereby films from pure starch are more heterogeneous and fibrillar than films with protein isolate addition (fig. 6.1.a,b). Even though cross section seems different, it does not influence the thickness of starch films and starch-whey protein films which are very similar (around 80 μm) and probably more related to dry matter content per unit area than to microstructure. After rapeseed oil addition the thickness decreased to ~28 μm. Thickness of the starch-oil-starch coating onto plum surface is also about 28 μm.

Photographs of coated plums showed good adhesion of the starch–oil–starch films (fig. 6.1e-j). In previous work, Basiak et al. (2015a) investigated 3-layer films contained from wheat starch (as the 1st layer), rapeseed oil (the 2nd layer) and wheat starch (the 3rd layer), that means from starch-based (the first layer), fat (the second layer) and starch-based (the third layer) (fig. 6.1e,f). The same structure was observed for coated plums: skin of plum (the first layer), wax onto the surface (the second layer), wheat starch coating (the third layer). These layers are presented on the fig 6.1. ESEM photographs displayed that films were prepared layer by layer. But, this technique did not give a 3-layer or multilayer structures as expected. The casting of the third layer wet the previous layers and partly solubilized the first one. In consequence it creates an emulsion (fig. 6.1d). Surface images show drops of oil dispersed in the starch matrix driven to an emulsified structure and not a multilayered (fig. 6.1d). Similar effect can be observed onto plum surface after coating process (fig. 6.1e-j). Plums possess a natural wax layer onto their surface which is nonpolar. In theory fruit with this kind of surface can be coated only by apolar substances. It is noteworthy that wax contains long chain and primary alcohols (Backer, 1982). Film-forming suspension contains polyol – glycerol. Probably hence covering of hydrophobic surface by hydrophilic suspension is possible. It is also not excluded that starch is able to establish hydrophobic bonds that permit adhesion onto the wax layer onto plums coatings. Moreover lipid aggregates are pretty small so they are properly integrated in the starch matrix as well as films. On the plum surface, the epicuticular wax layer is in a crystalline form (fig. 6.1e,f). Light and polarizing microscopies observations prove that after fruit dipping in the film-forming suspension this wax structure still exists (fig. 6.1g,h). Crystalline wax can melt, for instance by touching by hands or by polishing due to temperature increase or due to mixture with other substances (Wisuthiphaet et al. 2014). Thus at the beginning you have crystalline wax form visible on the surface. During the drying process this crystalline wax layer melted with other components (fig. 6.1g-j) and then the coating is no more visible (fig.6.1i,j).

Colour parameters (*L,a,b, ΔE,* C_{colour}) given in table 6.2 show a significant differences in a value and ΔE between raw (control) and coated plums after drying process (2 hours). Redness parameter is 1.5 and 2 times higher for starch-whey protein (80/20) and starch coatings, respectively. Higher redness is positively perceived by consumers, which is the main asset. Thus colour difference for starch-whey protein (80/20) coatings is below the noticeable difference (1.53), while difference for starch coating is marked by inexperienced customers (2.49). Data presented for films, it is coatings dried not onto plum, but onto Petri dishes revealed not significant differences between them and white control plate and significant differences between films and coated plums. It means both starch and starch-whey protein films (80/20) are transparent. Wisuthiphaet et al. (2014) used same varieties of plums *Prunus domestica* – cv. *Hanita* and cv. *Ortenauer* in their experiments. One of the goals of this work was to compare colour parameters of untreated and polished plums, where epicuticular wax layer was removed manually. Results for colour parameters obtained by these authors show that plum species play a significant role. Anyway, *L*, *a* and *b* values for untreated fruit were lower than that for cv. *Jojo* plums, i.e. 37.3, 2.35, -13.9, respectively. The values after taking out the epicuticular wax were even less for lightness: 28.1 and higher for redness 2.86 and blueness -5.0 than for untreated plums of the same variety. Total colour difference (ΔE) is under the value 2.3, so the just noticeable difference (JND) in colour is not visible for human eyes (Mahy et al., 1994). This fact also confirms lack of influence of coating and films on plum colour parameters. Consequently, it should not affect negatively the acceptance by consumers. However colour chroma C_{colour} parameters do not significantly differ. Untreated plums, coated plums with starch and starch-whey protein solutions have same C_{colour} value (no significant difference). Study of Valero et al. (2013), who measured the effect of alginate coating on colour parameters in four plum cultivars during the postharvest storage shows the colour chroma changed of few units, but not significantly. The variety of the plums has the main influence on the colour parameters. Indeed, fruit colour chroma changed from 13 to 15 (cv. *Blackamber*), from 13 to 14 (cv. *Larry Ann*), from 44 to 45 (cv. *Golden Globe*), from 43 to 45 (cv. *Songold*) in the first day after coating.

Table 6.2 Colour parameters of the surface of non-coated plums (control), of starch films, starch-whey protein (80/20) films, starch coated plums, of starch-whey protein (80/20) coated plums. Thicknesses of films and coatings were similar (around 30 µm).

Surface	L	a	b	ΔE	C_{colour}
Uncoated plums (control)	54.43 ± 4.90^{a}	2.23 ± 0.37^{b}	-8.18 ± 1.73^{a}	-	8.48^{b}
Starch film	96.20 ± 0.31^{b}	0.32 ± 0.10^{a}	3.72 ± 0.61^{b}	1.52^{a}	4.02^{a}
Starch-whey protein (80-20) films	95.13 ± 0.32^{b}	0.46 ± 0.11^{a}	4.36 ± 0.73^{b}	2.59^{b}	5.08^{a}
Starch coated plums	54.45 ± 11.04^{a}	4.61 ± 1.37^{c}	-8.93 ± 3.31^{a}	2.49^{b}	10.04^{b}
Starch-whey protein (80-20) coated plums	54.01 ± 11.42^{a}	3.64 ± 1.21^{c}	-7.76 ± 3.34^{a}	1.53^{a}	8.57^{b}

6.1.3. Surface and wettability properties

Table 6.3 contains the data related to properties of starch films, starch – whey protein (80/20) films and starch films containing oil. The contact angles and surface tension values are given in table 6.3, displaying differences between the 3 type of films studied. While a new surface was created, the highest disruptions of intermolecular bonds were observed for starch based films, because starch surface is more hydrophilic than starch-whey protein (80/20) matrix, affinity to water is higher so moisturizing properties are higher and thereby comes to bigger ruptures. It is confirmed by the value of contact angle which behave in opposite to the surface tension. It describes good wettability properties of pure starch surface. Biomaterials which contain oil have the most stable creations of new surface, so to compare pure saccharide and saccharide – protein films with saccharide – fat matrix have the highest value of wetting angle and thus the best barrier properties. Scanning microscope observations revealed rounded shape drops on the surface. The same round shape drops of oil are visible onto starch films with rapeseed oil and onto plum surface few hours after coating in starch and starch –whey protein film forming solutions (fig. 6.1d,i). So, it probably means starch and starch-protein coatings onto fruit surface interacted with the wax covering the plum peel and they behave like starch films with oil addition. A few minutes after coating, polarizing photographs and light microscopy photographs revealed homogeneous layer on the plum surface, typical for the starch solution (contact angle is low, fig. 6.1g,h). In the next 1 to 1.5 hours on the plum surface appeared round drops related to oil. The contact angle of water on the plum surface is much higher than directly after coating. It is also noteworthy that fruit skin has evidently apolar character after treatment whereas the pure starch films have an almost optimal balance between dispersive and polar components. This balance is upset in starch-whey protein films. In starch films with oil addition dispersive component represents nearly 2/3 of total surface tension, thus in starch and starch-whey protein films is twice reduced.

Colocation between starch-glycerol systems and waxes explains why coating of two opposite materials (polar and non-polar) is possible. Moreover surface tension is the lowest for starch films with oil addition, which thereby means fat causes the higher stability than in case of wheat starch and whey protein systems. It is also confirmed by absorption. Value for starch matrix is about 1/3 higher than for starch-oil matrix and films with proteins. Either it means films with oil addition have lower water affinity than pure starch films and starch films with 20% of proteins addition (Basiak et al., 2015b,c,d). Significantly difference between starch –

oil and pure starch or starch-whey protein films is visible for work of adhesion and cohesion. These parameters are higher for films without fat, so in starch and starch-protein systems phases have higher possibility for connecting.

Table 6.3 Physico-chemical characteristics of films: surface tension of liquids (γ_L), surface tension of films (γ_S) and their dispersive (γ_S^D) and polar (γ_S^P) components, adhesion (W_A), cohesion (W_C) and spreading coefficients (W_S), swelling index, water content, solubility in water, water vapour and oxygen permeabilities at 25°C.

Film characteristics			Film composition		
			Starch-whey protein (80-20)	Starch	Starch-oil-starch (3-layer)
Thickness (μm)			79.7 ± 11.2^b	80.8 ± 12.59^b	27.7 ± 5.69^a
Surface properties	Contact angle (°)	Water (γ_L=72.8 mNm^{-1})	61^b	43^a	77^c
	Surface free energy (mNm^{-1})	γ_S^D	$38.0^{a,b}$	35.4^a	40.6^b
		γ_S^P	22.9^b	24.9^b	15.5^a
		γ_S	60.9^b	60.3^b	56.1^a
	W_A (mJ m^{-2})		125.86^b	126.80^b	115.12^a
	W_C (mJ m^{-2})		121.80^b	120.56^b	112.12^a
	W_S (mJ m^{-2})		-4.06^a	-6.24^b	-3.00^a
Transport and solubility properties	Swelling index (%)		53.49 ± 1.86^c	39.20 ± 1.43^b	34.91 ± 1.39^a
	Water content (kg $_{water}$ kg$^{-1}_{dm}$)		2.04 ± 0.05^a	3.24 ± 0.50^c	$2.69 \pm 0.21^{b,c}$
	Solubility in water (%)		13.06 ± 0.18^b	19.67 ± 0.17^c	10.70 ± 0.72^a
	Water vapour permeability (10^{-10} g m^{-1}s^{-1}Pa^{-1})	33-0% RH	4.99 ± 0.06^b	5.24 ± 0.26^b	0.57 ± 0.46^a
		75-30% RH	6.37 ± 1.03^c	7.70 ± 0.85^c	3.55 ± 2.80^b
		100-30% RH	7.16 ± 0.54^c	7.87 ± 0.65^c	3.40 ± 0.31^b
	Oxygen permeability (10^{-14} cm^{3}m^{-1}s^{-1}Pa^{-1})	53% RH	7.44 ± 1.07^c	7.23 ± 1.00^c	0.96 ± 0.02^a
		75% RH	11.69 ± 0.86^d	7.41 ± 1.39^c	1.12 ± 0.05^b

Values having the same letter for a parameter are not significantly different at p level < 0.05

Looking on sorption isotherms (fig. 6.2) it is noteworthy starch films increase sharply water content above a water activity of 0.60 whilst 20% of protein addition to the structure causes water content increase first around 0.78. Even so slight addition of proteins as 1/5 of total dry film-forming powder mass can reduce adverse chemical, physical and enzymatic reactions and growth of microorganisms, which appeared above 0.60 of water activity (halophilic bacteria, xerophilic yeast and filamentous fungi may show growth at significantly reduced in the 0.60-0.75 water activity range. Whey protein isolate contains very hydrophobic amino acids as valine, leucine, less hydrophobic as proline and amino acid that is part hydrophobic - lysine. Anyway, 16 of 22 protein molecules have a hydrophobic core. Whereas the hydrophobic amino acids residue to build the core of polar and charged amino acids favourably cover the surface of the molecule and are exposed to solvents due to their ability to create hydrogen bonds (by donating or accepting a proton from an electronegative atom). Thus hydrophobic character of these amino acids reduces affinity to water. In turn, oil addition can reduce water activity and all unfavourable processes above 0.8 water activity. That has particular importance in case of food products containing a lot of water and stored in high relative humidity, as plums. In case of starch films water occurs (up to 0.60 of water activity) in the form of bound water. Above this value water exists as free water and hence water content is higher. The same dependence concerns starch-whey protein films, where bound water occurs up to ~ 78%. Fat addition reduces not only the swelling index and solubility in water but also the water content above 0.80 water activity. Hydrophobic oil drops located outside provide more effective barrier for vapour than pure starch or starch-whey protein systems.

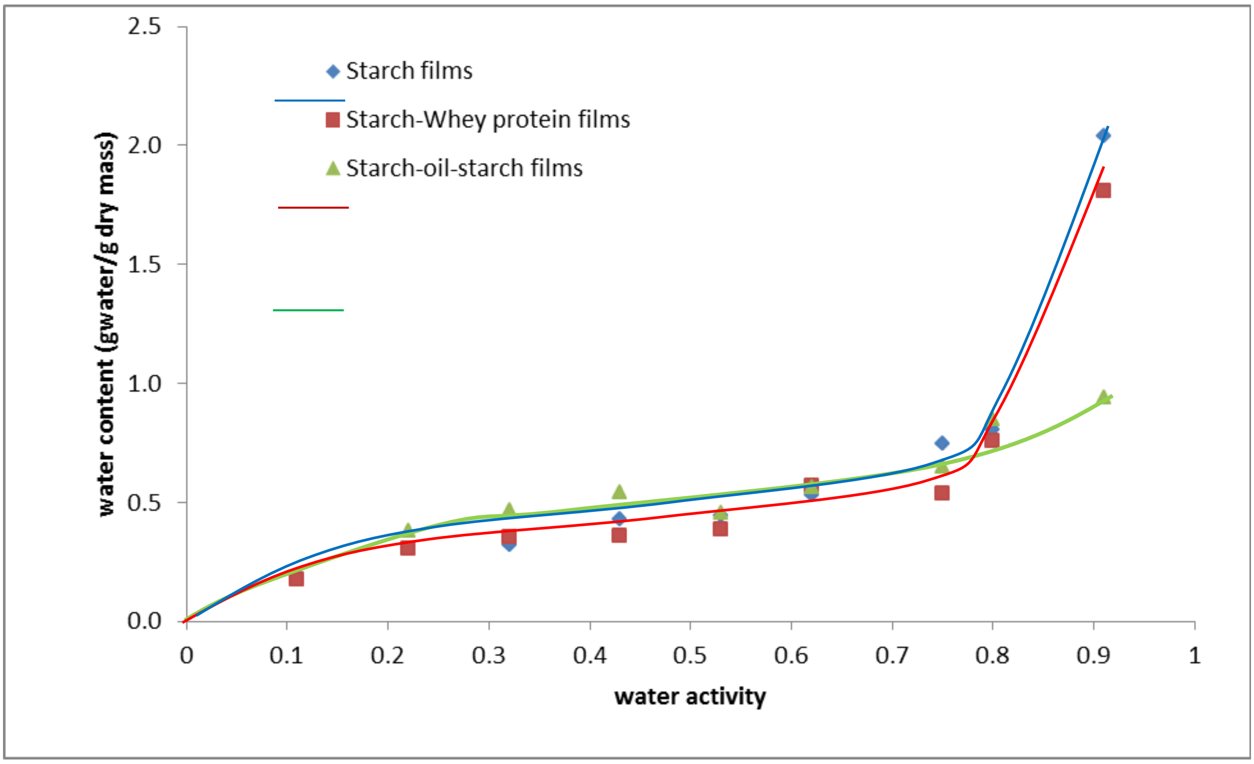


Figure 6.2 Moisture sorption isotherms of starch films, of starch- whey protein (80-20) films and of starch-oil-starch 3-layer films (g water/g dry matter).

Table 6.3 gives the values of water vapour permeability (WVP). In 0-33% relative humidity differential, the WVP for starch films and starch – whey protein (80/20) films were even 10 times higher than that for starch-oil systems. In turn, for 30-75 and 30-100% of RH, water vapour permeability of 3-layer films (starch-oil-starch) was about twice lower than that of starch films and starch-whey protein (80/20) films. Fat molecules acted as effective barrier for water permeation. So, even a slight addition of oil can reduce WVP about one order of magnitude. As observed for WVP, solubility in water is significantly reduced for the films containing oil. Oxygen permeability of films is displayed in table 6.3. Starch-oil emulsions have almost 8 times higher permeability to oxygen at 53% RH than for films without fats, and up to 10 times at 75% RH. This could be a disadvantage for oil emulsions treatment for fruit because reduced oxygen permeability would induce modified atmosphere conditions with the advantage for longer shelf life of plum fruit. Oxygen is known to be highly soluble in lipids, which explain the increase of oxygen permeability when fat added into the starch based films.

6.1.4. Molecular interactions involved in coatings and films related to surface properties

The most important interaction between molecules which describes wheat starch system is hydrogen bond (Liang and Ludescher, 2015). The FTIR spectra of wheat starch powder, wheat starch-glycerol films, whey protein powder, whey protein-glycerol films, starch-oil-glycerol films and glycerol are presented in figure 6.3 and show location and shifts due to bonding and interactions involved in matrices. In previous work Basiak et al. (2015d,e) described spectra of starch powder and starch films with 50% of glycerol (w/v). At 3430 cm^{-1} (not showed onto the graph) is located a broadband with very strong character. OH-groups come from the absorbed starch polymer, from water and from glycerol corresponding with this peak. Thus, broad bands with wavenumber 2928 cm^{-1}, 1655 cm^{-1} and 1373 cm^{-1} either have weak character and they assigned with hydrogen bond, the δCH and the C=O, respectively. Peaks with wavenumbers of 1165 cm^{-1} and 1084 cm^{-1} are attributed to CH and δCO groups, but in contrast to previous bonds they have stronger energy. Also peak located at 980 cm^{-1} has energy and it associates with δC-O stretching vibrations (Zaleska et. al, 2001). The region quite typical for saccharides is located at 1165–980 cm^{-1} (Souza and Andrade, 2002). In turn, on whey protein isolate powder and films, the wave number is located (not presented on the graph) at 3450 cm^{-1} in the spectrum which is assigned to free N–H groups (Stuart, 2004). The peak located at 3293 cm^{-1} in the spectrum of whey protein isolate is corresponding to hydrogen bonded N–H stretching (Farjamia et al., 2015). Wavenumbers at 1700-1600 cm^{-1} are characterised for amide I. So, as well peak at 1643 cm^{-1} correspond to the C=O stretching vibrations of amide I (Casal et al., 1988; Pelton and McLean, 2000). The N−H stretching vibration is attributed to peak at 1565cm^{-1}. Aggregated proteins show a peak assigned to 1600 cm^{-1} which is characteristic of intermolecular β-sheets (Barth, 2007). Peak located at wavenumber 1541 cm^{-1} is attributed to the C−N stretching and N−H bending of vibrations from amide II (Farjamia et al. 2015; Jackson and Mantsch, 1995). Thus C=O and N−H peaks in the amide I and II regions of whey proteins are assigned to hydrogen bonds (Mohammadian and Madadlou, 2016). Wavenumbers at 1400–1200 cm^{-1} attributed to N−H bending and C−H stretching vibrations it is amide III region (Barth, 2007). Thus the wavenumbers between 1250 and 1220 cm^{-1} are assigned to β-sheet structures (Hussain et al. 2012).

Spectrum FT-IR of pure glycerol reveals bands at wavenumbers 850, 925 and 995 cm^{-1} which are associated to the vibration of the skeleton C-C. The broadband at 1045 cm^{-1} corresponds to the stretching of the C-O linkage in C1 and C3 and the peak at 1117 cm^{-1} is attributed to the stretching of C-O in C2 (Guerrero et al., 2010).

The effect of glycerol, water and oil can be analysed by comparing the spectra of wheat starch powder and glycerol to the spectra of starch films with 50% of glycerol and spectra of wheat starch powder, whey protein powder and glycerol with starch-whey protein films with 50% of glycerol. Comparing wheat starch and whey protein isolate powders to the films made from 5% of starch and 5% of starch + whey protein (in ratio 80/20), both matrices with 2,5% of glycerol, as seen on figure 6.3, the characteristic peaks for saccharides region are increased. Bands at 1014 cm^{-1} can be attributed to C-O stretching, are shifted to 1020 cm^{-1} (all kinds of films). Moreover, the peak located at 2908cm^{-1} and corresponds to C-H vibrations also shifted to a higher position at 2932 cm^{-1} for starch and whey protein films, and to 2938 cm^{-1} for the films with oil. Broadband assigned to O-H bond at wavenumber 3320 cm^{-1} is shifted at 3338 cm^{-1} for starch and starch-whey protein films and to 3356 cm^{-1} for oil starch systems. Addition of glycerol influences the hydrogen bonding interactions among starch and glycerol, starch-whey protein and glycerol and starch-oil and glycerol systems. Accordingly, the decreased intensity of the peak at 3293 cm^{-1} is attributed to reactions between groups of starch and NH groups of whey proteins which shifted the number of NH groups (Barth, 2007).

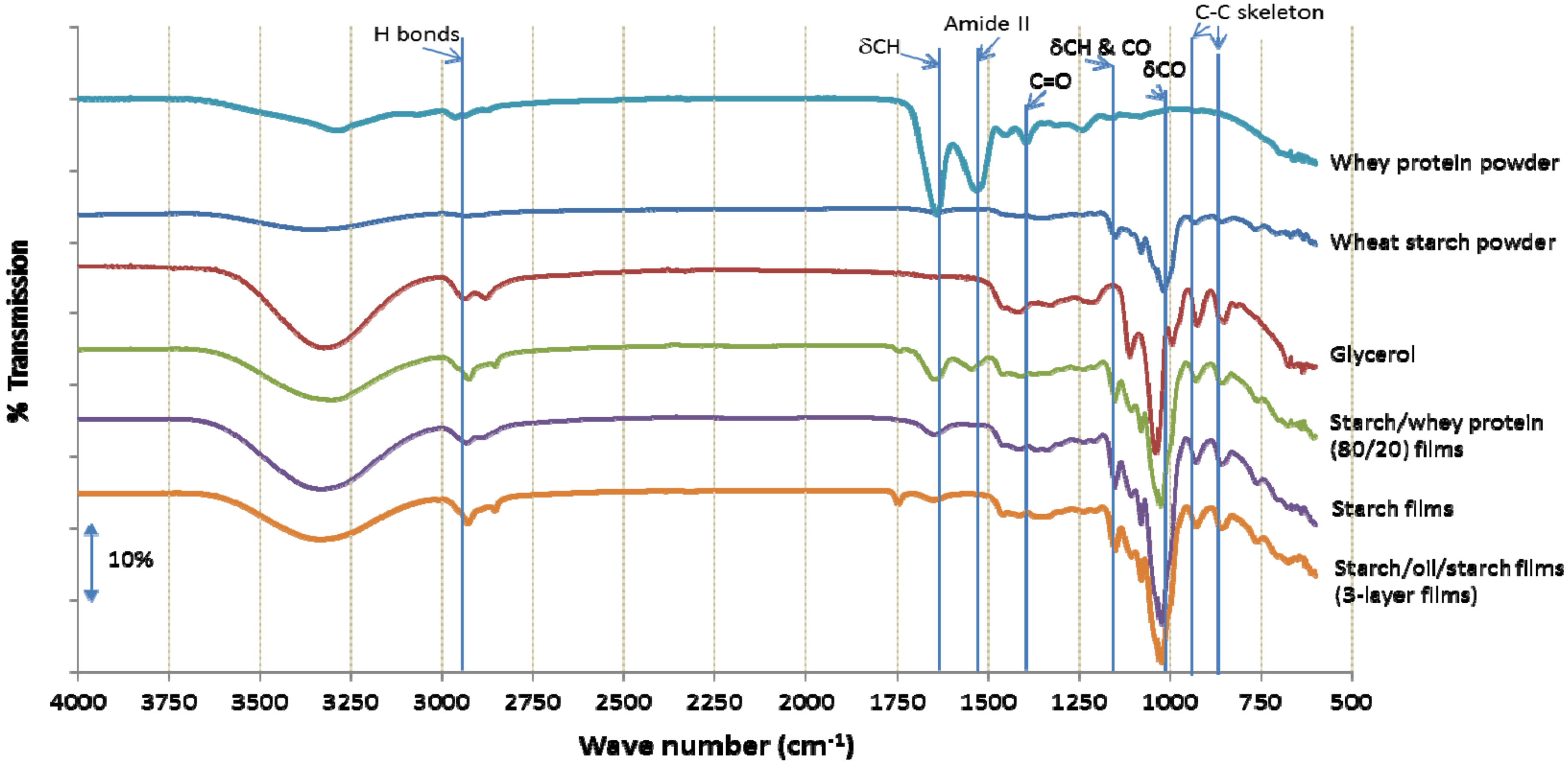


Figure 6.3 FTIR spectra of whey protein powder, of wheat starch power, of pure glycerol, of starch-whey protein (80-20) films, of starch films and of starch-oil-starch films (3-layer) at 50% RH and 25°C.

6.1.5. Conclusions

Coating process does not affect appearance an additional layer. By alcohol groups epicuticular crystal wax mixes together with starch-glycerol film-forming suspension. ESEM, light microscopy and thermographic pictures confirm that layers structure gets loss. Epicuticular wax crystal coating is not visible a few hours after coating, but still exists dispersed in starch-glycerol-water system. By this way on the fruit surface appeared not a 3rd layer structure as expected, but thin (~30µm) emulsion. Resistance for oxygen and water vapour permeability is improved. Coated by starch and/or starch-whey protein film-forming suspension can influence positively on receive by customers, who prefer shiny surface of fruit than a dull surface.

6.2. Dynamic behaviour of coated plum as a model of food system (Basiak E., Linke M., Debeaufort F., Lenart A., Geyer M. (2016). Submitted to, April 2016).

6.2.1. Abstract and introduction

Quantitative detection of thermal barrier coating thickness based on simple equations and measurements was carried out using thermographic camera system connected with logger, which measured environment conditions. The principle of this calculation method was described. The used method focuses onto the second Fick's law. Comparing to the other methods thickness and mass loss can be calculated much more precise and quicker than using for instance SEM photographs and to measure using a caliper. This method is very sensitive and exact and can be used even for very thin layers applied onto materials. The model materials used for experiments were plums coated by two kind of solution: starch and starch-whey protein (80/20) in two different relative humidities. Starch coatings are thicker than starch-whey protein (80/20) film-forming suspensions. Any significant difference in transpiration resistance, layer resistance and boundary resistance was observed.

In the 21st century civilization and technological progress are growing at a dizzying pace. Thus, not only the choice of products but consumer awareness increases. Demand for health, fresh, minimally processed, natural, nutritious and ready to eat products increasing in a logarithmic rate too. To follow the consumer expectations non-destructive processes should dominate the market. The excellent alternative to preserve the quality and freshness of minimally processed food and prolongation their shelf-life is using of edible coatings (Chiumarelli and Hubinger, 2012). Biodegradable/ edible coatings function as barriers to gas exchange and protection of water loss. Depending on constituent material membranes have different physical and chemical properties. In low relative humidity (under 75%) polysaccharides and proteins can successively replace plastic materials (Debeaufort, Quezada-Gallo and Voilley, 1998). Above this value in matrix is a lot of free water and quickly comes to swelling of the food surface. It is caused of high hydroscopicity of hydrocolloid materials and in consequence it constituting poor barrier to water vapour (Basiak, Debeaufort and Lenart, 2015). However, edible coatings due to its numerous advantages can be successively used in food industry. Edible film-forming solutions are able to act as carriers for fungicides, growth regulators and improve fruit gloss (Llanos Navarro-Tarazaga et al., 2011). The next of primary quality of using biodegradable membranes is that couple active ingredients can be incorporated into the biopolymer system and consumed with the food product, thus enhancing the adaptation to fast lifestyle (Rojas-Graü, 2009). Additionally food-grade ingredients cause they are saved for human consumption (Llanos Navarro-Tarazaga et al., 2011).

Surface coatings are applied to many food products, especially fruits and vegetables to increase skin resistance to gases diffusion (O_2, CO_2), and thereby to keeping high index of firmness, and reduce mass lost (transpiration losses) by retardation of the natural physiological ripening process (Banks et al., 1993; Valero et al., 2013; Valero et al., 2003). The effectiveness of coatings depends on fruit type and cultivar. Llanos Navarro-Tarazaga et al. (2011) worked with plum Prunus domestica cv. ‘Angeleno’. They admitted the coatings did not reduce mass loss. For that they have a valid effect maintaining flesh firmness and reducing juice bleeding.

The main goal of this work was to calculate coating thickness, weight loss by using thermographic camera and limiting mass transfer and therefore increasing the shelf-life of the plums coated by starch and mixture of starch and whey protein solutions. Used method and equations are not only novelty but several times more precisely than current method used in food industry.

6.2.2. Influence of the coatings on transpiration behaviour on plums

On the figure 6.4 is presented transpiration behaviour on plums before and after coating process at 33.9% of relative humidity. The part 0 presents mass loss of plum before covering in film-forming suspension. The line of water evaporation and its prolongation (dashed line) shows the mass lost is the highest. Fruit covering in film-forming suspension reduce transpiration and connected of that mass loss. The more layers is the lowest mass lost and higher transpiration resistance is. But as you can see from the table 6.4 every next layer does not cause reduce of the mass loss of the same value. With every additional layer the effect is lower, it is for instance the effect of the first layer is 8.80, of the single second layer is 7.80 and of the single third layer is 6.70 (s cm^{-1}), but they are summed together, so the effect after addition of the second layer is 16.60, after the third 23.30 (s cm^{-1}). As well as you can see from the same table 6.4 resistance with every next layer also increases. In relative humidity equals 33.9% transpiration resistance of plums coated by starch material rises of 16.6% (after the first layer), 34.6% (after the second layer) and 51% (after the third layer), and of 31, 53 and 65.6% for starch - whey protein (80/20) material, respectively. Values of boundary layer are very similar. For Lady Red are 2 times lower than for Angeleno variety. It is caused the weight of plums - around 42 g for Lady Red and 75 g for Angeleno.

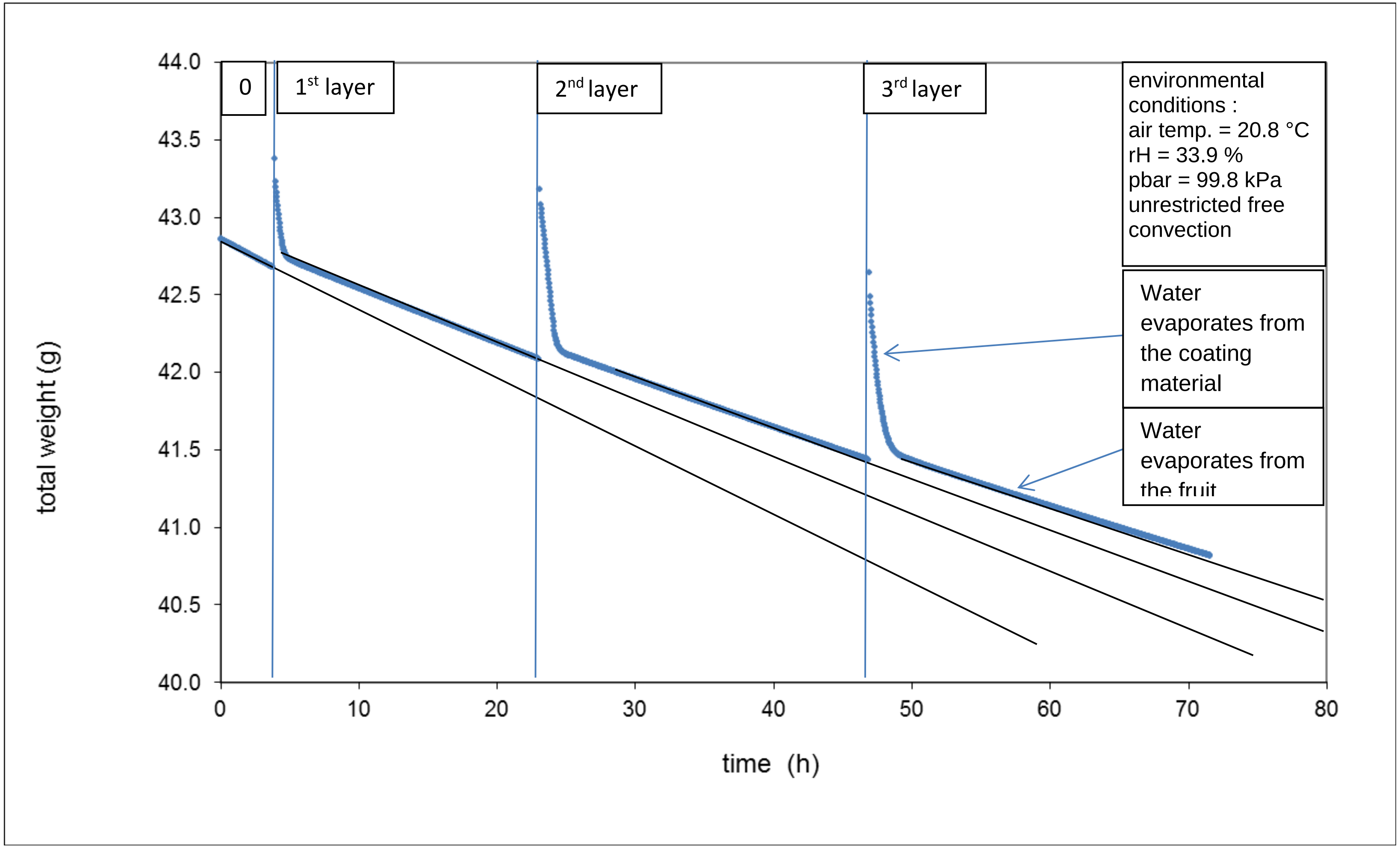


Figure 6.4 Transpiration behaviour on plums before and after coating process at 33.9% of relative humidity.

Table 6.4 Resistances and thickness of starch and starch-whey protein (80/20) coatings.

STARCH	1st plum				2nd plum				3rd plum			
Results:	rT [s cm^{-1}]	all layers rLges [s cm^{-1}]	single layers rL [s cm^{-1}]	rB [s cm^{-1}]	rT [s cm^{-1}]	all layers rLges [s cm^{-1}]	single layers rL [s cm-^{1}]	rB [s cm^{-1}]	rT [s cm-^{1}]	all layers rLges [s cm^{-1}]	single layers rL [s cm^{-1}]	rB [s cm^{-1}]
uncoated	32,30				52,00				50,70			
1 layer	41.10	8.80	8.80	2.46	62.70	10.70	10.70	2.59	56.90	6.10	6.10	2.37
2 layer	48.90	16.60	7.80	2.43	69.10	17.10	6.30	2.52	61.70	10.90	4.80	2.29
3 layer	55.60	23.30	6.70	2.36	77.50	25.50	8.50	2.46	67.00	16.20	5.30	2.54
Climate data:	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness
uncoated	20.89	35.58	99.59		20.34	73	100.05		20.73	33.17	100.02	
1 layer	21.13	35.95	99.52	6.49	20.97	33.94	99.75	6.52	20.49	32.49	100.06	6
2 layer	20.96	34.71	99.59	6.3	20.68	34.35	99.83	6.4	20.51	32.3	100.1	6.6
3 layer	20.84	33.9	99.68	6.5	20.66	34.52	99.95	6.6	20.14	30.41	99.98	6.48

PROTEIN	1st plum				2nd plum				3rd plum			
Results:	rT [s cm^{-1}]	all layers rLges [s cm^{-1}]	single layers rL [s cm^{-1}]	rB [s cm^{-1}]	rT [s cm^{-1}]	all layers rLges [s cm^{-1}]	single layers rL [s cm-^{1}]	rB [s cm^{-1}]	rT [s cm-^{1}]	all layers rLges [s cm^{-1}]	single layers rL [s cm^{-1}]	rB [s cm^{-1}]
uncoated	31.87				31.20				52.33			
1 layer	44.88	13.02	13.02	5.88	38.06	6.86	6.86	5.75	68.37	16.04	16.04	6.01
2 layer	51.25	19.38	6.37	5.72	45.56	14.36	7.51	5.59	79.56	27.23	11.18	5.95
3 layer	55.30	23.43	4.05	5.56	48.88	17.68	3.32	5.42	87.11	34.78	7.55	5.84
Climate data:	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness
uncoated	20.34	35.03	100.05		20.68	31.78	100.36		21.47	32.20	100.82	
1 layer	20.62	35.93	100.07	5.5	20.82	30.81	100.36	5.66	21.58	31.81	100.05	4.82
2 layer	20.51	36.18	100.05	4.4	21.08	29.79	101.49	4.68	21.29	31.70	98.50	5.54

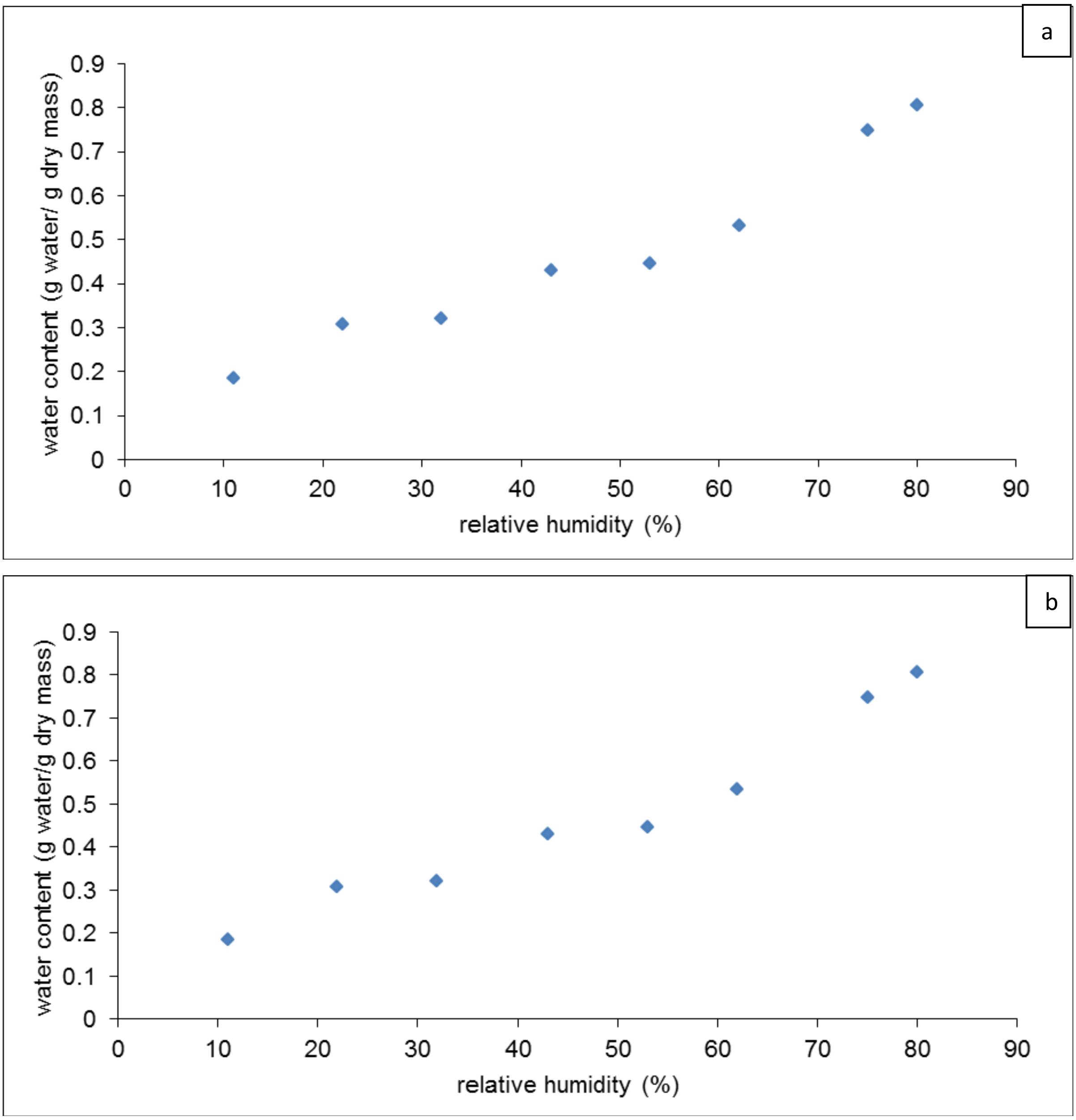
3 layer	20.63	35.42	100.01	5.8	20.91	33.54	101.45	6.2	21.74	31.78	98.26	5.2

STARCH 80	1st plum				2nd plum				3rd plum			
		all layers	single layers			all layers	single layers			all layers	single layers	
Results:	rT	rLges	rL	rB	rT	rLges	rL	rB	rT	rLges	rL	rB
	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm-^{1}]	[s cm^{-1}]	[s cm-^{1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]
uncoated	53.94				76.79				95.97			
1 layer	57.35	3.41	3.41	6.29	81.27	4.48	4.48	5.94	97.51	1.54	1.54	6.56
2 layer	58.33	4.39	0.98	6.30	83.16	6.36	1.89	5.98	98.74	2.77	1.23	6.58
Climate data:	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness
uncoated	20.11	80.99	100.37		19.97	81.13	99.98		20.16	81.22	98.46	
1 layer	20.18	81.14	99.79	6.8	20.07	81.10	99.17	6.2	20.21	81.36	98.65	5.81
2 layer	20.09	81.42	99.87	7.1	20.07	81.31	98.74	7.8	20.26	81.23	99.29	8.2
3 layer	20.04	81.49	100.42	5.2	20.02	81.52	98.48	5.12	20.43	81.44	99.29	10.2

PROTEIN 80	1st plum				2nd plum				3rd plum			
		all layers	single layers			all layers	single layers			all layers	single layers	
Results:	rT	rLges	rL	rB	rT	rLges	rL	rB	rT	rLges	rL	rB
	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm-^{1}]	[s cm^{-1}]	[s cm-^{1}]	[s cm^{-1}]	[s cm^{-1}]	[s cm^{-1}]
uncoated	40.41				43.02							
1 layer	41.25	0.84	0.84	6.14	53.71	10.69	10.69	6.22	-	-	-	6.19
2 layer	42.35	1.93	1.10	6.13	56.72	13.70	3.01	6.21	-	-	-	6.10
Climate data:	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness	air temp. [°C]	rel. humidity [%]	bar. pressure [kPa]	thickness
uncoated	19.87	77.76	101.13		20.07	80.72	100.35		19.99	81.17	100.09	
1 layer	20.15	77.90	101.25	10.50	20.67	77.23	100.42	8.60	20.09	81.15	100.07	9.82
2 layer	20.01	80.11	101.21	11.20	20.07	76.64	100.61	13.40	20.02	81.18	100.06	11.51
3 layer	19.99	80.42	100.81	9.04	19.96	77.60	101.02	11.00	20.02	81.30	100.06	10.32

6.2.3. Isotherm sorption and equilibrium water content and thickness

For starch and starch – whey protein coatings the equilibrium water content at 33.94% was 0.410 g dry mass/ g water. Knowing this value it was possible to describe moment, when thickness is constant.



Figures 6.5a,b Isotherm sorption and equilibrium water content a) starch coating, b) starch-whey protein coating.

Results of constant thickness, it is thickness after water evaporation from the coating solution, are presented in table 6.4 and mean values in table 6.5. They are calculated for each layer. As well as you are able to see the thickness of starch coating is thicker than thickness of starch – whey protein (80/20) systems. It is especially noticed in higher relative humidity (80%). The mean thickness of starch coatings for one layer in 35% of RH is 6.43 ± 0.19 µm, in 80% of RH is 6.94 ± 1.63 µm, thus starch-protein coatings in 35% of RH have thickness 5.31 ± 0.58 µm, and in 80% of RH 4.97 ± 0.73 µm. Thickness of all 3 layers is also the highest for starch coatings and the lowest for starch-whey protein film-forming suspensions. Previous experiments onto films also confirmed that incorporation of proteins reduces thickness and systems with protein addition are denser than pure starch matrices (Basiak et al., 2016). On the figure 6.6 are presented changes of thickness, directly after coating process. The curve of changes is exactly the same as modelling curve presented on the figure 6.4 (curve of drying process).

Table 6.5 Starch and starch-whey protein (80/20) coating thickness

	S coating (µm)	S-WP coating (µm)	S coating in 80% (µm)	S-WP coating in 80% (µm)
1st layer	6.4 ± 0.1	5.2 ± 0.7	6.4 ± 1.0	5.1 ± 1.0
2nd layer	6.5 ± 0.1	5.5 ± 0.8	6.4 ± 1.3	4.5 ± 0.6
3rd layer	6.4 ± 0.3	5.2 ± 0.4	8.1 ± 2.2	5.3 ± 0.6
3 layers	19.3 ± 0.1	15.9 ± 0.2	20.8 ± 1.0	14.9 ± 0.4

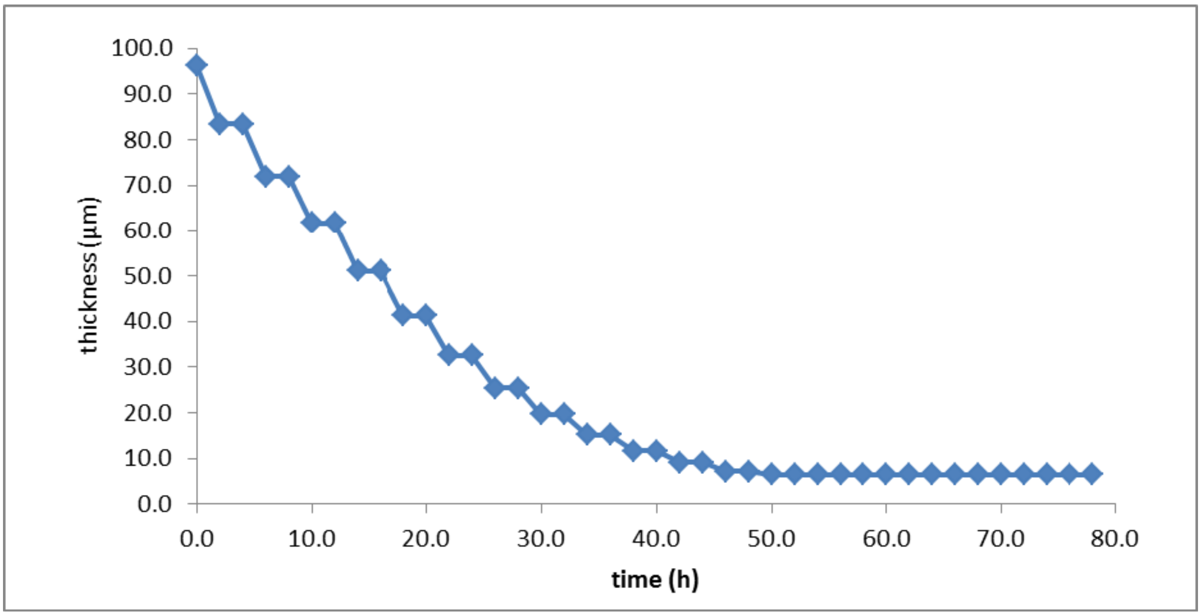


Figure 6.6 Changes of thickness in time directly after coating process

6.2.4. Conclusions

Quantitative detection of coating thickness based on the second Fick's law was conducted. Thermographic camera system linked with logger were used. The basic algorithm has been carried out. Comparing to currently used method the thermographic camera method is very precise and can be used for different surfaces. Obtained results show a uniform distribution of coating material onto the surface. Fruit covered by starch solution are thicker than these ones with protein addition (80/20). The transpiration resistance, resistance of each layer and boundary layer resistance exhibit not significant difference.

6.3. Impact of biodegradability materials on quality of plums (Basiak E., Linke M., Debeaufort F., Lenart A., Geyer M. (2016). Submitted to Postharvest Biology and Technology, July 2016).

6.3.1. Abstract and introduction

Edible starch-based coatings have the positive effect on quality parameters. Plums (Prunus domestca cv. *Jojo*) were divided onto 5 groups: control probe, plums treated with starch and starch-whey protein (80/20) coating materials and plums wrapped in starch and starch-whey protein films. Biodegradable materials, especially coating treatment in no negative manner influence on colour parameters. After 28 days of conducted tests firmness was the highest of starch and starch-whey protein (80/20) films thus of coated materials on not significantly lowest level than of control probe. Transpiration resistance of wrapped and coated plums is higher than untreated fruits, for starch materials (both coating and film) even two times. Respiration resistance is the greatest of plums wrapped in starch-based films and of coated starch films. For bio-packaging materials with whey protein is also higher than for control sample. After conducted trials weight loss of not treated plums was on the level 10%, thus 5% of weight loss was noticed for plums wrapped in starch matrices, around 6% of the other materials. It means customers can consume plums even of 1/3 of their shelf-life longer.

Plums are seasonal fruit with a short postharvest storage life due to acceleration of quality parameters loss such as firmness, balance, colour, total acidity (TA) and total soluble solids (TSS). Method which inhibits fruit ripening by reducing ethylene production, softening, pigment changes, respiration and transpiration rates, increasing in TSS and the reduction in TA is a cold storage (Díaz-Mula et al., 2011; Díaz-Mula et al., 2009). However, cold storage it is not enough to stop the perishable nature of plums and to preserve the fruit quality during transportation, storage and marketing. Therefore additional postharvest technologies as coating and wrapping in biodegradable packages are needed (Díaz-Mula et al., 2011; Valero et al., 2013).

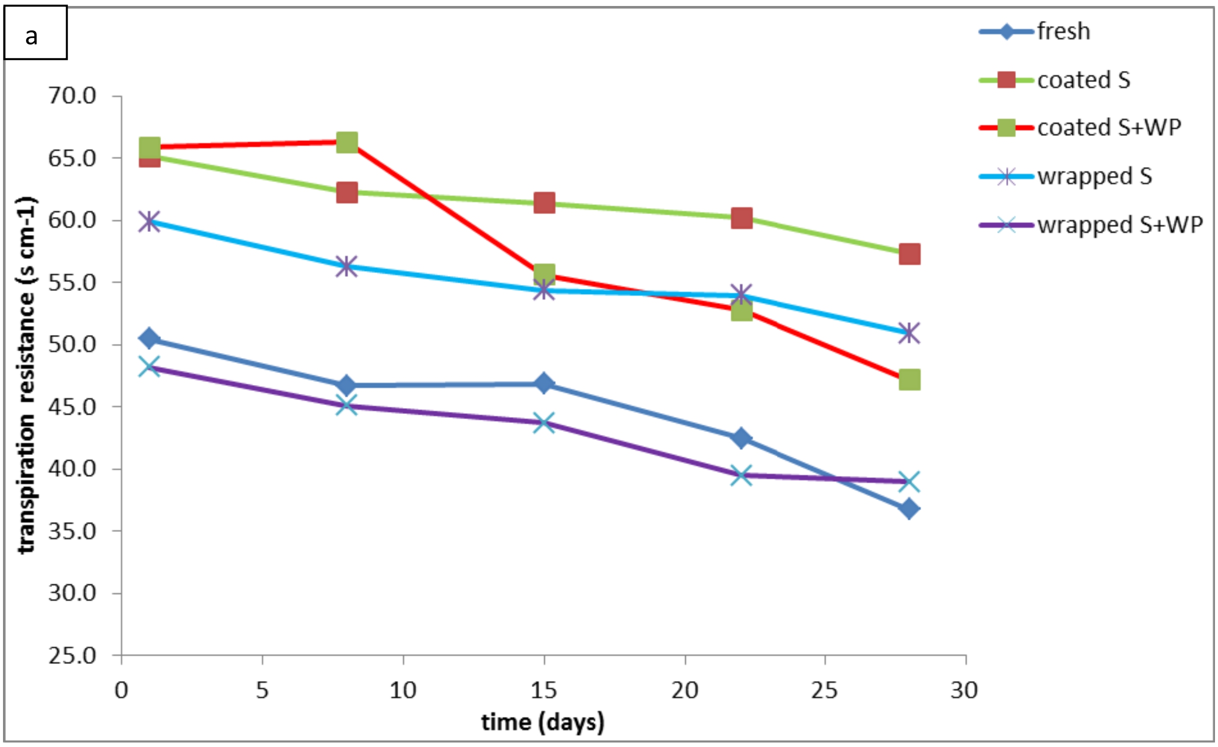
Transpiration is substantially evaporation of water. Temperature and relative humidity strongly have effect on this parameter. Respiration is an exchange of gas with an environment. During this exothermic redox reaction oxygen is absorbed, energy, carbon dioxide and water vapour permeability are released. Instead to the other metabolic processes as photosynthesis respiration takes place all the time. In consequence fruits and vegetables constantly reduce the weight. These kind of changes negatively decrease firmness, influence on titratable acidity, microbiological and other parameters. It is negatively perceived by consumers. Quickly putrefy vegetables and fruits caused not only economical losses, but also of a case developing microbes negatively affects human health (in particular moulds).

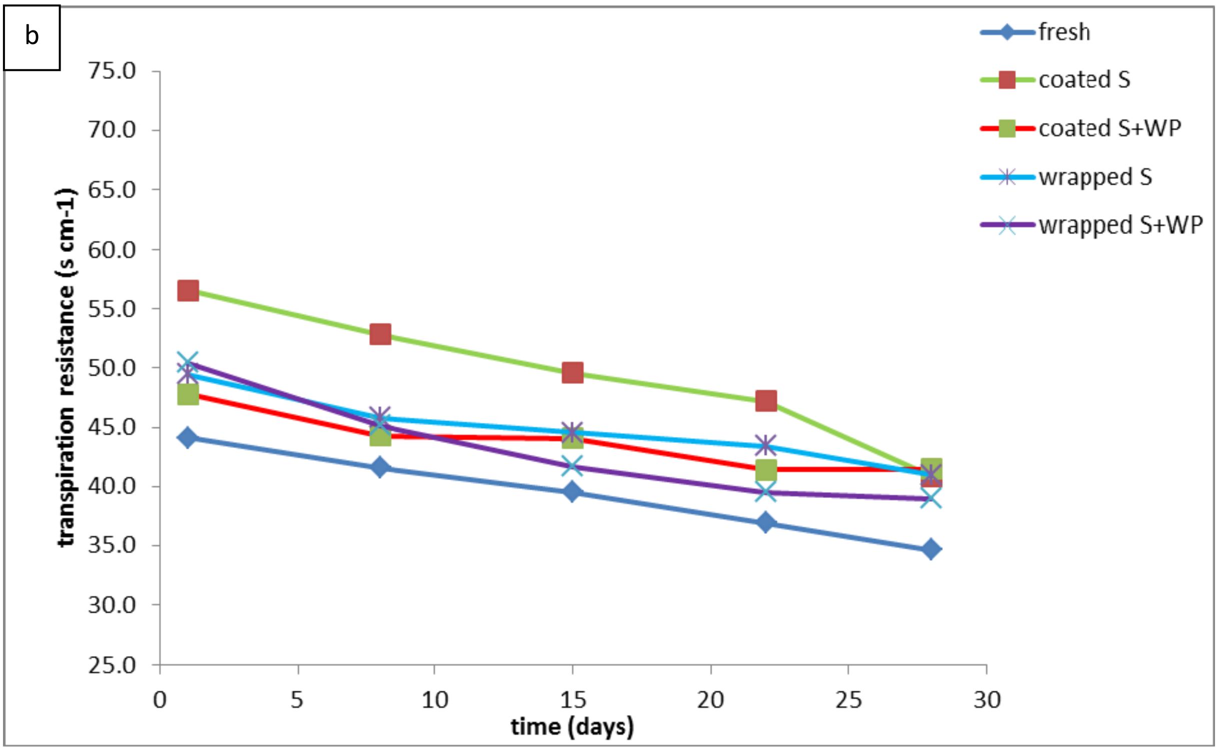
The aim of this work was to limiting mass transfer and therefore increasing the shelf-life of the plums coated made from starch and mixture of starch and whey protein (80-20).

6.3.2. Physiological parameters: respiration, transpiration and mass loss

On the figures 6.7a and 6.7b you are able to see transpiration resistance results of Jojo plum during 28 days of experiment. Figure 6.7a shows the values of fruit where transpiration was measured after few hours from taking samples out from cooling room. Thus figure 6.7b presents transpiration values imitated grocery store conditions. As well as you can see between both storage conditions is a significant difference. Higher temperature of storage decreased respiration resistance, especially in the third and fourth weeks of storage. In case of plum coated by starch suspension the difference is equal almost 25% and ~8% of plums coated by starch-whey protein (80/20) suspension, wrapped by starch film, starch-whey protein (80/20) film and fresh non treated plum after the first 2 weeks of storage. After 4 weeks the difference in the storage condition is following: 29%, 13%, 20%, 0%, 6% for starch coated, starch-whey protein (80/20) coated, starch wrapped, starch-whey protein (80/20) wrapped and fresh plums respectively. The storage temperature has significant meaning especially in case of fruit coated by starch suspension (figures 6.7a,b). Anyway between fresh, coated and wrapped plums in the same temperature of storage meaningful differences were either observed. The highest resistance of transpiration (figure 6.7a) was noticed for coated plums, wherein after one week of storage fruit coated by starch suspension with whey protein addition significantly decreased of 17% and 24% after 4 weeks. Thus starch coatings after one week of storage decreased of 5% and 11% after 28 days. However, comparing to fruit in films and untreated fruit starch and starch-whey protein (80/20) coatings have the highest transpiration resistance. Transpiration rate of fruit wrapped in starch films were comparable to coatings (the same homogenous group). What can be interesting: starch films with 20% addition of proteins significantly decreased resistance of gases exchange. It can be explained by oxygen permeability of starch and starch-whey protein films (80/20). For starch films permeability is equal 81 x 10^{14} ($cm^3m^{-1}s^{-1}Pa^{-1}$) and 117 x 10^{14}($cm^3m^{-1}s^{-1}Pa^{-1}$) for starch films with whey proteins addition (80/20). So, higher permeability increases water movement from fruit to environment. Thus thickness of both kinds of films is comparable: 80.8µm and 79.7µm for starch and starch/whey protein (80/20) films respectively. Very similar results as for plum packaged in starch films were obtained for fresh fruit, but during the last week of observation transpiration resistant significantly decreased of almost 9%. In turn in greengrocer store conditions the lowest resistance was observed for fresh fruit. Plum packaged in starch-whey protein (80/20) systems had similar resistance as plums coated by starch/whey protein material. Thus starch-based films and coatings had the highest resistance of transpiration. Independently on storage temperature these materials have supreme transpiration resistance for fruits and vegetables, even with very high water content.

Anyway, the gradient of water vapour pressure between the plum and the environment conditions determined transpiration process. Cuticle and epidermal cell layer usually reduced transpiration. Hence, different plum varieties have different surface, volume, cuticle structure and epidermis. Thus comparing physiological parameters as transpiration with different varieties should not contain obtained values but only fruit behaviour. Moreover biodegradable coatings and films act as a layer which covers the stomata leading to diminish in transpiration rate. Thus coatings and films can be used for different fruits and vegetables varieties (Valero et al., 2013).

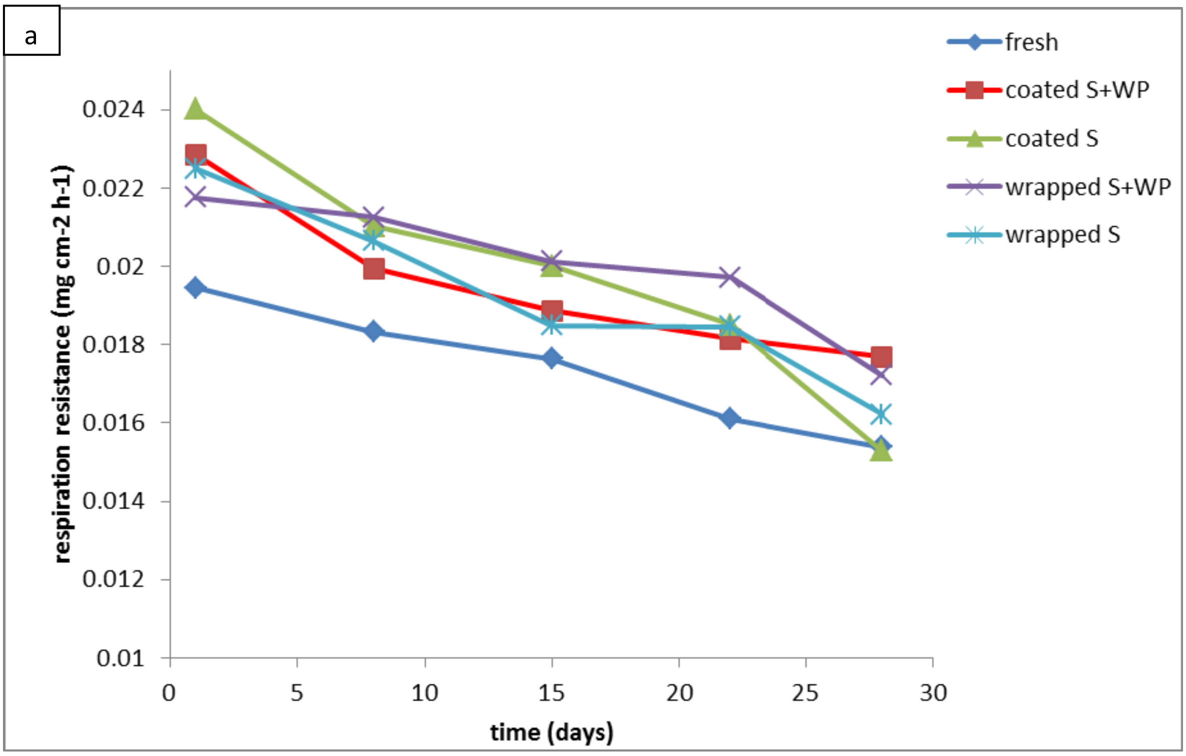


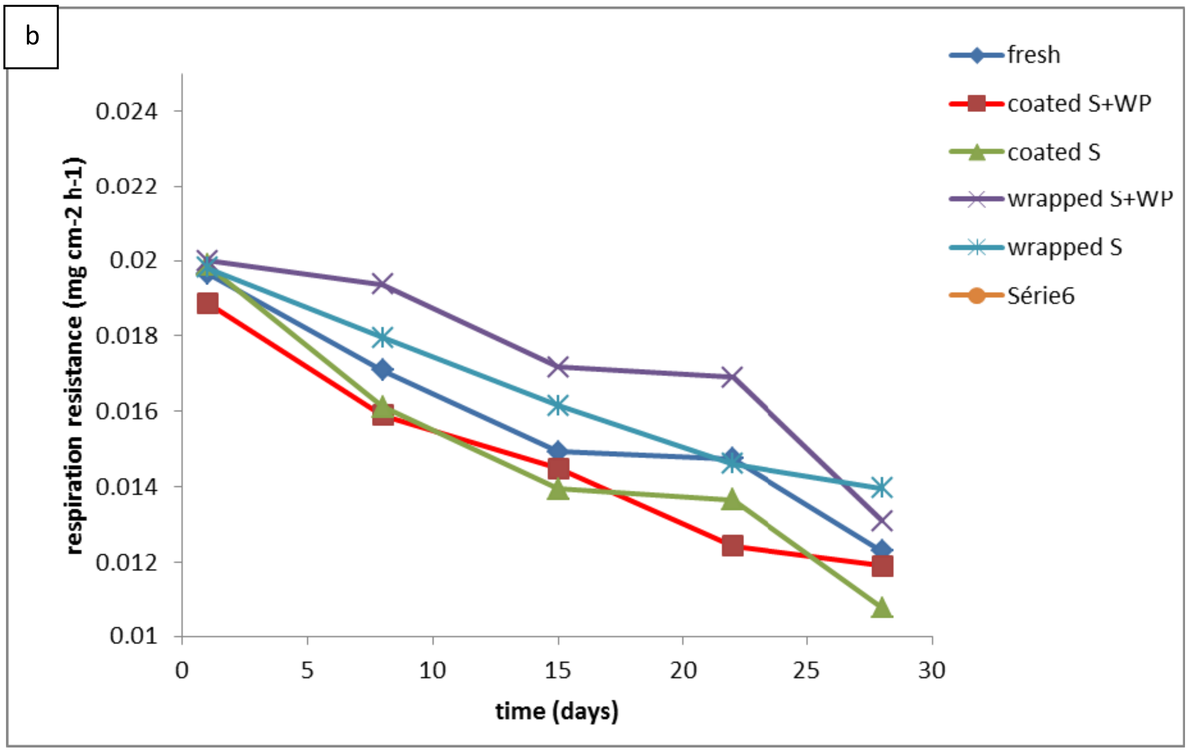


Figures 6.7a,b Transpiration resistance of fresh, coated in starch suspension, coated in starch/whey protein suspension (80/20), wrapped in starch film and wrapped in starch/whey protein (80/20) film plums in 3.5°C and 22°C, respectively.

Respiration resistances of plums moved from cooling room the same day and for plum kept in conditions similar to grocery store are presented on the figures 6.8a and 6.8b, respectively. Respiration resistance of plums stored in cooling room shows the significant impact of biodegradable materials (both films as coatings) on gas changing reduction. It means wrapped and coated plums in starch and starch-whey protein (80/20) materials can be held longer than fresh fruit. Anyway, the highest respiration resistance in the first week was noticed for starch coated plum, but in the third week this value radically decreased. Very similar behaviour was observed for wrapped in starch films fruit. The most stable respiration resistance was for plum stored on trays wrapped in starch-whey protein (80/20) films. Results obtained for fruit kept in greengrocer conditions relevant lower respiration resistance of all samples, especially of coated plum. When the temperature of environment is higher the higher amount of ethylene is produced because the metabolic processes increase, the demand of energy is higher and respiration rate rises up. This accelerates the ripening of fruit and thereby the higher amount of produced gases. However, plum stored on plastic trays and wrapped by starch and starch-whey protein (80/20) films had the highest respiration resistance. When fruits are kept in a plastic box with permeable film cover they produce carbon dioxide and they absorb oxygen.

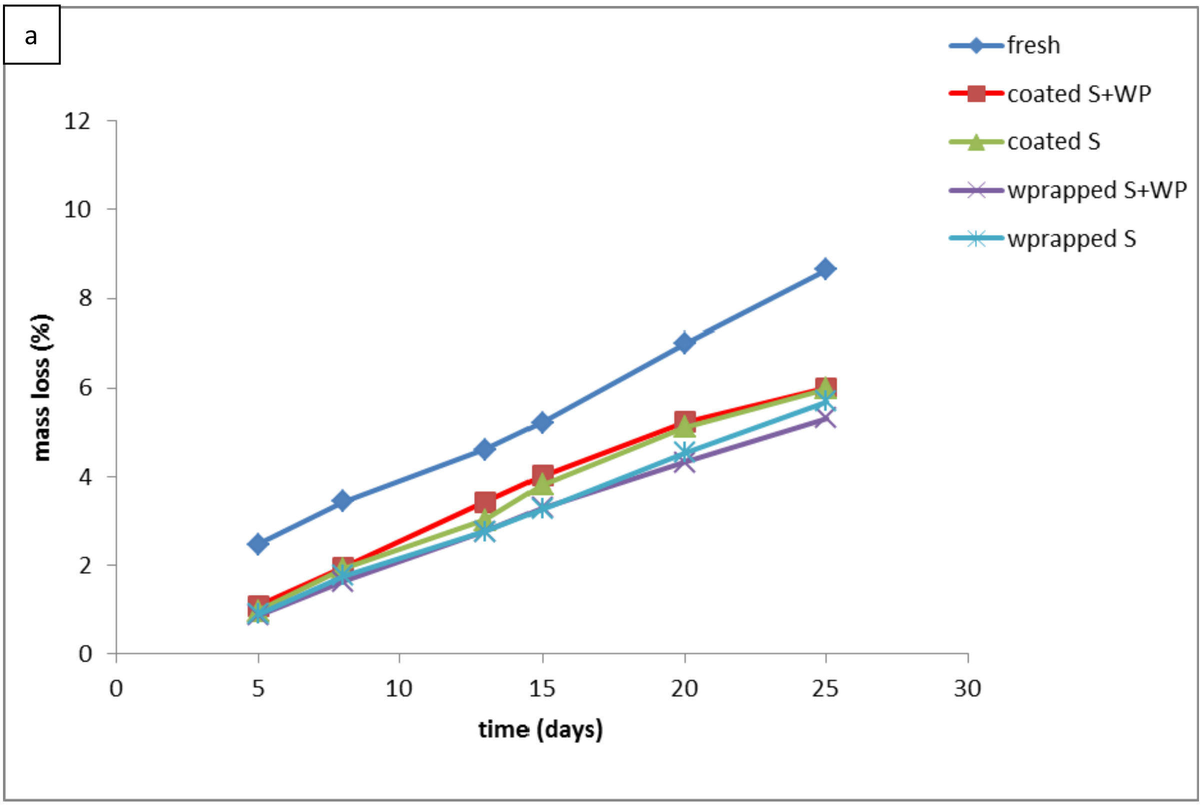
Emitted ethylene reacts with condensate water which comes from outside (biodegradable films are permeable for water vapour permeability). The concentration of atmosphere gases is controlled. In consequence carbon dioxide is emitted, the production of oxygen is reduced and transpiration rate is reduced either. Thus, between fruit surface and coating material does not exists any free spare, so the exchanging of gases is reduced, because starch and starch whey protein (80/20) coatings have very high barrier of oxygen. Also carbon dioxide is emitted lower than in case of fruit without coating layer. Hence ethylene production it is not at any way blocked, thus respiration rate in covered fruit is higher. These data has confirmation in literature. Choi et al. (2016) packaged plum *Prunus salicina* L. variety Formosa in edible films containing essential oils in hydroxypropyl methylcellulose. After 14 days of storage in room conditions respiration of fresh fruit was 15.41 mL CO_2 kg^{-1} h^{-1}, of wrapped fruit was 6.88 mL CO_2 kg^{-1} h^{-1} and in 5°C and 7.06 mL CO_2 kg^{-1} h^{-1} for fresh samples and 2.91 mL CO_2 kg^{-1} h^{-1} for wrapped plums.

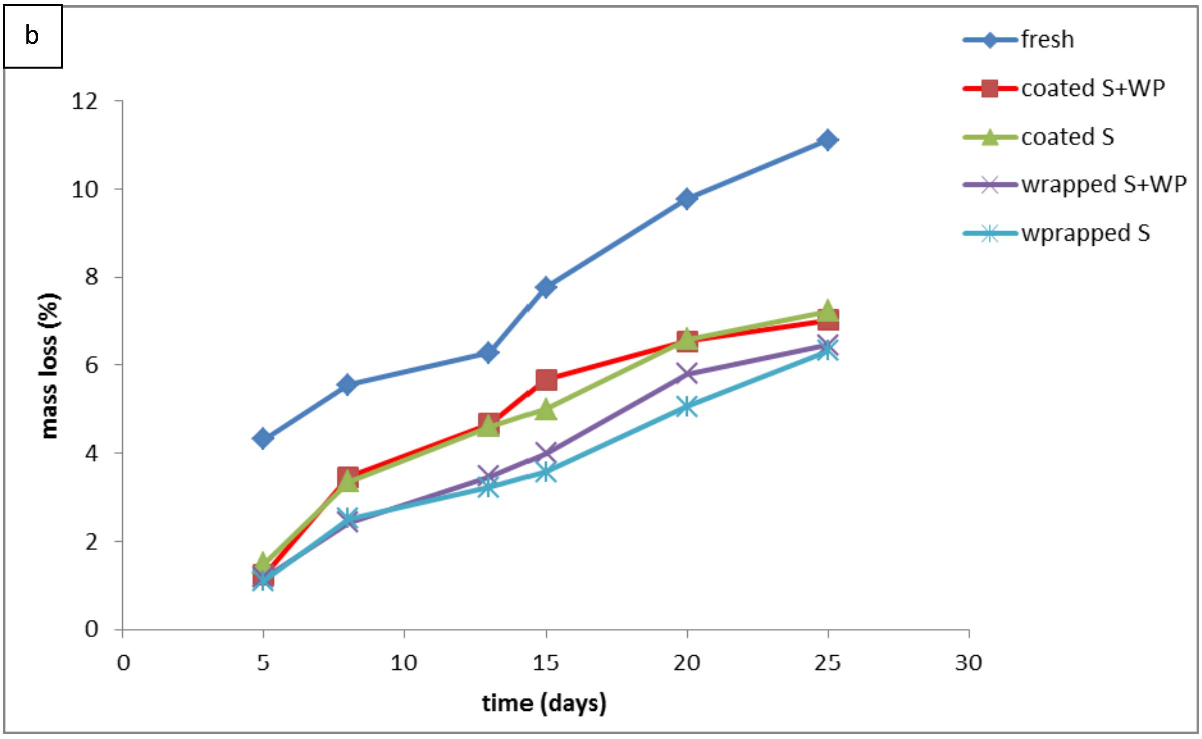




Figures 6.8a,b Respiration resistance of fresh, coated in starch suspension, coated in starch/whey protein suspension (80/20), wrapped in starch film and wrapped in starch/whey protein (80/20) film plums in 3.5°C and 22°C, respectively.

During respiration and transpiration processes comes to exchange of gases and water. Fruit lost part of their weight. These mass loss changes are presented on figures 6.9a for plums stored in 3.5°C and 35%RH and 6.9b for plums stored in 22°C and 35%RH. As well as you can compare both figures mass loss depends on factors as temperature. Higher temperature caused emission of higher amounts of energy. Metabolic processes are faster hence the mass loss is higher. Independently from the storage temperature fresh fruits lost the highest amount of mass. Valero et al. (2013) worked with 4 varieties of European plums Prunus salicina L. They noticed that after 35 days of storage in 2°C mass loss of Blackamber variety sample was of 10.8% reduced, of coated in alginate solution of 5.8%, thus in 20°C of 16.2% and 10.2% respectively. However, coating process significantly decreased weight loss for all plum cultivars. Brasil et al. (2012), Chien, Sheu, & Yang (2007), Chiumarelli & Hubinger (2014), Das et al. (2013), Gol, Patel, & Rao (2013), Guerra et al. (2016), Hong et al. (2012), Poverenov et al. (2014) and others covered tomatoes, papayas, mangoes, apples, strawberries, grapes, guavas, red bell peppers, respectively, in different coating materials. Obtained results showed the coated fruits and vegetables had lower mass loss than uncoated. The lowest losses of plum mass are noticed for both kinds of films. It has confirmation in transpiration and respiration results. For biodegradable matrices respiration and transpiration resistance are the lowest, so mass loss is the lowest as well. After 25 days of storage the weight loss of fruit was 1.8 and 1.6 times lower for wrapped fruits and 1.6 and 1.5 times lower for coated fruit than for fresh fruit in room and cooling room conditions, respectively. Fail et al. (2016), Mistriotis et al. (2016), Rux et al. (2015), Sousa-Gallagher et al. (2013), Vázquez-Celestino et al. (2016) packaged carrots, cherry, tomatoes and peach, mushrooms, strawberries and mangoes respectively, in plastic boxes and wrapped by different biodegradable films. Received values also show the wrapped vegetables and fruits have several percent lower mass loss than unpackaged.





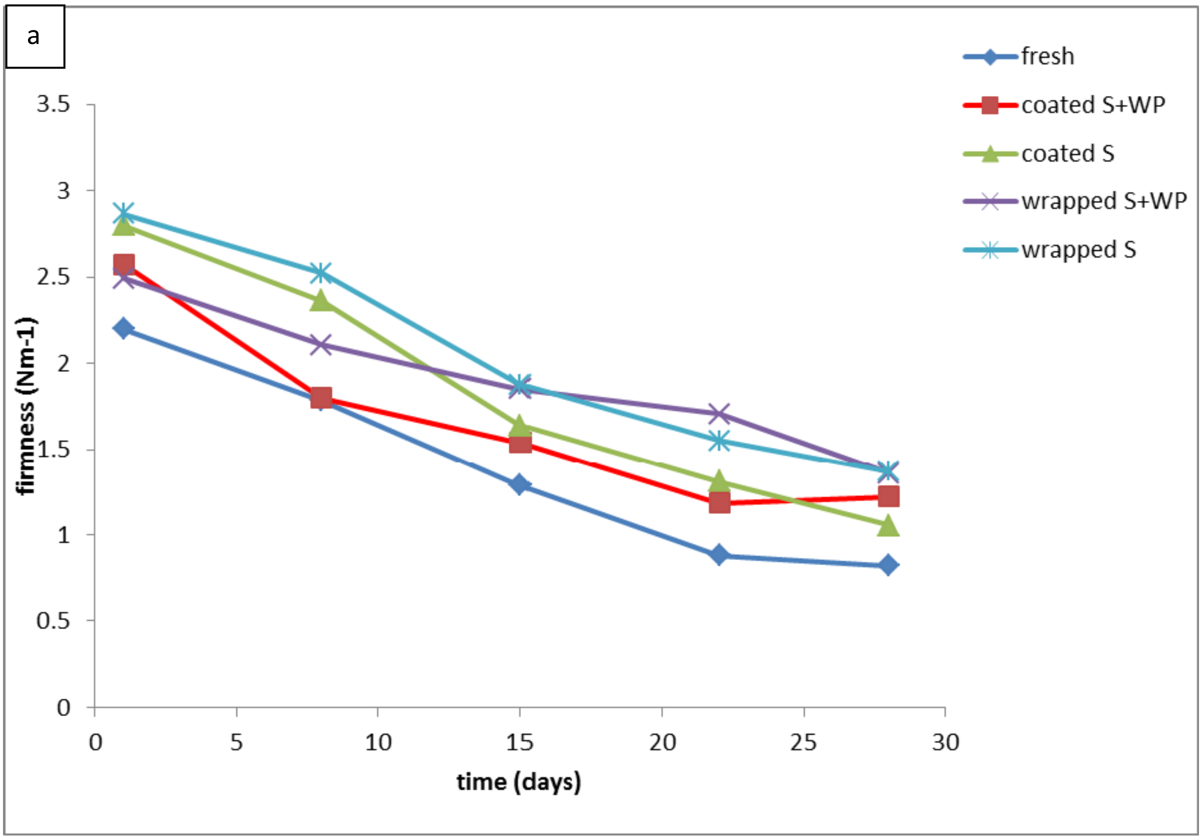
Figures 6.9a,b Mass loss of fresh, coated in starch suspension, coated in starch/whey protein suspension (80/20), wrapped in starch film and wrapped in starch/whey protein (80/20) film plums in 3.5°C and 22°C, respectively.

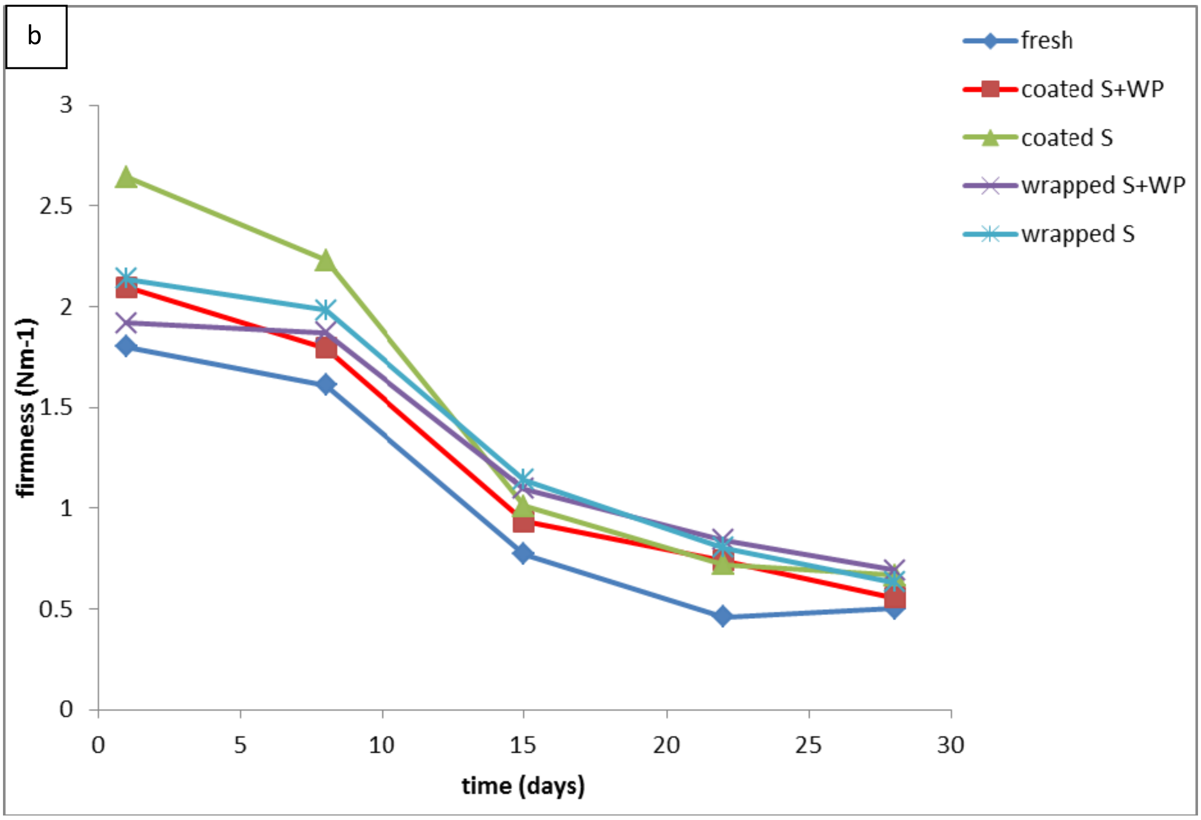
6.3.3. Firmness

One of the crucial factors affecting fruits and vegetables shelf life is flesh firmness, which is directly related to postharvest products ripeness (Valero et al., 2013). Firmness of plums stored in 3.5°C and 22°C are presented on the figures 6.10a and 6.10b respectively. In the first two weeks of storage the highest firmness was noticed of wrapped by starch material fruit and of plum coated by starch based solution in both temperatures. After the next two weeks the firmness was the highest of wrapped in starch-whey protein (80/20) films. During the whole storage process the lowest terseness was admitted of fresh plum. Independently of storage temperature fresh fruit loss the firmness the fastest.

Firmness depends on factors as fruit maturity, harvesting time and variety of fruits, so values in literature for different kinds of plums in different maturity stage are not comparable with these ones presented in this study, but the trend is the same (Mukhtar et al., 2014).

Results demonstrated by Hussain et al. (2015) show the coating process significantly prolong firmness of agriculture products. Prunus domestica L. cv. *Santa Rosa* covered by carboxymethyl cellulose coatings after 12 days of storage had firmness even 3 times higher than fresh fruit. Liu et al. (2014) worked with plums Prunus salicina L. cv. *Sanhuali*. Part of fruit were kept 20 days in plastic baskets wrapped by chitosan films, part was coated by chitosan solution. After 3 weeks of storage in 5°C and 90% of RH firmness of fresh fruit decreased more than 5 times, thus firmness of coated and wrapped Japanese plums were 2 and 2.5 lower for treated plums. Also Choi et al. (2016) revealed the plum Prunus salicina L. cv. *Formosa* coated by hydroxypropyl methylcellulose with essential oil addition have higher firmness in storage and in room conditions. After 14 days of storage in 23°C firmness of fresh plum were approximately 5.33 Nm^{-1} and 9.58 Nm^{-1} for HPMC coating with essential oil addition. Results obtained after 14 days of storage in 5°C showed higher value of firmness than in room conditions. In 5°C respiration is lower so mas and water losses are lower as well. Firmness for control plums in 5°C was 11.68 Nm^{-1} and 15.45 Nm^{-1} for HPMC coating.





Figures 6.10a,b Firmness of fresh, coated in starch suspension, coated in starch/whey protein suspension (80/20), wrapped in starch film and wrapped in starch/whey protein (80/20) film plums in 3.5°C and 22°C, respectively.

6.3.4. Titratable acidity and total soluble solid

The effect of coating and wrapping on titratable acidity (TA) and total soluble solid (TSS) of plum kept in 3.5°C and 22°C is given in table 6.5. Titratable acidity decreased according the storage time. Values obtained in the same week of measurements did not affect significant difference nor in 3.5°C neither in 22°C. Valero et al. (2013) coated 4 varieties of European plums in alginate solution. Presented results showed the titratable acidity depending on plum cultivar. Anyway, edible coatings based on alginate delayed titratable acidity decrease in Blacamber, Lary Ann, Golden Globe and Songold varieties. Díaz-Mula et al. (2011) presented similar trend of yellow and purple European plum cultivars wrapped in MAP packages.

Table 6.5 Titratable acidity and total soluble solid of fresh, coated in starch suspension, coated in starch/whey protein suspension (80/20), wrapped in starch film and wrapped in starch/whey protein (80/20) film plums in 3.5°C and 22°C, respectively.

time (days)		1	8	15	22	28
TA (g/100mL) in 3.5°C	control	0.75 ± 0.03^{c}	0.69 ± 0.01^{c}	0.68 ± 0.02^{c}	0.62 ± 0.01^{b}	0.64 ± 0.01^{b}
	coated S+WP	0.74 ± 0.02^{c}	0.70 ± 0.03^{c}	0.69 ± 0.02^{c}	0.68 ± 0.03^{c}	0.63 ± 0.01^{b}
	coated S	0.69 ± 0.03^{c}	$0.66 \pm 0.03^{b.c}$	0.64 ± 0.03^{b}	0.61 ± 0.02^{b}	0.60 ± 0.01^{b}
	wrapped S+WP	0.71 ± 0.03^{c}	0.68 ± 0.02^{c}	0.63 ± 0.04^{b}	0.62 ± 0.02^{b}	0.58 ± 0.01^{a}
	wrapped S	0.69 ± 0.02^{c}	0.67 ± 0.03^{c}	$0.65 \pm 0.03^{b.c}$	0.61 ± 0.02^{b}	$0.59 \pm 0.02^{a.b}$
TA (g/100mL) in 22°C	control	0.76 ± 0.04^{c}	0.72 ± 0.03^{c}	0.69 ± 0.01^{c}	0.67 ± 0.02^{b}	$0.62\pm 0.01^{a.b}$
	coated S+WP	0.76 ± 0.04^{c}	0.73 ± 0.02^{c}	0.71 ± 0.03^{c}	0.65 ± 0.02^{b}	0.59 ± 0.01^{a}
	coated S	0.72 ± 0.02^{c}	$0.68 \pm 0.03^{b.c}$	0.66 ± 0.03^{b}	$0.62 \pm 0.03^{a.b}$	0.58 ± 0.01^{a}
	wrapped S+WP	0.73 ± 0.03^{c}	$0.70 \pm 0.03^{b.c}$	0.67 ± 0.03^{b}	0.64 ± 0.02^{b}	$0.61 \pm 0.02^{a.b}$
	wrapped S	0.75 ± 0.03^{c}	$0.69 \pm 0.04^{b.c}$	0.66 ± 0.02^{b}	0.64 ± 0.03^{b}	$0.60 \pm 0.03^{a.b}$
TSS (°Bx) in 3.5°C	control	15.82 ± 0.34^{c}	18.15 ± 0.27^{e}	20.16 ± 0.21^{f}	20.70 ± 0.20^{f}	20.40 ± 0.15^{f}
	coated S+WP	12.50 ± 0.25^{a}	15.01 ± 0.15^{c}	18.55 ± 0.27^{e}	17.92 ± 0.29^{e}	18.23 ± 0.19^{e}
	coated S	15.70 ± 0.26^{c}	15.02 ± 0.12^{c}	15.64 ± 0.14^{d}	14.88 ± 0.22^{c}	15.84 ± 0.23^{d}
	wrapped S+WP	12.81 ± 0.21^{a}	14.10 ± 0.19^{b}	18.29 ± 0.32^{e}	13.07 ± 0.22^{a}	16.03 ± 0.17^{d}
	wrapped S	12.81 ± 0.32^{a}	14.00 ± 0.17^{b}	$15.13 \pm0.26^{c.d}$	14.54 ± 0.26^{b}	15.00 ± 0.12^{c}
TSS (°Bx) in 22°C	control	15.07 ± 0.36^{b}	19.20 ± 0.41^{e}	21.91 ± 0.50^{g}	20.81 ± 0.32^{f}	21.15 ± 0.28^{g}
	coated S+WP	12.50 ± 0.24^{a}	18.18 ± 0.31^{d}	19.42 ± 0.19^{e}	20.70 ± 0.21^{f}	20.34 ± 0.29^{f}
	coated S	13.05 ± 0.35^{a}	19.05 ± 0.35^{e}	18.92 ± 0.24^{e}	19.01 ± 032^{e}	20.10 ± 0.27^{f}
	wrapped S+WP	15.19 ± 0.22^{b}	16.16 ± 0.31^{c}	16.57 ± 0.18^{c}	15.28 ± 0.23^{b}	16.30 ± 0.26^{c}
	wrapped S	18.09 ± 0.40^{d}	16.25 ± 0.28^{c}	18.34 ± 0.19^{d}	18.22 ± 0.16^{d}	18.20 ± 0.21^{d}

In both temperatures total soluble solid increased faster in fresh fruit. This means that untreated plum ripened quicker than coated and wrapped fruits. Changes of wrapped fruits are lower than of coated, but the trend during the storage time was increased. However, comparing to fresh fruit starch and starch-whey protein (20/20) coatings and films significantly delayed the rising in total soluble solid. It means using biodegradable films for packaging fresh material can keep the total soluble solid longer, what is better received by consumers. The same wrapping and coating processes also prolongate the shelf-life of storage postharvest crops. These data are in agree with results received by Liu et al. (2014) of plum Prunus salicina L. cv. *Sanhuali* coated and packaged in chitosan solution and films, respectively and with data published by Choi et al. (2016) for plum Prunus salicina L. cv. *Formosa* covered in hydroxypropyl methylcellulose. Authors obtained the same rising trend with values from around 14 to 16 °Bx in the first two weeks of storage at 23°C.

6.3.5. Surface colour

Surface colour changes of plums were observed following in the fresh, coated and wrapped plums. Obtained results are presented in table 6.6 only for fruit storage in 3.5°C. In higher temperature (22°C) no significant differences were observed. Significant differences were admitted between fresh and coated and wrapped plums. During the storage time fruit colour changed from violet to navy violet. Coating materials and films delayed this process. After 28 days of storage time *L* parameter decreased of 18% in fresh plum and in coated in starch and starch-whey protein (80/20) plums and wrapped in starch and starch-whey protein (80/20) plums only of half of this value, it is of 9%. In *a* and *b* parameters no significant changes were noticed. Anyway, coated and wrapped fruits showed slower rates of change in peels of colour than control plum. Choi et al. (2016), Liu et al. (2014), Valero et al. (2013) obtained similar results for coated and wrapped European and Japanese varieties of plums.

Table 6.6 Colour parameters of fresh, coated in starch suspension, coated in starch/whey protein suspension (80/20), wrapped in starch film and wrapped in starch/whey protein (80/20) film plums in 3.5^{o}C and 22^{o}C, respectively.

Time	Parameter	control	coated S	coated S+WP	wrapped S	wrapped S+WP	film S	film S+WP
1st day	L	38.82 ± 1.23^{c}	40.01 ± 1.51^{d}	40.43 ± 1.60^{d}	39.91 ± 1.32^{c,d}	42.34 ± 1.22^{e}	95.47 ± 0.34^{h}	94.75 ± 0.50^{g}
	a	13.06 ± 3.02^{b}	13.41 ± 2.01^{b}	11.92 ± 1.02^{a}	12.59 ± 1.34^{a}	11.99 ± 1.87^{a}	0.24 ± 0.06^{b}	0.55 ± 0.11^{b}
	b	6.99 ± 0.76^{c}	5.87 ± 0.98^{c}	6.29 ± 1.89^{c}	7.08 ± 1.57^{c}	6.91 ± 1.78^{c}	3.18 ± 0.28^{b}	4.27 ± 0.75^{b}
	ΔE	-	1.67 ± 0.52^{c,d}	2.09 ± 0.14^{d}	1.19 ± 0.32^{c}	3.68 ± 1.14^{e}	1.46 ± 0.43^{c}	2.78 ± 0.73^{d}
8th day	L	37.02 ± 1.03^{b}	38.11 ± 1.36^{c}	39.42 ± 1.32^{c,d}	38.22 ± 1.46^{c}	40.94 ± 1.87^{d}	95.12 ± 0.14^{h}	94.05 ± 0.41^{g}
	a	12.74 ± 2.75^{a,b}	13.01 ± 1.71b	11.24 ± 1.74^{a}	12.08 ± 1.85^{a}	11.31 ± 1.48^{a}	0.23 ± 0.05^{a}	0.49 ± 0.90^{b}
	b	6.66 ± 0.73^{c}	5.67 ± 1.49^{c}	6.00 ± 1.17^{c}	6.88 ± 1.07^{c}	6.76 ± 2.02^{c}	2.98 ± 0.17^{a,b}	4.03 ± 0.82^{b}
	ΔE	-	1.50 ± 0.03^{c}	2.91 ± 0.21^{d}	1.39 ± 0.26^{c}	4.17 ± 0.44^{f}	0.40 ± 0.22^{a}	0.74 ± 0.18^{b}
15th day	L	36.01 ± 1.17^{b}	39.00 ± 1.39^{c,d}	38.06 ± 1.06^{c}	38.97 ± 1.85^{c}	39.21 ± 1.65^{c,d}	94.97 ± 0.19^{g}	93.33 ± 0.43f,^{g}
	a	12.39 ± 3.20^{a,b}	12.97 ± 1.58^{a,b}	10.75 ± 1.36^{a}	11.59 ± 1.34^{a}	11.01 ± 1.74^{a}	0.22 ± 0.13^{a}	0.43 ± 0.10^{b}
	b	6.39 ± 0.51^{c}	5.39 ± 0.54^{c}	5.87 ± 1.52^{c}	6.35 ± 1.79^{c}	6.41 ± 1.48^{c}	2.49 ± 0.15^{a}	3.69 ± 0.74^{b}
	ΔE	-	3.12 ± 0.15^{e}	2.01 ± 0.14^{d}	1.25 ± 0.26^{b}	3.48 ± 0.41^{e}	0.84 ± 0.13^{b}	1.54 ± 0.24^{c}
28th day	L	34.36 ± 1.11^{a}	36.74 ± 1.12^{a,b}	36.98 ± 1.41^{b}	35.92 ± 1.51^{a,b}	38.11 ± 1.21^{c}	94.12 ± 0.45^{g}	92.82 ± 0.37^{f}
	a	12.11 ± 2.45^{a,b}	12.72 ± 3.00^{a,b}	10.14 ± 1.51^{a}	11.47 ± 1.36^{a}	10.82 ± 2.02^{a}	0.21 ± 0.25^{a}	0.41 ± 0.23^{b}
	b	6.01 ± 0.41^{c}	5.20 ± 0.78^{c}	5.69 ± 1.76^{c}	6.14 ± 1.37^{c}	6.01 ± 1.52^{c}	2.13 ± 0.78^{a}	3.44 ± 0.56^{b}
	ΔE	-	2.59 ± 0.23^{d}	3.29 ± 0.54^{e}	1.69 ± 0.48^{c,d}	3.96 ± 1.22^{e,f}	1.71 ± 1.22^{c}	2.10 ± 0.95^{c,d}

6.3.6. Conclusions

European plum Prunus domestica cv. *Jojo* were covered and wrapped in coatings and films made from starch and starch-whey protein (80/20) suspensions. Fresh and packaged samples were storaged 28 days in 3.5ºC. Every week physiological tests were done in cooling and room temperature. Comparing to control sample in plums covered and wrapped in films delayed changes in colour parameters were observed. Moreover, total soluble solid increased lower as well in treated fruits. Thus titratable acidity increased according to storage time in all conducted samples. Transpiration resistance of wrapped and coated plums is higher than untreated fruits, even two times. The highest respiration resistance is noticed for fruit wrapped in films, for coating materials is either high but only in cooling room temperature conditions. Mass loss of treated fruits is even 1.8 times higher of wrapped plums and 1.6 times higher of coated samples. Fruits packaged in starch-based biomaterials can be storage even 1/3 of their shelf-life longer.

GENERAL CONCLUSIONS

and

PERSPECTIVES

7. GENERAL CONCLUSIONS

Up to the eighteenth century, the food products were carried and stored in natural raw materials. With the appearance of industrial revolution, these habits have been changed. Few decades later with the advent of plastic, a lot of food products were wrapped, packaged, conditioned in various plastic polymer materials such as low density polyethylene, polyethylene terephthalate to satisfy consumer new habits (figure 7.1).

Figure 7.1 Food packages nowadays.

During the last ten years, the amount of plastic material production was higher than in the whole period since plastic packaging materials were invented. Crude oil products are convenient for users, relatively not expensive. Crude oil decay, increasing price and very long decomposition time (up to 1 millenary) that induces visible pollution and environmental disturbances, are the main drawbacks. Although the very first plastic materials invented were biodegradable (Cellophan® from cellulose and Bakelite® from milk proteins), it appears the need of reducing plastic materials by producing new biodegradable packages. These ecological friendly materials can be applied onto food (as coatings) or used separately (as films). Decomposition time of these new materials should be even several magnitude orders shorter than for plastic, which can help in reduction of troubles caused by plastic materials.

Many bio-sourced raw-materials can be envisaged for the production of biodegradable packaging. The most commonly used materials are polysaccharides, then proteins and fats; which the most suitable, abundant, renewable and low-cost are starches, particularly from potato, corn and wheat. That's why we selected these 3 types of starch to make films and coatings for application onto fruits.

Films were thus prepared from corn starch (27% amylose), wheat starch (25% amylose) and potato starch (20% amylose) with relatively low glycerol content (30% to the starch basis) to better display the effect of starch nature. Amylose/ amylopectin ratio of each starch tested was considered for the explanation of observed film behaviours. This ratio strongly influenced the microstructure because the viscosity of the film-forming suspension increased with amylopectin content affected the network retraction during the film drying and then the final thickness. Indeed, the corn-starch films were the more viscous and the thickest for a same amount of dry matter per surface unit deposit in Petri dishes. The film colour and transparency also depended on the thickness. Indeed, the thinnest potato starch films were transparent, while corn and wheat starch matrices are more white and opaque. The amylose/amylopectin ratio also influences the hydration characteristic. The lowest solubility in water, water vapour and oxygen permeabilities were obtained for the potato starch films and wheat starch films corresponding to the lowest amylopectin content. Surface and mechanical properties are also improved. The lower the amylose content was the greater were the barrier, the surface resistance to moisture absorption and the mechanical properties. In case of corn and potato starch-based films, the swelling of surface (figure 7.2) and high moisture sensitivity were observed. This suggested a limitation for the application for products with high water content or storage in high relative humidity. This first study on starch-based films demonstrated the better suitability of the wheat starch for producing edible films and coatings. However, the properties of wheat starch films did not completely comply with the requirements for applications. Hence, some strategies dealing with starch and glycerol content optimisations, protein addition, and rapeseed oil lamination onto starch layer or between starch layers have been envisaged prior validation onto plums.

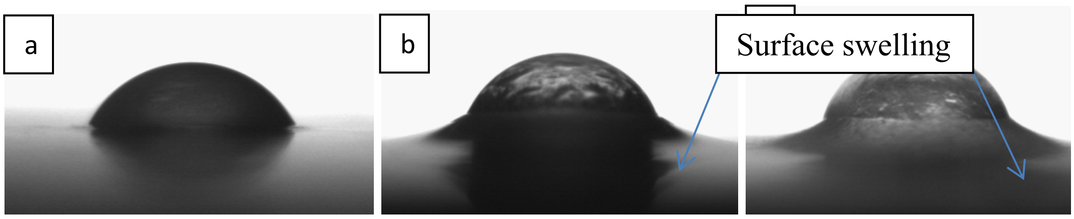


Figure 7.2 Starch surface after 2 minutes of contact with water drop: a) wheat starch, b) corn starch, c) potato starch.

Starch-based films were then produced in several concentrations of starch. Film forming solutions containing less than 3% of starch did not allowed making films with expected properties. Films were too thin and too brittle, with a lot of defects such as cracks and holes. The optimized content of 5% starch in the film forming solution was determined from resulting thickness, mechanical and barrier properties.

Glycerol content and moisture level strongly influenced all the film structure and properties. Wettability, water content, swelling index, solubility in water, water vapour and oxygen permeabilities increased with the glycerol content. The overall surface tension and its polar component also rose whereas the dispersive component of the surface tension, the works of adhesion and cohesion diminished for the highly plasticized systems. The moisture diffusivity and the permeabilities to gases and water vapour were twice times reduced in 30% of glycerol films compared to those of the films containing 50% of glycerol. The lower the glycerol content was, the lower the plasticity and the higher the Young modulus and the tensile strength tend to be. However, the increase from 30% to 50% glycerol content to starch dry basis did not changed significantly the mechanical properties in moderate relative humidity (50%RH). The effect of glycerol content might be exacerbated at higher relative humidities as suggest from the sorption isotherms that displayed a greater sorption of moisture in 50% glycerol films at water activities higher than 0.8. In such conditions, the cumulative effect of water and glycerol plasticization would strongly deteriorate the mechanical properties of films containing the higher glycerol concentration. Finally, films and coatings with the lower plasticizer content can be used preferentially for high water activity products without losing their mechanical properties as water may act as plasticizer instead of glycerol.

Starch-based films have weak networks that could be strengthen by mixing with other biopolymers able to interact with amylose or amylopectin chains. Proteins, because of their multiple chemical groups and amino acids, are able to establish various types of bonds and interactions. Moreover, they can provide a better nutritional value to starch coatings.

Thus whey protein isolate was added to starch in film forming suspension at ratios from 20% to 100% (to dry biopolymer content) by 20% steps. Whey protein addition allowed to make film for all ratios tested, in most of the case with better optical properties and visual appearance. The less the starch content, the more homogenous and dense the films were. The addition of whey protein allowed reducing the surface wettability by increasing the water contact angle from about 43 up to 93° according the protein content. Starch films, whey protein films and 80S-20WP mixture films were less hydrophilic as they had higher dispersive component of the surface energy than other blends. However, even if the film surface hydrophilicity was reduced by protein presence, the moisture sorption isotherms were not modified. Interactions involved between starch and whey proteins modified the surface properties, probably with preferential orientation of hydrophobic amino-acids toward the air during the film drying. The protein addition at all ratios, also contributed to slightly reduce water vapour permeability of starch films, whatever the RH gradient used. The physical-chemical interactions involved explain the increase in density of the film structure and thus slowed the water mobility in film. Whey proteins also contributed to limit the absorption of typical flavour compounds of fruits and vegetables. This was interesting information to keep in fruits their aromas when coated with starch-whey proteins suspensions.

However, the reduction of moisture sensitivity is still required, even with controlled concentration of glycerol or addition of whey proteins. Then, films were formulated with oil.

Rapeseed oil is a common vegetal oil having a good ratio of Ω3 to Ω6 (around 1:1) triacyl-glycerols and also containing healthy sterols. It is odourless and cheap; that's why it was selected. From literature analysis, it appeared that a dispersion of oil in biopolymer matrix does not significantly improve the film moisture barrier efficiency or water sensitivity. Thus, the starch films in presence of rapeseed oil have been prepared as multilayer systems, by applying an oil layer (at different amounts) onto a first starch layer and then covered by a second starch-based film forming layer. However, after film drying, the microstructure observations by ESEM displayed that films did not have a multilayered structure as expected from the 3layers step process, but they look as emulsions. Indeed, the rapeseed oil formed large droplets at the film surface, disturb the starch network organisation favouring a stronger retraction inducing a twice reduced thickness. Oil addition induced thus a denser structure without modifying so much the film colour or appearance. Surface wettability was also reduced by increasing the dispersive component of the surface tension and water contact angle from about 40° to 70° and thus reducing the polar component of the surface properties. Oil addition did not alter significantly the mechanical properties of films. However, as expected, both the water solubility and the moisture sorption were diminished proportionally to the oil content. Increasing the film hydrophobicity and decreasing its moisture sensitivity allowed to twice decrease the water vapour permeability and almost 10 times the oxygen permeability at high relative humidity (>75% RH).

The better knowledge of the physical, chemical and functional properties of films as well as the structure and the interactions involved between components, few recipes of films-forming suspensions were selected for validation as coating or wrapping onto plums. Starch and starch–whey protein (80/20) suspensions were used for European and Japanese plums coatings, and a recipe with oil to improve moisture retention in fruits as well as coating adherence on plums. The coatings applied onto plum were effective. Coatings reduced physiological parameters related to fruit ripeness and ageing (transpiration and respiration rates) and kept firmness longer compared to uncoated plums stored in same conditions. Our work considered the effect of natural skin, natural wax produced by the plums at its surface and of course the coatings based on starch and protein or oil on fruit shelf life. Finally, the coated plums get freshness for at least a quarter longer times which is significant for the producers, the grocers and the consumers.

Even if the formulation of starch-oil-protein based films and coatings need to be again improved, it is also important to consider the technologies of applications. Thus, to finalize the validation on real food, test should be done at industrial scale or pilot-plant scale on various fruits and vegetables to compare with our lab-scale tests on plums. Moreover, one of the main reason for fruit coating application, is to entrap at the surface (skin contact) some preservatives like antifungals. But, recent studies displayed the increasing and deleterious consequences of mycotoxins (ochratoxins, aflatoxins) on human health (several types of cancers). Now it appears that most of mycotoxins are produced by fungi grown onto fruit skin. Thus, starch-based edible coatings are known to be efficient systems for encapsulation of active compounds such as natural antimycotoxins. Thus, it could be very interesting to study and understand the behaviour of starch-based films containing these natural antimycotoxins (eg. parietina) or the microorganism that produces the antimicotoxin (*X*anthoria parietina).

LIS OF REFERENCES

8. LIST OF REFERENCES

1. Abdorreza M.N., Cheng L.H., Karim A.A. (2011). Effects of plasticizers on thermal properties and heat sealability of sago starch films. *Food Hydrocolloids,* 25, 56-60.
2. Acosta S., Jiménez A., Chiralt A., González-Martinez Ch., Cháfer M. (2013). Mechanical, barrier and microstructural properties of films based on cassava starch gelatin blends: effect of aging and lipid addition. *Inside Food Symposium, Brussels*, 9-12 April 2013, Belgium.
3. Adhikari B., Chaudhary D.S., Clerfeuille E. (2010) Effect of plasticizers in the moisture migration behavior of low-amylose starch films during drying. *Drying Technology,* 28, 468-480.
4. Al-Hassan A.A., Norziah M.H. (2012). Starch-gelatin edible films: Water vapour permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids,* 26, 108-117.
5. Allen L., Nelson A.I., Steinberg M.P., McGill J.N. (1963). Edible corn-carbohydrate food coatings. II. Evaluation of fresh meat products. *Food Technology*, 17(11), 104–108.
6. Al-Muhtaseb A.H., McMinn W.A.M., Magee T.R.A. (2004). Water sorption isotherms of starch powders. Part 2: Thermodynamic characteristics, *Journal of Food Engineering*, 62 (2) 135-142.
7. Alves, V.D., Mali, S., Beléia, A., Grossman, M.V.E. (2007). Effect of glycerol and amylose enrichment on cassava starch film properties. *Journal of Food Engineering* 78, 941-946.
8. Alvin, K.L., Price, C.E. (Eds), The Plant Cuticle. Academic Press, 115-123.
9. Anker M., Berntsen J., Hermansson A.M, Stading M. (2002). Improved water vapour barrier of whey protein films by addition of an acetylated monoglyceride, *Innovative Food Science and Engineering Technologies, 3 (1)* 81-92.
10. Anonim (1999). Colour and quality. Heidelberg Druckmaschinen AG, Kurfursten-Anlage, 52-60, (in polish).
11. Arancibia M., Giménez B., López-Caballero M.E., Gómez-Guillén M.C., Montero P. (2014). Release of cinnamon essential oil from polysaccharide bilayer films and its use

for microbial growth inhibition in chilled shrimps. *LWT - Food Science and Technology*, 59(1), 989–995.

12. Araujo-Farro P., Podadera G., Sobral P.J.A., Menegalli F. C. (2010). Development of films based on quinoa (Chenopodium quinoa, Willdenow) starch. *Carbohydrate Polymers*, *81*, 839-848.
13. Arvanitoyannis I.S., Tserkezou P. (2009). Waste: A Handbook for Management. In: Letcher TM, Vallero D, editor Academic Press, USA.
14. ASTM (1995). Standard test method for tensile properties of thin plastic sheeting, method D 882-95. *American Society for Testing and Materials*, Philadelphia.
15. ASTM (1980). Standard Test Methods for Water Vapor Transmission of Materials, method E96-80. *American Society for Testing and Materials*, Philadelphia.
16. Baker E.A. (1982). Chemistry and morphology of plant epicuticular waxes. In Cutler D.J., Alvin K.L., Price C.E. (eds). *The Plant Cuticle*, Academic Press, London, 139-165.
17. Baldwin E.A., Hagenmaier R., Bai J. (2012). Edible coatings and films to improve food quality. (2nd ed). United States of America: CRC Press.
18. Banks, N.H., Dadzie, B.K., Cleland D.J. (1993). Reducing gas exchange of fruits with surface coatings. *Postharvest Biology and Technology,* 3, 269-284.
19. Barth A. (2007). Infrared spectroscopy of proteins. *BBA-Gen Subjects,* 1767*,* 1073–1101.
20. Basiak E., Lenart A., Debeaufort F. (2015a). Effect of oil lamination and starch content on structural and functional properties of starch-based films. *Food Chemistry*, 195, 56–63.
21. Basiak E., Lenart A., Debeaufort F. (2015b). Effects of carbohydrate/protein ratio effects on microstructure, barrier and sorption properties, of wheat starch – whey protein blend edible films. *Journal of the Science of Food and Agriculture, doi: 10.1002/jsfa.7807*.
22. Basiak E., Galus S., Lenart, A. (2015c). Characterization of composite edible films based on wheat starch and whey-protein isolate. *International Journal of Food Science and Technology,* 50, 372-380.
23. Basiak E., Lenart A., Debeaufort F. (2015d). Role of starch nature on the physico-chemical properties of edible films. Submitted.
24. Basiak E., Lenart A., Debeaufort F. (2015e). Glycerol and water contents affect the structural and functional properties of starch-based edible. Submitted.

25. Basiak E., Gayer M., Linke M., Debeaufort F., Lenart A. (2016). Relevance of interactions between starch-based coatings and plum fruit surfaces: a physical-chemical analysis Submitted.

26. Bergo P., Moraes I.C.F., Sobral P.J.A. (2012). Effects of plasticizer concentration and type on moisture content in gelatin films. *Food Hydrocolloids*, 32, 2, 412-415.
27. Berlanstein, L.R. (1992). *The Industrial Revolution and work in nineteenth-century Europe*. London and New York: Routledge.

28. Berski W., Ptaszek A., Ptaszek P., Ziobro R., Kowalski G., Grzesik M., Achremowicz B. (2011). Pasting and rheological properties of oat starch and its derivatives. *Carbohydrate Polymers,* 83, 665-671.
29. Bertuzzi M.A., Armada M., Gottifredi J.C. (2007). Physicochemical characterization of starch based films. *Journal of Food Engineering*, 82, 17–25.
30. Białpiotrowicz T. (2003). Wettability of starch gel films. *Food Hydrocolloids,* 17, 141-147.
31. Białopiotrowicz T., Janczuk B. (2002). Surface properties of gelatin films. *Langmuir,* 18 9462-9468.
32. Bickelmann U. (2008). Marketing standards for fruit and vegetables. AID, Germany. (In German).
33. Bohdan M. (2007). Influence of packaging on food safety. *Przemysł Spożywczy,* 2, 24-27.
34. Bonilla J., Atarés L., Vargas M., Chiralt A. (2013). Properties of wheat starch film-forming dispersions and films as affected by chitosan addition. *Journal of Food Engineering*, 114, 303-312.
35. Brasil I.M., Gomes C., Puerta-Gomez A., Castell-Perez M.E., Moreira R.G. (2012). Polysaccharide-based multilayered antimicrobial edible coating enhances quality of fresh-cut papaya. *LWT – Food Science and Technology*, 47, 39–45.
36. Caleb O.J., Mahajan P.V., Opara U.L., Withhuhn C.R. (2012). Modelling the respiration rates of pomegranate fruit and arils. *Postharvest Biology and Technology,* 64, 49-54.
37. Cagri A., Ustunol Z., Ryser E.T. (2004). Antimicrobial edible films and coatings. *Journal of Food Proteins*, 67(4), 833-848.
38. Cao N., Fu Y., He J. (2007). Preparation and physical properties of soy protein isolate and gelatin composite films. *Food Hydrocolloids,* 21 (7), 1153-1162.

39. Casal H.L., Kohler U., Mantsch H.H. (1988). Structural and conformational changes of beta-lactoglobulin B: an infrared spectroscopic study of the effect of pH and temperature. *BBA- Protein Structure and Molecular Enzymology,* 957, 11–20.
40. Cerqueira M.A., Souza B.W.S., Teixeira J.A., Vicente, A.A. (2012). Effect of glycerol and corn oil on physicochemical properties of polysaccharide films – A comparative study. *Food Hydrocolloids,* 27, 175–184.
41. Chambi H., Grosso C. (2006). Edible films produced with gelatin and casein cross-linked with transglutaminase. *Food Research International,* 39 (4), 458-466.
42. Chang Ch., Nickerson M.T. (2013). Effect of protein and glycerol concentration on the mechanical, optical and water vapour barrier properties of canola protein-based edible films. *International Journal of Food Science and Technology,* 21(1), 33-44.
43. Chang P.R., Jian R., Zheng P., Yu J., Ma X. (2010). Preparation and properties of glycerol plasticized-starch (GPS)/cellulose nanoparticle (CN) composites, *Carbohydrate Polymers,* 79, 301-305.
44. Chang Y.P., Cheah P.B., Seow C.C. (2000). Plasticizing-antiplasticizing effects of water on physical properties of tapioca starch films in the glassy state. *Journal of Food Science,* 65(3). 445-451.
45. Chang Y.P., Karim-Abda A., Seow C.C. (2006). Interactive plasticizing-antiplasticizing effects of water and glycerol on the tensile properties of tapioca starch films. *Food Hydrocolloids,* 20, 1-8.
46. Chen Ch.H., Kuo W.S., Lai L.S. (2009). Effect of surfactants on water barrier and physical properties of tapioca starch/decolorized hsian-tsao leaf gum films. *Food Hydrocolloids*, 23, 714-721.
47. Chen J., Feng M., Gonzalez Y., Pugnaloni L.A. (2008). Application of probe tensile method for quantitative characterisation of the stickiness of fluid foods. *Journal of Food Engineering*, 87, 281-290.
48. Chen P., Yu L., Kealy T., Chen L. Li L. (2007). Phase transition of starch granules observed by microscope under shearless and shear conditions. *Carbohydrate Polymers*, 68(3), 495-501.
49. Chen Y., Cao X., Chang P., Huneault M.A. (2008). Comparative study on the films of poly(vinyl alcohol)/pea starch nanocrystals and poly(vinyl alcohol)/native pea starch. *Carbohydrate Polymers,* 73, 8-17.
50. Chien P.J., Sheu F., Yang F.H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering,* 78 (1) 225–229.

51. Chiumarelli M., Hubinger M.D. (2014). Evaluation of edible films and coatings formulated with cassava starch, glycerol, carnauba wax and stearic acid. *Food Hydrocolloids,* 38, 20-27.
52. Chiumarelli M., Hubinger M.D. (2012). Stability, solubility, mechanical and barrierproperties of cassava starch – Carnauba wax edible coatings to preserve fresh-cut apples. *Food Hydrocolloids,* 28, 59-67.
53. Cho S.Y., Rhee Ch. (2002). Sorption characterictic of soy protein films and their relation to mechanical properties. *LWT - Food Science and Technology,* 35, 151–157.
54. Ciurzyńska A., Lenart A. (2010). Rehydration and sorption properties of osmotically prepared freeze-drying strawberries. *Journal of Food Engineering*, 97, 267-274.
55. Ciurzyńska A., Lenart A., Kawka P. (2013). Influence of chemical composition and structure on sorption properties of freeze-dried pumpkin. *Drying Technology* 31, 655-665.
56. Cooper C.L., Dubin P.L., Kayitmazer A.B., Turksen S. (2005). Polyelectrolyte-protein complexes. *Current Opinion in Colloid and Interface Science,* 10, 52-78.
57. Coupland J.N., Shaw N.B., Monahan F.J., O'Riordan E.D., O'Sullivan M. (2000). Modeling the effect of glycerol on the moisture sorption behavior of whey protein edible films, *Journal of Food Eng*ineering, 43, 1, 25-30.
58. Crank J. (1975). The Mathematics of Diffusion. Oxford Science Publications Oxford Science Publications Clarendon Press. Oxford.
59. Cutter C.N., Sumner S.S. (2002). Application of edible coatings on muscle foods. In Gennadios A. (ed.) *Protein-based films and coatings*, CRC Press LLC, Boca Raton, Floryda.
60. Das D.K., Dutta H., Mahanta Ch.J. (2013). Development of a rice starch-based coating with antioxidant and microbe-barrier properties and study of its effect on tomatoes stored at room temperature. *LWT - Food Science and Technology*, 50, 1, 272–278.
61. Debeaufort F., Martin-Polo M., Voilley A. (1993). Polarity, homogeneity and structure affect water vapour permeability of model edible films. *Journal of Food Science,* 58, 426–429.
62. Debeaufort F., Quezada-Gallo J.A., Voilley A. (1998). Edible films and coatings: tomorrow packaging. *Critical reviews in Food Science,* 38, 299-313.
63. Debeaufort F.A. Voilley A. (1995). Effect of surfactants and drying rate on barrier properties of emulsified films. *International Journal of Food Science and Technology*, 30, 183–190.

64. Descours E., Hambleton A., Kurek M., Debeaufort F., Voilley A., Seuvre A.M. (2013) Aroma behaviour during steam cooking within a potato – starch based model matrix, *Carbohydate Polymers,* 95 (1), 560-568.
65. Dias A.B., Müller C.M.O., Larotonda F.D.S., Laurindo J.B. (2010) Biodegradable films based on rice starch and rice flour, *Journal of Cereal Science,* 51, 213–219.
66. Díaz-Mula H.M., Martínez-Romero D., Castillo S., Serrano M. (2011). Modified atmosphere packaging of yellow and purple plum cultivars. 1. Effect on organoleptic quality. *Postharvest Biology and Technology*, 61, 2–3, 103–109.
67. Dong Z., Wang Q., Du Y. (2007). Alginate/gelatin blend films and their properties for drug controlled release. *Journal of Membrane Science,* 280 (1-2), 37-44.
68. Donhowe I.G., Fennema O.R. (1993). Water vapour and oxygen permeability of wax films. *Journal of the American Oil Chemists' Society,* 70 (9), 867-871.
69. Dupre A. (1869). Théorie Mécanique de La Chaleur (The Mechanical Theory of Heat). Gauthier-Villan, Paris. (In French).
70. Dury Brun C., Cayot N., Karbowiak T., Savary G., Voilley A. (2008). In Robertson G.L., (ed.) *Food Packaging*. New York. Principles and Practice. CRC Press, Taylor and Francis Group.
71. Embuscado M.E., Huber K.C. (2009). *Edible films and coatings for food applications.* USA, Springer, New York.
72. Ercelebi E.A., Ibanoglu E. (2007). Influence on hydrocolloids on phase separation and emulsion properties of whey protein isolate, *Journal of Food Engineering,* 80, 454-459.
73. Eum H.L., Hwang D.K., Linke M., Lee S.K. Zude M. (2009). Influence of edible coating on quality of plum (Prunus salicina Lindl. cv. ‘Sapphire’). *European Food Research and Technology,* 229, 427–434.
74. Fabra M.J., Chambin O., Voilley A., Gay J.P., Debeaufort F. (2012). Influence of temperature and NaCl on the release in aqueous liquid media of aroma compounds encapsulated in edible films. *Journal of Food Engineering,* 108, 30-36.
75. Fabra M.J., Talens P., Chiralt A. (2009). Microstructure and optical properties of sodium caseinate films containing oleic acid-beeswax mixtures. *Food Hydrocol*loids, 23(3), 676-683.
76. Farahnaky A., Saberi B., Majzoobi M. (2013). Effect of glycerol on physical and mechanical properties of wheat starch edible films. *Journal of Texture Studies,* 44, 176-186.

77. Farjamia T., Madadloua A., Labbafia M. (2015). Characteristics of the bulk hydrogels made of the citric acid cross-linked whey protein microgels. *Food Hydrocolloids,* 50, 159–165.
78. Fenaroli G. (1975). *Fenaroli's handbook of flavour ingredients.* (2nd ed.), Cleveland, Ohio: CRC Press.
79. Fengwei X., Halley P.J., Avérous L. (2012). Rheology to understand and optimize processibility, structures and properties of starch polymeric materials. *Progress in Polymer Science*, 37, 595-623.
80. Ferreira C.O., Nunes C.A., Delgadillo I., Lopes-da-Silva J.A. (2009). Characterization of chitosan-whey protein films at acid pH, *Food Research International,* 42, 807-813.
81. Flores S., Famá L., Rojas A.M., Goyanes S., Gerschenson L. (2007). Physical properties of tapioca-starch edible films: Influence of filmmaking and potassium sorbate. *Food Research International,* 40, 257-265.
82. Fu Z., Wang L., Li D., Wi Q. Adhikari B. (2011). Effects of high-pressure homogenization on the properties of starch-plasticizer dispersions and their films, *Carbohydrate Polymers,* 86, 202-207.
83. Galus S., Lenart A. (2013). Development and characterization of composite edible films based on sodium alginate and pectin. *Journal of Food Engineering,* 115, 159-165.
84. Galus S., Lenart A. (2012). Influence on fat emulsion on optical properties of whey protein films. *Acta Agrophysica,* 19 (1), 29-36. (In Polish).
85. Galus S., Lenart A., Voilley A., Debeaufort F. (2013). Effect of potato oxidized starch on the physico-chemical properties of soy protein isolate-based edible films. *Food Technology and Biotechnology,* 51(3), 403-409.
86. Galus S., Mathieu H., Lenart A., Debeaufort F. (2012a). Effect of modified starch or maltodextrines incorporation on the barrier and mechanical properties, moisture sensitivity and appearance of soy protein isolate-based edible films. *Innovative Food Science and Emerging Technologies,* 16, 148-154.
87. Galus S., Turska A., Lenart A. (2012b). Sorption and wetting properties of pectin edible films. *Czech Journal of Food Science,* 30(5), *446-455.*
88. Ganzevles R.A., Zinoviadou K., van Vliet T., Stuart M.A.C., Jongh H.H.J. (2006). Modulating surface rheology by electrostatic protein/polysaccharide interactions. *Langmuir,* 22, 10089-10096.

89. García M.A., Martino M.N., Zaritzky N.E. (2000). Lipid addition to improve barrier properties of edible starch-based films and coatings. *Journal of Food Science,* 65, 941-947.
90. Garcia M.A., Martino M.N., Zaritzky N.E. (1999). Edible Starch Films and Coatings Characterization: Scanning Electron Microscopy, Water Vapor, and Gas Permeabilities, 21 (5), 348-353.
91. Garcia L.C., Pereira L.M., De Luca Sarantopoulos C.I.G., Hubinger M.D. (2010). Selection of an Edible Starch Coating for Minimally Processed Strawberry. *Food Bioprocess Technology,* 3, 834-842.
92. García N.L., Ribba L., Dufresne A., Aranguren M., Goyanes S. (2011). Effect of glycerol on the morphology of nanocomposites made from thermoplastic starch and starch nanocrystals. *Carbohydrate Polymers,* 84, 203-210.
93. Gennadios A., Weller C.L., Hanna M.A., Froning G.W. (1996). Mechanical and barrier properties of egg albumen films. *Journal of Food Science,* 61, 585-589.
94. Gennadios A., Weller C.L., Hanna M.A., Froning G.W. (1996). Edible coatings and films to improve food quality. CRC Press, Taylor & Francis Group, USA.
95. Ghanbarzadeh B., Almasi H., Entezami A.A. (2011). Improving the barrier and mechanical properties of corn starch-based edible films. Effect of citric acid and carboxymethyl cellulose. *Industrial Crops and Products,* 33, 229-235.
96. Ghanbarzadeh B., Almasi H., Entezami A.A. (2010). Physical properties of edible modified starch/carboxymethyl cellulose films. *Innovate Food Science and Emerging Technologies*, 11, 697-702.
97. Ghanbarzadeh B., Musavi M., Oromiehie A.R., Rezayi K., Rad E.R., Milani J. (2007). Effect of plasticizing sugars on water vapour permeability, surface energy and microstructure properties of zein films. *LWT.* 40, 1191-1197.
98. Ghosh V., Ziegler G.R., Anantheswaran R.C. (2005). Moisture migration through chocolate-flavored confectionery coatings. *Journal of Food Engineering,* 66, 177-186.
99. Gillgren T., Blennow A., Pettersson A.J., Stading M. (2011). Modulating rheo-kinetics of native starch films towards improved wet-strenght. *Carbohydrate Polymers*, 83, 383-391.
100. Godbillot L., Dole P., Joly C., Rogé B., Mathlouthi M. (2006). Analysis of water binding in starch plasticized films. *Food Chemistry,* 96, 380-386.
101. Goel P.K., Singhal R.S., Kulkarni P.R. (1999). Studies on interactions of corn starch with casein and casein hydrolysates. *Food Chemistry,* 64, 383-389.

102. Gol N.B., Rao T.V.R. (2011). Banana fruit ripening as influenced by edible coatings. *International Journal of Fruit Science,* 11, 119–135.
103. Gontard D., Duchaz C., Cuq J., Guilbert S. (1994). Edible composite films of wheat gluten and lipids: Water vapour permeability and other physical properties. *International Journal of Food Sciences and Technology,* 29, 39-50.
104. Gontard N., Guilbert S., Cuq J.L. (1994). Edible wheat gluten films: Influence of the main process variables of the film properties using response surface methodology. *Journal of Food Science,* 57, 190-195.
105. Gounga M.E., Xu S.Y., Wang Z. (2007). Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio, pullulan addition in film formation. *Journal of Food Engineering*, *83*, 521-530.
106. Griffin E. (2010). *Short History of the British Industrial Revolution*. Palgrave.
107. Guckain S., Dwyer C., O'Sullivan M., O'Riordan E.D., Monahan F.J. (2006). Properties of and mechanisms of protein interactions in films formed from different proportions of heated and unheated whey protein solutions, *European Food Research and Technology,* 223, 91-95.
108. Guerrero P., Garrido T., Leceta I., de la Caba K. (2013). Films based on proteins and polysaccharides: Preparation and physical–chemical characterization. *European Polymer Journal*, 49(11), 3713–3721.
109. Guerrero P., Retegi A., Gabilondo N., de la Caba K. (2012). Mechanical and thermal properties of soy protein films processed by casting and compression*. Journal of Food Engineering,* 100, 145-151.
110. Guettler B.E., Moresoli Ch., Simon L.C. (2013). Contact angle and surface energy analysis of soy materials subjected to potassium permanganate and autoclave treatment. *Industrial Crops and Products,* 50, 219-226.
111. Guilbert S., Gontard N., Gorris L.G.M. (1996). Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. *LWT – Food Science and Technology*, 29, 10-17.
112. Guilbert S. (1986). Technology and application of edible protective films. In Mathlouthi M. (ed.) *Food Packaging and Preservation. Theory and Practice*. London, England: Elsevier Applied Science.
113. Gutiérrez T.J., Tapia M.S., Pérez E., Famá L. (2014). Edible films based on native and phosphate 80:20 waxy:normal corn starch. *Starch/Stärke,* 66, 1-8.

114. He G.J., Liu Q., Thompson M.R. (2013). Characterisation of structure and properties of thermoplastic potato starch film surface cross-linked by UV irradiation. *Starch/Stärke,* 65, 304-311.

115. Hall D.J., Sorenson D. (2006). Washing, wahing and color-adding. In Wardowski W.F., Miller W.M., Hall D.J., Grierson W. (eds.), *Fresh Citrus Fruits*. 2nd. Longboat Key, Fl: Florida Science Source, 421-450.

116. Hambleton A., Debeaufort F., Bonnotte A., Voilley A. (2009). Influence of alginate emulsion-based films structure on its barrier properties and on the protection of microencapsulated aroma compound. *Food Hydrocolloids*, 23, 2116-2124.

117. Han J.H. Gennadios A. (2005). Edible films and coatings: a review. In: Han J.H. (ed.), *Innovations in food packaging*. Elsevier Academic Press, San Diego.

118. Han J.H., Floros J.D. (1997). Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *Journal of Plastic Film & Sheeting,* 13, 287-298.

119. Hernández O., Emaldi U., Tovar J. (2008). In vitro digestibility of edible films from various starch sources. *Carbohydrate Polymers*, 71, 648-655.

120. Heydari A, Alemzadeh I., Vossoughi M. (2013). Functional properties of biodegradable corn starch nanocomposites for food packaging applications. *Materials and Design,* 50, 954-961.

121. Hong K., Xie J., Zhang L., Sun D., Gong D. (2012). Effects of chitosan coating on postharvest life and quality of guava (Psidium guajava L.) fruit during cold storage. *Scientia Horticulturae,* 144, 172–178.

122. Hoover R., Hughes T., Liu Q. (2010). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International,* 43, 399-413.

123. Hu G., Chen J., Gao J. (2009). Preparation and characteristics of oxidized potato starch films. *Carbohydrate Polymers,* 76, 291-298.

124. Hussain R., Gaiani, C., Jeandel, C., Ghanbaja, J., Scher, J. (2012). Combined effect of heat treatment and ionic strength on the functionality of whey proteins. *Journal of Dairy Science,* 95(11), 6260–6273.

125. Hussain P.R., Suradkar P.P., Wani A.M., Dar M.A. (2015). Retention of storage quality and post-refrigeration shelf-life extension of plum (Prunus domestica L.) cv. Santa Rosa using combination of carboxymethyl cellulose (CMC) coating and gamma irradiation. *Radiation Physics and Chemistry,* 107, 136–148.

126. Jackson M., Mantsch, H.H. (1995). The use and misuse of FTIR spectroscopy in the determination of protein structure. *Critical Reviews in Biochemistry and Molecular Biology,* 30, 95–120.
127. Jagadish R.S., Raj B. (2011). Properties and sorption studies of polyethylene oxide-starch blended films. *Food Hydrocolloids,* 25, 1572-1580.
128. Janjarasskul T., Rauch D.J., McCarthy K.L., Krochta J.M. (2014). Barrier and tensile properties of whey protein – candelilla wax film/sheet. *LWT – Food Science and Technology,* 56, 377-382.
129. Janjarasskul T., Krochta J.M. (2010). Edible packaging materials. *The Annual Review of Science and Technology,* 1, 415-448.
130. Janssen L., Mościcki L. (2009). *Thermoplastic Starch*, Wiley-VCH Verlag GmbH & Co. KGaA.
131. Jayakody L., Hoover R., Liu Q., Donner E. (2009). Studies on tuber starches III. Impact of annealing on the molecular structure, composition and physicochemical properties of yam (*Dioscorea* sp.) starches grown in Sri Lanka. *Carbohydrate Polymers*, 76, 145-153.
132. Jayakody L., Hoover R. (2008). Effect of annealing on the molecular structure and physicochemical properties of starches from different botanical origins – A review. *Carbohydrate Polymers*, 74, 691-703.
133. Jiménez A., Fabra M.J., Talens P., Chiralt A. (2012). Effect of re-crystallization on tensile, optical and water vapour barrier properties of corn starch films containing fatty acids. *Food Hydrocolloids*, 26, 302-310.
134. Jiménez A., Fabra M.J., Talens P., Chiralt A. (2013a). Phase transitions in starch based films containing fatty acids. Effect on water sorption and mechanical behaviour. *Food Hydrocolloids*, 30, 408-418.
135. Jiménez A., Fabra M.J., Talens P., & Chiralt A. (2013b). Physical properties and antioxidant capacity of starch – sodium caseinate films containing lipids. *Journal of Food Engineering,* 3, 116, 695-702.
136. Jiménez A., Sánchez-González L., Desobry S., Chiralt A., Arab Tehrany E. (2913c). Influence of nanoliposomes incorporation on properties of film forming dispersions and films based on corn starch and sodium caseinate. *Food Hydrocolloids,* 35, 159-169.

137. Jiménez A., Fabra M.J., Talens P., Chiralt A., (2013d). Effect of sodium caseinate on properties and ageing behaviour of corn starch based films. *Food Hydrocolloids,* 29, 265-271.
138. Joshi M., Aldred P., McKnight S., Panozzo J.F., Kasapis S., Adhikari R., Adhikari B. (2013). Physicochemical and functional characteristics of lentil starch. *Carbohydrate Polymers*, 92, 1484-1496.
139. Jost V., Langowski H.Ch. (2015). Effect of different plasticizers on the mechanical and barrier properties of extruded cast PHBV films. *European Polymer Journal*, 68, 302–312.
140. Kairytė A., Vėjelis S. (2015). Evaluation of forming mixture composition impact on properties of water blown rigid polyurethane (PUR) foam from rapeseed oil polyol. *Industrial Crops and Products,* 66, 210-215.
141. Kalin M., Polajnar M. (2013). The correlation between the surface energy, the contact angle and the spreading parameter, and their relevance for the wetting behaviour of DLC with lubricating oils. *Tribology International,* 66, 225-233.
142. Karbowiak T., Debeaufort F., Voilley A. (2006). Wetting properties at the surface of iota carrageenan-based edible films. *Journal of Colloid and Interface Science,* 294(2), 400–410.
143. Kaur L., Singh J., Singh N., (2005). Effect of glycerol monostearate on the physic-chemical, thermal, rheological and noodle making properties of corn and potato starches. *Food Hydrocolloids,* 19, 839-849.
144. Kibar E.A.A., Us F. (2013). Thermal, mechanical and water adsorption properties of corn starch–carboxymethylcellulose/methylcellulose biodegradable films. *Journal of Food Engineering,* 11, 123–131.
145. Kim S.R.B., Choi Y.Ch., Kim J.Y., Lim S.T. (2015). Improvement of water solubility and humidity stability of tapioca starch film by incorporating various gums. *LWT-Food Science and Technology,* 64, 1, 485-482.
146. Kim Y.J., Lee H.M., Park O.O. (1995). Processabilities and mechanical properties of Surlyn-treated starch/LDPE blends. *Polymer Engineering Science,* 35, 1652-1657.
147. Koch K., Gillgren T., Stading M., Andersson R. (2010). Mechanical and structural properties of solution-cast high amylose maize starch films. *International Journal of Biological Macromolecules*, 46, 13-19.
148. Kokoszka S., Lenart A. (2007). Edible coatings, characteristics and use – a review. *Polish Journal of Food and Nutrition Sciences*, 57, 4, 399-404.

149. Kong X., Bao J., Corke H. (2009). Physical properties of *Amaranthus* starch. *Food Chemistry*, 113 (2), 371-376.

150. Koupantsis T., Kiosseoglou V. (2009). Whey protein-carboxymethylcellulose interaction in solution and in oil-in-water emulsion systems. Effect on emulsion stability. *Food Hydrocol*loids, 23, 1156-1163.

151. Kowalczyk D., Pikula E. (2010). Influence of edible protein – waxy coating on quality of storage grapes (Vitisvinifera L.). *Żywność, Nauka, Technologia, Jakość,* 5 (72), 66-76.

152. Kowalska H., Domian E., Janowicz M., Lenart A. (2006). Water adsorption kinetic by selected food powder aglomerates. Agricultural Engineering, 3/78, 143-153. (In polish).

153. Krogars K., Heinämäki J., Karjalainen M., Niskanen A., Leskelä M., Yliruusi J. (2003). Enhanced stability of rubbery amylose-rich maize starch films plasticized with a combination of sorbitol and glycerol. *International journal of pharmaceutics,* 251, 205-208.

154. Krysińska P., Gałkowska D., Fortuna T. (2008). Characteristics of modify starch systems obtained from wax corn. *Żywność. Nauka. Technologia. Jakość,* 5 (60), 9-23. (In Polish).

155. Labuza T.P., Altunakar B. (2007). Water activity prediction and moisture sorption isotherms. In: Barbosa-Canovas G.V., Fontana A.J., Schmidt S.J., Labuza, T.P. (eds), *Water activity of foods - fundamentals and applications*, Blackwell Publishing, 109-154.

156. Landois-Garza J., Hotchkiss J.H. (1987). Plastic packaging can cause aroma sorption, Journal of *Food Engineering,* 59, 39-42.

157. Laohakunjit N., Noomhorm A. (2004). Effect of plasticizers on mechanical and barrier properties of rice starch films. *Starch/ Stärke,* 22, 348-356.

158. Laufer G., Kirkland Ch., Cain A.A. Grunlan J.C. (2013). Oxygen barrier of multilayer thin films comprised of polysaccharides and clay. *Carbohydrate Polymers*, 95, 299–302.

159. Lawton J.W., Fanta G.F. (1994). Glycerol-plasticized films prepared from starch-poly (vinyl alcohol) mixtures: Effect of poly (ethylene-co-acrylic acid). *Carbohydrate Polymers,* 23, 276-280.

160. Lelievre J., Husbands J. (2002). Effects of sodium caseinate on the rheological properties of starch pastes. *Starch/Stärke, 41,* 236-238.

161. Li M., Liu P., Zou W., Yu L., Xie F., Pu H., Liu H., Chen L. (2011). Extrusion processing and characterization of edible starch films with different amylose contents. *Journal of Food Engineering,* 106, 95-101.

162. Li Y., Shoemaker Ch.F., Ma J., Shen X., Zhong F. (2008). Paste viscosity of rice starches of different amylose content and carboxymethylcellulose formed by dry heating and the physical properties of their films. *Food Chemistry,* 109, 616-623.

163. Li X., Qiu Ch., Ji N., Sun C., Xiong L., Sun Q. (2015). Mechanical, barrier and morphological properties of starch nanocrystals-reinforced pea starch films. *Carbohydrate Polymers,* 121, 155–162.

164. Liang J., Ludescher R.D. (2015). Effects of glycerol on the molecular ability and hydrogen bond network in starch matrix. *Carbohydrate Polymers,* 115, 401-407.

165. Lin Q., Xiao H., Fu X., Tian W., Li L., Yu F. (2011). Physico-chemical properties of flour, starch, and modified starch of two rice varieties. *Agricultural Sciences in China*, 10(6), 960-968.

166. Linke, M., Geyer, M. (2013). Condensation dynamics in plastic film packaging of fruit and vegetables. *Journal of Food Engineering,* 116 (1), 144-154.

167. Liu K., Yuan Ch., Chen Y., Liu H. (2014). Combined effects of ascorbic acid and chitosan on the quality maintenance and shelf life of plums. *Scientia Horticulturae*, 176, 45–53.

168. Liu P., Xie F., Li M., Yu L., Halley P.J., Chen L. (2011). Phase transitions of maize starches with different amylose contents in glycerol-water systems. *Carbohydrate Polymers,* 85, 180-187.

169. Liu Z. (2005). Edible films and coatings from starches. In Jung Han (ed.), *Innovations in Food Packaging*, Elsevier Academic Press, 319-351.

170. Llanos Navarro-Tarazaga M., Massa A., Pérez-Gago M.B. (2011). Effect of beeswax on hydroxypropyl methylcellulose-based edible film properties and postharvest quality of coated plums (Cv. Angeleno). *LWT – Food Science and Technology*, 44, 2328-2334.

171. Lourdin D., Valle G., Colonna P. (1995). Influence of amylose content on starch films and foams. *Carbohydrate Polymers,* 27, 275-280.

172. Louw E.D., Theron K.I. (2012). Volatile dynamics during maturation, ripening and cold storage of three Japanese plum cultivars (Prunus salicina Lindl.). *Postharvest Biology and Technology,* 70, 13-24.

173. López O.V., García M.A., Zaritzky N.E. (2008). Film forming capacity of chemicaly modified corn starches. *Carbohydrate Polymers,* 73, 573-581.

174. Lubbers S., Landy P., Voilley A. (1998). Retention and release of aroma compounds in foods containing proteins. *Food Technology,* 52, 68-72.

175. Maftoonazad N., Ramaswamy H.S., Moalemiyan M., Kushalappa A.C. (2007). Effect of pectin-based edible emulsion coating on changes in quality of avocado exposed to Lasiodiplodiatheobromae infection. *Carbohydrate Polymers*, 68, 341–349.

176. Mahieu A., Terrié C., Youssef B. (2015). Thermoplastic starch films and thermoplastic starch/polycaprolactone blends with oxygen-scavenging properties: Influence of water content. *Industrial Crops and Products,* 72, 192-199.

177. Mahy M., Van Eycken L., Oosterlinck A. (1994). Evaluation of Uniform Color Spaces Developed after the Adoption of CIELAB and CIELUV. *Color Research & Application,* 19(2), 105–121.

178. Majdzadeh-Ardakani K., Navarchian A.H., Sadeghi F. (2010). Optimization of mechanical properties of thermoplastic starch/clay nanocomposites. *Carbohydrate Polymers*, 79, 547-554.

179. Mali S., Grossmann M.V., García M.A., Martino M.N., Zaritzky N.E. (2002). Microstructural characterization of yam starch films. *Carbohydrate Polymers,* 50, 379-386.

180. Mali S., Grossmann M.V., García M.A., Martino M.N., Zaritzky N.E. (2006). Effects of controlled storage on thermal, mechanical and barrier properties of plasticized films from different starch sources. *Journal of Food Engineering,* 75, 453-460.

181. Mali S., Laram L.B., Pereira Ramos L., Grossmann M.V.E. (2004). Relationships among the composition and physicochemical properties of starches with the characteristics of their films. *Journal of Agriculture Food Chemistry,* 52, 7720-7725.

182. Mali S., Sakanaka L.S., Yamashita F., Grossmann M.V.E. (2005). Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect. *Carbohydrate Polymers,* 60, 283–289.

183. Martins J.T., Cerqueira M.A., Bourbon A.I., Pinheiro A.C., Souza B.W.S., & Vicente A.W. (2012). Synergistic effects between [kappa]-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. *Food Hydrocolloids*, 29, 280-289.

184. Martins J.T., Cerqueira M.A., Vicente A.W. (2010). Influence of α-tocopherol on chitosan-edible film properties. Presented on *International conference of food innovation*, Food Innova2010, Spain.

185. Mastromatteo M., Del Nobile M.A. (2011). A simple model to predict the oxygen transport properties of multilayer films. *Journal of Food Engineering*, 102, 170-176.

186. Mathew A.P., Dufresne A. (2002). Morphological investigation of nanocomposites from sorbitol plasticized starch and tunicin whiskers. *Biomacromolecule,* 3, 609-617.

187. McHugh T.H., Avena-Bustillos R., Krochta J.M. (1993). Hydrophilic edible films: modified procedure for water vapour permeability and explanation of thickness effects. *Journal of Food Science,* 58 (4), 899-903.

188. McHugh T.H., Krochta J.M. (1994). Water vapour permeability properties of edible whey protein-lipid emulsion films. *Journal of the American Oil Chemists' Society*, 71, 307-312.

189. McMinn W.A.M., Magee T.R.A. (1999). Studies of the effect of temperature on the moisture sorption characteristics of potatoes. *Journal of Food Process Engineering*, 22, 113-128.

190. Mehyar G.F., Han J.H. (2004). Physical and mechanical properties of high-amylose rice and pea starch films as affected by relative humidity and plasticizer. *Journal of Food Science,* 69 (9), 449–454.

191. Mieliwodzka M. (2006). Strechable foils in food packaging. *Przemysł Spożywczy,* 8, 90-95. (In Polish).

192. Mistriotis A., Briassoulis D., Giannoulis A., D'Aquino S. (2016). Design of biodegradable bio-based equilibrium modified atmosphere packaging (EMAP) for fresh fruits and vegetables by using micro-perforated poly-lactic acid (PLA) films. *Postharvest Biology and Technology,* 111, 380–389.

193. Mitrus M., Wojnowicz A., Mościcki L. (2009). Biodegradable polymers and their practical utility in thermoplastic starch. A green material for various industries. In Janssen L., Mościcki L. (eds), *Thermoplastic starch*, Viley-VCH, Weinheim, 1-30.

194. Mohammadian M., Madadlou A. (2016). Characterization of fibrillated antioxidant whey protein hydrolysate and comparison with fibrillated protein solution. *Food Hydrocolloids,* 52, 221–230.

195. Molenda M., Stasiak M., Horabik J., Fornal J., Błaszczak W., Ornowski A. (2006). Microstructure and mechanical parameters of five types of starch. *Polish Journal of Food and Nutrition Sciences*, 15/56 (2), 161-168.

196. Monedero F.M., Fabra M.J., Talens P., Chiralt A. (2009). Effect of oleic acid-beeswax mixtures on mechanical, optical and Water barrier properties of soy protein isolate based films. *Journal of Food Enginering,* 91(4), 509-515.
197. Morillon V., Debeaufort F., Blond G., Capelle M., Voilley A. (2002). Factors affecting the moisture permeability of lipid-based edible films: a review. *Critical Reviews in Food Sciences and Nutrition,* 42(1), 67-89.
198. Mukhtar A., Damerow L., Blanke M. (2014). Non-invasive assessment of glossiness and polishing of the wax bloom of European plum. *Postharvest Biology and Technology*, 87, 144–151.
199. Murillo-Martínez M.M., Pedroza-Islas R., Lobato-Calleros C., Martínez-Ferez A., Vernon-Carter E.J. (2011). Designing $W_1/O/W_2$ double emulsions stabilized by protein and polysaccharide complexes for producing edible films: Rheological, mechanical and water vapour properties. *Food Hydrocolloids*, 25, 577-585.
200. Muscat D., Adhikari B., Adhikari R., Chaudhary D.S. (2012). Comparative study of film forming behaviour of low and high amylose starches using glycerol and xylitol as plasticizers. *Journal of Food Engineering,* 109, 189-201.
201. Muscat D., Adhikari R., McKnight S., Guo Q., Adhikari B. (2010). The physicochemical characteristics and hydrophobicity of high amylose starch–glycerol films in the presence of three natural waxes. *Journal of Food Engineering*, 119, 205-219.
202. Müller C.M.O., Laurindo J.B., Yamashita F. (2009). Effect of cellulose fibers on the crystallinity and mechanical properties of starch-based films at different relative humidity values. *Carbohydrate Polymers***, 77**, 293-299.
203. Müller C.M.O., Yamashita F., Laurindo J.B. (2008). Evaluation of the effects of glycerol and sorbitol concentration and water activity on the water barrier properties of cassava starch films through a solubility approach. *Carbohydrate Polymers,* 72(1), 82-87.
204. Navarro-Tarazaga M.L., Massa A., Pérez-Gago M.B. (2011). Effect of beeswax content on hydroxypropyl methylcellulose-based edible film properties and postharvest quality of coated plums (*Cv. Angeleno*). *LWT - Food Sciences and Technology,* 44, 2328-2334.
205. NurHanani Z.A., McNamara J., Roos Y.H., Kerry J.P. (2013). Effect of plasticizer content on the functional properties of extruded gelatin-based composite films. *Food Hydrocolloids*, 31, 264–269.

206. Nwokocha L.M., Aviara N.A., Sean Ch., Williams P.A. (2009). A comparative study of some properties of cassava (*Manihot esculenta*, Crantz) and cocoyam (*Colocasia esculenta*, Linn) starches. *Carbohydrate Polymers,* 76, 362-367.

207. Ogonek A., Lenart A. (2002). Influence of eddible coatings in osmotic rehydration of freezen strawberies. *Żywność. Nauka. Technologia. Jakość,* 3, 32, 116-126. (In Polish).

208. Oh E., Luner P.E. (1999). Surface free energy of ethylcellulose films and the influence of plasticizers. *International Journal of Pharmaceutics,* 188, 203-219.

209. Olivas G.I., Barbosa-Canovas G.V. (2008). Alginate-calcium films: Water vapour permeability and mechanical properties as affected by plasticizer and relative humidity. *LWT- Food Science and Technology,* 41, 359-366.

210. Olivia V., López O.V., Ninago M.D., Soledad Lencina M.M., García M.A., Andreucetti N.A., Ciolino A.E., Villara M.A. (2015). Thermoplastic starch plasticized with alginate–glycerol mixtures: Melt-processing evaluation and film properties. *Carbohydrate Polymers,* 126, 83-90.

211. Ollé R.C.P., Jagus R.J., Gerschenson L.N. (2014). Effect of natamycin, nisin and glycerol on the physicochemical properties, roughness and hydrophobicity of tapioca starch edible films. *Materials Science and Engineering: C,* 40, 281–287.

212. Osés J., Fernández-Pan I., Mendoza M., Maté J.I. (2009). Stability of the mechanical properties of edible films based on whey protein isolate during storage at different relative humidity. *Food Hydrocolloids*, 23, 125-131.

213. Owens D.K., Wendt R.C. (1969). Estimation of the surface free energy of polymers. *Journal of Applied Polymer Science,* 13(8), 1741–1747.

214. Ozdemir M., Floros J.D. (2008). Optimization of edible whey protein films containing preservatives for water vapour permeability, water solubility and sensory characteristics. *Journal of Food Engineering,* 86, 215-224.

215. Pal J., Singhal R.S., Kulkarni P.R. (2002). Physicochemical properties of hydroxypropyl derivative from corn and amaranth starch. *Carbohydrate Polymers,* 48, 49-53.

216. Palviainen P., Heinamaki J., Myllarinen P., Lahtinen R., Yliruusi J., Forssell P. (2001). Corn starches and film formers in aqueus-based film coating. Pharmaceutical Development and Technology, 6, 353-361.

217. Parker R., Ring S.G. (2000). Aspects of the physical chemistry of starch. *Journal of Cereal Science,* 34, 1-17.

218. Pelton J.T., McLean L.R. (2000). Spectroscopic methods for analysis of protein secondary structure. *Analytical Biochemistry,* 277, 167–176.
219. Pérez-Gallardo A., Bello-Pérez L.A., García-Almendárez B., Montejano-Gaitán G., Barbosa-Cánovas G.V., Regalado C. (2011). Effect of structural characteristics of modified waxy corn starches on rheological properties, film-forming solutions, and on water vapour permeability, solubility, and opacity of films. *Starch/Stärke,* 64, 27-36.
220. Petersen K., Nielsen V.P., Bertelsen G., Lawther M., Olsen M.B., Nilsson N.H., Mortensen G. (2011). Potential of biobased materials for food packaging. *Trends Food Science Technology*, 10, 52-68.
221. Phan The D., Debeaufort F., Luu D., Voilley A. (2005). Functional properties of edible agar-based and starch-based films for food quality preservation. *Journal of Agricultural and Food Chem*istry, 53, 973-981.
222. Phan The D., Debeaufort F., Luu D., Voilley A. (2008). Moisture barrier, wetting and mechanical properties of shellac/agar or shellac/cassava starch bilayer bio-membrane for food applications. *Journal of Membrane Science,* 325, 277–283.
223. Phan The D., Debeaufort F., Peroval C., Despré D., Courthaudon J.L., Voilley A. (2002). Arabinoxylan-lipid-based edible films: 3 influence of drying temperature on emulsion stability and on film structure and functional properties. *Journal of Agriculture and Food Chemistry,* 50(8), 2423-2428.
224. Phan The D., Debeaufort F., Voilley A. (2009). Biopolymer interactions affect the functional properties of edible films based on agar, cassava starch and arabinoxylan blends. *Journal of Food Engineering,* 90, 548-558.
225. Phan The D., Debeaufort F., Voilley A., Luu D. (2009). Influence of hydrocolloid nature on the structure and functional properties of emulsified edible films. *Food Hydrocolloids,* 23, 691–699.
226. Piyada K., Waranyou S., Thawien W. (2013). Mechanical, thermal and structural properties of rice starch films reinforcesd with rice starch nanocrystals. *International Food Research Journal,* 20 (1), 439-449.
227. Pommet M., Redl A., Morel M., Guilbert S. (2003). Study of wheat gluten plasticisation with fatty acids. *Polymer,* 44(1), 115-122.
228. Prakash Maran J., Sivakumar V., Sridhar R., & Prince Immanuel V. (2013). Development of model for mechanical properties of tapioca starch based edible films. *Industrial Crops and Products*, 42, 159-168.

229. Prakash Maran J., Sivakumar V., Thirugnanasambandham K., Kandasamy S. (2013). Modeling and analysis of film composition on mechanical properties of maize starch based edible films. *International Journal of Biological Macromolecules,* 62, 565-573.
230. Pushpadass H.A., Marx D.B., Hanna M.A. (2008). Effects of extrusion temperature and plasticizers on the physical and functional properties of starch films. *Starch/Stärke,* 60, 527-538.
231. Ramirez C., Gallegos I., Ihl M., Bifani V. (2012). Study of contact angle, wettability and water vapour permeability in carboxymethylcellulose (CMC) based film with murata leaves (*Ugni molinae* Turcz) extract. *Journal of Food Engineering,* 109, 424-429.
232. Rao M.S., Kanatt S.R., Chawla S.P., Scharma A. (2010). Chitosan and guar gum composite films: Preparation physical, mechanical and antimicrobial properties. *Carbohydrate Polymers,* 81, 1243-1247.
233. Rattanapnoe N., Plotto A., Baldwin E. (2007). Effect of edible coatings and surface treatments on pericarp color of Thai Lychee cultivars. *Proceedings of the Florida State Horticultural Society*, 120, 222-227.
234. Reinoso E., Mittal G.S., Lim L.-T. (2008). Influence of whey protein composite coatings on plum (Prunus domestica L.) fruit quality. *Food Bioprocess Technol*ogy, 1, 314-325.
235. Reis K.C., Smith J.P., Carvalho C.W.P., Wellner N., Yakimetes I. (2008). Characterization of polyhydroxybutyrate-hydroxyvalerate (PHB-HV)/maize starch blend films. *Journal of Food Engineering,* 89, 361-369.
236. Rhim J.W., Shellhammer T.H. (2005). Lipid-based edible films and coatings. *Innovations in Food Packaging.* Amsterdam. Elsevier Science & Technology Books.
237. Ribeiro C., Vicente A. A., Teixeira J. A., Miranda C. (2007). Optimization of edible coating composition of retard strawberry fruit senescence. *Postharvest Biology and Technology*, 44, 63-70.
238. Rivero S., García M.A., Pinotti A. (2010). Correlations between structural, barrier, thermal and mechanical properties of plasticized gelatin films. *Innovative Food Science and Emerging Technologies*, 11, 369-375.
239. Rodríguez M., Osés J., Ziani K., Maté J.I. (2006). Combined effect of plasticizers and surfactants on the physical properties of starch based edible films. *Food Research International*, 39, 840-846.

240. Rojas-Graü M.A., Soliva-Fortuny R., Martín-Belloso O. (2009). Edible coatings to incorporate active ingreadiens to fresh-cut fruits. *Trends in Food Science and Technology,* 20, 438-447.
241. Roy S., Gennadios A., Weller C. (2000). Water vapour transport parameters of a cast wheat gluten film. *Biological Systems Engineering*, 1, 43-50.
242. Rux G., Mahajan P.V., Geyer M., Linke M., Pant A., Saengerlaub S., Caleb O.J. (2015). Application of humidity-regulating tray for packaging of mushrooms. *Postharvest Biology and Technology*, 108, 102–110.
243. Savadekar N.R., Mhaske S.T. (2012). Synthesis of nano cellulose fibers and effect of thermoplastics starch based films. *Carbohydrate Polymers,* 89, 146-151.
244. Schmidt V.C.R., Porto L.M., Laurindo J.B. (2013). Water vapour barrier and mechanical properties of starch films containing steric acid. *Industrial Crops and Products*, 41, 227-234.
245. Shen X.L., Wu J.M., Chen Y., Zhao G. (2010). Antimicrobal and physical properties of sweet potato starch films incorporated with potassium sorbate or chitosan. *Food Hydrocolloids,* 24, 285-290.
246. Shi A., Wang L., Li D., Adhikari B. (2013). Characterization of starch films containing starch nanoparticles. Part 1: Physical and mechanical properties. *Carbohydrate Polymers*, 92 (2), 593.
247. Shi L., Yang J., Huang Z., Li J., Tang Z., Li Y., Zheng Q. (2013). Fabrication of transparent, flexible conducing graphene thin films via soft transfer printing method. *Applied Surface Science,* 276, 437-446.
248. Silva J.B.A., Nascimento T., Costa L.A.S., Pereira F.V., Machado B.A., Gomes G.V.P., Assis D.J., Druzian J.I. (2012). Effect of source and interaction with nanocellulose cassava starch, glycerol and the properties of films bionanocomposites. *Materials Today: Proceedings*, 2, 1,200-207.
249. Sikora M., Kowalski S., Tomasik P., Sady M. (2007). Rheological and sensory properties of dessert sauces thickened by starch-xantan gum combinations. *Journal of Food Engineering,* 79, 1144-1151.
250. Singh N., Belton P.S., Georget D.M.R. (2009). The effects of iodine on kidney bean starch: films and pasting properties. *International Journal of Biological Macromolecules*, 45, 116-119.

251. Singh J., Kaur L., McCarthy O. J. (2007). Factors influencing the physicochemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – a review. *Food Hydrocolloids,* 21 (1), 1-22.

252. Slavutsky A.M., Bertuzzi M.A. (2014). Water barrier of starch films reinforced with cellulose nanocrystals obtained from sugarcane bagasse. *Carbohydrate Polymers,* 110, 53-61.

253. Smits A.L.M., Kruiskamp P.H., van Soest J.J.G., Vligenthart J.F.G. (2003). The influence of various small plasticizer and malto-oligosaccharides on the retrogradation of (partly) gelatinised starch. *Carbohydrate Polymers*, 51, 417-424.

254. Soazo M., Rubiolo A.C., Verdini R.A. (2011). Effect of drying temperature and beeswax content on moisture isotherms of whey protein emulsion film. *Procedia Food Sci*ence, 1, 210-215.

255. Sobral P.J., dos Santos J.S., Garcia F.T. (2005). Effect of protein and plasticizer concentration in film forming solutions on physical properties of edible films based on muscle proteins of a Thai Tilapia. *Journal of Food Engineering*, 70, 93-100.

256. Soliman E.A., Furuta M. (2014). Influence of phase behavior and miscibility on mechanical, thermal and micro-structure of soluble starch-gelatin thermoplastic biodegradable blend films. *Food and Nutrition Sciences*, 5, 1040-1055.

257. Song B., Springer J. (1996a). Determination of Interfacial Tension from the Profile of a pendant Drop Using Computer-Aided Image Processing**:** 1. Experimental. *Journal of Colloid and Interface Science,* 184(1), 77-91.

258. Song B., Springer J. (1996b). Determination of Interfacial Tension from the Profile of a Pendant Drop Using Computer-Aided Image Processing: 2. Experimental. *Journal of Colloid and Interface Science,* 184(1), 77-91.

259. Sothornvit R., Krochta J.M. (2000). Water vapour permeability and solubility of films from hydrolyzed whey protein, *Journal of Food Science,* 65 (4), 700-703.

260. Sothornvit R., Pitak N. (2007). Oxygen permeability and mechanical properties of banana films. *Food Research International,* 40, 3, 356-370.

261. Souza R.C.R., Andrade C.T. (2002). Investigation of the gelatinization and extrusion processes of corn starch. *Advances in Polymer Technology,* 21(1), 17–24.

262. Souza B.W.S., Cerqueira M.A., Teixiera J.A., Vicente A.A. (2010). The use of electric fields for edible coatings and films development and production: A review. *Food Engineering Review,* 2, 244-255.

263. Staroszczyk H., Tomasik P., Janas P., Poręda A. (2007). Estrification of starch with sodium selenite and selenade. *Carbohydrate Polymers*, 69, 2, 299-304.

264. Stuart B. (2004). Polymers. Infrared spectroscopy: Fundamentals and applications, John Wiley & Sons, New York, 113–135.

265. Su J.-F., Huang Z., Yuan X.-Y., Li M. (2010). Structure and properties of carboxymethyl cellulose/soy protein isolate blend edible films crosslinked by Maillard reactions. *Carbohydrate Polymers,* 79 (1), 145-153.

266. Subedi D.P. (2011). Contact angle measurement for the surface characterisation of solids. *Himalayan Physics,* 2, 2, 1-4.

267. Sun Q., Sun C., Xiong L. (2013). Mechanical, barrier and morphological properties of pea starch and peanut protein isolate blend films. *Carbohydrate Polymers,* 98, 1, 630-637.

268. Sun Q., Xiong C.S.L. (2014). Functional and pasting properties of pea starch and peanut protein isolate blends. *Carbohydrate Polymers,* 101, 1134-1139.

269. Suppakul P., Chalernsook B., Ratisuthawat B., Prapasitthi S., Munchukangwan N. (2013) Empirical modelling of moisture sortion characteristics and mechanical and barrier properties of cassava flour film and their relation to plasticizing-antiplasticizing effects. *LWT – Food Science and Technology,* 50, 290-297.

270. Suzuki M. (1993). Fundamentals of adsorption. USA: Kluwer Academic Publishers.

271. Talja R.A., Helén H., Roos Y.H., Jouppila K. (2008). Effect of type and content of binary polyoil mixtures on physical and mechanical properties of starch-based edible films. *Carbohydrate Polymers,* 71, 269-276.

272. Talja R.A., Helén H., Roos Y.H., Jouppila K. (2007). Effect of various polyols and polyol contents on physical and mechanical properties of potato starch-based films. *Carbohydrate Polymers,* 67, 288-295.

273. Tavassoli-Kafrani E., Shekarchizadeh H. Masoudpour-Behabadi M. (2016). Development of edible films and coatings from alginates and carrageenans. *Carbohydrate Polymers*, 137, 360–374.

274. Tederko A. (1995). Edible packaging. *Przemysł Spożywczy*, 9, 343-345. (In Polish).

275. Teodoro A.P., Mali S., Romero N., Carvalho G.M. (2015). Cassava starch films containing acetylated starch nanoparticles as reinforcement: Physical and mechanical characterization*.* *Carbohydrate Polymers,* 126, 9–16.

276. Tharanathan R.N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technology*, 14, 71-78.

277. Thirathumthavorn D., Charoendrin S. (2006). Thermal and pasting properties of native and acid-treated starches derivatized by 1-octenyl succinic anhydride. *Carbohydrate Polymers,* 66, 258-265.

278. Thirathumthavorn D., Charoenrein S. (2007). Aging effects on sorbitol- and non-crystallizing sorbitol-plasticized tapioca starch films. *Starch/Stärke,* 59, 493-497.

279. Tihminlioglu F., Doğan Atik I., Özen B. (2010). Water vapour nad oxygen-barrier performance of corn-zein coated polypropylene films. *Journal of Food Engineering,* 96, 342-347.

280. Timmermann E.O., Chirife J., Iglesias H.A. (2001). Water sorption isotherms of foods and foodstuffs: BET or GAB parameters? *Journal of Food Engineering*, 48, 19-31.

281. Tomasik P. (2000). Polisaccharides – treasure of the next century. *Niedziałki,* 34 (1), 2-7. (In Polish).

282. Tongdeesoontorn W., Mauer L.J., Wongruong S., Rachtanapun P. (2009). Water vapour permeability and sorption isotherms of cassava starch based films blended with gelatin and carboxymethyl cellulose. *Asian Journal of Food & Agro-industry,* 2 (4), 501-514.

283. Torres F.G., Troncoso O.P., Torres C., Diaz D.A., Amaya E. (2011). Biodegradability and mechanical properties of starch films from Andean crops. *International Journal of Biological Macromolecules*, 48, 603-606.

284. Valero D., Díaz-Mula H.M., Zapata P.J., Guillén F., Martínez-Romero D., Castillo S., Serrano M. (2013). Effects of alginate edible coating on preserving fruit quality in four plum cultivars during postharvest storage. *Postharvest Biology and Technology*, 77, 1-6.

285. Valero D., Fai1 A.E.C., de Souza M.R.A., de Barros S.T., Bruno N.V., Ferreira M.S.L., de Andrade Gonçalves E.C.B. (2016). Development and evaluation of biodegradable films and coatings obtained from fruit and vegetable residues applied to fresh-cut carrot (*Daucus carota* L.). *Postharvest Biology and Technology,* 112, 194–204.

286. Van den Berg C. (1984). Description of water activity of foods for engineering purposes by means of the GAB model of sorption. In: McKenna B.M. (ed.), *Engineering and Food.* New York: Elsevier Applied Science, 311-321.

287. Vázquez-Celestino D., Ramos-Sotelo H., Rivera-Pastrana D.M., Vázquez-Barrios M.E., Mercado-Silva E.M. (2016). Effects of waxing, microperforated polyethylene bag, 1-methylcyclopropene and nitric oxide on firmness and shrivel and weight loss of

‘Manila’ mango fruit during ripening. *Postharvest Biology and Technology,* 111, 398–405.

288. Villalobos R., Hernández-Muñoz P., Chiralt A. (2006). Effect of surfactants on water sorption and barrier properties of hydroxypropyl methylcellulose films. *Food Hydrocolloids,* 20, 502-509.
289. Viña S.Z., Mudgridge A., García M.A., Ferreya R.M., Martino M.N., Chaves A.R., Zaritzky N.E. (2007). Effects of polyvinylchloride films and edible starch coatings on quality aspects of refrigerated Brussels sprouts. *Food Chemistry*, 103, 701-709.
290. Viollaz P.E., Rovedo C.O. (1999). Equilibrium sorption isotherms and thermodynamic properties of starch and gluten. *Journal of Food Engineering*, 40, 287-292.
291. Wu Y., Geng F., Chang P.R., Yu J., Ma X. (2009). Effect of agar on the microstructure and performance of potato starch film. *Carbohydrate Polymers,* 76, 299-304.
292. Wan Y.Z., Luo H., He F., Liang H., Huang Y., Li X.L. (2009). Mechanical, moisture adsorption, and biodegradation behaviours of bacterial cellulose fibre-reinforced starch biocomposites. *Composites Science and Technology,* 69, 1212-1217.
293. Wang Z., Zhou J., Wang X., Zhang N., Sun X., Ma Z. (2014). The effects of ultrasonic/microwave assisted treatment on the water vapour barrier properties of soybean protein isolate-based oleic acid/stearic acid blend edible films. *Food Hydrocolloids,* 35, 51-58.
294. Wisuthiphaet N., Damerow L., Blanke M.M. (2014). Non-destructive detection of the wax bloom on European plum during post-harvest handling. *Journal of Food Engineering*, 140, 46–51.
295. Wu H.X., Liu C.H., Chen J.G., Chang P.R., Chen Y., Anderson D.P. (2009). Structure and properties of starch/α-zirconium phosphate nanocomposite films. *Carbohydrate Polymers*, 77, 358-364.
296. Wu Y., Geng F., Chang P. R., Yu J., Ma X. (2009). Effect of agar on the microstructure and performance of potato starch film. *Carbohydrate Polymers,* 76, 299-304.
297. Xie F., Flanagan B.M., Li M., Sangwan P., Truss R.W., Halley P.J., Shamshina J.L., Rogers R.D., McNally T. (2014) Characteristics of starch-based films plasticized by glycerol and by the ionic liquid 1-ethyl-3methylimidazolium acetate: A comparative study. *Carbohydrate Polymers,* 111, 841-848.

298. Xie F., Halley P.J., Avérous L. (2012). Rheology to understand and optimize processibility, structures and properties of starch polymeric materials. *Progress in Polymer Science*, 37, 4, 595-623.

299. Yan Q., Hou H., Guo P., Dong H. (2012). Effects of extrusion and glycerol content on properties of oxidized and acetylated corn starch-based films. *Carbohydrate Polymers*, 87, 707-712.

300. Yang L., Paulson A.T. (2000). Mechanical and vapor barrier properties of edible gellan films. *Food Research International*, 33, 563-570.

301. Yin Y.J., Yao K.D., Cheng G.X., Ma J.B. (1999). Properties of polyelectrolyte complex films of chitosan and gelatin. *Polymer International,* 48(6), 429–432.

302. Yoshida C.M.P., Antunes A.J. (2004). Characterisation of whey protein emulsion films. *Brazilian Journal of Chemistry and Engineering,* 21, 247-252.

303. Young, T. (1805). An Essay on the Cohesion of Fluids. *Philosophical Transactions of the Royal Society*, 95, 65-87.

304. Yuan Y., Zhang L., Dai Y., Yu J. (2007). Physicochemical properties of starch obtained from *Dioscorea nipponica* Makino comparison with other tuber starches. *Journal of Food Engineering,* 82, 436-442.

305. Yuryev V., Tomasik P., Bertoft E. (2007). Starch: Achievements in understanding of structure and functionality. Nova Science Publishers, New York.

306. Zaleska H., Ring S., Tomasik P. (2001). Elektrosynthesis of potato starch-whey protein isolate complexes. *Carbohydrate Polymers,* 45, 89-94.

307. Zhanga Y., Thompson M., Liu Q. (2011). The effect of pea fiber and potato pulp on thermal property, surface tension, and hydrophilicity of extruded starch thermoplastics. *Carbohydrate Polymers,* 86, 700–707.

308. Zavareze E.R., Pinto V.Z., Klein B., El Halal S.L.M., Cardoso Elias M., Prentice-Hernández C., Guerra Dias A.R. (2011). Development of oxidised and heat–moisture treated potato starch film. *Food Chemistry*, 132, 1, 1344-350.

309. Zavareze E.R., Storck C.R., Suita de Castro L.A., Schirmer M.A., Guerra Dias A.R. (2010). Effect of heat-moisture treatment on rice starch of varying amylose content. *Food Chemistry,* 121, 2, 358-365.

310. Ziemnicka-Niewczas J. (2007). Packaging as source of success. *Opakowanie 2,* 30-32. (In Polish).

311. Zisman W.A. (1964). Contact angle, wettability and adhesion. Washington: American Chemical Society, 1-51.
312. Zobel H.F. (1988). Molecules to granules: a comprehensive starch review. *Starch/Stärke*, 4, (20), 44-45.
313. Zullo R., Iannace S. (2009). The effects of different starch sources and plasticizers on film blowing of thermoplastic starch: Correlation among process, elongational properties and macromolecular structure. *Carbohydrate Polymers*, 77, 376-383.
314. Żenkiewicz M., Richert J. (2008). Permeability of polylactide nanocomposite films for water vapour, oxygen and carbon dioxide. *Polymer Testing*, 27, 835-840.

315. http://www.environmentalhealthnews.org/
316. http://blog.michalgosk.com/opakowania/historia-opakowan-czesc-1/ (in Polish)
317. http://blog.michalgosk.com/opakowania/historia-opakowan-czesc-2/ (in Polish)
318. www.biolinscientific.com
319. www.chemspider.com

RESUME EN FRANCAIS

ENGLISH ABSTRACT

STRESZCZENIE W JĘZYKU POLSKIM

RÉSUMÉ FRANÇAIS

INTRODUCTION

Depuis l'aube de la civilisation moderne, les gens doivent être en mesure de stocker leurs aliments. Cependant, des méthodes telles que la congélation ou le séchage ne sont pas toujours possibles partout dans le monde, le transport n'étant pas forcément suffisamment développé ou adéquat. De plus, certains aliments se dégradent très rapidement, ce qui entraîne de nombreuses contaminations pouvant entrainer des décès. Le développement des premiers emballages a été une étape importante dans le stockage et la conservation des aliments. La véritable révolution est survenue au début du XXe siècle. Avec la découverte de polyéthylène, l'ensemble de la chaîne logistique de production, le transport et le stockage des aliments était alors initié. Les emballages en plastique ne sont pas seulement pratiques, fonctionnels mais aussi peu chers. Les nombreux avantages des plastiques ont induit le remplacement progressif des autres matériaux d'emballage. Même les paniers en osier, si populaires et utilisés par les consommateurs sur les marchés, ont été remplacés par des sacs en matière. Chaque année, une seule personne en Europe consomme 450 sacs en plastique. 500 000 tonnes sont ainsi produites dans le monde. Chaque année, un habitant européen génère 250 kg de déchets. La production de l'emballage donne 80 millions de tonnes de déchets plastiques par an. Une large partie des déchets est constituée par les emballages dont une fraction importante n'est ni triée ni détruite et finit dans les océans, tuant ou contaminant les animaux marins. Ces inconvénients concernent la chaine alimentaire jusqu'à l'homme, induisant des désordres endocriniens, des maladies et une hausse du prix des produits halieutiques. Au XXIe siècle, même la laitue, les oranges, le brocoli, les biscuits sont emballés individuellement dans des films plastiques. Sur la base de ces tendances de consommation, des conséquences graves se produiront parce que la superficie exploitable (cultivable) de la terre est limitée et l'homme est fragile. En outre, les emballages issus du pétrole ont besoin jusqu'à 1000 ans pour leur dégradation / décomposition. Selon les données rapportées par l'UE, en 2050 la terre comptera 10 milliards d'habitants. Cela correspondra aussi au début de l'épuisement des ressources de pétrole, il faudra alors plus de surfaces pour la production alimentaire. Une solution pour ces problèmes peut être l'utilisation d'emballages biodégradables ou comestibles. Ils peuvent être produits à partir de ressources naturelles comme des plantes et / ou des produits d'origine animale. L'un des polysaccharides le plus abondant est l'amidon. C'est un constituant de réserve des plantes supérieures, disponible presque partout. L'amidon ne provoque pas d'allergies; il est insipide, inodore, peut être mis en forme, imprimable et peut jouer non seulement la fonction d'emballage, mais peut aussi être utilisé comme une étiquette. L'amidon utilisé sous la forme d'enrobage comestible peut être un emballage moderne et futuriste. Les protéines, quant à elles, sont des hydrocolloïdes utilisés pour la fabrication des bio-emballages. Elles apportent des éléments nutritionnels précieux et en combinaison avec l'amidon peuvent réduire sa perméabilité à la vapeur d'eau. L'addition d'huile permet une réduction importante des propriétés barrières à la vapeur d'eau, qui est le principal inconvénient de l'amidon. Conformément au protocole de Kyoto en 1997, l'utilisation des matériaux biodégradables a également l'avantage de baisser la production de dioxyde de carbone. Les biopolymères ont une influence positive sur l'environnement et plus directement sur les aliments. Aujourd'hui, l'espérance de vie est 2,5 fois plus élevée qu'il y a 30 ans ; les gens attendent donc une nourriture commode, peu transformée et sûre. Pour atteindre ces objectifs, les enrobages et les films peuvent être envisagés comme une des solutions.

Le domaine d'application de matières biodégradables dans l'industrie alimentaire est très large. Les films réalisés à partir d'amidon avec addition de glycérol présentent des propriétés mécaniques voisines de celles des plastiques. Ses propriétés mécaniques élevées doivent être maintenues. La résistance à la traction des films produits doit être de l'ordre de 3,5 MPa, valeur de référence exigée pour les matières plastiques. De plus, les polymères d'amidon peuvent être facilement utilisés pour le conditionnement et le transport des aliments, comme des sacs pour le shopping, des barquettes pour la viande, le poisson, le fromage, les fruits, etc. En dessous de cette valeur de résistance mécanique, les films à base d'amidon doivent être utilisés comme enrobages. Sinon, le maintien de l'allongement, du module d'Young et de résistance à la traction sont obligatoires pour la réalisation des films. Les propriétés de mouillabilité des films d'amidon font partie des critères principaux de l'évaluation de leur hydrophilie. Elles sont directement liées à l'adsorption de l'eau, qui, pour des produits alimentaires doit être faible. Par conséquent, les isothermes de sorption de l'eau, la perméabilité à la vapeur d'eau, la solubilité dans l'eau, l'indice de gonflement, les cinétiques d'absorption et la diffusivité, et de propriétés de surface comme les angles de contact, la tension superficielle critique, les forces d'adhésion, de cohésion, etc. doivent être mesurés. Ces propriétés conditionnent ou résultent des échanges d'humidité entre le produit et l'environnement ambiant pouvant provoquer des modifications dans les denrées alimentaires et les propriétés du film. La connaissance des isothermes de sorption d'humidité des films et de l'activité de l'eau jouent un rôle important pour prédire les changements de qualité et la durée de vie des bioproduits et produits alimentaires. Par goniométrie, il est possible d'évaluer la mouillabilité de surface du film par une mesure direct de l'angle de contact de liquides tels que l'eau ou le di-iodomethane. La tension superficielle critique informe sur le caractère hydrophile ou hydrophobe et influe également sur les performances barrière à l'eau du film. Au-delà des propriétés d'humidité, des caractéristiques optiques sont également impliqués dans les qualités fonctionnelles des films. Dans l'acte d'achat, les consommateurs utilisent en premier lieu le sens de la vue. Par conséquent, les paramètres de couleur jouent un rôle important. Avec le vieillissement, les caractéristiques sensorielles telles que l'aspect, la couleur, l'odeur d'un emballage peuvent changer. Le vieillissement physique peut aussi causer la délamination, la séparation des phases, la migration du glycérol depuis le biopolymère et conduire à des propriétés réduites voire indésirables de l'emballage.

Le choix de l'emballage ou d'un enrobage biodégradable adéquat et optimisé peut réduire considérablement la quantité de produits en plastique comme les sachets et sacs de caisse. L'une des applications les plus prometteuses concerne la durée de conservation des fruits et légumes de saison, par exemple.

Objectifs de la thèse

Les objectifs du travail de thèse étaient de trouver un matériau filmogène le plus approprié, de déterminer les concentrations en plastifiants, composés hydrophobes, additifs introduits dans les films et enrobages à base d'amidon, tout autant que structure monocouche ou multicouche, pour une application dans l'industrie alimentaire (conservation des fruits et légumes frais). Pour atteindre ces objectifs, la compréhension des interactions entre les composants, les propriétés physico-chimiques des films, les propriétés interfaciales des films au contact du produit et des enrobages déposés sur la peau des fruits, la structure des films, les mécanismes de transferts de masse doivent être étudiés et compris.

Quinze types de films ont été préparés, tous à base d'amidon, en combinaison ou non avec des protéines ou des lipides. Les propriétés physiques, chimiques, optiques, mécaniques, thermiques, structurales, de sorption et les caractéristiques de surface ont été mesurées. En fonction des résultats physico-chimiques obtenus, quelques recettes de films ont été choisies pour être testés sur des produits alimentaires réels (prunes) sous forme d'enrobage ou de films d'emballage. Des tests physiques, physiologiques, chimiques ont été réalisés sur des prunes en fonction du temps de stockage. Le développement de nouvelles méthodes pour évaluer l'épaisseur de l'enrobage, la perte de poids des fruits via des mesures thermographiques et les équations mathématiques associées a été mis en place.

Cette thèse est organisée en 3 sections principales (synthèse bibliographique, matériel et méthodes, et résultats-discussion) subdivisées en 11 chapitres.

Le chapitre 1 (section 1) donne une synthèse et une analyse détaillée de la littérature scientifique sur les biomatériaux, en particulier l'amidon comme matière filmogène. Il repose sur 4 publications.

La section Matériel et Méthodes (chapitre 2) est la compilation des parties « matériel et méthodes » de toutes les publications qui constituent la section 3 des résultats et discussions.

La section 3 « résultats et discussion » est composée de 9 publications acceptées ou soumises : les premières portant sur les propriétés physico-chimiques des films à base d'amidon, les dernières concernent l'application sur les produits alimentaires réels (les prunes). Le chapitre 3 est relatif à l'étude de plusieurs amidons, la sélection des films les plus appropriés pour leur combinaison avec d'autres constituants et à l'application finale. Ensuite, l'effet de la concentration en glycérol (chapitre 4) a été étudié pour trouver la concentration du plastifiant la plus adéquate sur les propriétés barrières et mécaniques et ayant la polarité pour un bon recouvrement (adhésion) de la surface des prunes. Les chapitres 5 et 6 sont centrés sur le mélange de protéines de lactosérum et d'amidon et l'examen de son impact sur les propriétés de structure, fonctionnelles et de surface des films. Les chapitres 7, 8 et 9 traitent de l'influence de l'incorporation de l'huile sur les propriétés physico-chimiques et de structure des films. Les chapitres 10 et 11 sont dédiés aux applications des enrobages sur les prunes ou de l'emballage des prunes dans les films à base d'amidon. Les propriétés de surface, les paramètres de physiologie, la prolongation de la durée de vie des prunes ont été analysées ainsi que la comportement de l'enrobage ou du film au contact des fruits.

Enfin, les conclusions générales de la thèse et les perspectives sont présentées avant la liste des références.

RESULTATS (traduction des résumés et conclusions des articles)

Chapitre 3: Role de l'origine de l'amidon et de sa concentration sur les propriétés physico-chimiques de films comestibles (Basiak E., Lenart A., Debeaufort F. 2015. Role of starch nature on the physico-chemical properties of edible films. Submitted to Journal of Food Science, December, 2015)

La conservation des aliments est principalement mise en oeuvre dans des emballages en matière plastique issue du pétrole, posant des problèmes environnementaux liés à la pollution. Mais cet inconvénient pourrait être limité par l'utilisation de films et d'enrobages comestibles ou biodégradables. Les propriétés physico-chimiques des films ont été évalués pour démontrer le rôle de la nature de l'amidon (blé, maïs ou pomme de terre), et donc celui du rapport amylose / amylopectine. Ce dernier influe sur l'épaisseur, la couleur, l'humidité, la mouillabilité et sur les propriétés mécaniques, thermiques et de surface. Le rapport amylose/amylopectine dans les films à base d'amidon joue un rôle important sur les propriétés physiques et chimiques. Ce rapport influe fortement sur la microstructure. En effet, l'amylopectine entraine une augmentation de la viscosité de la solution filmogène qui affecte la rétraction du réseau pendant le séchage du film et donc l'épaisseur finale. La couleur du film et la transparence dépendent de l'épaisseur. En effet, les films à base d'amidon de pomme de terre sont transparents, alors que les matrices d'amidon de maïs et de blé sont plus opaques et blanchâtres. Le ratio amylose/amylopectine influence également les propriétés d'hydratation. De plus faibles solubilités dans l'eau et perméabilités à la vapeur d'eau et à l'oxygène sont observées pour les films d'amidon de pomme de terre, correspondant à une teneur en amylopectine plus basse. Plus la teneur en amylose est grande, plus la résistance superficielle est accrue, comme les propriétés mécaniques et barrières. Des teneurs en amylose supérieures dans les films induit une plus grande sensibilité à l'humidité, et ainsi affecte les propriétés mécaniques et de barrière. Dans le cas des fruits et légumes, des films ou des enrobages en couches minces à base d'amidon n'inhibent pas les processus physiologiques (respiration et transpiration) mais ils les retardent. Les films fabriqués à partir d'amidon de pomme de terre constituent une meilleure barrière pour l'oxygène et la vapeur d'eau. Mais leurs propriétés mécaniques sont plus faibles que celle des films à base d'amidon de blé ou de maïs. Les films fabriqués à base d'amidon à haute teneur en amylose ont des propriétés de mouillabilité plus faibles et une meilleure résistance mécanique, de sorte qu'ils peuvent être utilisés pour les produits ayant une activité d'eau plus élevée comme le fromage, les fruits et les légumes, etc.

Chapitre 4: Influence des teneurs en glycérol et en eau sur la structure et les propriétés fonctionnelles de films comestibles à base d'amidon (Basiak E., Lenart A., Debeaufort F. 2015. Effects of carbohydrate/protein ratio effects on microstructure, barrier and sorption properties of wheat starch – whey protein blend edible films. Submitted in Innovative Food Science and Emerging Technologies, July 2015)

Produit peu cher, l'amidon est largement disponible et est en mesure de remplacer 1/3 des plastiques pour fabriquer des films et des contenants biodégradables. En outre, l'amidon pourrait être envisagé comme enrobage comestible pour prolonger la durée de conservation des aliments. Des films d'amidon de blé avec deux teneurs en glycérol ont été formulés. Leurs propriétés structurales et fonctionnelles ont été caractérisées. Les propriétés de transfert de films d'amidon contenant 33% de plastifiant (tels que le coefficient de diffusion, les perméabilités à l'oxygène et à la vapeur d'eau à 2 gradients d'humidité différents, la composante polaire de la tension de surface, les forces d'adhérence et de cohésion, et la sorption d'humidité) sont réduites comparées aux valeurs des films contenant 50% de glycérol. La teneur en glycérol ne joue pas un rôle important sur la couleur ou sur les propriétés mécaniques. Ce travail montre que le glycérol peut toutefois fortement affecter les autres propriétés fonctionnelles des enrobages et des films à base d'amidon. Les principales conclusions peuvent être tirées de la manière suivante: les films contenant 33% de glycérol ont une capacité d'absorption d'humidité plus faible que des films avec une teneur plus élevée en glycérol. Lorsque la concentration des plastifiants augmente, la résistance à la traction et le module d'élasticité diminuent, alors que l'allongement à la rupture augmente. Plus la teneur en glycérol est élevée, plus les perméabilités à l'oxygène et à la vapeur d'eau et la diffusivité de l'eau sont accrues. Les interactions entre l'amidon, le glycérol et l'eau se produisent entre les groupes C-O et OH impliquant principalement des liaisons hydrogène.

Chapitre 5: Caractérisation de films comestibles composites à base d'amidon et de protéines de lactosérum (Basiak E., Galus S., Lenart A. 2015. Characterisation of composite edible films based on wheat starch and whey-protein isolate. International Journal of Food Science and Technology, 50, 372-380).

L’amidon de blé et les isolats protéiques de lactosérum sont d'excellents matériaux pour faire des films comestibles. Des films composites ont été préparés par casting de solutions filmogènes à base d’amidon de blé et de protéines de lactosérum dans des proportions de 100-0, 75-25, 50-50, 25-75, 0-100% et ont été caractérisés d’un point de vue physico-chimique. La combinaison des deux hydrocolloïdes a donné des films continus et homogènes. Plus la teneur en amidon augment, plus les films sont ternes, blanchâtres et opaques. Tous les films étudiés, purs ou composites, ont une structure continue et homogène. Seul l’aspect de la surface entre films à base d’amidon pur ou de protéines pures diffère. En effet, la brillance du film augmente avec la teneur en protéines. L'épaisseur finale du film dépend de la composition des solutions filmogènes. Les valeurs les plus élevées de l'épaisseur et de l'allongement à la rupture ont été observées pour les films composites produits à base du mélange amidon-protéines. L'adsorption d'eau est la plus importante pour les films à base de protéines de lactosérum et la plus faible pour les films d'amidon de blé, avec des teneurs en eau respectives de 0,324 et de 0,264 $g_{eau}/g_{matière\ sèche}$. Les cinétiques de sorption de vapeur d’eau prouvent le caractère hydrophile des deux hydrocolloïdes, aussi bien l’amidon que les protéines. Avec la teneur croissante de protéines de lactosérum, l'indice de gonflement et la résistance à la rupture des films a augmenté de 34,31 à 71,01% et de 2,29 à 8,90 MPa, respectivement. Les valeurs de perméabilité à la vapeur d'eau dépendent des conditions d'humidité, mais elles diminuent avec la concentration croissante en protéines de lactosérum. Lorsque le gradient d'humidité relative (HR) augmente, la perméabilité à la vapeur d’eau augmente d’autant plus que la teneur en amidon est réduite. La résistance à la traction augmente avec la concentration en protéines des films.

Chapitre 6: Influence du ratio polyosides/protéines sur la microstructure et sur les propriétés de sorption et barrières de films comestibles à base d’amidon de blé et de protéines de blé (Basiak E., Lenart A., Debeaufort F. 2015. Glycerol and water contents affect the structural and functional properties of starch-based edible. Submitted in Food Technology and Biotechnology, July 2015)

De l'amidon et des protéines de lactosérum, et leurs mélanges ont été utilisés pour fabriquer des films comestibles. Les isothermes de sorption d'humidité, la perméabilité à la vapeur d'eau, la sorption de composés d'arômes, la microstructure, les angles de contact de l'eau et les propriétés de surface ont été étudiées. Avec l'augmentation de la teneur en protéines, la microstructure a changé vers une plus grande homogénéité. En effet, les films d'amidon sont plus hétérogènes et moins denses que les films protéiques. Plus la teneur en amidon est élevée, plus les films sont homogènes et denses. Les propriétés de surface varient selon le rapport amidon/protéines de lactosérum et sont principalement liées à la composante polaire de l'énergie de surface. En effet, la composante dispersive de l’énergie de surface n'a pas varié. 80% d'amidon - films de protéines 20% de lactosérum ont des surfaces plus hydrophobes que les autres films en raison d'interactions spécifiques. L'énergie de surface, les forces d'adhésion, de cohésion et d'étalement sont plus faibles pour les films mono-composants et pour les films 80S-20WP contrairement aux autres mélanges. Pour tous les échantillons, l’hygroscopie des films augmente avec la teneur en amidon.

La perméabilité à la vapeur d'eau s’accroit avec à la fois le gradient d'humidité et la teneur en amidon.

La sorption de butyrate d'éthyle décroit avec la concentration en amidon alors que la perméabilité à la vapeur d’eau augmente. L'augmentation de l'hydrophobie coïncide avec une diminution de la volatilité des composés d’arômes. Ceci induit une absorption réduite pour le composé plus hydrophobe comme pour l'hexanoate d'éthyle et d'octanoate d'éthyle. Cela signifie que les films d'amidon de blé seront de moins bonnes barrières aux composés d’arôme hydrophobes.

Chapitre 7: **Influence de l’ajout d’huile de colza sur les propriétés optiques de films à base d’amidon** (Basiak E., Lenart A. 2013. Wpływ oleju rzepakowego na właściwości optyczne filmów skrobiowych. Postępy Techniki Przetwórstwa Spożywczego, 23/42, 1, 23-26 Influence of rapeseed oil on optical properties of starch films, Development of Technic and Food Industry, 23/42, 1, 23-26).

Le but de ce travail était d'analyser l'effet de l'addition d'huile de colza sur les propriétés optiques des films d'amidon de blé. Des films ont été préparés à partir de solutions aqueuses filmogènes d'amidon (5g/L) avec 50% de glycérol (% du poids d’amidon) utilisé comme plastifiant. L'huile de colza a été ajoutée à la solution à une concentration de 0, 1, 2, 3%. Toutes les caractéristiques colorimétriques ont été mesurées sur la base du système international CIEL : L*a*b, différence de couleur totale, la saturation des couleurs et de l'indice de saturation. De plus l'opacité à la longueur d'onde de 600 nm a été calculée. Une modification très significative des paramètres de couleur et une diminution de l'opacité des films d'amidon a été constatée avec addition d'huile de colza.

Après séchage, des zones grasses ou huileuses ont été observées sur la surface des films. Avec l'augmentation de la teneur en huile (1-3%), l'huile est d'autant plus visible sur la surface. L'huile de colza des films d'amidon réduit la luminosité (L) mais augmente la valeur b. Quoi qu'il en soit, changer la concentration d'huile dans une plage de 1 à 3% ne provoque pas de modification significative des paramètres colorimétriques des films en dehors de l'opacité qui est améliorée avec la concentration en huile.

Chapitre 8: **Effet sur les propriétés des films de l'ajout d'huile par laminage entre des couches d'amidon plastifié** (Basiak E., Lenart A., Debeaufort F. 2016. Effect of oil lamination and starch content on structural and functional properties of starch-based films. Food Chemistry,195, 56-63.)

Depuis la fin des années 80, la plupart des publications traitent des films multicouches contenant des graisses solides ou des huiles hydrogénées ou des cires. Cependant, les couches de matière grasse solide sont souvent très fragiles et n'adhérent pas à des couches de polysaccharides ou de protéines. De plus, leurs caractéristiques sensorielles sont délétères pour les aliments sur lesquels ils sont appliqués sous forme d'enrobages. Pour réduire le caractère hygroscopique des films biodégradables à base d'amidon, de l'huile de colza a été incorporé par laminage (amidon-huile-amidon par une technique 3 couches). L'huile liquide a été choisie pour éviter ces inconvénients, même si nous supposions que la structure finale diffère probablement d'un véritable système 3 couches. La stratification supposée après le protocole de fabrication par dépôts successifs de 3 couches a finalement conduit à obtenir des films de type émulsion.

Les films composites sont plus opalescents et plus brillants que les films lipidiques purs. Pour tous les films, bien que leur surface semble uniforme, la structure est hétérogène dans la section transversale des films. L'ajout de matières grasses a induit une diminution de la résistance à la traction du fait de zone de rupture préférentielle. L'analyse thermogravimétrique n'a pas montré de différences entre les films avec et sans huile. L'addition de lipide réduit l'absorption d'humidité en particulier aux fortes humidités relatives, ainsi que l'indice de gonflement de la surface lors du contact avec des gouttelettes d'eau.

L'hydrophobie de surface est augmentée et de ce fait l'affinité de la surface des films pour les liquides moins polaires que l'eau est accrue avec l'addition d'huile de colza. L'addition d'huile de colza réduit de manière significative la perméabilité à l'oxygène et la vapeur d'eau. Cependant, l'augmentation de la teneur en amidon dans la solution filmogène pour accroitre la viscosité et limiter l'effet de mélange (émulsion) a modifié les propriétés de surface mais très peu l'efficacité barrière.

Chapitre 9: Importance des interactions entre les enrobages à base d'amidon et la surface de prunes : approche physico-chimique (Basiak E., Linke M., Geyer M., Debeaufort F., Lenart A. 2016. Submitted to Journal of Food Engineering in February 2016)

Des enrobages à base d'amidon de blé, d'isolat de protéines de lactosérum, ou à base de mélanges amidon-protéines de lactosérum (en rapport 80-20%) ont été préparés et appliqués pour prolonger la durée de conservation des fruits, pour améliorer l'apparence et garder toutes les propriétés nutritionnelles des prunes. Les films ont également été produits pour évaluer certaines propriétés qui ont aidé à comprendre les phénomènes qui se produisent à la surface des prunes enrobées. L'épaisseur des films d'amidon obtenus à partir du mélange d'amidon et de protéines de lactosérum (80/20) était similaire. L'addition d'huile de colza à l'amidon a entrainé une réduction de l'épaisseur des films de 80 à 28µm. Lorsqu'ils sont appliqués sur les prunes, les enrobages à base d'amidon ou à base du mélange (80/20) ont la même épaisseur. Les enrobages à base d'amidon additionnés d'huile ou de protéines ont de meilleures propriétés barrières que les films d'amidon seul. L'addition d'huile double la valeur de l'angle de contact et de la composante dispersive de la tension superficielle des enrobages déposés sur les prunes. De même, les forces d'adhérence, de cohésion et d'étalement, l'absorption de vapeur d'eau et la solubilité des films et l'enrobage dans l'eau sont diminuées. Les nouvelles liaisons ou interactions établies entre l'amidon, les protéines de lactosérum, l'eau, le glycérol et l'huile ont été identifiées par l'analyse FTIR. L'addition influence fortement les propriétés physiques et chimiques de l'enrobage. Les caractéristiques colorimétriques des fruits enrobés ont été améliorées par les couches protectrices. Ce travail a révélé les interactions entre les enrobages et la surface des prunes et l'efficacité des enrobages pour des prunes.

Chapitre 10: Comportement dynamique de prunes enrobées (Basiak E., Linke M., Geyer M., Debeaufort F., Lenart A. (2016). Dynamic behaviour of coated plum as example. In preparation).

L'épaisseur des enrobages deposés sur des prunes a été déterminée par une méthode nouvelle basée sur la détection quantitative par camera thermographique qui mesure l'environnement autour du fruit. Le principe de calcul est présenté dans cette publication. La méthode s'appuie sur la loi de Fick. Comparée aux methodes plus classiques comme la microscopie électronique à balayage, l'observation par loupe binoculaire ou la mesure directe par pied-à-coulisse, la détection par caméra thermographique est plus rapide, et plus précise et exacte pour mesurer l'épaisseur d'enrobage fin sans détruire l'échantillon sur lequel il est appliqué. Les système étudiés étaient des prunes enrobées par deux solutions filmogènes - l'une à base d'amidon, l'autre à base d'un mélange amidon-protéines de lactosérum (80/20) – et stockées à deux humidités différentes. Malgré une teneur en matière séche identique des solutions filmogènes, l'épaisseur des films à base d'amidon s'avère plus importante que celle du mélange avec les protéines. Aucun effet sur les résistances à la transpiration, de l'enrobage ou de la couche limite non agitée n'a été observé.

Chapitre 11: Application de films et enrobages à base d'amidon et de protéines de lactosérum pour la conservation de prunes (Basiak E., Linke M., Geyer M., Debeaufort F., Lenart A. 2016. Impact of biodegradability materials on quality of plums. In preparation for Journal of Food Science and Technology).

Des enrobages comestibles à base d'amidon ont montré un effet positif sur la qualité de de prunes (Prunus domestica cv. Jojo). Les échantillons de prunes ont été répartis en 5 groupes: témoins (prunes non traitées), prunes enrobées par la solution d'amidon, prunes enrobées par la solution filmogène amidon-protéines de lactosérum (80-20) et des prunes emballées soit dans un film comestible à base d'amidon ou dans un films d'amidon et protéines de lactosérum. Seuls les enrobages ont une incidence sur la couleur des prunes, sans pour autant dégrader leur apparence. Après 28 jours de stockage en conditions contrôlées, les prunes enveloppées dans les films présentent une fermeté nettement supérieure à celles des prunes enrobées qui reste légèrement plus fermes que celle des prunes témoins. La transpiration des fruits enrobés et enveloppés dans les films comestibles est significativement réduite comparée aux témoins, jusqu'à deux fois plus pour les films et enrobages à base d'amidon. La même évolution est constatée pour la respiration des prunes. Les formules à base de protéines et amidon restent effective bien que moindre que pour l'amidon. La perte d'eau des fruits non traité est supérieur à 10% alors qu'elle est réduite à 5% pour les films et enrobages à base d'amidon et de l'ordre de 6% pour les mélanges amidon-protéines. La finalité de ce travail montre que la durée de vie des prunes est ainsi prolongée d'au moins un tiers par l'application d'un enrobage à base d'amidon ou d'enveloppement dans un film comestible à base d'amidon.

CONCLUSIONS

Jusqu'au dix-huitième siècle, les produits alimentaires ont été transportés et stockés dans des matières premières naturelles (bois, papier, cellophane). Avec la révolution industrielle, ces habitudes ont été modifiées. Quelques décennies plus tard, avec l'avènement du plastique, beaucoup de produits alimentaires ont été enveloppés, emballés, conditionnés dans divers matériaux polymères plastiques tels que le polyéthylène basse densité, le polyéthylène téréphtalate pour satisfaire aux nouvelles habitudes de consommation (figure 11.1).

Figure 11.1 Les emballages alimentaires de nos jours.

Au cours des dix dernières années, la production de matière plastique a été plus élevé que dans l'ensemble de la période écoulée depuis que les matériaux d'emballage en plastique ont été inventés. Produits à partir du pétrole brut, les plastiques sont pratiques pour les utilisateurs, et surtout relativement peu chers. La durée excessive de désintégration des plastiques issus du pétrole brut qui induit la pollution visible et les perturbations environnementales qui s'ensuivent, et l'augmentation de son prix sont les principaux inconvénients. Bien que les premières matières plastiques inventées étaient biodégradables (Cellophane□ obtenue à partir de cellulose de bois ou de paille, Bakelite□ produite à partir des protéines du lait), il est nécessaire de réduire les matières plastiques en produisant de nouveaux emballages biodégradables. Ces matériaux respectueux de l'écologie peuvent être appliqués sur la nourriture (comme enrobages) ou utilisés séparément (comme les films). Leurs temps de décomposition sera même de plusieurs ordres de grandeur plus courts que ceux des plastiques, ce qui peut aider à la réduction des nuisances causées par les matières plastiques.

Beaucoup de matières premières bio-sourcés peuvent être envisagées pour la production d'emballages biodégradables. Les matériaux les plus couramment utilisés sont des polysaccharides, puis les protéines et les graisses. Les plus appropriés, abondants, renouvelables et à faible coût sont les amidons, en particulier de pomme de terre, de maïs et de blé. Voilà pourquoi nous avons sélectionné ces 3 types d'amidon pour faire des films et enrobages destiné à être appliqués sur les fruits.

Les films ont ainsi été préparés à partir d'amidon de maïs (27% d'amylose), d'amidon de blé (25% d'amylose) et d'amidon de pomme de terre (20% d'amylose) avec une relativement faible teneur en glycérol (30% de la masse de l'amidon) pour mieux mettre en évidence l'effet de la nature de l'amidon. Le rapport amylose / amylopectine de chaque amidon testé a été considéré pour l'explication des comportements des films. Ce rapport a fortement influencé la microstructure parce que la viscosité de la solution filmogène augmente avec la teneur en amylopectine. Cela a affecté la rétraction du réseau pendant le séchage du film et l'épaisseur finale. En effet, les films d'amidon de maïs ont été les plus visqueux et sont plus épais pour une même quantité de matière sèche déposée par unité de surface dans des boîtes de Petri. La couleur du film et sa transparence dépendent aussi de l'épaisseur. En effet, les plus minces films d'amidon de pomme de terre sont transparents, tandis que les matrices à base d'amidon de maïs et de blé sont plus blanches et opaques. Le ratio amylose / amylopectine influence également les caractéristiques d'hydratation. Les plus faibles solubilités dans l'eau, perméabilités à la vapeur d'eau et à l'oxygène ont été obtenues pour les films d'amidon de pomme de terre et les films d'amidon de blé correspondent à la plus faible teneur en amylopectine. La surface et les propriétés mécaniques sont également améliorées. Plus la teneur en amylose est élevée, plus la barrière est efficace, la résistance superficielle à l'absorption d'humidité et les propriétés mécaniques sont accrues. Dans le cas de films à base d'amidon de maïs et de pommes de terre, le gonflement de la surface (figure 11.2) et une plus haute sensibilité à l'humidité ont été observées. Cela suggère une limitation de l'application pour les produits ayant une teneur élevée en eau ou le stockage dans une humidité relative élevée. Cette première étude sur des films à base d'amidon a montré la meilleure adéquation de l'amidon de blé pour la production de films et enrobages comestibles. Cependant, les propriétés des films à base d'amidon de blé ne sont pas complètement conformes aux exigences des applications. Par conséquent, des stratégies d'amélioration des films à base d'amidon de blé, comme la teneur en glycérol, l'addition des protéines ou de l'huile de colza, la stratification (multicouche amidon/huile/amidon) ont été envisagées au préalable à la validation sur les prunes.

Les films à base d'amidon ont été réalisés à plusieurs concentrations d'amidon. Les solutions filmogènes contenant moins de 3% d'amidon n'ont pas permis de faire des films avec des propriétés souhaitées. Les films étaient trop minces et trop cassants, avec beaucoup de défauts tels que des fissures et des trous. La teneur optimale de 5% d'amidon dans la solution filmogène a été déterminée à partir des épaisseurs et des propriétés mécaniques et barrières résultantes.

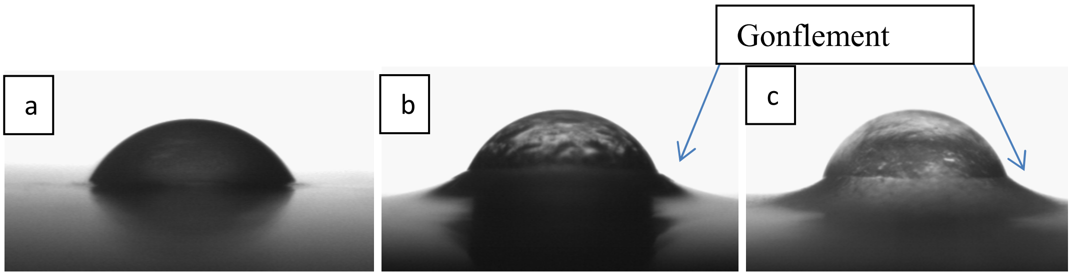


Figure 11.2 : surface des films d'amidon après deux minutes de contact avec une gouttelette d'eau: a) amidon de blé, b) amidon de maïs, c) amidon de pomme de terre.

La teneur en glycérol et le niveau d'humidité influencent fortement la structure du film et toutes ses propriétés. La mouillabilité, la teneur en eau, l'indice de gonflement, de solubilité dans l'eau, les perméabilités à la vapeur d'eau et à l'oxygène augmentent avec la teneur en glycérol. La tension de surface globale et sa composante polaire ont également augmenté alors que la composante dispersive de la tension de surface, les forces d'adhérence et de cohésion ont diminué pour les films hautement plastifiés par le glycérol. La diffusivité de l'humidité et la perméabilité aux gaz et à la vapeur d'eau ont été réduites deux fois pour les films ayant 30% de glycérol par rapport à celles des films contenant 50% de glycérol. Plus la teneur en glycérol est élevée, plus la plasticité et plus le module d'Young et la résistance à la traction ont tendance à être diminués. Toutefois, l'augmentation de 30% à 50% de teneur en glycérol rapporté à la base séche d’amidon n'a pas changé de manière significative les propriétés mécaniques à une humidité relative modérée (50% HR). L'effet de la teneur en glycérol peut être exacerbée à des humidités relatives plus élevées, ce que suggèrent les isothermes de sorption qui présentent une plus grande sorption d'humidité à des activités d'eau supérieure à 0,8 pour les films avec 50% de glycérol. Dans ces conditions, l'effet cumulatif de l'eau et du glycérol sur la plastification détériorent fortement les propriétés mécaniques des films contenant la concentration de glycérol supérieure. Enfin, des films et des enrobages avec la teneur en plastifiant inférieure peuvent être utilisés de préférence pour les produits alimentaires ayant une activité élevée de l'eau sans perdre leurs propriétés mécaniques.

Les films à base d'amidon ont des réseaux de faible cohésion qui pourraient être renforcés par mélange avec d'autres polymères capables d'interagir avec l’amylose ou l’amylopectine. Les protéines, en raison de leurs multiples groupes chimiques et les acides aminés, sont en mesure d'établir divers types de liaisons et d'interactions. En outre, ils peuvent fournir une meilleure valeur nutritionnelle aux enrobages à base d'amidon.

Ainsi, les isolats protéiques de lactosérum ont été ajoutés à l'amidon dans une solution filmogène à des taux de 0 à 100% (de la teneur en biopolymère sec) par pas de 20%. L’addition de protéines de lactosérum a permis de faire des films avec tous les ratios testés, dans la plupart des cas avec de meilleures propriétés optiques et d’aspect visuel amélioré. Plus la teneur en amidon était faible, plus les films étaient homogènes et denses. L'addition de protéines de lactosérum a permis de réduire la mouillabilité de la surface en augmentant l'angle de contact avec l'eau de 40 à 93 °, suivant la teneur en protéines. Des films d'amidon et de protéines de lactosérum seuls et des films issus du mélange 80S-20WP sont moins hydrophiles car ils ont la composante dispersive de l’énergie de surface plus élevé que celle des autres mélanges. Cependant, même si le caractère hydrophile de la surface du film a été réduit par la présence de protéines, les isothermes de sorption d'humidité ne sont pas modifiées. Les interactions entre les protéines de lactosérum et l’amidon modifient les propriétés de surface, probablement avec une orientation préférentielle des acides aminés hydrophobes orientés vers l'air pendant le séchage du film. L'ajout de protéines à tous les ratios, a également contribué à réduire légèrement la perméabilité à la vapeur d’eau des films d'amidon, quel que soit le gradient RH utilisé. Les interactions physico-chimiques impliquées expliquent l'augmentation de la densité de la structure du film et donc ralentissent la mobilité de l'eau dans le film. Les protéines de lactosérum ont également contribué à limiter l'absorption des composés d’arômes (ester d’éthyle) typiques des fruits et légumes. Cette information était intéressante pour retenir les arômes dans les fruits revêtus de films composés d’amidon et de protéines de lactosérum.

Cependant, la réduction de la sensibilité à l'humidité est encore nécessaire, même avec une concentration contrôlée de glycérol ou l'addition de protéines de lactosérum. Les films ont ainsi été formulés avec de l'huile de colza.

L'huile de colza est une huile végétale commune ayant un bon rapport de triacylglycérols Ω3 à Ω6 (environ 1:1) et contenant également des stérols. Elle est inodore et peu chère, voilà pourquoi elle a été choisie. D'après l'analyse de la littérature, il est apparu qu'une dispersion d'huile (structure en émulsion) dans la matrice de biopolymère ne permet pas d'améliorer significativement l'efficacité de film barrière à l'humidité ou la sensibilité à l'eau. Ainsi, les films d'amidon en présence d'huile de colza ont été préparés comme des systèmes multicouches, par application d'une couche d'huile (à diverses quantités) sur une première couche d'amidon, puis recouverte par une couche à base d'amidon formant une troisième couche. Cependant, après séchage du film, les observations de la microstructure par ESEM montrent que les films n’ont pas une structure multicouche comme prévu par le procédé en 3 couches, mais ils se présentent sous forme d'émulsions. En effet, l'huile de colza forme de grosses gouttelettes sur la surface du film, perturbe l'organisation du réseau d'amidon qui favorise une rétraction plus forte induisant une épaisseur deux fois plus réduite. L'huile entraine ainsi une structure plus dense sans modifier la couleur du film ou son apparence. La mouillabilité de surface a également été réduite grâce à la composante dispersive de la tension de surface et un angle de contact avec l'eau accru de 40° à 70°, réduisant aussi la composante polaire des propriétés de surface. L’addition d'huile n'a pas modifié de manière significative les propriétés mécaniques des films. Cependant, comme prévu, à la fois la solubilité dans l'eau et l'absorption d'humidité sont réduites proportionnellement à la teneur en huile. L'augmentation de l'hydrophobicité du film et la réduction de sa sensibilité à l'humidité ont permis de diminuer par deux la perméabilité à la vapeur d'eau et de diminuer de près de 10 fois la perméabilité à l'oxygène à une humidité relative élevée (> 75% d'humidité relative).

La meilleure connaissance des propriétés physiques, chimiques et fonctionnelles des films ainsi que la structure et les interactions entre les composants a permis de sélectionner quelques recettes de solutions filmogènes. Elles ont été sélectionnés pour être testées en tant qu'enrobage ou comme emballage sur les prunes. Des formulations à base d'amidon, ou avec des protéines de lactosérum (80/20) ont été utilisées sur des prunes européennes et japonaises sous forme d'enrobage, et une recette avec de l'huile a été mise au point pour améliorer la rétention d'humidité dans les fruits ainsi que l'adhésion de l'enrobage sur les prunes. Les enrobages appliqués sur la prune ont été efficaces. Les enrobages et les films ont permis une diminution des paramètres physiologiques liés à la maturité des fruits et à leur vieillissement (transpiration et respiration) et le maintien de la fermeté par rapport aux prunes non enrobées stockées dans les mêmes conditions. Notre travail a examiné l'effet de la peau, de la cire naturelle produite par les prunes à sa surface et bien sûr des enrobages à base d'amidon et de protéines ou d'huile sur la durée de vie des fruits. Les prunes enrobées présentent une fraicheur et donc une durée de conservation augmentée de 25%, ce qui est important pour les producteurs, les détaillants et les consommateurs.

Même si la formulation de films et enrobages à base d'amidon-huile-protéines a besoin d'être encore améliorée, il est important de tenir compte des technologies d'application. Ainsi, pour finaliser la validation sur des produits alimentaires réels, des tests doivent être effectués à l'échelle pilote et industrielle sur divers fruits et légumes afin de valider les essais en laboratoire sur les prunes. En outre, l'une des principales raisons pour l'application d'enrobage de fruits est de piéger à la surface (contact avec la peau) certains conservateurs comme des antifongiques. Des études récentes ont affiché les conséquences croissantes et délétères des mycotoxines (ochratoxines, aflatoxines) sur la santé humaine (lien avec plusieurs types de cancers). Or, il apparaît que la majeure partie des mycotoxines sont produites par des champignons cultivés sur la peau du fruit. Les enrobages comestibles à base d'amidon sont connus pour être des systèmes efficaces pour l'encapsulation de composés actifs tels que des antimycotoxines naturelles. Ainsi, il pourrait être intéressant d'étudier et de comprendre le comportement des films à base d'amidon contenant ces antimycotoxines naturelles (par exemple la parietina) ou le micro-organisme qui produit l'antimycotoxine (*xanthoria parietina*).

STRESZCZENIE W JĘZYKU POLSKIM

WPROWADZENIE

Aż do XVIII wieku produkty żywnościowe były transportowane i przechowywane w materiałach pochodzenia naturalnego. Wraz z pojawieniem się rewolucji przemysłowej zwyczaje te uległy zmianie. Kilka dekad później, kiedy to odkryto plastik materiał ten zrewolucjonizował wiele gałęzi gospodarek. Także wiele produktów spożywczych było pakowanych i owijanych przy użyciu różnych kopolimerów jak polietylen niskiej gęstości, politereftalan etylenu w celu zaspokojenia potrzeb konsumentów.

Z każdym rokiem wzrastała liczba produkowanych odpadów, w szczególności tych z plastiku. W ciągu pierwszych dziesięciu lat wyprodukowano więcej tworzyw sztucznych niż w przeciągu całego ubiegłego tysiąclecia. Staje się to poważym problemem dla środowiska naturalnego i dla gospodarek wielu państw. Redukcja terenów zielonych, produkcja coraz większej ilości spalin i gazów cieplarnianych nie tylko negatywnie wpływają na zdrowie, ale i zaburzają socjalną i ekonomiczną stopę życia kilku miliardów ludzi. Rozwiązaniem tych ekologicznych i ekonomicznych problemów mogą okazać się filmy i powłoki do żywności. Są to jadalne i/lub biodegradowalne materiały nakładane bezpośrednio na żywność lub produkowane poza nią. Są one wytwarzane z sacharydów, białek i tłuszczów. Najbardziej popularnym polisacharydem występującym w naturze jest skrobia. W przyrodzie występuje ona jako materiał zapasowy u roślin. Nie tylko jest łatwo dostępna, ale jest przede wszystkim niealergenna, bezzapachowa, z reguły przezroczysta (z fantastycznymi możliwościami barwienia lub zadrukowywania) i relatywnie niedroga. Otrzymywane z niej filmy z odpowiednim dodtkiem plastyfikatora są elastyczne, nie pękają, nie łamią się i nie kruszą. Z kolei powłoki tworzą jednolitą warstwę na produkcie, która w żaden negatywny sposób nie wpływa na parametry sensoryczne powleczonej nimi żywności. Także dodatek innych substancji powłokotwórczych jak białka czy tłuszcze korzystnie wpływa na parametry fizyczne, chemiczne i funcjonalne opakowań.

Choć filmy i powłoki znane są od kilkuset lat, zainteresowanie nimi rozpoczęło sie dopiero na początku XXI wieku. Wtedy to właśnie populacja odczuła negatywne skutki wynikające z produkcji i utylizacji materiałów plastikowych. Coraz szybsze tempo życia wpłynęło również na zmianę diety, rozwój chorób cywilizacyjnych. Fakty te dały społeczeństwu podstawę do zmian. Jak się okazuje biodegradowalne filmy i powłoki mogą zastąpić opakowania do żywności nawet o 1/3 ich całkowitej produkcji. Mogą być także nośnikiem aromatów, mikro- i makroelementów, witamin itd. Dodatkowo dzięki możliwości przedłużenia życia produktu, którą zapewniają, promują żywność świeżą i nieprzetworzoną. Powłoki mogą być konsumowane razem z żywnością lub można zmyć je z powierzchni produktu pod bieżącą wodą. Czas rozkładu biodegradowalnych filmów jest znacznie krótszy niż plastiku, co znacząco ułatwia organizację zagospodarowania odpadami.

Opakowania biodegradowalne są stosowane na szeroką skalę w przemyśle spożywczym. Można używać je jako tacki do mięsa, owoców, warzyw, zastąpić nimi plastikowe otoczki do sera, mogą być używane w charakterze opakowań do jajek, folii do pieczywa, słodyczy, nabiału itd. Asortyment pakowanych produktów jest również bardzo szeroki. Może to być żywność o niskiej, jak i wysokiej zawartości wody. Jednak nad pakowaniem produktów spożywczych o zawartości wody powyżej 75% trwają liczne badania, gdyż wciąż są obserwowane braki i wady w tych materiałach. Nawet włączenie tłuszczu do matrycy redukujące istotnie przepuszczalność dla pary wodnej, tlenu i aromatów nie pomaga w całkowitej eliminacji niedostatków związanych z właściwościami barierowymi w tych biopolimerach. Dotyczy to przede wszystkim przemysłu owocowo-warzywnego.

Wiele owoców i warzyw występuje sezonowo. Śliwkami europejskimi konsumenci mogą cieszyć się zaledwie maksymalnie osiem tygodni w ciągu roku. A szkoda, bo zawierają wiele cennych witamin, i pierwiastków dla naszego zdrowia. Są bogate w polifenole. Dzięki zawartemu w nich błonnikowi usprawniają pracę jelit, zmniejszają zagrożenie zachorowania na raka jelita grubego, wzbudzają apetyt, działają korzystnie na trawienie, przeciwmiażdżycowo – hamują wchłanianie się cholesterolu itd. Stąd w celu przedłużenia ich unikalnych zalet i smaku, redukcji ilości metod przetwarzania filmy i powłoki mogą okazać się idealnym opakowaniem będącym w stanie zastąpić plastik i dodatkowo przedłużyć dostępność tych owoców na półkach sklepowych.

HIPOTEZA

Filmy i powłoki na bazie skrobi mają zróżnicowane właściwości fizyczne, chemiczne i funkcjonalne. Ich zmienność uwarunkowana jest składem chemicznym oraz technologią wytwarzania.

CEL

Cel pracy stanowił znalezienie właściwego materiału powłokotwórczego na bazie skrobi, w takiej koncentracji i przy takim stężeniu plastyfikatora, które zapewnią otrzymywanie filmów i powłok zgodnych z oczekiwaniami konsumentów i producentów. Biodegradowalne opakowania powinny w szczególności mieć praktyczne zastosowanie w przechowywaniu owoców i warzyw. W produktach tych zawartość wody jest zazwyczaj powyżej 90%, stąd opakowania powinny charakteryzować się odpowiednimi właściwościami barierowymi. W celu osiągnięcia tych założeń zrozumienie i wyjaśnienie interakcji pomiędzy komponentami, właściwości fizycznych i chemicznych filmów, reakcji między fazami filmów i powłok a powierzchnią owoców, struktury filmów, mechanizmów wymiany masy jest kluczowym elementem tej pracy.

ZAKRES

Piętnaście rodzajów filmów na bazie skrobi w połączeniu z tłuszczem lub białkami zostało przygotowanych. Właściwości fizyczne, chemiczne i funkcjonalne, w tym geometryczne, sorpcyjne, optyczne, mechaniczne, cieplne, strukturalne, barierowe i powierzchniowe zostały zbadane. Następnie kilka z nich zostało wybranych do walidacji na żywności (śliwki) w postaci powłok i filmów. Testy fizjologiczne, chemiczne i fizyczne zostały przeprowadzone na śliwkach w wyznaczonych okresach przechowywania owoców. Rozwój i wprowadzenie nowych, pionierskich metod badania grubości powłok, utraty masy owoców i warzyw orazpowiązanych z tym równań matematycznych zostały utworzone i zastosowane w praktyce.

Praca jest przedstawiona w postaci 3 głównych sekcji podzielonych na 13 rozdziałów. Sekcja pierwsza (rozdział pierwszy) składająca się z czterech publikacji przedstawia ogólny i szczegółowy przegląd literatury dotyczący materiałów biodegradowalnych, w szczególności skrobi jako nowoczesnego materiału powłokotwórczego. Sekcja druga (rozdział drugi) obejmująca materiały i metody jest zbiorem materiałów i metod ze wszystkich zaprezentowanych w niniejszej pracy publikacji w czasie omówienia i dyskusji wyników. Na sekcję trzecią składa się dziewięć publikacji (wydrukowanych bądź wysłanych do redakcji), a tym samym dziewięć kolejnych rozdziałów. Zaprezentowano w nich rezultaty i dyskusję wyników. Pierwsze publikacje dotyczą fizyko-chemicznych właściwości filmów, kolejne skupiają się na aplikacji na żywności (jako powłoki) i do żywności (jako filmy). Rozdział trzeci (w części sekcja druga) dotyczy badań nad kilkoma typami skrobi i wyborze jednego rodzaju do dalszych testów i do aplikacji na żywności. Wybór stężenia glicerolu został przedstawiony w rozdziale czwartym. Efekt kilku stężeń plastyfikatora był analizowany w celu ustalenia optymalnej ilości i polarności materiału, którym zostanie pokryta powierzchnia owoców. Rozdziały piąty i szósty koncentrują się na dodatku do roztworu powłokotwórczego skrobi izolatu białek serwatkowych w kilku stężeniach i ich wpływie na właściwości strukturalne, funkcjonalne i powierzchniowe filmów. Rozdziały siódmy, ósmy i dziewiąty odnoszą się do wpływu dodatku oleju na właściwości fizyko-chemiczne i strukturalne. Zaś rozdział dziesiąty i jedenasty jest powiązany z aplikacją materiałów biodegradowalnych w postaci powłok i foli na śliwki. Właściwości powierzchniowe, parametry fizjologiczne, wydłużenie okresu przechowywania i inne parametry zostały zanalizowane w powlekanych i pakowanych owocach. Na końcu pracy w rozdziale dwunastym przedstawiono wnioski i konkluzje. Rozdział trzynasty stanowi spis literatury.

MATERIAŁY I METODY

Materiały

Do badań zostały użyte 3 rodzaje skrobi: kukurydziana (27% amylozy), pszenna (25% amylozy) i ziemniaczana (20% amylozy). Wszytskie skrobie pochodziły od tego samego producenta Hortimex (Konin, Polska). Izolat białek serwatkowych (~90% białek) BiPRO pozyskano z Davisco Foods International Inc. (Le Sueur, MN., USA), a olej rzepakowy z Bouton d’Or (Tourcoing, Francja) i z Kruszwicy (Kujawski, Polska). Bezwodny glicerol (99.9% czystości) zakupiono w Sigma-Aldrich (Niemcy) i w Chempur (Polska), 10 nasyconych roztworów soli Prolabo (Fontenay-sous-Bois, France) zostało użytych w celu otrzymania 10 różnych wilgotności względnych w 25°C: chlorku wapnia (~3% RH), chlorku litu (11%), octanu potasu (22%), chlorku magnezu (33%), węglanu potasu (43%), azotanu magnezu (53%), azotanu sodu (65%), chlorku sodu (75%), siarczanu amonu (81%) i difosforanu amonu (93%). Cztery estry etylu (Aldrich Chemical Company and Saint-Quentin

Fallavier, Francja) zostały wybrane jako model związków aromatycznych (Fenaroli, 1975) i pozwoliły na szeroki zakres badań nad lotnością i rozpuszczalnością.

Metody technologiczne

Przeprowadzono badania wstępne, na podstawie których wybrano 3 rodzaje skrobi i dwa stężnia substancji powłokotwórczej oraz dwa stężenia glicerolu i jedno stężenie dla białek serwatkowych.

Przygotowanie powłok i filmów

Roztwory powłokotwórcze wszystkich filmów zostały przygotowane w ten sam sposób. 3 g lub 5 g suchej substancji powłokotwórczej (np. skrobi pszennej) rozpuszczono w wodzie destylowanej (100 ml). W przypadku mieszanin skrobia - białko każdy z roztworów był przygotowany oddzielnie. Roztwór/ roztwory zostały podgrzane w łaźni wodnej w 85°C przez 30 min., przy obrotach mieszadła 700 rpm. Następnie mieszaniny ochłodzono do 40ºC i dodano plastyfikator (30% lub 50% glicerolu względem suchej masy) i wymieszano. W celu otrzymania filmów o pożądanej grubości zdefiniowana objętość roztworów powłokotwórczych została wylana na płytki Petriego. Roztwory suszono w 25°C i 30% wilgotności względnej przez co najmniej 48 godzin w komorze klimatycznej (KBF 240, Binder, Niemcy). Wysuszone filmy zostały zdejmowane z płytek, a w przypadku laminatów kolejna warstwa była nanoszona (olej), a po 24 godzinach filmy pokryto drugą warstwą skrobi i suszono. Filmy przechowywano w 53±1% wilgotności względnej i 25±1°C w dygestoriach z chlorkiem magnezu przez conajmniej 7 dni przed rozpoczęciem właściwych testów.

Metody pomiarowe

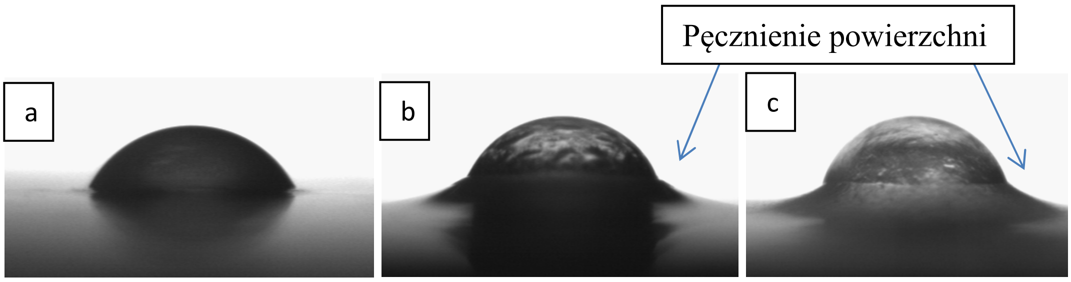
1. Właściwości transportowe i rozpuszczalność: zawartość wody, indeks pęcznienia, rozpuszczalność w wodzie, izotermy sorpcji, dyfuzja wody, sorpcja komponentów aromatów.

2. Właściwości strukturalne i optyczne: obserwacje pod mikroskopem elektronowym, obserwacje pod mikroskopem polaryzacyjnym, obserwacje pod mikroskopem świetlnym, obserwacje przy użyciu kamery termowizyjnej (pomiary temperatury, model transpiracji dla świeżych i powlekanych owoców), spektroskopia fourierowska ze spektroskopią osłabionego całkowitego odbicia w podczerwieni.
3. Właściwości geometryczne: grubość filmów, grubość powłok.
4. Właściwości powierzchniowe: determinacja kąta zwilżania, krytyczne napięcie powierzchniowe, napięcie powierzchniowe, siły adhezji, kohezji, współczynnik rozprzestrzeniania.
5. Właściwości barierowe: przepuszczalność pary wodnej, przepuszczalność tlenu.
6. Właściwości mechaniczne.
7. Właściwości optyczne filmów i powłok.
8. Analiza termograwimetryczna.
9. Właściwości fizjologiczne: transpiracja, respiracja, kwasowość i całkowita masa substancji nierozpuszczalnych.

Opracowanie metod statystycznych przy pomocy programu Statgraphics Plus wersja 5.0 (Manugistics Corp., Rockville, MD, U.S.A.). Analiza wariancji (ANOVA), Fisher’s LSD test wielokrotnego porównania zostały wykorzystane w celu odnalezienia istotnych różnic między filmami. Poziom istotności wynosił 0,001.

OMÓWIENIE

Filmy zostały przygotowane na bazie skrobi kukurydzianej, pszenicznej i ziemniaczanej z relatywnie niewielką ilością plastyfikatora (30% w stosunku do skrobi) w celu lepszego zrozumienia efektów wynikających z natury skrobi. Stosunek amyloza/ amylopektyna w każdej badanej skrobi dał podstawę do wyjaśnienia zaobserwowanych zachowań w filmach. Stosunek formy rozgałęzionej do nierozgałęzionej silnie wpływa na mikrostrukturę, ponieważ lepkość roztworów powłokotwórczych wzrasta z zawartością amylopektyny powodując tym samym zmiany w sieci podczas suszenia, co ostatecznie wpływa na grubość filmów. I tak, roztwór skrobi kukurydzianej jest najbardziej lepki i powłoki są najgrubsze, przy tej samej ilości użytych komponentów, tj. roztworu powłokotwórczego użytego na jednostkę powierzchni. Barwa filmów i przezroczystość również zależą od grubości. Dowodem na to są folie ze skrobi ziemniaczanej, która jest najcieńsza i przezroczysta, podczas gdy skrobia kukurydziana i pszeniczna jest bardziej biała i nieprzejrzysta. Stosunek amylozy do amylopektyny wpływa na właściwości hydratacyjne. Najmniejsza rozpuszczalność w wodzie, przepuszczalność pary wodnej i tlenu zostały otrzymane dla skrobi ziemniaczanej i pszennej, to jest dla tych, które zawierają najmniej amylopektyny. Właściwości powierzchniowe i mechaniczne także zostały zbadane. Im mniejsza zawartość amylozy tym lepsza bariera, większy opór powierzchni do absorpcji wilgoci i właściwości mechanicznych. W przypadku skrobi kukurydzianej i ziemniaczanej pęcznienie powierzchni (rysunek 1) i większa wrażliwość na wilgoć zostały zaobserwowane. Sugeruje to ograniczenia w aplikacji dla produktów z większą zawartością wody lub tych przechowywanych w pomieszczeniach z wysoką wilgotnością względną. Badania dotyczące natury skrobi pokazują lepsze dopasowanie skrobi pszennej do produkcji jadalnych filmów i powłok. Jednakże właściwości skrobi pszennej nie do końca odpowiadają oczekiwaniom producentów do potencjalnej aplikacji. Stąd pewne strategie związane ze skrobią i z zawartością glicerolu, dodatkiem białek i oleju rzepakowego jako warstwy laminatu, lub pomiędzy warstwami skrobi zostały wzięte pod dalszą uwagę przy powlekaniu śliwek.



Rysunek 1: Powierzchnia skrobi po 2 minutach kontaktu z naniesioną na nią kroplą wody: a) skrobia pszenna, b) skrobia kukurydziana, c) skrobia ziemniaczana.

Filmy skrobiowe zostały utworzone w kilku stężeniach skrobi. Roztwory powłokotwórcze zawierające poniżej 3% skrobi nie pozwalały na utworzenie filmów o oczekiwanych właściwościach. Filmy były zbyt cienkie i zbyt kruche z wieloma defektami jak pękanie czy dziury. Optymalną ilością okazało się 5% skrobi względem roztworu powłokotwórczego. Stężenie to ustalono na podstawie analizy grubości, właściwości mechanicznych i barierowych otrzymanych powłok.

Zawartość glicerolu i poziom wilgoci silnie wpływa na strukturę i właściwości filmów. Parametry takie jak: zwilżalność, zawartość wody, indeks pęcznienia, rozpuszczalność w wodzie, przepuszczalność pary wodnej i tlenu rosną wraz ze wzrostem glicerolu. Napięcie powierzchniowe i jego polarny komponent również rosną, podczas gdy komponent dyspersyjny napięcia powierzchniowego, adhezja i kohezja ulegają zmniejszeniu dla systemów wysoce plastycznych. Dyfuzja wilgoci i przepuszczalność dla gazów i pary wodnej zostały zredukowane dwukrotnie, kiedy zamiast 50% glicerolu było użyte tylko 30%. Im mniejsza jest zawartość glicerolu tym mniejsza plastyczność i większy moduł Younga oraz siła rozciągania. Jednakże wzrost zawartości plastyfikatora z 30 do 50% względem suchej masy skrobi nie powoduje znaczących zmian właściwości mechanicznych przy umiarkowanej wilgotności względnej (50%). Jak pokazują izotermy sorpcji efekt zawartości glicerolu może mieć dobry wpływ w większej wilgotności względnej. Izotermy wykazują większą absorpcję wilgoci w filmach z 50% dodatkiem glicerolu i aktywności wody wyższej niż 0,8. W takich warunkach efekt kumulacyjny wody i glicerolu jako plastyfikatora silnie pogarsza właściwości mechaniczne filmów zawierających większą ilość glicerolu. Filmy i powłoki z mniejszą zawartością plastyfikatora mogą być wykorzystane z korzyścią do produktów z wysoką zawartością wody bez utraty właściwości mechanicznych, ponieważ może ona oddziaływać jako plastyfikator zastępując tym samym glicerol.

Filmy na bazie skrobi posiadają słabe właściwości barierowe, które mogą zostać poprawione poprzez dodatek innych biopolimerów zdolnych do interakcji z łańcuchami amylozy i amylopektyny. Białka zawierają różne grupy chemiczne i aminokwasy, stąd są w stanie tworzyć różne typy wiązań i interakcji. Ponadto mogą one zapewnić lepszą wartość odżywczą powłok skrobiowych. Dodatek izolatu białek serwatkowych do skrobi w stosunku od 20 do 100% (względem masy suchej substancji) pozwala na tworzenie filmów w większości przypadków z lepszymi właściwościami optycznymi i wizualnymi. Im mniejsza zawartość skrobi tym bardziej jednolite i gęste filmy są otrzymywane. Oprócz tego dodatek białek serwatkowych pozwala na redukcję zwilżalności na powierzchni poprzez wzrost kąta zwilżalności od około 43 do 93^{o}, zgodnie z zawartością białek. Filmy skrobiowe, filmy białkowe i ich mieszanina w stosunku 80-20 (skrobia-białko) są mniej hydrofilowe i mają wyższą wartość współczynnika dyspersji powierzchni niż pozostałe mieszaniny. Jednakże, nawet jeśli hydrofilowość na powierzchni filmu została zredukowana poprzez obecność białek izotermy sorpcji nie uległy zmianie. Interakcje pomiędzy skrobią i białkami modyfikują właściowści powierzchniowe prawdopodobnie z uprzywilejowaną orientacją hydrofobowych aminokwasów zgodnie z kierunkiem ruchu powietrza podczas suszenia folii.

Dodatek białka we wszystkich stosunkach przyczynił się także do niewielkiego zmniejszenia przepuszczalności pary wodnej folii skrobiowych. Interakcje fizyko-chemiczne pozwalają na wyjaśnienie wzrostu gęstości struktury filmów a tym samym spowolnienie ruchliwości wody w filmie. Białka serwatkowe również przyczyniają się do ograniczenia wchłaniania związków smakowo-zapachowych typowych dla owoców i warzyw. Wiedza z tego zakresu pozwoliła na utrzymanie w owocach ich aromatów, po pokryciu roztworami skrobi z białkami serwatkowymi powierzchni owoców.

Natomiast nawet przy kontrolowanej zawartości glicerolu i protein redukcja właściwości sorpcyjnych jest nadal pożądana. Stąd filmy zostały utworzone z dodatkiem oleju.

Olej rzepakowy jest olejem roślinnym z najlepszym stosunkiem kwasów Ω3 do Ω6 (blisko 1:1), zawiera triacyloglicerole i sterole. Jest pozbawiony zapachu i relatywnie niedrogi. Są to główne powody, dla których został wybrany. Z analizy literatury wynika, że dyspersja oleju w biopolimerowej matrycy nie wpływa znacząco na poprawę bariery wilgotności czy wrażliwości na wodę. W związku z tym filmy skrobiowe zostały przygotowane jako systemy wielowarstwowe: warstwa skrobi, warstwa oleju, warstwa skrobi. Jednakże po wysuszeniu filmów zdjęcia mikroskopowe pokazały, iż otrzymane filmy nie mają struktury wielowarstwowej jak się spodziewano, lecz przypominają one emulsje. Oprócz tego duże krople oleju rzepakowego zostały zaobserwowane na powierzchni matryc zakłócając organizację sieciową skrobi, a tym samym sprzyjając silniejszej redukcji grubości filmów. Dodatek oleju wpływa zatem na gęstszą strukturę bez dodatkowych modyfikacji koloru i wyglądu. Zwilżalność powierzchni również została zmniejszona przez zwiększenie składnika dyspersyjnego napięcia powierzchniowego i kąta zwilżania wody od około 40 do 70°, a tym samym został zmniejszony polarny składnik wpływający na właściwości powierzchniowe. Dodatek oleju nie miał istotnego wpływu na właściwości mechaniczne folii. Tak jak oczekiwano, zarówno rozpuszczalność w wodzie jak i sorpcja wilgoci były zmniejszone proporcjonalnie do zawartości oleju. Zwiększenie hydrofobowości błony i zmniejszenie wrażliwości na wilgoć mogą dwukrotnie zmniejszyć przepuszczalność pary wodnej i prawie 10 razy przepuszczalność tlenu w warunkach wysokiej wilgotności względnej (> 75% wilgotności względnej).

Na podstawie uzyskanej wiedzy o właściwościach fizycznych, chemicznych i funcjonalnych filmów, jak struktury i oddziaływania pomiędzy komponentami filmów, kilka metod tworzenia zostało wybranych do walidacji jako powłoki lub opakowania do śliwek. Roztwory powłokotwórcze skrobi i skrobi z białkami serwatkowymi (w stosunku 80-20) użyto do powlekania śliwek europejskich i japońskich, a receptura z olejem miała za zadanie poprawić zatrzymywanie wilgoci w owocach, jak również poprawę przylegania materiału do śliwek. Powłoki nakładane na śliwki okazały się bardzo efetywnymi. Błony te obniżają parametry fizjologiczne związane z dojrzewaniem owoców i czasem przechowywania (transpiracja i respiracja) i utrzymują twardość (jędrność) owoców dłużej niż śliwki niepowlekane, przechowywane w tych samych warunkach. Niniejsza praca była związana z pokrywaniem skórki owoców błonami, i ich oddziaływaniem z powłokami skrobiowymi i skrobiowo-białkowymi na przedłużenie okresu przechowywania owoców. Otrzymane rezultaty pokazały, że świeżość i okres przydatności owoców może zostać wydłużonym nawet o ¼, co jest bardzo dużym atutem dla konsumentów i dla producentów.

PODSUMOWANIE I WNIOSKI

Przeprowadzone testy fizyczne, chemiczne i fizjologiczne filmów i powłok na bazie skrobi pozwoliły na zrozumienie interakcji pomiędzy komponentami biopolimerów i określenie ich właściwości funkcjonalnych do dalszego zastosowania w przemyśle opakowaniowym i spożywczym. Wstępne badania dotyczące rodzajów skrobi wykazały, że niezależnie od źródła pochodzenia tego materiału zapasowego wszystkie skrobie mają silne właściwości hydrofilowe. Co więcej spośród trzech odmian skrobi wybranych do analizy, tylko powierzchnia filmu ze skrobi pszennej po bezpośrednim kontakcie z kroplą wody nie pęczniała. Oznacza to, iż skrobia kukurydziana i ziemniaczana nie powinny być stosowane do materiałów o dużej zawartości wody. Ich zaletą są bardzo dobre właściwości mechaniczne i optyczne. Skrobia ziemniaczana może posłużyć w charakterze folii uwydatniających wygląd pakowanych produktów, zaś ze skrobi kukurydzianej mogą być produkowane wytrzymałe mechanicznie materiały do produkcji np. reklamówek i siatek na zakupy. Wybrany udział procentowy skrobi gwarantował homogeniczność i elastyczność filmów. Przy 3 i 5% dodatku suchej masy skrobi względem wody nie zaobserwowano żadnych defektów, takich jak pęknięcia, dziury, gródki, a filmy nie łamały się i zachowywały się jak ciała sprężyste.

Dodatek plastyfikatora w ilości 30 i 50% względem suchej masy skrobi również nie powodował łamliwości, kruchości, przebarwień. Oznacza to, że bio-opakowania mogą z łatwością zastąpić wiele rodzajów folii, tacek i pojemników. Dodatek białek serwatkowych miał za zadanie zredukowanie silnych właściwości sorpcyjnych, w szczególności wysokiej przepuszczalności dla pary wodnej. Powłoki i filmy skrobiowe z dodatkiem białek są w szczególności rekomendowanymi do żywności o ubogich wartościach odżywczych. W celu redukcji właściwości sorpcyjnych dla pary wodnej o kilka rzędów dodano olej rzepakowy. Silny charakter hydrofobowy tego tłuszczu miał za zadanie zwiększenie bariery dla gazów i aromatów. Kilkuprocentowy dodatek oleju zwiększył wartości barierowe jak oczekiwano, jednak nie do takiego stopnia jak w opakowaniach plastikowych. Dodatek tłuszczu spowodował, że grubość filmów uległa znacznej redukcji.

Spośród 15 rodzajów biodegradowalnych filmów i powłok wybrano dwa rodzaje do powlekania i pakowania owoców, na przykładzie śliwek. Testy laboratoryjne dały pozytywne wyniki, pokazały, że zarówno filmy jak i powłoki wytworzone na bazie skrobi przedłużają trwałość owoców, jędrność, spowalniają utratę masy w czasie i nie powodują żadnych negatywnych zmian barwy i wyglądu zewnętrznego. Również są bezpieczne, łatwe i tanie w użyciu. Mogą być stosowane w wielu różnych postaciach, w wielu formach i do wielu różnych produktów, także tych z wysoką zawartością wody.

Pomimo tak wielu pozytywnych wyników pilotażowe testy przemysłowe powinny być również przeprowadzone na kilku rodzajach owoców i warzyw w celu porównania otrzymanych informacji. Co więcej, jednym z głównych powodów aplikacji powłok na powierzchnię może okazać się dodatek preparatów antymikrobiologicznych. Jest to potencjalny cel dalszych badań. Wzrasta liczba szkodliwych konsekwencji wywołanych przez mykotoksyny (ochratoksyny i aflatoksyny). Ma to negatywny wpływ na zdrowie człowieka (mykotoksyny powodują wiele rodzajów nowotworów). Większość mykotoksyn jest produkowana przez grzyby rozwijające się na skórce owoców. Powłoki jadalne na bazie skrobi są znane jako skuteczne nośniki substancji aktywnych. Również mogą służyć do kapsułkowania substancji aktywnych, takich jak naturalne antymykotoksyny. Tak więc dodatek naturalnych antymykotoksyn lub mikroorganizmów, które wytwarzają antymykotoksyny może okazać się bardzo pomocnym narzędziem nie tylko w przedłużaniu trwałości owoców, ale także w zwiększaniu ich bezpieczeństwa.