Fakulta zemědělská a technologická
Faculty of Agriculture and Technology
Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE
FACULTY OF AGRICULTURE AND TECHNOLOGY

Department of Plant Production

Application of selected plant materials for the preparation of biodegradable plastics

Master thesis

Author: Bc. Jana Tůmová

Supervisor: doc. Ing. Jan Bárta, Ph.D.

Consultant: Ing. Markéta Jarošová

České Budějovice

2023

Declaration

I hereby declare that I have done this thesis entitled Application of selected plant materials for the preparation of biodegradable plastics independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to citation rules of the faculty of agriculture and technology.

In České Budějovice 14. 4. 2023

....

Bc. Jana Tůmová

Acknowledgments

I would like to express my gratitude to my supervisor, doc. Ing. Jan Bárta, Ph.D. (Department of Plant Production, Faculty of Agriculture and Technology, University of South Bohemia in České Budějovice), who guided me throughout the writing of my master thesis, for his patience and helpful comments. Also, Ing. Markéta Jarošová and Ing. Adéla Stupková, Ph.D. for their guiding hand through my experiments in the laboratory premises of the Department of Plant Production. Finally, I would like to thank my friends, and family who gave me a big support.

Abstract

In the past decades, there has been a growing public demand to reduce the use of plastics and their derivatives in packaging materials for a wide range of products, including food. For this purpose, there is a desire to use natural, easily biodegradable, or recyclable materials. These include natural polymers such as starch, plant proteins and cellulose derived from the harvested products of crops and the by-products of their processing.

The aim of this thesis is the preparation of model biodegradable plastics in the form of films using selected materials such as flour (wheat, flaxseed, soybean full fat) and plasticizer (sorbitol). After the optimization of the conditions for the preparation of these films by the casting method, the qualitative and functional parameters of the obtained biodegradable films were subsequently evaluated. Their characteristics (thickness, visual and tactile properties), colour, transparency, water solubility and biodegradability through enzymatic degradation were determined. It was found that biofilms made from wheat, flaxseed and soybean full fat flours are high soluble in water and a biodegradable in aqueous environment with enzyme pronase from *Streptomyces griseus* that normally occurs in the soil. Therefore, these biofilms could be further studied under the conditions of decomposition in soil. Subsequent researches also need to be carried out to incorporate additives that can improve the barrier and mechanical properties of the films.

Keywords: flours, natural polymers, casting method, food packaging, biodegradable

Abstrakt

V posledních dekádách vzrůstá celospolečenský požadavek na omezování používání plastů a z nich odvozených výrobků v oblasti obalových materiálů pro nejrůznější výrobky včetně potravin. Pro tento účel je snaha uplatňovat přírodní snadno rozložitelné či recyklovatelné materiály. Patří mezi ně také přírodní polymery, jako je škrob, rostlinné bílkoviny a celulóza odvozené ze sklizňových produktů polních plodin a vedlejších produktů vznikajících při jejich zpracování.

Cílem této práce je příprava modelových biodegradabilních plastů ve formě filmů s využitím vybraných materiálů typu mouka (pšeničná, lněná, sójová plnotučná) a změkčovadlu (sorbitol). Po optimalizaci podmínek pro přípravu těchto filmů odlévací metodou byly následně hodnoceny kvalitativní a funkční parametry získaných biodegradabilních filmů. Byly stanoveny jejich charakteristiky (tloušťka, vizuální a hmatové vlastnosti), barva, rozpustnost ve vodě a biodegradabilita prostřednictvím enzymového odbourávání. Bylo zjištěno, že biofilmy vyrobené z pšeničné, lněné a sójové plnotučné mouky jsou vysoce rozpustné ve vodě a biologicky rozložitelné ve vodném prostředí s enzymem pronáza ze *Streptomyces griseus*, bakterie, která se přirozeně vyskytuje v půdě. Proto by tyto biofilmy mohly být dále studovány na základě jejich rozkladu v půdních podmínkách. Následné výzkumy by bylo také vhodné provádět za účelem začlenění dalších aditiv, které mohou zlepšit ochranné a mechanické vlastnosti těchto filmů.

Klíčová slova: mouky, přírodní polymery, metoda odlévání, potravinové obaly, biologicky odbouratelné

Content

List of tables

List of figures

1 Introduction

Bioplastics still represent less than one percent of the more than 390 million tonnes of plastic produced annually. However, contrary to a slight decrease in the overall global plastic production, bioplastics market has continuously grown (European Bioplastics e.V., 2022).

Almost every conventional plastic material can be replaced by bioplastic alternatives. In recent years, biodegradable polymers (plant proteins, polysaccharides, and lipids) from renewable resources, such as agro-industrial by-products, waste, or flours which naturally contain these polymers in the matrix, are being used for preparing biobased films for food packaging as an alternative to petroleum derived polymers (Peron-Schlosser et al., 2021).

Using one of the above-mentioned biomaterials the edible coating or film can be created whether by wet (casting) or dry (extrusion) processes, mostly combined with other biopolymers with added plasticizers (sorbitol, glycerol) . The advantages of using the casting method include, among others, ease of handling without a specialized equipment, homogenous casted solution, fewer defects on films and optical purity (Suhag et al., 2020).

Polysaccharide-based edible films possess good oxygen barriers. However, these films, as well as protein-based edible films, have poor moisture barriers because of their hydrophilic nature. On the contrary, lipid-based edible films have good moisture barrier, but low mechanical properties due to their hydrophobicity (Barrett et al., 2010).

Packaging films and coatings made of natural biopolymers are particularly interesting due to their biodegradability, since most of these products have a relative short service life and end up in landfills (Peelman et al., 2013). For plastic product to be considered as biodegradable is needed to be fragmented i.e., shortening and weakening of polymer chains under the influence of heat, moisture, sunlight, and/or enzymes and mineralized i.e., complete assimilation of plastic fragments by the microbial population in the disposal environment (Havstad, 2020).

The agricultural by-products with composition rich in proteins and carbohydrates e.g., wheat bran, germ, low-grade flours have also a high potential to be used in biotechnological processes and biodegradable packaging development and thus present promising biobased agents of further studies (Peron-Schlosser et al., 2021).

2 Literature review

2.1 Biopolymers

Biopolymers are the types of polymers that are produced by living organisms. These polymeric biomolecules that we get from crops and the sea (plants and animals) are the ones most commonly used. Furthermore, they can come from some bacteria strains (Yadav et al., 2015).

According to the method of production or their source, biopolymers are 3 groups. First group are polymers derived directly from vegetal or animal biomass such as polysaccharides and proteins. Secondly, polymers produced by classical chemical synthesis using renewable bio-based monomers such as polylactic acid (PLA) as raw materials. And third group: polymers synthesized by microorganisms such as polyhydroxyalkanoates (PHAs), cellulose, xanthan, and pullulan (Nair et al., 2016). Polysaccharides (cellulose, starch, and chitin) and proteins (casein, whey, collagen, and soy) are examples of the biopolymers that are mostly used for preparing biodegradable materials (Yadav, 2015).

The biodegradability of polymers is consider depending on capability of undergoing decomposition into CO_2, CH_4, H_2O, inorganic compounds, or biomass through predominantly the enzymatic action of microorganisms. Some of these polymers can also be compostable, which means decomposition takes place in a compost site at a rate consistent with known compostable materials (Peelman et al., 2013).

The advantage of biodegradability finds an assertion in many industries from which the most important are food production, packaging, and medicine. Some bioplastic materials from biopolymers can even directly replace synthetically derived materials in traditional applications, whereas others possess unique properties that could open up a range of new commercial opportunities. In recent years, biodegradable polymers, captured from renewable resources, such as agro-industrial like orange waste (Bátori et al., 2019), potato peel and sweet lime pomace (Borah et al., 2017), sugar beet and bagasse (Šimkovic et al., 2017), cassava bagasse (de Carvalho et al., 2019), or marine resources like chitin extract from shrimp shells (Lopez et al., 2014), have been used as an alternative to petroleum derived polymers. Another alternative to biodegradable film production is to use flours, which are complex mixtures where starch, protein, lipids, and fibers are naturally present in the matrix (Daudt et al., 2016; Orsuwan & Sothornvit, 2018). The excellent characteristics of these films stem from the natural and intrinsic molecular interactions taking place between their components (Peron-Schlosser et al., 2021).

2.1.1 Plant polymers

Plants naturally produce a number of structural and carbonreserve polymers. Polysaccharides are estimated to make up to 70% of all organic matter. The main content represents cellulose for about 40 % of total organic matter, lignin which comprises 15–25% of a typical woody plant, and starch which is also a major component of global biomass (Miyata, 1994). A number of natural polymers have been exploited for commodity manufacturing, and most of these products retain the inherent biodegradability of their carbohydrate building blocks.

2.1.1.1 Cellulose

Cellulose is a linear polymer of β-1,4-linked D-glucose. In the case of quantity cellulose is the most widely spread natural polymer on Earth (Mooney, 2009). It is a structural polysaccharide derived by a delignification from wood pulp or cotton linters for industrial processes (Peelman et al., 2013). In addition to higher plants, acetic acid bacteria can also synthesize cellulose but unlike plants it is pure while cellulose from plants is typically mixed with lignin, hemicelluloses, and pectin.

Addition of the biopolymer cellulose to biobased films can improve properties in the way of chemical and mechanical properties such as high crystallinity, a high degree of polymerization, high water-absorbing and water-holding capacities, high tensile strength, high elasticity, excellent biocompatibility, and biodegradability. Cellulose is the main raw material for paper, cardboard, and textiles made of cotton, flax, or other plant fibres production. It is also used as a component of fibres, films, and cellulose derivatives. In fact, the first industrial polymers (celluloid, cellophane) were based on cellulose (Miyata, 1994). The cellophane is prepared from wood pulp using NaOH to break down the crystalline structure, followed by gelling with acid. Despite being mostly replaced by synthetics, it is still used in some food packaging applications, where, for example, the cellophane is impregnated with an antibacterial peptide, nisin, and used to wrap fresh meat. At the other hand, the field of cellulose-based materials is today still not fully explored (Mooney, 2009).

There is a chemical similarity between starch and cellulose thus they can provide a strong interaction e.g., incorporated cellulose is well cemented in the plasticized starch polymer matrix. Due to highly crystalline and hydrophobic character of the natural fibres it causes a positive effect on the water permeability e.g., decrease the water vapor transmission by increase the diffusion path length through the film (tortuous path). However, this effect can reverse on adding too many fibres, causing congregation (Peelman et al., 2013).

Nowadays paper and board, based on cellulose, are the most widely used renewable packaging materials. According to cellulose-based films, they are produced commercially, of which cellulose acetate is the most commonly used for packaging several food products e.g., fresh produce, baked goods (Pothet, 2014).

2.1.1.2 Starch

After cellulose, starch is the most widely spread organic compound found in nature. Starch is a carbohydrate, a polysaccharide that serves as an energy reserve in plants such as oats, barley, wheat, potato, or pea. Starch consists of two major components, amylose (10-30 %) and amylopectin (70-90 %) (Sone Aung et al., 2018). Amylose is linear polysaccharide, while amylopectin is highly branched, so starch can be regarded as a crystalline material (Nair et al., 2016).

As starch is one of the cheapest and most abundant agricultural products, completely degradable, these properties lead to exploring starch as a polymer for various applications. The major of commercial starch is derived from corn, potato, wheat, rice, and barley, because these plants contain large amounts of starch, usually between 60% and 90% of the dry weight (Miyata, 1994). But due to poor mechanical properties (such as brittleness) and hydrophilicity, better characteristics are achieved if the starch is blended with more waterproof polymers or if it is chemically modified. One of the key issues in the processing of starch-based materials is also low thermal stability. The thermal decomposition and stability depend on starch microstructures i.e., amylose/amylopectin content and molecular weight, as well as on modification and processing conditions (such as open, sealed, and shear stress). Therefore, to produce a starch-based film, high water content or plasticizers (e.g., glycerol, sorbitol) are necessary. These plasticized materials are called thermoplastic starch (TPS) and constitute an alternative for polystyrene (Peelman et al., 2013).

Applications of starch-based materials include products for loose-fill packaging, various types of bags and sacks e.g., shopping bags, refuse sacks, and bags for biowaste storage. Another material from starch is for flexible and rigid packaging e.g., nets for fresh fruit and vegetables, thermoformed trays and containers. Mulching film, plant pots or hygiene products and cosmetics products e.g., nappies and sanitary products can also include starch (Platt, 2006).

2.1.1.3 Plant proteins

Proteins are heteropolymers whose monomer units are α-amino acids. The combinations of the twenty amino acids of which proteins are composed provide an almost unlimited number of different polymer chains, with diverse physicochemical properties (Hernández-Muñoz et al., 2005). Therefore, plant proteins can offer the best prospects of all the existing biopolymers for the production of packaging materials (Reddy & Yang, 2013).

Proteins contain a great variety of functional groups which make it possible to alter them enzymatically, chemically or physically, varying the properties of the materials obtained in order to adjust them to the specific needs of each application. The properties for packaging can include good optical effects (gloss and transparency), excellent fat barrier properties, a high oxygen and organic vapour barrier at low and intermediate relative humidities and selective permeability to gases (high CO_2/O_2 permeability relationship in comparison with other synthetic polymers) and moderate mechanical properties (Hernández-Muñoz et al., 2005). In contrast, the main drawback of materials made from plant proteins is their great sensitivity to water, which can affect their mechanical properties e.g., oxygen barrier and even their integrity. However, the hydrophilic attribute of these materials can be used positively for the development of active packaging by supporting the release of functional compounds into the material (López-Rubio et al., 2004).

Among proteins of plant origin, corn zein, wheat gluten, soya protein, and peanut protein are the most commonly used substances for preparing a biodegradable material. For example, soya protein was mostly used as s filler, which reduced the price of plastics based on oil. Today, it is still used, this time with the purpose of increased biodegradability of plastics. In comparison to plastics from casein, zein, and glycine, soya protein is also economically competitive (Yamada et al., 2020).

2.2 Biobased films production

2.2.1 Formation of films

Edible materials are developed from various types of biopolymers such as polysaccharides, proteins, lipids, and composite material (Suhag et al., 2020). Polysaccharide-based materials such as e.g., starch and cellulose, proteins like corn zein, soy protein and lipid-based materials such as polyethylene wax, rice bran wax are used to develop coatings or films to enhance shelf life, improve the post-harvest quality of fruits and vegetables and other food products (Barrett et al., 2010).

Using one of the above-mentioned biomaterials the edible coating or film can be created whether alone or combined with other biopolymers with added plasticizer. Commonly used plasticizers are glycerine, propylene glycol, sorbitol, sucrose, polyethylene glycol, and corn syrup (Kumar & Neeraj, 2019). It is a necessary ingredient for edible films made from polysaccharides and proteins, due to the extensive interactions among polymeric chains, in order to make materials more flexible and processable (Suhag et al., 2020).

The chemical and structural properties of film forming biopolymers and plasticizers, or other additives should be understood well and adapted for future specific applications. However, it is always meant to be flavourless, colourless and should not interfere with sensory prospects of the food product (Viana et al., 2018).

2.2.1.1 Casting method

One process for obtaining an edible film is called casting method. It is the most commonly used method for a film formation at laboratory and pilot scales and was also chosen for the practical part of this thesis. It is based on three steps, solubilization of biopolymer in a suitable solvent as a first step, casting of the solution in the mould and at last drying of casted solution resulting in easily peeled edible films with an excellent mechanical strength, barrier properties, thermal stability, and uniform microstructure (Fakhouri et al., 2013). The thickness, transparency, opacity, swelling degree, thermal stability, mechanical strength, oxygen transmission rate (OTR), water vapor permeability (WVP), and biological characteristics are the most important parameters of edible films (Khanzadi et al., 2015).

The key advantages of casting method are e.g., easy handling without a specialized equipment at low cost, homogenous casted solution, causing small and fewer defects on films, optical purity. There are also some drawbacks of this method e.g., different evaporative levels and temperatures can lead to films with different features being produced or challenges how to convert film production from laboratory to production scale because many variables, like, heating, combination of speed and temperature, could cause quality differences and prevent the constant development for commercial scales (Suhag et al., 2020).

2.2.1.2 Extrusion method

Extrusion method is another method which can be used for preparing polymeric films and it represents one of the major techniques currently in use at commercial scale (Hernandez-Izquierdo & Krochta, 2008). It is based on three zones: the feeding zone, the kneading zone, and the heating zone, respectively. This method uses a minimum content of water or solvents; therefore, it is also called a dry process. As well as in casting method, to increase film flexibility, plasticizers are also needed. Polyethylene glycol, or sorbitol are the main plasticizers used for extrusion. The strain, temperature, and density of the mixture increase when the ingredients pass to the kneading zone. Some parameters e.g., moisture content of the film components, screw speed, temperature, pressure, energy input are critical for the process in order to achieve final products with desirable properties (Fitch-Vargas et al., 2016).

Compared to casting method, there several advantages e.g., short time of processing with a low energy consumption, enhanced mechanical and optical properties, high performance, generation of wide range of forms. On the other hand, the disadvantages of extrusion method are higher initial cost of specialized equipment and a higher maintenance cost affect the usage of this process, and also limitations of raw material blends features, that means they should be temperature tolerant and have a low level of moisture (Suhag et al., 2020).

2.3 Food packaging

2.3.1 Application of bioplastics

The most used application techniques are (A) dipping, (B) spreading, (C) spraying, and (D) wrapping, as illustrated in figure 1. Edible coatings can be directly applied on the surface on fruits, vegetables, and other food products, while edible films are used as a wrapping packaging material (Díaz-Montes & Castro-Muñoz, 2021). The various types of edible coatings have been already applied to enhance or extend shelf life as well as to improve post-harvest physiological properties of food products such as fruits and vegetables, fresh meat cuts or meat and fish products (Sone Aung et al., 2018).

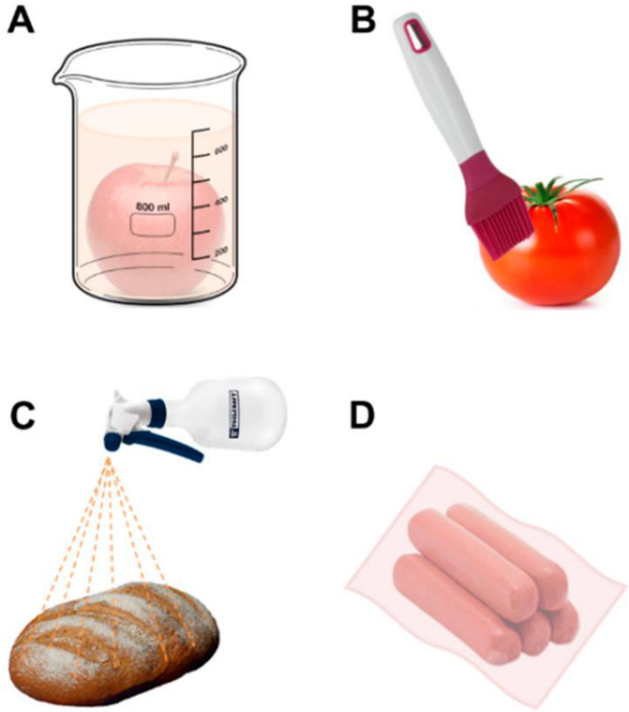

Figure 1. Main techniques used for food coating (Díaz-Montes & Castro-Muñoz 2021).

2.3.2 Biodegradation

Most of biobased packaging materials are biodegradable and biocompatible. These packaging materials can be degradable by microbial, enzymatic, or chemical process (Nair et al., 2016). In general, biodegradation consists of two stages: fragmentation and mineralization. As fragmentation are considered i.e., shortening and weakening of polymer chains under the influence of heat, moisture, sunlight, and/or enzymes. Otherwise, mineralization represents complete assimilation of plastic fragments by the microbial population in the disposal environment (Miyata, 1994).

The course of biodegradability depends not only on the external environment but also on the on the raw materials e.g., chemical composition, and the structure of the final product.

For plastic product to be considered as biodegradable is needed to be both fragmented and mineralized. Moreover, the plastic fragments should not represent risks in the environment unless they are completely assimilated by the microbial populations present in the disposal system in a relatively short period (Havstad, 2020).

2.3.3 Limitations

Besides the fact that application of biopolymers based edible coating on food products acts as a barrier layer against gas diffusion, water migration, aroma changes and different solute exchange, there are, some limitations related to their properties (Sone Aung et al., 2018). Because of the hydrophilic nature of starch and cellulose, packaging materials based on these materials have a low water vapor barrier, which causes a limited long-term stability and poor mechanical properties (sensitive to moisture content). Other drawbacks are bad processability, brittleness and vulnerability to degradation. Brittleness (due to high glass transition and melting temperatures), stiffness, poor impact resistance and thermal instability are finally also factors limiting the application of biobased films as food packaging. Therefore, many studies also focus on improving the functionality of bioplastics. These improvements include e.g., additional thin layer of another material on top of the biobased film or incorporation of nanoparticles (Peelman et al., 2013).

Most of these characteristics of edible films and coatings are relevant; however, the biological protection of food is one of the most important since it directly affects the shelf- life of the product (Díaz-Montes & Castro-Muñoz, 2021). Therefore, it is necessary to inhibit or eliminate bacterial or fungal microorganisms (as well as their derivatives) that can cause or accelerate putrefaction in food due to the action of their enzymes and by-products produced from their metabolism (e.g., gases) (Rawat, 2015). Moreover, epidemiological studies have analysed different bioactive molecules (e.g., flavonoids and phytoestrogens), which in fact have been recognized by their antioxidant, antimutagenic, anti-inflammatory, anti-cancer, apoptotic, and anti-cholesterol effects. Thus, various researches have been specifically focused on incorporating a wide variety of these bioactive compounds into edible films (Castro-Muñoz et al., 2020).

2.3.4 Environmental footprint

Edible film is also known as eco-friendly packaging material, which replaces synthetic or plastic packaging material and reduces post-harvest losses of agricultural products (Suhag et al., 2020). According to the United States Department of Agriculture (USDA, 2020), wheat processing is one of the most prominent among the various agro-industrial segments. The agro-industrial waste or by products coming from e.g, wheat, soybean or any other plant production are generally used for the processing of animal feed or adhesive in manufacturing industry, even with its composition rich in proteins and carbohydrates with high potential for use for biotechnological application (Frantz et al., 2019), and biodegradable packaging development (Drakos et al., 2018).

3 Aims of the Thesis

The main aim of the thesis was to evaluate the feasibility of the production of sustainable and biodegradable films for food packaging made from raw plant materials using casting method.

Specific objectives were:

a) Optimization of the steps of the casting method for films preparation based on the use of different types of flours, determination of their physical properties (colour, cohesiveness, elasticity).
b) Preparation of biobased plastic material from selected types of flours, determination of their physical and mechanical properties (visual and tactile aspects, colour, thickness, moisture content).
c) Investigation of the biodegradability of prepared materials based on enzymatic degradation in water suspension and water itself.

4 Materials and Methods

4.1 Raw material

The wheat flour (Europasta SE), soybean (full fat) (Paleta, spol. s.r.o.) and soy (defatted) (Jím Dobře – Ekoprodukt, s.r.o) flours were bought via grocery stores and the information of crucial nutritional values is given in the table 1. Flaxeed flour was prepared in the University of South Bohemia in České Budějovice laboratory from the Agriol variety of oilseed flax (harvested in 2018) with a fraction size below 0.315 mm. The nutritional information has been recalculated from the dry matter determination. Sorbitol (Thermo Fisher Scientific, 97 %), was used as plasticizer and distilled water as a solvent.

Table 1. Crucial nutritional values of different types of flours used.

Types of flour	Proximate composition of (per 100 g)		
	protein (total)	fat (total)	carbohydrate
Wheat	11.0 g	1.0 g	71.0 g
Soybean full fat	34.5 g	20.7 g	25.6 g
Flaxseed	33.1 g	30.3 g	29.2 g

4.2 Testing of materials and optimization of bioplastics preparation

In order to obtain suitable plastic materials with ideal properties, several types of flours were tested and also a desirable value of concentrations were set. Moreover, some techniques of the casting method have been modified.

4.3 Casting technique

Films were prepared by the casting technique. An aqueous suspension of flour (8.0% w/w), sorbitol (4.0% w/w) was stirred and heated up to 80 °C on the magnetic stirring hotplate (Heidolph MR 3001 K – 800 W, Germany) with set conditions of 300 °C and 1000 rpm. Next, the suspension was spilled into 50 ml centrifuge tubes and put into the centrifuge (Rotina 420R) with set up conditions of 4500 rpm for 10 minutes. Then, the unsettled portion was poured into a 500 mL beaker and the suspension was stirred. After that, silicone plates (128 x 10 mm) were prepared and the 34 $\pm$ 2 g of suspension was poured and spread on them. Finally, the films were dried at 35 °C in a dryer (Memmert, Germany) for 48 h, peeled from the plates, and stored at 25 °C in desiccators before the analysis.

4.4 Characterization of films

4.4.1 Thickness, visual, and tactile aspects

A digital micrometer was used to assess the thickness of the films. The values were obtained by measuring from seven random points on the surface of the material and their arithmetic average including standard deviation was recorded.

The visual and tactile qualities of the films were evaluated subjectively. The focus was on handleability (handling capacity of the film without breaking it); continuity (the absence or presence of breaks in the film); homogeneity (uniformity of the film); adhesivity (if the film is sticky or not), and transparency (how clear is the film).

4.4.2 Colour

The colour analysis was detected using a pre-calibrated colorimeter ColorEye XTH (X-Rite (Gretag-Macbeth), USA) operating in the CIELab system (L*, a*, b*) using calibrated colorimeter operating in this system. Each sample was placed on a white paper background and measurements were taken at three random points on the film surface. White standard was L* = 97.14, a* = -0.26, b* = 2.6.

4.4.3 Moisture content

The moisture content was determined by the drying method at 90 °C for 24 h (dryer Memmert, Germany). Samples from each type of flour and their repletion measures (A, B, C, D, E) were cut, their weight was measured using analytical balances (before and after drying and resulting values were used for calculation according to Equation (1):

$$S(\%) = \frac{W_i - W_f}{W_i}$$

4.5 Biodegradability

4.5.1 Solubility in water

The prepared samples of edible films were cut into squares (20 x 20 mm). The samples were weighed (W_i) and placed in 10 mL of distilled water thermoplastic vessel. Then the samples were stored at 25 °C in thermostat for 24 h. After 24 h of storage, the samples were dried again for 24 h under 90 °C and the dry residue was weighed (W_f). Finally, solubility (S) was calculated according to Equation (2):

$$S(\%) = \frac{(W_i - W_f) * 100}{W_i}$$

4.5.2 Solubility in the water with added enzyme

The solubility in water with added enzyme: pronase E from *Streptomyces griseus* – 4000 000 PU/g (Merck, Germany) was determined by the drying method at 90 °C for 24 h (dryer Memmert, Germany). Before drying, the samples were dissolved in 10 mL destilled and 1 mL of 20% and stored in thermostat for 24 h. After drying the samples were weighed again and solubility (S) was calculated according to Equation (2).

4.6 Statistical analysis

The results were evaluated by one-way analysis of variance (ANOVA) using the software Statistica 13.0 (TIBCO Statistica Ultimate Academic). Fisher LSD test was performed to compare the results, expressed by mean (±) standard deviation, at the level of significance of 0.05.

5 Results

5.1 Testing of materials and optimization of bioplastics preparation

Flours from cereals - wheat

The first testing on wheat flour was mainly based on the difference in the resulting properties when sorbitol and glycerol were used at the same time and only sorbitol was used. The concentrations were as follows: 6% c_f; 3% c_s; 1% c_g for variation one and 6% c_f; 4% c_s for variation two. The techniques of casting method which changed during other testing included: stirring the suspension for 1 minute, water bath for 10 minutes (80 °C), pouring 20 g and 10 g of suspension on plastic petri dishes (86 x 10 mm), and 24 hours in dryer (35 °C). The result was a material that was difficult to peel off and brittle, less translucent, not cellular, with cracks. Nevertheless, a better result was obtained in a variation of the suspension with sorbitol only, when 20 g were poured onto petri dish.

Second testing included concentrations: 8% c_f; 4% c_s. Instead of heating the suspension in a water bath, it was heated up to 80 °C and stirred on the magnetic stirring hotplate (Heidolph MR 3001 K – 800 W, Germany), and 23 g of suspension poured on plastic petri dishes (86 x 10 mm). The drying temperature has not changed (24 hours in dryer (35 °C)). The result was a material with good properties but non-transparent.

Therefore, a centrifuge was used in the third measurement. The suspension of the given concentrations (8% c_f; 4% c_s) was centrifuged after heating and mixing and 34 ± 2 g of unsettled solution was poured onto silicon moulds (128 x 10 mm). It stayed in the dryer for 48 hours (35 °C) because of its larger volume. Resulting material was easily peelable, with good transparency, flexible, no adhesiveness, and excellent continuity. Thus, wheat flour was selected for further testing of its repetitions and bioplastic material properties were discovered.

Flours from cereals - rice and corn

First, testing was carried out under the same conditions of concentration values and steps of the casting method as for wheat in the second measurement, except for rice where, in addition to rice flour, flaxseed mucilage was added to the suspension (3% c_s; 1% of mucilage). In a comparison of the resulting materials, rice flour with added mucilage was more flexible. However, both materials were still poorly peelable, fragile, inflexible, but with good transparency.

For the second time, these materials were tested on silicone moulds without added slimes under the same concentration conditions and casting method steps as for wheat in the third measurement. The results showed in both cases very thin , brittle materials that could not be separated from the mould. Therefore, none of these materials were further used.

Flours from oilseeds – flaxseed

Flaxseed flour was tested the same way as wheat flour and its properties of resulting films were also very similar therefore flaxseed was used to further determination of its films.

Interest was also directed to the linseed mucilage itself. The mucilage was tested separately under suspension concentrations of 1% c_f; 4% c_s, heating up to 80 °C on the magnetic stirring hotplate (Heidolph MR 3001 K – 800 W, Germany) then pouring 23 g of solution on plastic petri dishes (86 x 10 mm) and dried for 24 hours at 35 °C in dryer. However, the resulting material was fragile, inflexible and poorly peelable therefore it was not used for further studies.

Flours from oilseeds – milk thistle and hempseed

Milk thistle flour was tested under concentration conditions of 8% c_f; 4% c_s. The steps of casting method were stirring and heating the suspension up to 80 °C on the magnetic stirring hotplate (Heidolph MR 3001 K – 800 W, Germany) then pouring 23 g of solution on plastic petri dishes (86 x 10 mm) and dried for 24 hours at 35 °C in dryer. These films were poorly peelable, very adhesive, inflexible, crackled, firm.

As for hempseed flour, secondly, the conditions such as suspension of the given concentrations (8% c_f; 4% c_s), centrifuging after heating and mixing, 34 ± 2 g of un-settled solution pouring onto silicon moulds (128 x 10 mm) and drying for 48 hours at 35 °C, were also tested. Resulting material was none peelable, transparent, nonflexible, very adhesive. Thus, neither milk thistle nor hempseed flour were used for further descriptions of feasibility of production biobased plastics.

Flours from oilseeds – soybean defatted and soybean full fat

Soybean defatted and soy full fat flours were tested under same conditions as third measurement of wheat flour. However, resulting films were different. Soybean full fat flour had suitable properties (easily peelable, good handleability, continuous, homogeneous, little adhesive, and transparent), on the other hand, soybean defatted flour was poorly peelable, very sticky, therefore it could not be possible to get material for further handling. Therefore, soybean full fat flour was chosen to additional testing.

Pea protein

Pea 100% protein was also a subject of observation in testing the production of bioplastics by the casting method. This testing included concentrations: 1.6% c_f; 4% c_s. Steps of casting method were stirring and heating the suspension up to 80 °C on the magnetic stirring hotplate (Heidolph MR 3001 K – 800 W, Germany) then pouring 23 g of solution on plastic petri dishes (86 x 10 mm) and dried for 24 hours at 35 °C in dryer. However, the resulting film had negative properties for further handling (non-peelable, very oily, and adhesive).

Potato starch and protein

The first testing on potato starch and protein was based on their mix 1:1. The concentrations were as follows: 6% (1:1) c_f; 3% c_s; 1% c_g for variation one and 6% (1:1) c_f; 4% c_s for variation two. The techniques of casting method included: stirring the suspension for 1 minute, water bath for 10 minutes (80 °C), pouring 20 g and 10 g of suspension on plastic petri dishes (86 x 10 mm), and 24 hours in dryer (35 °C). The result was a material that was difficult to peel off and brittle, less translucent, not cellular, with cracks.

Secondly, potato starch and protein were used separately under concentrations: 8% c_f; 4% c_s (potato starch) and 1,6% c_f; 4% c_s for potato protein. The suspension was heated up to 80 °C and stirred on the magnetic stirring hotplate (Heidolph MR 3001 K – 800 W, Germany), and 23 g of suspension poured on plastic petri dishes (86 x 10 mm). Then, it dried 24 hours under 35 °C in the dryer. Resulting materials were poorly peelable, fragile, inflexible (potato starch) and oily, sticky, greasy, inseparable (potato protein).

Third measurement of these components also involved their mix (1:1) but under concentrations of 8% (1:1) c_f; 4% c_s and with same conditions, which were used in the third measurement for wheat flour. Even though silicon forms were used, the final film was very fragile, therefore, neither potato starch nor potato protein or their mix were further studied.

After evaluating all the optimization results, the following flours were selected for film formation: wheat, flaxseed and soybean full fat.

5.2 Visual and tactile aspects

Wheat flour

Considering the visual appearance, materials made from wheat flours showed the best properties i.e., good handleability, no breaks in the film, excellent homogeneity, no adhesiveness, excellent transparency and whitish.

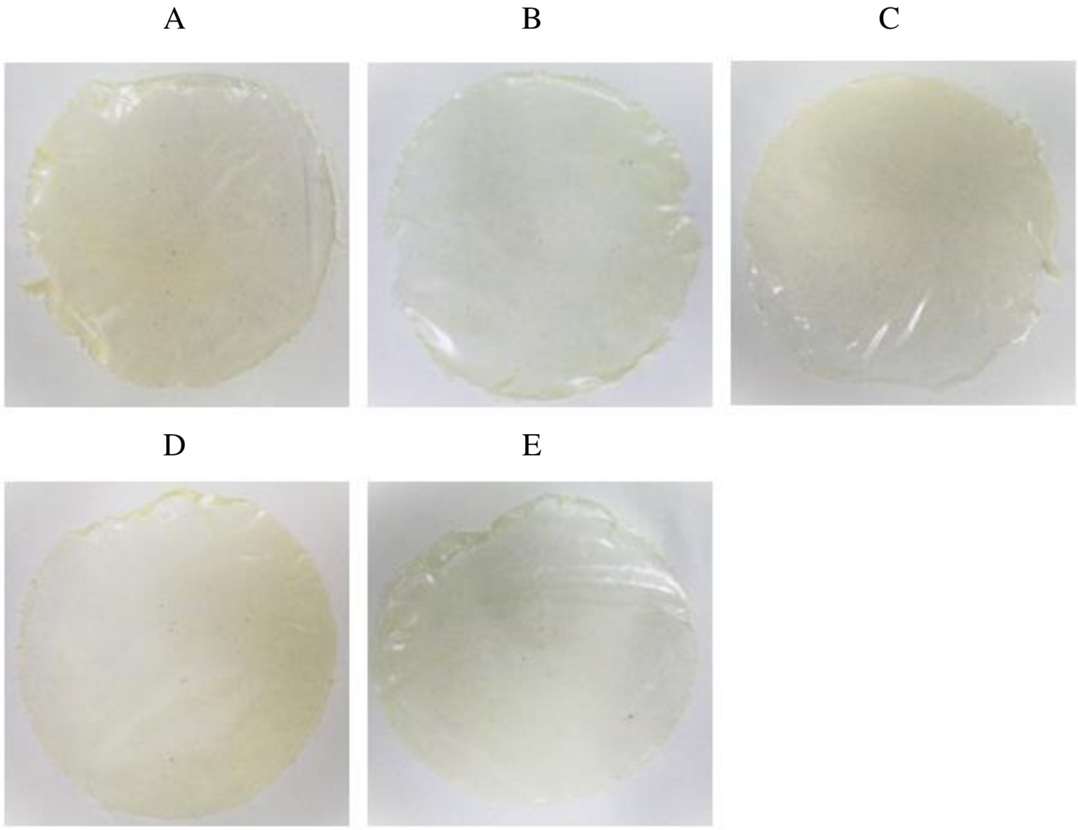

Figure 2. Five repetitions (A, B, C, D, E) of films made from wheat flour.

Flaxseed flour

Excellent handleability, no breaks, but several residues appeared on the surface of the film, excellent homogeneity, no adhesiveness, excellent transparency and yellowish colour were determined as visual and tactile properties of flaxseed flour biobased films.

A B C

D E

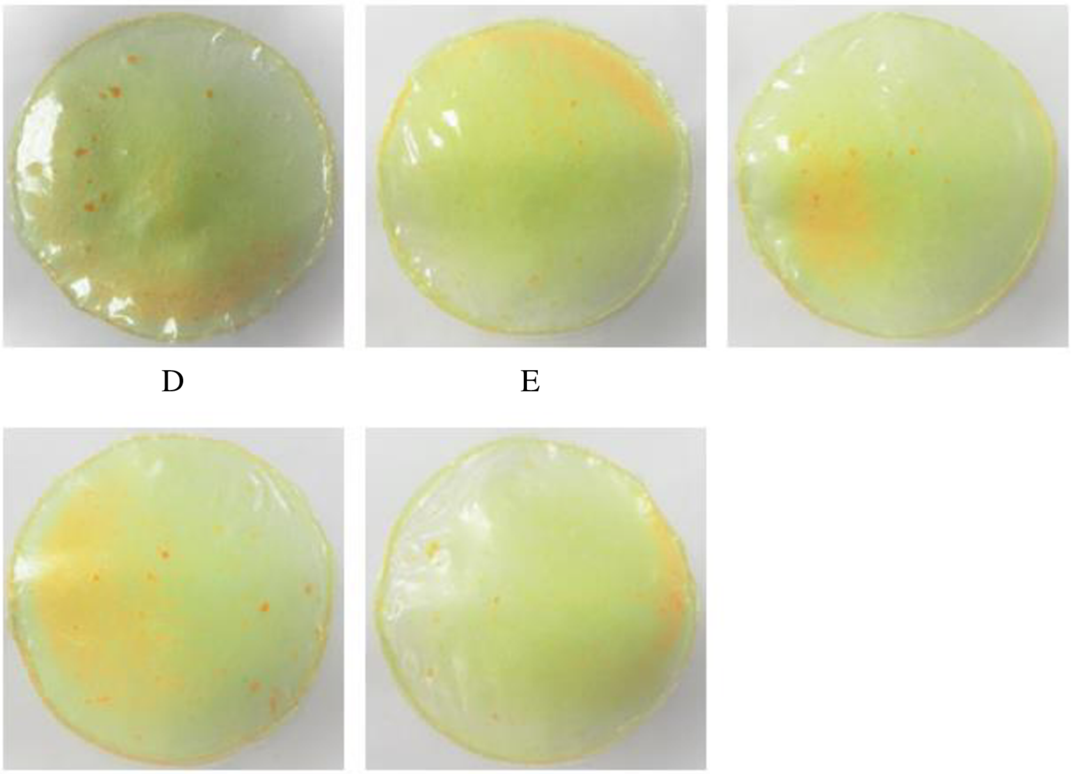

Figure 3. Five repetitions (A, B, C, D, E) of films made from flaxseed flour.

Soybean flour – full fat

The properties of soybean full fat flour films included excellent handleability, excellent continuity, excellent homogeneity, adhesiveness, good transparency, and yellowish colour.

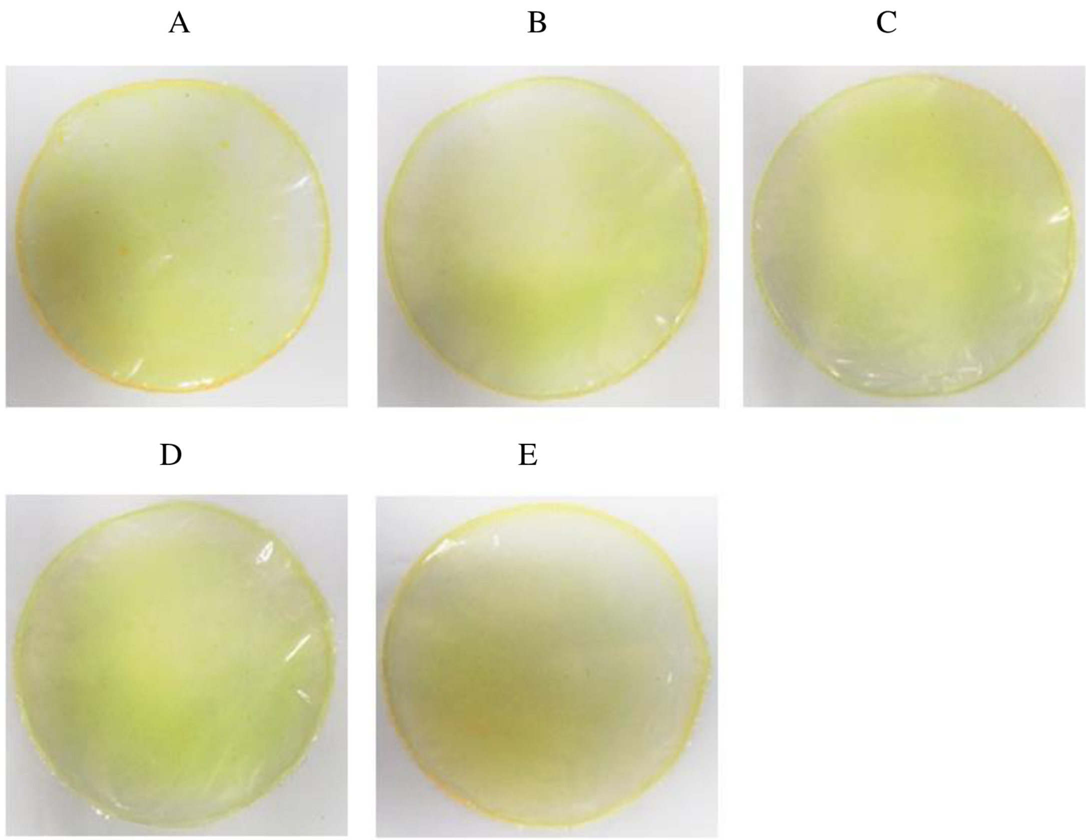

Figure 4. Five repetitions (A, B, C, D, E) of films made from soy full fat flour.

5.2.1 Colour

Table 2. Colour design of individual samples according to L*, a*, b* values.

Run	Wheat flour	Flaxseed flour	Soybean flour - full fat
A	L*= 92.29 a*= 2.42 b*= -9.19	L*= 90.92 a*= -2.20 b*= 4.93	L*= 89.08 a*= -2.58 b*= 10.19
B	L*= 92.76 a*= 2.40 b*= -9.35	L*= 90.54 a*= -3.45 b*= 9.06	L*= 90.25 a*= -1.86 b*= 7.64
C	L*= 92.19 a*= 2.31 b*= -8.71	L*= 89.27 a*= -4.48 b*= 14.16	L*= 90.13 a*= -2.14 b*= 7.64
D	L*= 92.63 a*= 2.48 b*= -9.72	L*= 90.10 a*= -3.95 b*= 11.14	L*= 89.80 a*= -2.16 b*= 7.89
E	L*= 93.07 a*= 2.71 b*= -10.36	L*= 89.79 a*= -4.20 b*= 12.48	L*= 89.36 a*= -2.46 b*= 10.01

Table 3. Analysis of colour attributes (L*, a*, b*) depending on the different films.

Film	**L* (mean ± SD)**	**a* (mean ± SD)**	**b* (mean ± SD)**
Wheat	92.59 ± 0.36^{a}	2.46 ± 0.15^{a}	-9.47 ± 0.62^{b}
Flaxseed	90.12 ± 0.64^{b}	-3.65 ± 0.90^{b}	10.35 ± 3.56^{a}
Soybean full fat	89.72 ± 0.50^{b}	-2.23 ± 0.27^{c}	8.70 ± 1.35^{a}
p	0,0000	0,0000	0,0000

Different superscript letters ($^{a\text{-}c}$) within the same column indicate significant differences between the films ($p < 0,05$).

The colour values of the obtained edible films are presented in Table 2 and subsequent analysis of the values are given in Table 3. The results show that the lowest brightness, 89.72 (parameter L*), and the darkest colour were found for the film made from soybean full fat flour. The highest value of the L* was obtained for the wheat flour edible film (92.59).

Positive values of the a* parameter indicate a greater proportion of red, while negative values indicate the presence of a greater proportion of green. The positive values for a* were observed in films made from wheat flour. On the other hand, the negative colours were found in films made from flaxseed and soybean full fat flour.

As for b* parameter, positive values indicate greater proportion of colour yellow and negative values colour blue. Negative values were found in wheat flour films, flaxseed and soybean flour films represent positive values of parameter b*.

The differences in the colours of the edible films are also proved by statistical analysis, that there is a significant difference between colour and type of flour used among all colour parameters (L*, a*, b*). For parameter L*, films made from wheat flour and soybean full fat flour films differed from each other, as did films made from flaxseed flour. Moreover, films made from flax and soybean full fat flour did not differ in parameter L*. For parameter a*, all the films differ from each other and as for parameter b*, the difference was the same as for parameter L*.

5.3 Physical properties

5.3.1 Thickness

A greater thickness was characterized for soybean full fat and wheat films (0.20 mm) than for flaxseed, which was 0.3 mm thinner on average (Table 4). Despite the statistical evaluation, no statistical significance was found among different film types.

5.3.2 Moisture content

The tested edible films had a moisture content of 6.32 – 14.74 %. Higher values were found in soybean full fat (14.74 %) and flaxseed flour films (10.95 %). The film made from wheat flour had the lowest moisture content (Table 4). Statistical evaluation did not show any statistical significance, however, according to LSD Fisher test, a significant difference was found between wheat flour and soybean full fat flour films and at the same time between soybean full fat flour and flaxseed flour films.

Table 4. Analysis of films related to their thickness and moisture content.

Film	Thickness mean ± SD (mm)	Moisture content mean ± SD (%)
Wheat	0.20 ± 0.05	6.32 ± 2.34^{b}
Flaxseed	0.17 ± 0.02	10.95 ± 4.59^{ab}
Soybean	0.20 ± 0.06	14.74 ± 7.49^{a}
p	0.5939	0.0758

Different superscript letters ($^{a-b}$) within the same column indicate significant differences between the films ($p < 0,05$).

5.4 Biodegradability

5.4.1 Water solubility

The solubility in water represented values from the lowest 78.12% (wheat flour films) to the highest 87.10% (flaxseed flour films) (Table 5). Despite the statistical evaluation, no statistical significance was found among different film types.

Table 5. Analysis of films under water solubility and solubility in water with pronase conditions

Film	Water solubility mean ± SD (%)	Solubility in water with pronase mean ± SD (%)
Wheat	78.12 ± 26.73	91.05 ± 3.95
Flaxseed	87.10 ± 3.48	90.36 ± 3.95
Soybean	86.65 ± 3.33	88.10 ± 3.76
p	0.6070	0.4098

5.4.2 Solubility in water with added enzyme

The effect of the pronase enzyme was reflected in the fact that the most soluble films were from wheat flour and the least soluble from soybean full fat flour. Despite these differences, statistical significance was not proven.

6 Discussion

6.1 Visual and tactile aspects

It has been shown that it depends on the nutritional composition of the individual flours. Since some flours, even though the plants from which they come, belong to the same plant family, it does not follow that they will have the same properties. For example, wheat, corn, and rice belong to the cereal family, but the films showed different properties in the resulting film. The problem could be found in the individual composition of the plants and also the type of plasticizer used. For example, gluten confers elastic properties owing to the presence of disulfide-linked glutenin chains. This may result in better handling of the material due to its greater flexibility compared to plants that do not contain gluten.

Moreover, the composition in terms of fats, carbohydrates and proteins is also important. It has been observed that proteins cause the stickiness of the resulting material, lipids create the cohesion of the material and sugars cause its strength. It is therefore important that materials have a balanced ratio of these nutrients. And that there is no predominance of one or the other. This has been observed using 100% pea protein, where the resulting film was very sticky and therefore unusable for further observation. As for the full-fat flour and defatted soybean, here the importance of lipids and proteins in film formation was observed. The defatted soya flour, which contained a minimum of lipids and more proteins than the full-fat flour, formed a material that was difficult to peel off from the mould, and when it was joined, it could no longer be peeled off.

In this research combination of glycerol with sorbitol and subsequently only sorbitol as a plasticizer was tested. It was observed, the combination of glycerol and sorbitol made cracks in the films. This confirms a study of Drakos et al. (2018), which says that films produced based on wheat flour had their mechanical properties decreased with increased concentration of glycerol. Furthermore, the difference between glycerol and sorbitol is in their chemical structure. Glycerol has only three hydroxyls to interact with the hydroxyls of polymers, on the other hand, sorbitol has six hydroxyls, allowing hydrogen bonds that are linearly oriented in the solution. This leads to more fragile structure due to the discontinuity of the chain in case of glycerol and better stable structure in case of sorbitol.

6.1.1 Colour

The colour of edible films is a very important parameter influencing the acceptance of products by consumers, therefore, transparent, bright and almost invisible films are mostly expected. The colour of the films obtained varied depending on the type of flour used (Mikus et al., 2021)

In order to achieve greater transparency of the material, centrifugation of the suspension was used and only the un-suspended portion was removed to produce the biofilm, which was reflected in the results. Without the use of the centrifugation method, the resulting film formed from the flour would have been almost opaque, as mentioned by research of Daudt et al. (2016). Transparent films can be considered desirable for foods which are not susceptible to reactions catalysed by light e.g., lipid oxidation, degradation of vitamins (Thorah et al., 2013).

In this research all the films had high values of L* > 89 comparable to the study of Peron-Schlosser et al. (2021). But the difference between this study and the Peron-Schlosser et al. (2021) research is that his team looked at the use of glue flour as a by-product of wheat flour production, and this study, on the other hand, used wheat flour as the main raw material.

Wheat flour films had a whitish colour which was also reflected in the values for colour parameters a* and b*, same situation showed films made from flaxseed and soybean full fat flours. In the other hand, they were yellowish and that is why they differed from wheat flour films, the parameter b* had positive values i.e., greater values for yellow colour. In general, the difference in the colours of the edible films depends on the type of flour used, which was proved by statistical significance.

6.2 Physical properties

6.2.1 Thickness

As research of Mikus et al. (2021) says ,,The differences in thickness of each type of films may have been due to the different densities of the prepared film-forming solutions, since viscous solutions tend to form thicker layers.'' Although this statement seems to be true, statistical significance of differences between flour types based on their thickness was not confirmed in this work. However, it must be considered that here the centrifugation method was used and only the unsettled part of the prepared homogeneous mixture was used as the film-forming solution.

6.2.2 Moisture content

Films that were higher in fat and protein and lower in carbohydrates had higher moisture content. On the other hand, films prepared from wheat flour, which had the highest carbohydrate content, had the lowest moisture content. Which leads to the finding and confirmation of the results of the study by Nouraddini et al. (2018) that the higher water content causes the high amount of hydrophilic components such as proteins, carbohydrates and fiber contained in the flour. The high content of these ingredients induces interactions with water molecules resulting in more water being retained in the film. Moreover, it is also necessary to consider the length of storage, when water loss may have occurred.

6.3 Biodegradability

6.3.1 Water solubility

The potential use of biodegradable films as food packaging material may require a high water resistance to increase the product integrity and to obtain a moisture resistant material especially for foods with higher water activity. However, portions of premeasured food (e.g., rice), which will be dissolved in water or heated, may require films with high solubility (Peron-Schlosser et al., 2021).

„Although glycerol increases the affinity of films with water molecules, plasticized films with glycerol do not easily dissolve in the presence of water. In turn, films plasticized with sorbitol are more soluble." In this work, we used only sorbitol as a plasticizer, so this information was also reflected in the results of the films, where the solubility in water was higher than 78%. If glycerol was added to the film-forming mixture, a lower solubility could be achieved. The most soluble material was soybean flour and the least soluble was wheat flour film. Wheat flour contained the most carbohydrates; therefore, it was proved that higher the amylose content of the flour the lower the solubility in water, a founding of Mikus et al. (2021) study.

6.3.2 Solubility in water with added enzyme

A demonstration of an environment in which biofilm degradation would occur was mediated as an aqueous environment containing the proteolytic enzyme pronase (EC 3.4.24.4) from *Streptomyces griseus*, a commonly occurring bacteria in soil. The solubility was higher than 88% and also, values of each film were higher than in case of solubility in water. Thus, it was proved that the effect of 2% pronase in aqueous environment is capable of breaking the crosslinks of polymers. The study of Yamada et al. (2020) also observed the positive effect of pronase (EC 3.4.24.4) on the degradability of soy protein films in case of structural change, such as a crack and transformation of biodegraded bioplastic.

7 Conclusions

In this study, different types of flour were tested for the production of bioplastics by casting method. The optimized conditions for obtaining biofilms were c_f 8% and c_s 4%, use of centrifugation of the film forming suspension during casting method to obtain transparent materials and use of silicone moulds for easier removal of the material. It was found that wheat, flaxseed and soybean full fat flours are promising alternative sources for production of biodegradable food packaging films. The properties of these films were excellent handleability, homogeneity, no adhesiveness and good transparency. Furthermore, all biofilms showed a high solubility in water and a biodegradable ability in aqueous environment with enzyme pronase from *Streptomyces griseus* that normally occurs in the soil. Therefore, these biofilms could be further studied under the conditions of decomposition in soil. Subsequent researches also need to be carried out to incorporate additives that could improve the barrier and mechanical properties of the films.

8 References

Barrett DM., Beaulieu JC., Shewfelt R. (2010). Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: Desirable levels, instrumental and sensory measurement, and the effects of processing. *Critical Reviews in Food Science and Nutrition*, 50: 369–389.

Bátori V., Lundin M., Åkesson D., Lennartsson PR., Taherzadeh MJ., Zamani A. (2019). The effect of glycerol, sugar, and maleic anhydride on pectin-cellulose thin films prepared from orange waste. *Polymers,* 11.

Borah PP., Das P., Badwaik LS. (2017). Ultrasound treated potato peel and sweet lime pomace based biopolymer film development. *Ultrasonics Sonochemistry,* 36: 11–19.

Castro-Muñoz R., Boczkaj G., Gontarek E., Cassano A., Fíla V. (2020). Membrane technologies assisting plant-based and agro-food by-products processing: A comprehensive review. *Trends in Food Science and Technology*, 95: 219–232.

Daudt RM., Avena-Bustillos RJ., Williams T., Wood DF., Külkamp-Guerreiro IC., Marczak LDF., McHugh TH. (2016). Comparative study on properties of edible films based on pinhão (Araucaria angustifolia) starch and flour. *Food Hydrocolloids*, 60: 279–287.

de Carvalho GR., Marques GS., de Matos Jorge LM., Jorge RMM. (2019). Cassava bagasse as a reinforcement agent in the polymeric blend of biodegradable films. *Journal of Applied Polymer Science*, 136: 1–7.

Díaz-Montes, E. and Castro-Muñoz, R. (2021). Edible films and coatings as food-quality preservers: An overview. *Foods*, 10: 1–26.

Drakos A., Pelava E., Evageliou V. (2018). Properties of flour films as affected by the flour's source and particle size. *Food Research International*, 107: 551–558.

European Bioplastics e.V. (2022). *Bioplastics market data*. [online] [cit. 8. 02. 2023]. Available at: https://www.european-bioplastics.org/market/

Fakhouri FM., Costa D., Yamashita F., Martelli SM., Jesus RC., Alganer K., Collares-Queiroz FP., Innocentini-Mei LH. (2013). Comparative study of processing methods for starch/gelatin films. *Carbohydrate Polymers*, 95: 681–689.

Fitch-Vargas PR., Aguilar-Palazuelos E., de Jesús Zazueta-Morales J., Vega-García MO., Valdez-Morales JE., Martínez-Bustos F., Jacobo-Valenzuela N. (2016).

Physicochemical and Microstructural Characterization of Corn Starch Edible Films Obtained by a Combination of Extrusion Technology and Casting Technique. *Journal of food science,* 81: e2224–e2232.

Frantz, S. C., Paludo, L. C., Stutz, H., & Spier, M. R. (2019). Pro- duction of amylases from Coprinus comatus under submerged culture using wheat-milling by-products: Optimization, kinetic parameters, partial purification and characterization. *Biocatalysis and Agricultural Biotechnology*, 17:82–92.

Havstad, M. R. (2020). Biodegradable plastics. In *Plastic waste and recycling* (pp. 97-129). Academic Press.Hernandez-Izquierdo, VM. and Krochta, JM. (2008). Thermoplastic processing of proteins for film formation - A review. *Journal of Food Science,* 73: 30–39.

Hernández-Muñoz P., Kanavouras A., Lagaron JM., Gavara R. (2005). Development and characterization of films based on chemically cross-linked gliadins. *Journal of Agricultural and Food Chemistry,* 53: 8216–8223.

Khanzadi M., Jafari SM., Mirzaei H., Chegini FK., Maghsoudlou Y., Dehnad D. (2015). Physical and mechanical properties in biodegradable films of whey protein concentrate-pullulan by application of beeswax. *Carbohydrate Polymers,* 118: 24–29.

Kumar, N. and Neeraj. (2019). Polysaccharide-based component and their relevance in edible film/coating: a review. *Nutrition and Food Science*, 49: 793–823.

López-Rubio A., Almenar E., Hernandez-Muñoz P., Lagarón JM., Catalá R., Gavara R. (2004). Overview of active polymer-based packaging technologies for food applications. *Food Reviews International,* 20: 357–387.

Lopez O., Garcia MA., Villar MA., Gentili A., Rodriguez MS., Albertengo L. (2014). Thermo-compression of biodegradable thermoplastic corn starch films containing chitin and chitosan. *Lwt,* 57: 106–115.

Mikus MM., Galus S., Ciurzynska A., Janowicz M. (2021). Development and characterization of novel composite films based on soy protein isolate and oilseed flours. Molecules 26 (e3738). DOI: 10.3390/molecules26123738.

Miyata, Y. (1994). Biodegradable Plastics. Page Nippon Nogeikagaku Kaishi.

Mooney, B. P. (2009). The second green revolution? Production of plant-based biodegradable plastics. *Biochemical Journal,* 418: 219–232.

Nair NR., Sekhar VC., Nampoothiri KM., Pandey A. (2016). Biodegradation of Biopolymers. Page Current Developments in Biotechnology and Bioengineering:

Production, Isolation and Purification of Industrial Products.

Nouraddini M., Esmaiili M., Mohtarami F. (2018). Development and characterization of edible films based on eggplant flour and corn starch. International journal of biological macromolecules, 120: 1639-1645.

Orsuwan, A. and Sothornvit, R. (2018). Effect of banana and plasticizer types on mechanical, water barrier, and heat sealability of plasticized banana-based films. *Journal of Food Processing and Preservation*, 42.

Peelman N., Ragaert P., De Meulenaer B., Adons D., Peeters R., Cardon L., Van Impe F., Devlieghere F. (2013). Application of bioplastics for food packaging. *Trends in Food Science and Technology,* 32: 128–141.

Peron-Schlosser B., Carpiné D., Matos Jorge RM., Rigon Spier M. (2021). Optimization of wheat flour by product films: A technological and sustainable approach for bio-based packaging material. *Journal of Food Science,* 86: 4522–4538.

Platt, D. (2006). Biodegradable polymers: market report. [online] [cit. 8. 02. 2023] Available from http://books.google.com/books?hl=en&lr=&id=Jtt2MFkYiP0C&oi=fnd&pg=PA1941&dq=Biodegradable+Polymers+Market+Report&ots=NDKXR5Fyb6&sig=Z2U9bQLrzMsLr9qAj0bcfMhvGyE.

Pothet, J. (2014). Introduction. Journal of cutaneous medicine and surgery [online] [cit. 8. 02. 2023] Available from http://www.ncbi.nlm.nih.gov/pubmed/22470909.

Rawat, S. (2015). Food Spoilage: Microorganisms and their prevention. Pelagia Res. Libr. *Asian J. Plant Sci. Res*, 5:47–56.

Reddy, N. and Yang Y. (2013). Thermoplastic films from plant proteins. *Journal of Applied Polymer Science,* 130: 729–738.

Šimkovic I., Kelnar I., Mendichi R., Bertok T., Filip J. (2017). Composite films prepared from agricultural by-products. *Carbohydrate Polymers,* 156: 77–85.

Sone Aung SP., Htet Shein HH., Aye KN., Nwe N. (2018). Environment-friendly biopolymers for food packaging: Starch, protein, and poly-lactic acid (PLA). Bio-based Materials for Food Packaging: Green and Sustainable *Advanced Packaging Materials*, 173–195.

Suhag R., Kumar N., Petkoska AT., Upadhyay A. (2020). Film formation and deposition methods of edible coating on food products: A review. *Food Research*

International, (e109582). DOI: 10.1016/j.foodres.2020.109582.

Thorat ID., Jagtap DD., MohapatraD., Joshi DC., Sutar R., Kapdi S. (2013). Antioxidants, their properties, uses in food products and their legal implications. *International Journal of Food Studies,* 2: 81–104.

United States Department of Agriculture. (2020). *World agricultural production*. [online] [cit. 8. 02. 2023]. Available at: https://apps.fas.usda.gov/psdonline/circulars/ production.pdf

Viana RM., Sá NMSM., Barros MO., Borges MF., Azeredo HMC. (2018). Nanofibrillated bacterial cellulose and pectin edible films added with fruit purees. *Carbohydrate Polymers,* 196: 27–32.

Yadav P., Yadav H., Shah VG., Shah G., Dhaka G. (2015). Biomedical biopolymers, their origin and evolution in biomedical sciences: A systematic review. *Journal of Clinical and Diagnostic Research*, 9: 21–25.

Yamada M., Morimitsu S., Hosono E., Yamada T. (2020). Preparation of bioplastic using soy protein. *International Journal of Biological Macromolecules* 149: 1077–1083.

Appendices

List of appendices

Appendix 1: Photographic illustrations of casting method

Figure 5. Weighting of raw materials for casting method (Tůmová 2023)

Figure 6. Heating, stirring and temperature control of the suspension (Tůmová 2023)

Figure 8. Centrifugation (Tůmová 2023)

Figure 7. Removal of unsettled solution (Tůmová 2023)

Figure 10. Storing the resulting material in glass petri dishes (Tůmová 2023)

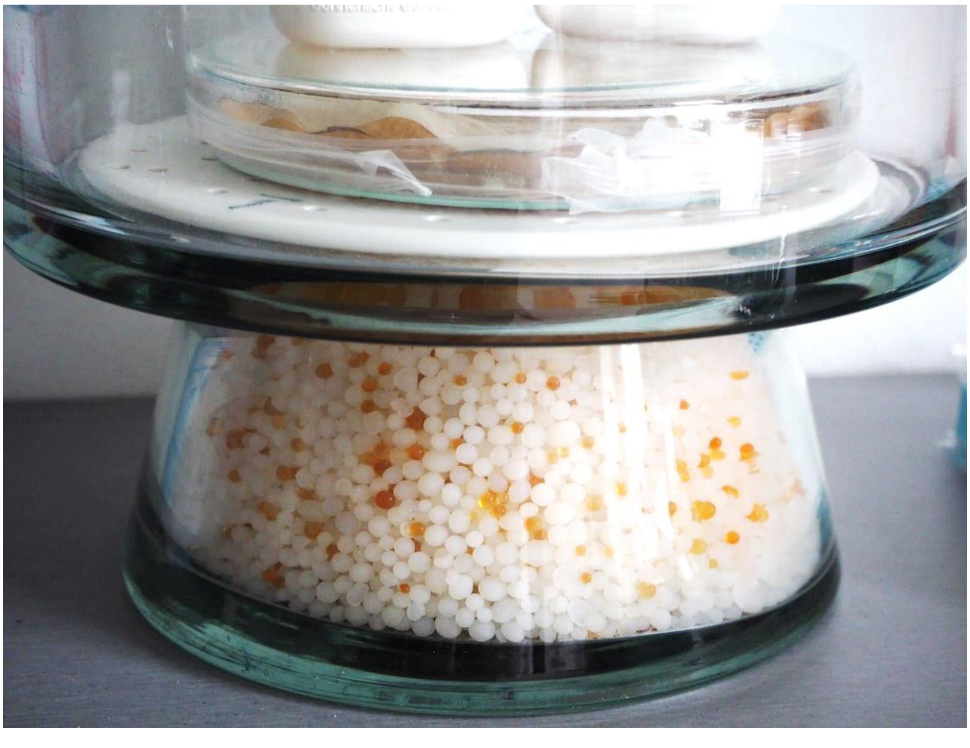

Figure 9. Placement in the desiccator (Tůmová 2023)

Appendix 2: Photographic illustrations of tested films

Figure 14. Biofilm from potato starch and protein (1:1), using glycerol (Tůmová 2023)

Figure 13. Biofilm from soy defatted flour (Tůmová 2023)

Figure 12. Biofilm from flaxseed without centrifugation (Tůmová 2023)

Figure 11. Biofilm from 100% pea protein (Tůmová 2023)

Figure 15. Biofilm from rice flour (Tůmová 2023)