Kaunas University of Technology
Faculty of Mechanical Engineering and Design

Effect of Essential Oils in Starch-Based Films on Fresh Food

Master's Final Degree Project

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Project author

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Supervisor

Kaunas, 2024

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Industrial Engineering and Management (6211EX018)

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Task of the Master's Final Degree Project

Given to the student – Dominyka Podoliankaitė

1. Title of the Project

Effect of Essential Oils in Starch-Based Films on Fresh Food

(In English)

Eterinių aliejų krakmolo pagrindo plėvelėse poveikis šviežiam maistui

(In Lithuanian)

2. Aim and Tasks of the Project

Aim: to develop starch-based film with different essentials oils

Tasks:

1. to investigate effect of different types of essentials oils in films;
2. to carry out tensile test of developed film;
3. to investigate the effect of selected essentials oils on food freshness;
4. to evaluate the economic value of the starch-based film with essentials oils.

3. Main Requirements and Conditions

The films will be made from potato starch with different kind of essentials oils: tea tree, oregano, rosemary, and clove. 3 different quantities of essentials oils will be added: 0,3 ml, 0,6 ml and 0,9 ml. Tensile test will be conducted with the 150 mm length and 15 mm width samples. 4 samples for testing will be prepared. Excel and QMAT software will be used. Colorimeter, micrometer, and microscope will be used.

4. Additional Requirements for the Project, Report and its Annexes

Not applicable

Project author	Dominyka Podoliankaitė		04-03-2024
	(Name, Surname)	*(Signature)*	*(Date)*
Supervisor	Laura Gegeckienė		04-03-2024
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Podoliankaitė Dominyka. Effect of Essential Oils in Starch-Based Films on Fresh Food. Master's Final Degree Project, supervisor assist. prof. dr. Laura Gegeckienė; Faculty of Mechanical Engineering and Design, Kaunas University of Technology.

Study field and area (study field group): Production and Manufacturing Engineering (E10), Engineering Sciences (E).

Keywords: starch; essential oils; food freshness; film; strawberry.

Kaunas, 2024. 58 p.

Summary

Since plastic is still one of the most widely used materials for packaging, alternatives are being sought to replace commonly used plastic packaging materials with more ecological, less polluting, biodegradable and functional packaging materials. Bioplastics are considered one of the alternatives that have attracted the attention of scientists and researchers. However, like any material, it has advantages and disadvantages. In this project, 12 films were made from potato starch, distilled water, glycerin, and essential oils: clove, oregano, rosemary, tea tree. In addition to all this, 1 control film was produced without essential oils. The study showed changes in the thickness of the packaging films, the structure of the films was examined, and tensile and color change tests were performed according to the type and amount of different essential oils. Daily weighing and visual inspections of strawberries packed in starch-based films were carried out over a 14-day period to evaluate differences in strawberry shelf life between the different additives used in the films. The obtained data showed that these films did not withstand high forces and showed minimal elongations, while increasing the amount of essential oils worsened their mechanical properties. However, essential oils such as clove and rosemary showed a positive effect on strawberry freshness and no mold or discoloration was observed using visual assessment, but tea tree and clove essential oils did not show a positive effect on strawberry freshness. These films are still not fully applicable in production for the packaging, but they are environmentally friendly, and after more detailed research and improvement of the mechanical properties of these materials, these materials can be considered as alternatives to replace traditional plastics. In addition, the use of essential oils could extend the shelf life of products from 4 to 14 days and reduce food waste by at least 3.5 times.

Podoliankaitė Dominyka. Eterinių aliejų krakmolo pagrindo plėvelėse poveikis šviežiam maistui. Magistro baigiamasis projektas, vadovė asist. dr. Laura Gegeckienė; Kauno technologijos universitetas, Mechanikos inžinerijos ir dizaino fakultetas.

Studijų kryptis ir sritis (studijų krypčių grupė): Gamybos inžinerija (E10), Inžinerijos mokslai (E).

Reikšminiai žodžiai: krakmolas; eteriniai aliejai; maisto šviežumas; plėvelė; braškės.

Kaunas, 2024. 58 p.

Santrauka

Kadangi plastikas vis dar yra viena plačiausiai naudojamų medžiagų pakavimui, tam yra ieškoma alternatyvų įprastai naudojamas plastikines pakavimo medžiagas pakeisti į labiau ekologiškas, mažiau teršiančias gamtą, biologiškai skaidžias ir atliekančias pakavimui skirtas funkcijas. Bioplastikas yra laikoma viena iš alternatyvų, patraukusių mokslininkų ir tyrėjų dėmesį. Tačiau, kaip ir kiekviena medžiaga, ši taip pat turi ir privalumų, ir trūkumų. Šiame baigiamajame projekte pagaminta 12 plėvelių, iš krakmolo, distiliuoto vandens, taip pat, glicerino ir eterinių aliejų: gvazdikėlių, raudonėlių, rozmarinų, arbatmedžių. Be viso to, buvo pagaminta 1 kontrolinė plėvelė nenaudojant priedų – eterinių aliejų. Tyrimas parodė pakavimo plėvelių storio pokyčius, ištirta plėvelių struktūra, atlikti tempimo bei spalvos pokyčio bandymai pagal skirtingų eterinių aliejų tipą ir kiekį. Atlikti kasdieniai braškių, supakuotų krakmolo pagrindu plėvelėse, svėrimai ir vizualinės inspekcijos 14 dienų laikotarpiu, siekiant įvertinti braškių galiojimo laiko skirtumus tarp naudotų skirtingų priedų plėvelėse. Gauti duomenys parodė, kad šios plėvelės neatlaikė didelės jėgos ir rodė minimalius pailgėjimus, o padidinus eterinių aliejų kiekį pablogėjo jų mechaninės savybės. Tačiau eteriniai aliejai, tokie kaip gvazdikėliai ir rozmarinai, parodė teigiamą poveikį braškių šviežumui, o naudojant vizualinį vertinimą nepastebėta pelėsių ar spalvos pakitimų, tačiau arbatmedžių ir gvazdikėlių eteriniai aliejai neparodė teigiamo poveikio braškių šviežumui. Šios plėvelės vis dar nėra pilnai pritaikomos gamyboje galutinių gaminių pakavimui, tačiau yra nekenksmingos aplinkai, o atlikus išsamesnius šių medžiagų mechaninių savybių tyrimus bei tobulinimą, šios medžiagos gali būti vertinamos kaip alternatyvos, pakeičiančios tradicinius plastikus. Be viso to eterinių aliejų naudojimas galėtų pailginti produktų galiojimo laiką nuo 4 iki 14 dienų laikotarpio ir sumažinti maisto švaistymą bent 3,5 karto.

Table of Contents

List of Figures

List of Tables

Introduction

Product packaging is an important part of the product, which performs protective and communication functions. Packaging is like a barrier between its surrounding and helps to protect the product from the surrounding environment. The packaging protects the product from foreign substances and contamination, such as dust or bacteria in the environment. This acts as a protective measure against damage caused by the consumer itself, such as touching the product, which can contaminate food, and accelerate the rate of rotting. Additionally, the packaging protects the product during transportation and handling, thus ensuring that the buyer receives it in a condition that is satisfactory to the buyer. Plastics remain the most popular packaging material on the market due to their versatility, ease of use and availability. Since plastics are made from petrochemicals such as crude oil, they are not recycled, they are not biodegradable, they pollute the environment by accumulating in green spaces. Additives in plastics, such as phthalates, also harm human health and, when accumulated in the environment, can pollute land, and water and contribute to climate change. Since the use of plastics is still not decreasing and remains the same, it poses significant challenges for their management. Some plastics are minimally recycled, their collection is not efficient, and it is very difficult to control their consumption, therefore it poses a great risk to the environment, humanity, and fauna. Since plastics are not environmentally friendly, there is a growing debate and interest in environmental conservation to replace traditional plastics. One of the alternative materials that are environmentally friendly are bioplastics. These plastics are obtained from natural materials, do not pollute the environment, are degradable and do not pose a threat of contamination. Additives such as essential oils that have antibacterial and antifungal properties can be added to these materials, which would extend the shelf life of food, natural pigments that would respond to environmental changes or product quality changes. Like all materials, these also have their own advantages and disadvantages. These materials, being environmentally friendly, are still not suitable for mass production. There are still questions about optimizing their production, storage, and packaging suitability. As the obtained data from the tensile strength showed that these materials are brittle and non-plastic and compared to traditional plastics do not show the same properties. To adapt these materials for packaging, research is still needed to investigate different additives that could improve the mechanical properties of these materials.

Strawberries are one of the most perishable fruits because of their thin skin, they are easily damaged and easily release moisture to the environment. Since they are perishable, the goal is to extend their shelf life, and in this way, it is possible to reduce the amount of wasted food, reduce the resources used, and save money. In this work, a study with strawberries and films made from starch and various essential oils used as additives showed a positive effect on the shelf life of strawberries.

Aim: to develop starch-based film with different essentials oils
Tasks:
1. to investigate effect of different types of essentials oils in films;
2. to carry out tensile test of developed film;
3. to investigate the effect of selected essentials oils on food freshness;
4. to evaluate the economic value of the starch-based film with essentials oils.

Hypothesis: properly selected essentials oils as additives in packaging film can prolong food freshness.

1. Food Safety, Food Packaging and Materials

Fruits and vegetables are one of the most important parts of the human diet. Fresh fruits and vegetables are rich in valuable substances beneficial to human health, such as vitamins, natural sugar and organic acids. There are scientific studies proving that the consumption of fresh fruits and vegetables every day improves human mental health [1]. A growing problem in the world is that fresh fruits and vegetables are transported from distant countries and by the time they reach the stores, the period during which they are fresh is shortened and most often they need to be consumed quickly after purchase. Also, this shortened period means that the abundance of minerals and vitamins and other important additives in the products is reduced. In order to keep food fresh as long as possible, scientists are looking for ways that could help extend the freshness of food without using any unnatural additives that could harm human health.

Food spoilage in general is a big problem facing the whole world, which includes not only fruits and vegetables, but all packaged raw products such as meat, fish. Because of spoilage, more and more food is thrown away. According to 2020 data, each resident of the European Union generated on average 130 kg of food waste. Italy generated the least food waster per person – 68 kg, and Cyprus the most – 397 kg (see Fig. 1.). Looking at this data, can be concluded, that extending the shelf life of fresh foods can help reduce food waste [2].

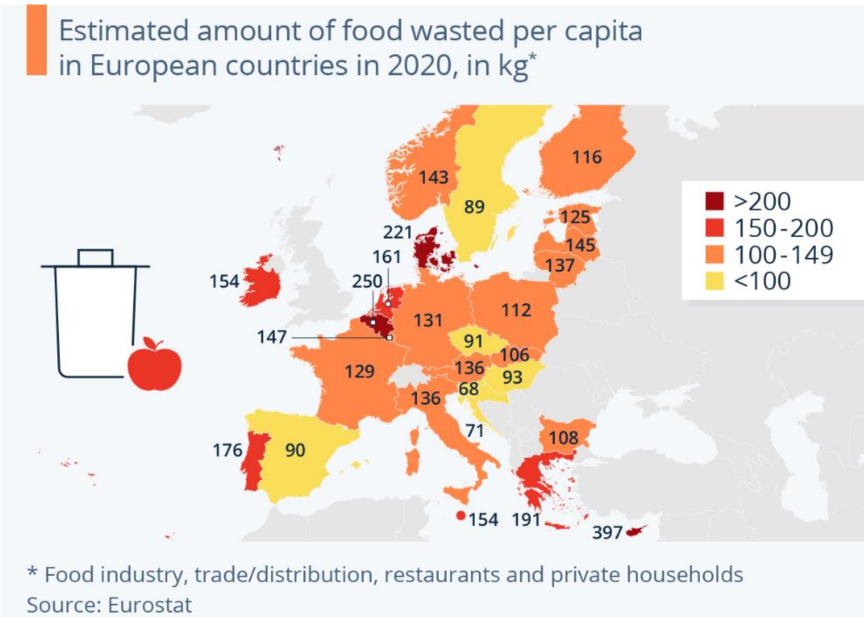


Fig. 1. Food waste per person (kg) in Europe countries in 2020 [2]

In 2015, the members of the United Nations adopted “17 Sustainable Development Goals” and undertake to implement them by 2030. The center of all these goals is sustainability, but the main goal of these obligations is to reduce climate change, preserve forests, oceans, and reduce hunger and poverty. By reducing food waste, we would directly contribute to the 12 goals "Responsible consumption and production" [3], which aims to ensure proper use of resources, sustainable infrastructure and provide a better quality of life. The twelfth goal has target 12.3, which states: "Halve global per capita food waste". It aims to reduce food waste from production to consumption [3]. This means that measures must be taken to reduce food waste.

1.1. Food Packaging

There are several main functions that packaging must meet and perform: containment, protective, availability and communication. Each product must be packed before it is transported from one place to another, and the packaging must protect the product during transportation so that it does not damage nor change its functions. The protective function of packaging is one of the main ones. The packaging must protect the product from external factors such as moisture, dust, odors, microorganisms, gases, etc. For example, if the package of a vacuum-packed product is damaged and oxygen gets into the package, it will shorten the shelf life of the product. Modern industrial societies bring important changes and create demand for new, better, smarter packaging, and industry and manufacturers must adapt to these changes. This can be from simplifying the package, improving it to making a change that would be attractive to the buyer. Packaging design is an integral part of innovation [4].

To date, the most popular packaging material remains plastic. Plastic has attractive properties that are perfect for transportation, protection, and its simplicity. This includes properties such as flexibility, which makes it easy to package a wide variety of products in various shapes and sizes. Lightness, which can reduce transportation costs by making possible to transport larger volumes with less weight. Transparency, because to this feature, buyers can see the packaged product and to evaluate its condition [5, 6]. According to 2021 data published by Plastic Europe, 90.2% of all plastics produced were fossil-based plastics and only 1.5% of all plastics were bio-based, while the remaining 8.3% were recycled plastics (see Fig. 2) [7].

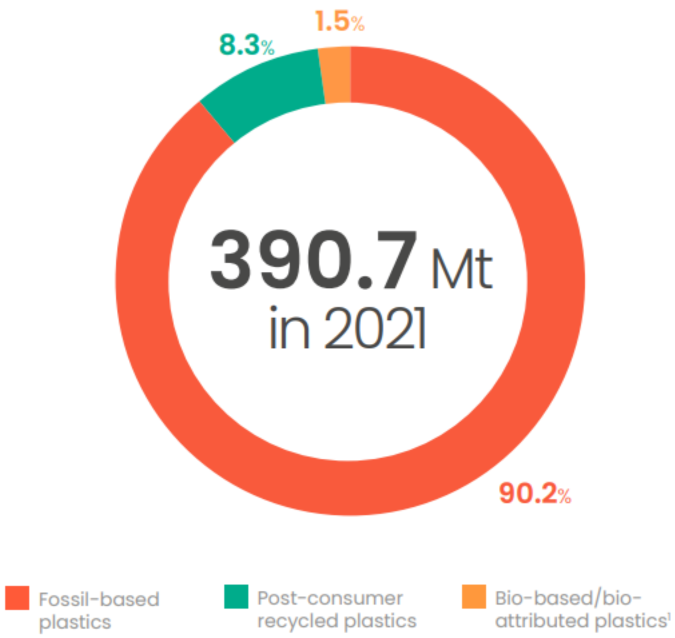


Fig. 2. Plastics produced in 2021 [7]

Plastic Soup announced 2021 according to data, in all countries of the European Union, as well as countries that have concluded agreements with the European Union, most of the plastic is used in packaging processes, the rest of the produced plastics are used in the machine industry, as interior or exterior parts, in the construction industry, as well as in the medical and furniture industries (see Fig. 3) [8] .

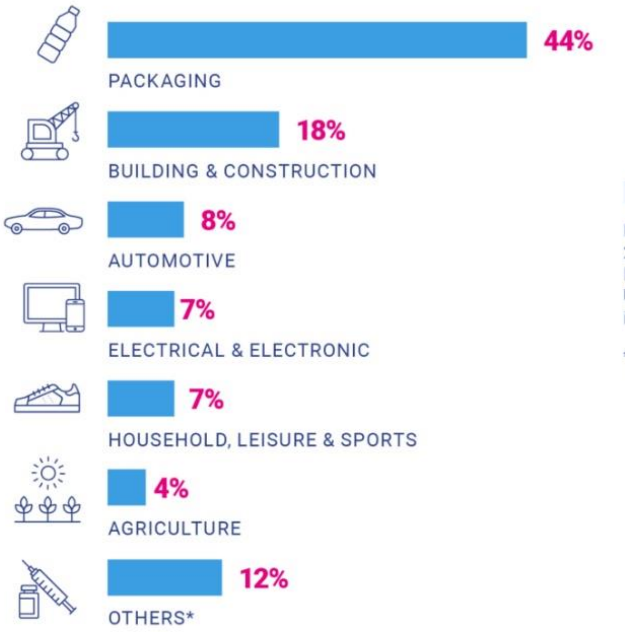


Fig. 3. Plastics application sector [8]

1.2. Starch Overview

Since plastics are unnatural and mostly derived from crude oil, they pollute the environment heavily. The majority of plastics are not discarded properly and therefore are not properly recycled and starts to build up in green spaces, polluting groundwater, oceans, contaminating the earth with toxins and contributing to global warming. It is for these reasons that scientists and researchers are looking for ways to replace plastics. Plastics that are obtained from natural materials and their properties have been studied for some time. It is one of the most talked about and researched alternatives that can replace non-naturally based plastics. Starch-based bioplastics are studied as a potential alternative to petroleum-based plastics.

Starch is a white, odourless, and tasteless powder. Green plants produce glucose from carbon dioxide through photosynthesis, it is used for its energy processes, synthesis and growth, and the surplus is stored as starch. Most starch is found in plant roots, potato tubers, grains, and fruits. In order to extract starch from these plants, they are crushed, washed with water, all dirt and impurities are washed away with water, the remaining impurities, proteins and lipids are separated by centrifugation, and the resulting residue is dried to specific moisture content.

Table 1. Physical properties of starch

Properties	**Value**
Solubility	Not soluble in cold water, or alcohol. Soluble in hot water, DMSO.
Taste	Tasteless
Colour	White
Form	Powder
Density (g/cm^3)	1,5
Particle diameter (μm)	5-25
Gelatinization temperature (°C)	55-60

Starch is a natural polysaccharide that is composed of two different polysaccharide molecules – amylose and amylopectin (see Fig. 4) [9]. Amylose is a linear polymer, which is responsible for chemical, mechanical and physical characteristics [10]. The increase in the amount of amylose in the structure of the film correlates with the improvement of the tensile strength, but the elongation value is decreasing. Amylopectin is a branched polymer and an increase in the amount of this polymer in the film correlates with decreasing mechanical properties [11]. Starch can be obtained from various sources as there are various sources such as corn, rice, wheat, and potatoes. However, it is worth noting that the ratios of amylose and amylopectin differ between these sources (see Table 2).

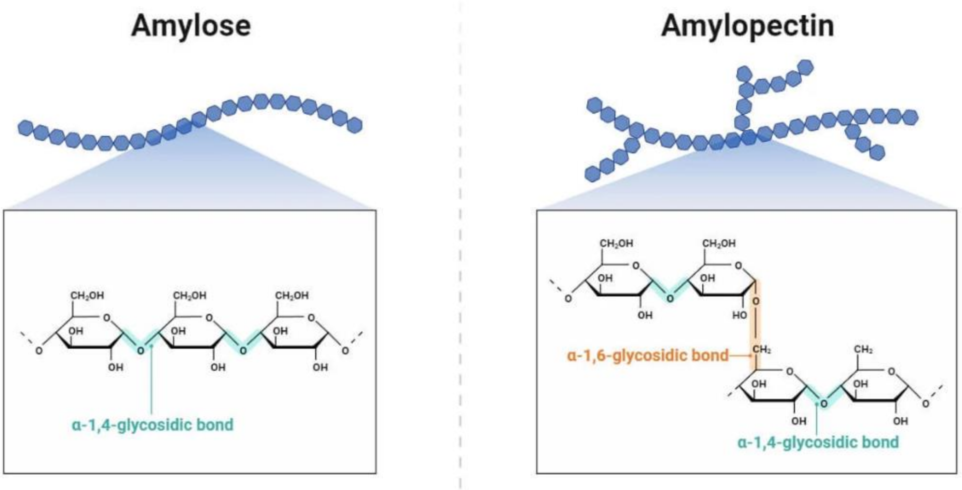


Fig. 4. Amylose and amylopectin chemical structure [12]

Table 2. Different ratios between polysaccharides based of the starch source [13]

Source	**Amylose (in %)**	**Amylopectin (in %)**
Arrowroot	20.5	79.5
Banana	17	83
Cassava	18.6	81.4
Corn	28	72
Potato	17.8	82.2
Rice	35	65
Tapioca	16.7	83.3
Wheat	20	80

Films made from this material have many potential applications as they can be used in various industries such as packaging, food, medical and automotive. Starch is also relatively cheap, natural and can be found in all plants and plant parts. However, films made from starch alone are too rigid and fragile. Therefore, the inclusion of plasticizers enhances the mechanical properties of the film. Plasticizers such as sorbitol, glycerol, fructose reduce hydrogen bonding between molecules and increase the intermolecular distance between polymers, making the film more flexible. The use of various plasticizers improves the water absorption properties of the film, and glycerol as a plasticizer, shows the best reduced water absorption compared to other plasticizers [14, 15]. To further improve starch-based films, essential oils can be incorporated into them. Studies have shown that some essential oils have antimicrobial, antibacterial and antifungal properties.

1.3. Starch-based Material Processing

– Film solution casting.

Film solution casting is one of the methods that is used to prepare the film that will be used as a packaging material (see Fig. 5). First, a solution must be prepared, which consists of water, starch, and other necessary additives such as plasticizers. According to the selected and required parameters, the prepared solution is heated for the required time until gelatinization of starch occurs. The resulting solution is poured as evenly as possible on a flat surface, such as glass or plastic sheet and left to dry at room temperature or slowly dried in a stove. When the solution dries, the obtained film removed carefully by peeling it from the flat surface. This method is widely used for preparing films from starch and several authors have recorded their methods for preparing biodegradable films [16]. However, this method has its limitations, it is difficult to apply it in industry, because it is necessary to make it exactly along the entire length and width of the film, so that its thickness does not differ and is as uniform as possible. Although it is widely applicable in the laboratory, it is somewhat more complicated in continuous production also due to the large amount of water used, the long drying time, which requires high energy consumption, and the drying film needs to have a separate designated place in the factory until it is dry and ready for use, which also requires additional costs [17, 18].

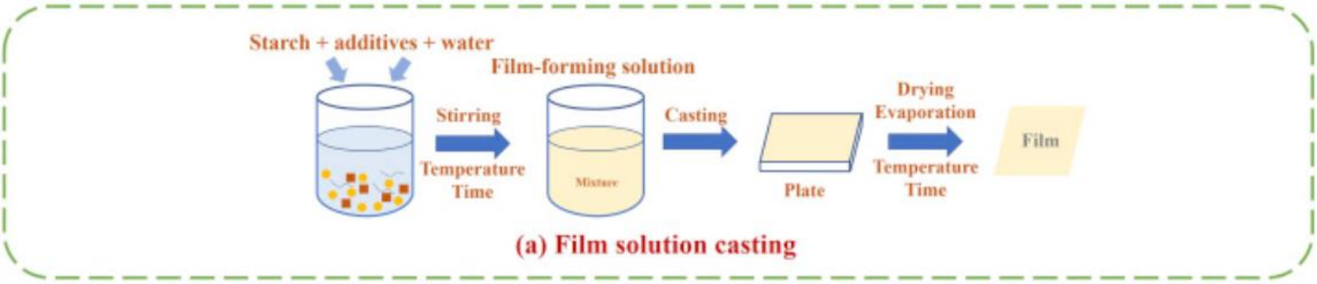


Fig. 5. Solution casting method [17]

– Foaming processing.

By using the foaming process, starch-based foam is being produced. This foam can be formed by 4 ways: heating, molding, extrusion, and supercritical fluid foaming (see Fig. 6). By using heating method, suspension of starch, material that would create foam and other necessary additives are poured into the cavity and heated in the oven. During this process, as the water evaporates, foam begins to form, and a starch paste is formed. The molding process is similar to the latter process, but this process requires a hydraulic press with temperature control. The prepared starch suspension with all the necessary additives, as well as the material that will form the foam, is placed in this press and foam is created by adjusting the pressure and temperature. The process of creating foam by using supercritical fluid, to form gas bubbles, uses supercritical carbon dioxide. Under pressure foam creating material is mixed with carbon dioxide, when the created pressure is released from the device, carbon dioxide forms the bubble and thus foamed material is formed. All the materials used in this method are mixed under pressure and then passed through an extruder. As with the above-mentioned method, when the pressure is released, foam is formed [17].

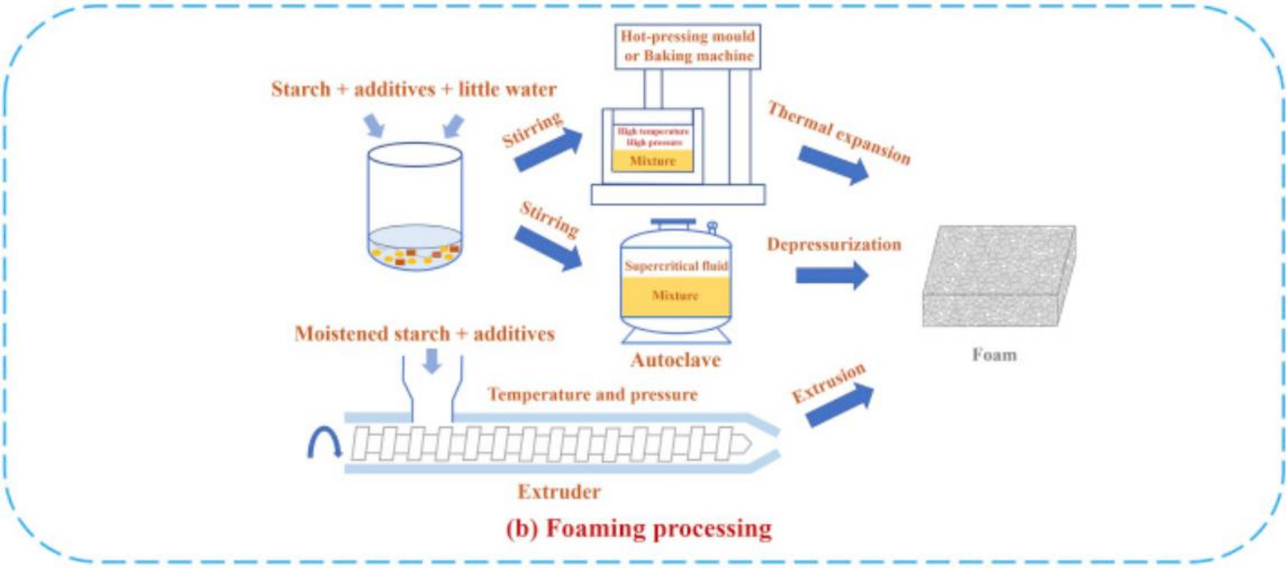


Fig. 6. Foaming method [17]

– Extrusion process.
The extrusion process consists of three types: using presses, blowing, and injection molding (see Fig. 7). The first raw materials, i.e. starch and additives, must be placed in a screw extruder, after which the resulting mass is crushed and prepared for use as needed. This process is dry because it did not requires water, this process uses dry and solid materials that have melting properties. The prepared pellets are poured into a screw extruder, which is heated while the material is pushed through the screw and melted. The molten mass is pushed through the head at the end of the extruder and the extrudate is processed to form a film, depending on the process. Blowing method, extrudate is blown, pressed, and then drying takes place. Heat pressing method involves hot presses that form an extrudate and cool it to form a film. Using this inject molding process, the materials are melted and then injected into the mold, where they are dried, until the product is obtained. This method is more attractive in industry, because when extracting the film in such ways, large quantities of the can be obtained in a short period of time. However, during the extraction of such starch, the degree of crystallinity decreases, which is directly related to the mechanical properties of the material [17, 18].

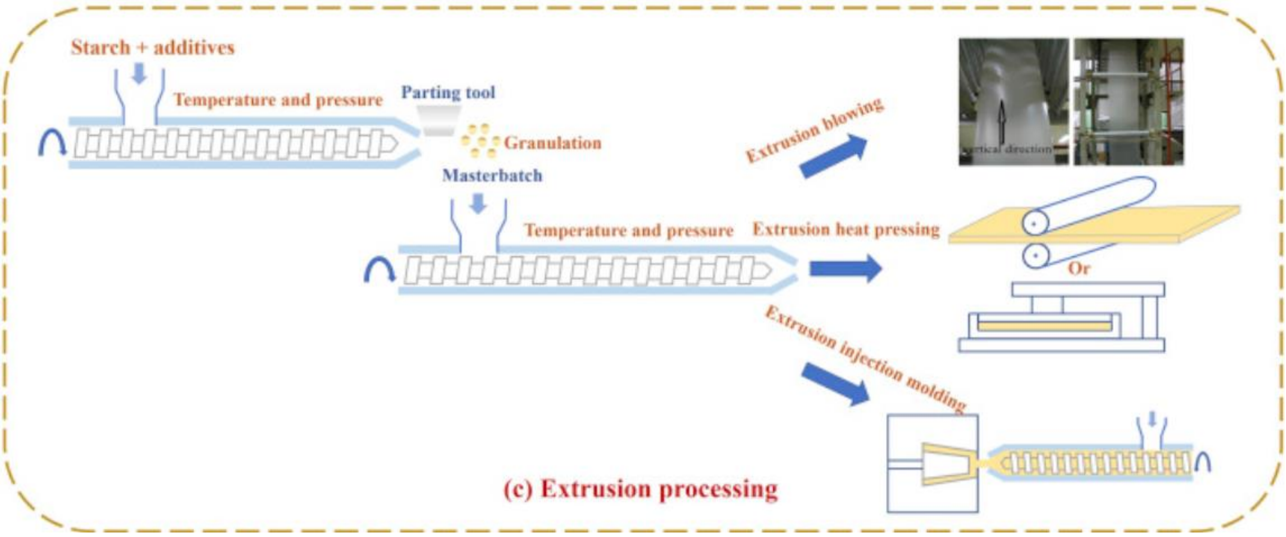


Fig. 7. Extrusion process [17]

– 3D printing.

3D technology is the pouring of material layer by layer on top of each other (see Fig. 8). 3D is a popular technology that is widely used in industry to produce complex designs or one-off parts, and is also widely used in the automotive, architectural, and aerospace industries. 3D printing can be used to print using starch-based materials. Studies have shown that wheat starch is the most suitable starch for this, as it has better extrudability and other properties compared to other starches. Since starch-based materials have good mechanical properties, gel like texture and high heat tolerance, they can be used as a filler in 3D printing. Since conventional plastic is now used for 3D printing, for environmental reasons it could be replaced by these biodegradable materials with a few changes and additives in the mixture [17].

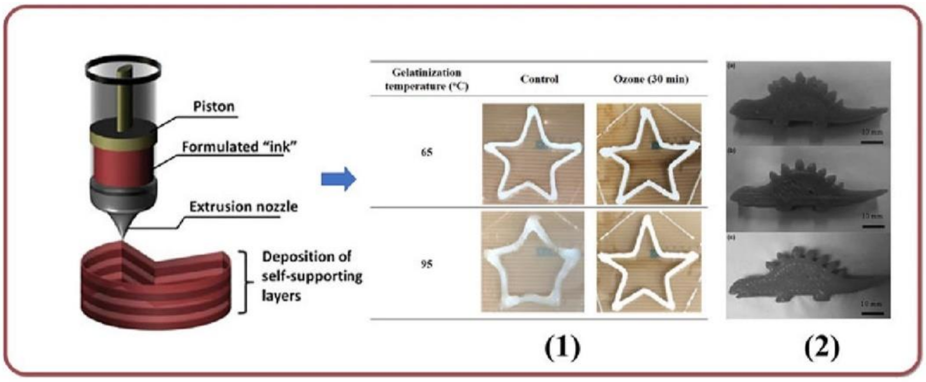


Fig. 8. 3D printing process [17]

1.4. Chapter Summary

Fruits and vegetables are essential to human nutrition, providing vitamins, minerals, and dietary fiber. However, the short expiration date of these fresh food can lead to food spoilage and lead to more generated waste. In 2015, the United Nations adopted "17 Sustainable Development Goals" to reduce food waste and contribute to responsible consumption and production. Packaging plays an important role in protecting products during transportation and ensuring their safety. Plastic is the most popular packaging material, mainly used in packaging, construction, automotive, electronics, household, agricultural and medical, mechanical and furniture industries. Starch-based bioplastics are one of the most talked about and researched alternatives that can replace non-naturally based plastics. Starch is a natural polysaccharide composed of amylose and amylopectin, the proportions of which vary depending on the source. Films made from starch can be used in various industries, but they are very stiff and brittle. The inclusion of plasticizers such as sorbitol, glycerol and fructose can improve the elongation, tensile strength, and young's module of the material. Essential oils can be used as additives to films made from starch to provide antimicrobial, antibacterial, and antifungal properties. Film solution casting is one of the simple methods that is used to prepare the film, but have some limitations, due to the need of control of thickness and evenness. Extrusion process is popular and relatively easy process and most suitable for starch-based film production.

2. Starch-Based Packaging: Material and Additives

Additives are added to packaging materials to improve their mechanical and various other properties. Different additives have different effects on the quality of the packaging material. Since there is an effort to replace conventional plastics with bioplastics, the latter do not always have the necessary properties to be a substitute for conventional plastics. Consequently, much interest has recently been focused on alternative materials that can be combined with bioplastics to achieve the best overall product.

2.1. Essentials Oils (EO)

Essential oils are aromatic liquids that are obtained from plants by steam distillation method. Essential oils are used in the cosmetics, perfume, pharmaceuticals, and food industries. These aromatic liquids have been known and used for a long time, the ancient Egyptians also used them in the perfumery, medical industry, and used them to prepare the bodies of the dead for embalming [19].

Different essential oils have different effects. Many works have been studied and described that the relevant essential oils have antifungal, antimicrobial, and antibacterial properties. In this work [20], the antifungal activity of cinnamon, clove and oregano essential oils was investigated. The film itself, into which the EOs were incorporated, was prepared from cassava starch and gelatine in a ratio of 1:1. The glycerol was used as plasticizer, in a ratio of 1:0.25 to the polymer. Using this basis, 3 more different films were prepared with cinnamon, clove, and oregano essential oils, in a ratio of 1:0,25 with the polymer. In vitro tests were chosen to investigate the antifungal efficacy of these three different essential oils. *Fusarium Oxysporum* and *Colletotrichum Gloeosporiodes* fungi were grown without films as control samples, which showed that within 24h the growing fungi covered 2/3 of the entire surface, and after 72h they covered the entire surface of the plate. The same plates with the same fungi were prepared but were covered with prepared films with different essential oils. The results showed that the effect of cinnamon essential oil was more pronounced against *Fusarium Oxysporum* fungus. Also, clove and cinnamon essential oils have shown efficacy against the fungus *Colletotrichum Gloeosporiodes*. Oregano essential oil showed the lowest efficacy against both fungi (see Table 3) [20].

Table 3. Effectiveness of different essential oils against fungi [20]

Essentian Oil	***Fusarium Oxysporum* at 48h (mm ± SD)**	***Fusarium Oxysporum at 72h (mm* ± SD*)***	***Colletotrichum Gloeosporiodes at 48h (mm* ± SD*)***	***Colletotrichum Gloeosporiodes at 72h (mm* ± SD*)***
Cinnamon EO	57 ± 4	48 ± 2	49 ± 3	38 ± 3
Clove EO	49 ± 2	46 ± 2	52 ± 2	45 ± 2
Oregano EO	42 ± 3	36 ± 2	41 ± 7	30 ± 7

Tea tree oil is reported to have anti-inflammatory, antibacterial and antiviral properties. *Staphylococcus aureus,* gram-positive bacterium is commonly found in products such as meat and meat products, eggs, potatoes, milk, and milk products. It can multiply in these products when their storage or preparation conditions are inappropriate, i.e. food is stored at an insufficiently low temperature or prepared at an insufficiently high temperature to provide suitable conditions for reproduction [21]. *Escherichia coli* is a gram-negative bacterium that can also cause foodborne disease. This bacterium can infect the human body through contaminated food, such as dairy products, raw meat products, as well as fruits and vegetables, such as spinach or lettuce. Also, if food is stored or not processed at the right temperatures, it can cause a greater risk of this bacteria multiplying [22]. Tea tree essential oil is obtained by steam distillation of *M. Alternifolia* leaves and branches [23]. In this work [24], the antimicrobial effect of tea tree essential oil against *Staphylococcus aureus* and *Escherichia coli* bacteria was investigated. To prepare the films, 50 ml of distilled water was prepared, and potato starch was dissolved in it, and the mixture of furcellaran and gelatin was prepared separately. After the starch was completely dissolved in water, the prepared furcellaran and gelatin mixture was poured into its solution and stirred for 15 minutes. After these 15 minutes, tea tree oil was added to the film solution. 4 different films were prepared with corresponding amounts of EO – 0%, 2%, 4% and 6%. These prepared mixtures were poured into 80 mm petri dishes and were put in the oven at 50 °C and dried in it for 48 hours. When the amount of TEO was 0%, no changes in the inhibitor area were observed. This essential oil showed positive effects against both bacteria at the lowest and highest levels in the film. The film with 6% TEO showed the greatest change in the inhibitor zone against *S. Aureus* bacteria, and with 4% it showed the best results against *E. Coli* bacteria (see Table 4) [24].

Table 4. Antimicrobial effect of Tea Tree Oil against selected bacteria [24]

Tea Tree Oil concentration (%)	**E. Coli (inhibitor zone mm ± SD)**	**S. Aureus (inhibitor zone mm ± SD)**
0	Not detected	Not detected
2	4.8 ± 0.78	9.3 ± 0.46
4	23.8 ± 1.04	10.6 ± 0.53
6	18.4 ± 0.2	27.8 ± 0.29

Lavender essential oil is obtained by steam distillation of these plants. Lavender essential oil is utilized in the cosmetics industry due to inflammation reducing properties, capability to relieve skin pain, and can be used in aromatherapy due to its pleasant smell. Reported to have antimicrobial, anti-inflammatory and antioxidant properties. Antimicrobial properties could be seen due to the composition of this EO, it is mostly composed of linalool and linalyl acetate, which show a positive effect against gram-positive bacteria [25]. This work investigated the effect of lavender essential oil against E. Coli and S. Aureus bacteria. The film was prepared by the method described above. 4 different films were prepared with corresponding amounts of Levander EO – 0%, 2%, 4% and 6%. Antimicrobial properties were determined using the agar disk diffusion test. This essential oil showed better properties against *S. Aures* bacteria but was sufficiently effective against *E. Coli*. The highest concentrations showed the highest efficacy in the inhibitor zone against both bacteria (see Table 5) [25].

Table 5. Antimicrobial effect of Lavender Essential Oil against selected bacteria [25]

Tea Tree Oil concentration (%)	**E. Coli (inhibitor zone mm ± SD)**	**S. Aureus (inhibitor zone mm ± SD)**
0	Not detected	Not detected
2	2.1 ± 0.1	8.9 ± 0.9
4	10.5 ± 0.5	10.4 ± 0.87
6	15.1 ± 0.65	24.5 ± 0.5

Like many other essential oils, eucalyptus essential oil has antibiotic, antioxidant, and anti-inflammatory properties [26]. This essential oil is used in the composition of personal hygiene products, cosmetics, cosmetics, and hair products. The smell of this EO oil is associated with cleanliness, so it can be used at home as a freshener. In this work, the antibiotic effect of Eucalyptus Globus EO against *E. Coli* and *S. Aureus* bacteria was investigated. Films were procced from thermoplastic starch and carrot nano fibers, which were mixed with water for 30 minutes, after this time glycerol and acetic acid were added and mixed until the mixture was fully gelatinized. The produced mass was dried in petri dishes for 2 days at room temperature. This method was used to prepare 5 different films with different amounts of Eucalyptus globulus EO – 0%, 2%, 3% and 4%. The obtained results show that against both bacteria EO showed positive results, Eucalyptus globus was more effective against *E. Coli* bacteria and the inhibitor zone was larger compared to *S. Aureus* inhibitor zone. The increasing amount of Eucalyptus globus in the film corresponded to an increasing inhibitor zone, which shows a positive effect against both bacteria (see Table 6) [27].

Table 6. Antimicrobial effect of Eucalyptus globus Essential Oil against selected bacteria [27]

Eucalyptus globus leaf concentration (%)	***E. Coli* (inhibitor zone mm ± SD)**	***S. Aureus* (inhibitor zone mm ± SD)**
0	0	0
1	49 ± 3.92	4 ± 1.68
2	61 ± 8.23	17 ± 2.79
3	104 ± 4.24	31 ± 2.85
4	139 ± 7.66	47 ± 2.97

2.2. Plasticizers

Plasticizer is a material that is used as an additive and gives flexibility to materials, increasing their plasticity, elasticity and processability. Many types and materials can be used as plasticizers. They can be classified according to their molecular weight – monomeric and polymeric plasticizers. Monomeric plasticizer is defined as a unique single structure such as citric acid, mono-alcohols, or monoesters. Polymeric plasticizer is defined as repeatable structures, such as polyesters. They can be classified by the mass that they as being used: primary and secondary plasticizers. Primary plasticizer means that this plasticizer is the main additive in a system, or the quantity is the biggest. Secondary plasticizer means that this plasticizer is found in the least amount in the total mass, and it was added to improve one or other properties [28].

Due to the chemical composition of starch, films made from this polysaccharide are characterized by brittleness and rigidity. Films made only from starch do not meet the standards for packaging materials, therefore plasticizers are added to their mixtures during the production of films. There are several widely studied plasticizers – glycerol and sorbitol. Although they perform the same function as materials, the results obtained are slightly different.

In this work [29] the effect of sorbitol and glycerol on the film made from corn starch was investigated. These films were prepared by preparing 100 g of water, dissolving prepared 10 g of corn starch in it, and heating this mixture at 85 °C for 20 minutes. After full gelatinization of the starch, three different plasticizers in three different amounts were added to the mixture (see Table 7).

Table 7. Different plasticizers used [29]

Name	Plasticizer	Concentration (%)
Control	-	-
S30%	Sorbitol	30
S45%	Sorbitol	45
S60%	Sorbitol	60
G30%	Glycerol	30
G45%	Glycerol	45
G60%	Glycerol	60
SG30%	Sorbitol/Glycerol	30
SG45%	Sorbitol/Glycerol	45
SG60%	Sorbitol/Glycerol	60

The control film which is made only of starch, without any plasticizer, tensile strenght was about 7 MPa, young's modulus about 60 MPa and fracture strain value about 100% of all these values shows that the material is not easily stretchable and that it is stiff. Comparing the results obtained for S30% and G30% specimens shows a huge difference. The S30% sample became stronger, but more resistant to stretching, it withstood approximately 14 MPa tensile strength, young's modulus was very similar to the control sample – 60 MPa, only the elongation at break was lower compared to the control sample – about 58%. Meanwhile, the G30% specimen withstood a lower tensile strength – approximately 5 MPa and showed a lower elongation at break result – approximately 43%, just like the young module obtained a lower value – approximately 20 MPa. As the amount of sorbitol in the film increased, the fracture strain value increased and the tensile strength decreased and the Youngs modulus value decreased, which indicates that the material became more and more plastic. Using glycerol as a plasticizer, the fracture strain value also increased and the tensile strength showed similar values. Using both glycerol and sorbitol gave the best results, as the samples had the highest elongation or break values compared to other glycerol and sorbitol samples. Hence, glycerol and sorbitol mix can be used to obtain the most plastic film. But depending on the desired results, both sorbitol and glyrecol can be used [29].

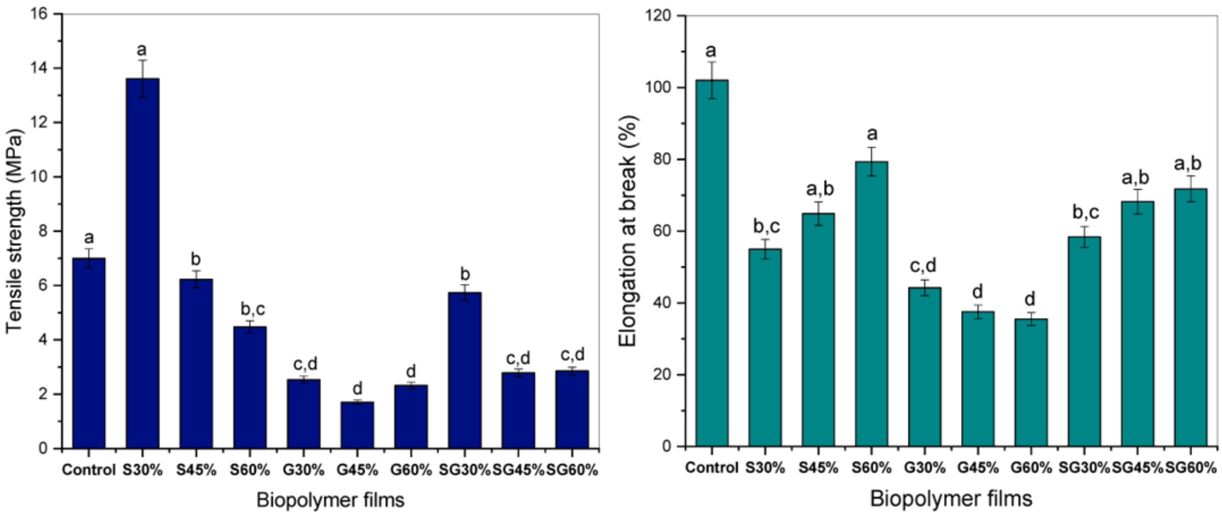


Fig. 9. Tensile strength and elongation at break values of specimens [29]

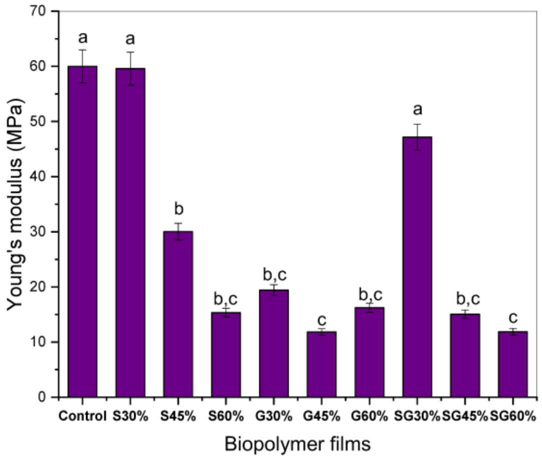


Fig. 10. Young's modulus value of specimens [29]

2.3. Natural Pigments

Due to increasing food waste, there is currently an interest in interactive packaging that can visually show the freshness of food. There are existing sensors that can monitor food from the time it is made until the customer buys the product [30]. Providing such information as food freshness and visualizing it not only gives the user a sense of security but can also reduce the amount of product waste.

Anthocyan is a natural pigment that is found in fruits and vegetables and can change color depending on the pH. Most of this natural pigment is found in red, blue, and purple fruits and vegetables (see Table 8.). Anthocyan extracted from different sources can show different color intensities due to differences in chemical composition and structures. Climate, temperature, light, season in area in which food was grown and harvested has an impact on this antioxidant content [31].

Table 8. Antyhocyan amount in different kind of food [31]

Name of the food	**Amount (mg) per 100 g**
Mulberries	1.4-704
Black chokeberries	46-558
Black elderberries	25-305
Sweet cherries	7-143
Blackberries	10-139
Lingonberries	4-49
Strawberries	4-48
Sour cherries	3-44
Red raspberries	5-38
Black grapes	3-39
Plums	5-34
Blueberries	11-26
Black beans	1-15
Red currants	2-11

Name of the food	Amount (mg) per 100 g
Red wine	4-10
Red onions	7

In this work [32], a natural pigment was isolated from the plant *Etlingera elatior*. The plant sample was crushed and freeze-dried for 2 days. The frozen samples were then ground to a powder. 5 g of this powder was used for extraction with 100 ml aqueous ethanol solution for 1 h under constant stirring. The resulting extract is filtered, and the filtrate is collected and stored in dark containers.

Films were prepared from distilled water, sago starch and glycerol, the mixture at 85 degrees was stirred for 45 minutes. After cooling the mixture to a temperature of 40 degrees, the obtained pigments were added and 4 films with different amounts of pigment – 10% (TGE1), 20% (TGE2) and 30% (TGE3) were prepared. One film was prepared as a control, without pigment (TGE0) (see Fig. 11.). 90g of the resulting solution was poured onto a plate made of glass and dried at 40 degrees for seven hours [32].

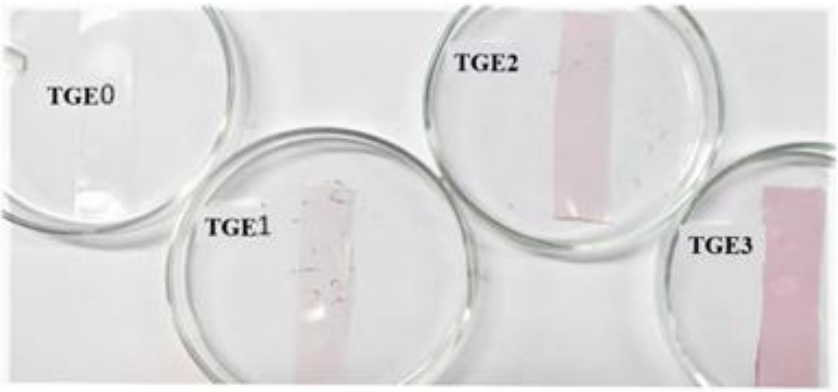


Fig. 11. Prepared films with different amount of pigment [32]

The pH change of the TGE3 film was prolonged with varied pH solutions – 4, 7 and 9. When the pH value was 7, the film did not change color at all, when the pH value was 4, the medium was acidic, the color changed to a pinkish color, and when the pH value was 9 and the medium was base film changed color to slightly bluish (see Fig. 12.). Hence, this shows that natural pigments respond to pH changes and can be adapted to monitor pH changes in food [32].

Fig. 12. Color changes depending on the pH [32]

2.4. Chapter Summary

The need to replace conventional plastics is more relevant than ever. Since bioplastics do not always have the required properties, additives can be added to them to improve their properties. It can be essential oils, plasticizers or natural pigments. Essential oils are aromatic liquids that are obtained from plants by the steam distillation method. There are many studies that have investigated and proven that essential oils have antibacterial, antifungal, antimicrobial, antibacterial properties. Their embedding in the film matrix can reduce microbial growth. Cinnamon, clove and oregano essential oils showed positive activity against *Fusarium Oxysporum* and *Colletotrichum Gloeosporiodes* fungi. Tea tree oil is reported to have anti-inflammatory, antibacterial and antiviral properties. This essential oil has shown positive effects against *E. Coli* and *S. Aureus* bacteria. Lavender essential oil is obtained by steam distillation of the lavender plant. Lavender essential oil is used in the cosmetics and perfumery industry, due to its pleasant smell. This essential oil was also tested against *E. Coli* and *S. Aureus* bacteria and thus showed a positive effect. Eucalyptus globulus EO, like many other EOs, has anti-inflammatory, antimicrobial, analgesic and antioxidant properties. When tested for its antibacterial activity, this essential oil also showed positive effects against *E. Coli* and *S. Aureus* bacteria, but against *E. Coli* bacteria, this essential oil was slightly more effective. Incorporation of plasticizers into starch-based film gives it plasticity, elasticity and makes it less brittle. The most widely and widely used plasticizers are glycerol and sorbitol. Both materials give stiff material plasticity, but at the same time the elongation at break, young's modulus and tensile strength properties change. The increasing amount of sorbitol in the matrix showed a decrease in tensile strength, but the value of elongation at break increased and the value of Young's modulus also decreased, which means that the material became more plastic compared to the material without plasticizer. Different amounts of glycerol showed similar values at tensile strength, but the value of elongation at break increased and the value of young's modulus remained similar with different amounts. Natural pigments such as anthocyanin extracted from fruits and vegetables can react to changing pH changes. Anthocyan pigment is extracted from the *Etlingera elatior* plant and changes its color as the pH of the medium changes.

3. Starch Based Film: Materials, Methods, Preparation

In the experimental part, starch films were made with different selected essential oils and the properties of these produced films were extended. A control film without any essential oils is a reference point against which other films containing different essential oils can be compared. This experiment went through several different stages:

- Preparation of films made from starch;
- Film thickness measurements;
- Film tension;
- Evaluation of film structure using a microscope;
- Evaluation of films color and lightness using a colorimeter;
- Effects of different essential oils as film additives on fresh food.

3.1. Materials Used

This work used potato starch, distilled water, glycerin and 4 different essential oils: tea tree, oregano, clove, and rosemary.

- Potato Starch
  Potato starch was purchased at a local store in Lithuania. Potato starch is a white coarse powder that has no smell or taste. Can be used as a thickener, filler or as a stabilizer or absorbent. In this work, it was used as the main material from which the material was formed. *Aloja* potato starch properties presented in Table 9 and nutrition composition presented in Table 10.

Table 9. Used potato starch properties [33]

Parameter	**Value**
Color	White
Smell and taste	None
Moisture content, %	15-20
pH in solution	6

Table 10. Nutrition values of potato starch

Nutrition Value, g	**per 100g**
Fat	0
Carbohydrates	80
Sugar	0
Protein	0.1
Salt	0

- Distilled water
  Distilled water was purchased at a local store in Lithuania. Distilled water is obtained by boiling water to its boiling temperature until it becomes steam, which is collected in another container during condensation. Distilled water is used when it is necessary not to contaminate the materials with unnecessary impurities that may harm the results.

– Glycerin

Glycerin was purchased at a local pharmacy in Lithuania. Glycerin is used as a plasticizer in this experiment. Since films made only from starch are very brittle and inflexible, they need additional additives to improve their properties. Glycerin is a widely used material in industries: medical industry, as a wetting agent, it is included in the composition of some medicines such as cough syrup and is also used in the formulation of medicinal tablets. In the food industry, it is used as a solvent, sweetener, and as a thickener in alcoholic beverages. In the cosmetics industry, it is used in products such as soap, hair gel, creams, shampoos, as it has moisture-retaining, attracting and softening properties. It is a viscous odorless and sweet tasting organic liquid. The property of glycerin is presented in Table 11.

Table 11. Glycerin properties [34]

Parameter	**Value**
Color	Clear
Taste	Sweet
Density, 20°C, g/cm^3	1.53
Boiling temperature, °C	167

– Essentials oils

All 4 essentials oils were purchased from “Kvapų namai” store in Lithuania. As analyzed above, essential oils can have a positive effect when added as film additives. These essential oils have antimicrobial, antifungal, anti-inflammatory and antioxidant properties. 4 different essential oils were used in this work: clove (*bot. Syzygium aromaticum*), oregano (*bot. Origanum vulgare L.*), rosemary (*bot. Rosmarinus officinalis L.*) and tea tree (*bot. Melaleuca alternifolia)*. Composition of these essential oils is presented in Table 12, 13, 14 and 15.

Table 12. Chemical composition of tea tree essential oil [35]

Chemical Component	**Amount, %**
Terpinen-4-ol	39.39
Gamma-Terpinene	19.38
Alpha-Terpinene	11.41
Eucalyptol	4.45
Terpinolene	3.74

Table 13. Chemical composition of clove essential oil [36]

Chemical Component	**Amount, %**
Eugenol	68.18
Eugenyl acetate	19.43
Caryophyllene	8.72

Table 14. Chemical composition of rosemary essential oil [37]

Chemical Component	**Amount, %**
Camphor	16.70
Bornyl acetate	13.23

Chemical Component	Amount, %
Alpha-Pinene	12.77
Eucalyptol	12.04

Table 15. Chemical composition of oregano essential oil [38]

Chemical Component	Amount, %
Carvacrol	64.34
Cymene	8.46
Gamma-Terpinene	6.42
Myrcene	2.23

3.2. Methods of Analysis

- Visual evaluation
  The visual evaluation is intended to inspect the obtained materials, to determine visible damage and color. Visual inspection of the films was performed when they were dry.
- Film thickness
  To evaluate thickness of films a Mitutoyo C112EXB micrometer (accuracy ± 0.001) was used. The micrometer consists of two main parts – the lower plane and the upper sliding pin. To measure film thickness, the upper pin is lifted up and the film is placed on the lower plane, the lower pin is lowered to touch the film and the value is recorded. For data accuracy, 10 different locations were measured.

Fig. 13. Mitutoyo micrometer

- Mechanical properties evaluation under tension
  A tensile test was performed to evaluate the stress and strain of the produced films. 4 samples of each film were prepared, their length was 150 mm, and the width was 15 mm. The Tinius Olsen H25KT tensile machine (see Fig. 13) was used for this test. The distance between the grippers was 100 mm and a constant stretching speed of 20 mm/min was set. The specimens were placed between two grippers, which moved in different axes at the start of the test and

stretched the specimen. QMAT software was used to capture the received data on a computer, which is then transferred and processed in Microsoft Excel.

Strain (Ɛ, %) was calculated using formula 1:

$$\varepsilon = \frac{\Delta L}{L_0} \quad (1)$$

ΔL – length difference, mm; L_0 – original length of the sample, mm.

Stress (σ, MPa) was calculated using formula 2:

$$\sigma = \frac{F}{S} \quad (2)$$

F – force, N; S – area, mm^2.

Fig. 14. Tinus Olsen tensile machine

– Film structure

A Keyence VHX-7000 microscope was used to evaluate the structure (see Figure 15). The formation of air bubbles in the film and the effect of essential oils on the film were evaluated. It is possible to observe with a microscope how different essential oils and their different amounts can affect the films.

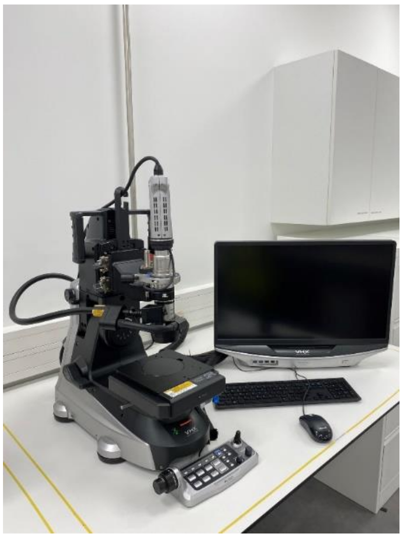


Fig. 15. Keyence microscope

– Colorimeter

A colorimeter is an optical device that determines the color coordinates of a material in the CIELAB color space. The CIELAB color space is also called L*a*b, the L* value shows the lightness of the material, a* and b* indicate which color quadrant the material is in. Quarters consist of 4 primary colors: red, green, blue, and yellow. A BYK Spectro-Guide 45/0 colorimeter was used for this test (accuracy ± 0.3) (see Fig. 16).

Fig. 16. Colorimeter used [39]

– Food freshness evaluation

To evaluate the effects of essential oils on food freshness, fresh strawberries purchased from a local fruit/vegetable store were wrapped in manufactured films with different essential oils. The samples were kept in a refrigerator at +5 °C and removed only for weighing and visual inspection. Wrapped strawberries were weighed daily to assess weight loss and visually observed for any change in appearance. The wrapped strawberries were weighed with a laboratory scale Mattler Toledo XS204 (accuracy ± 0.0001 g)

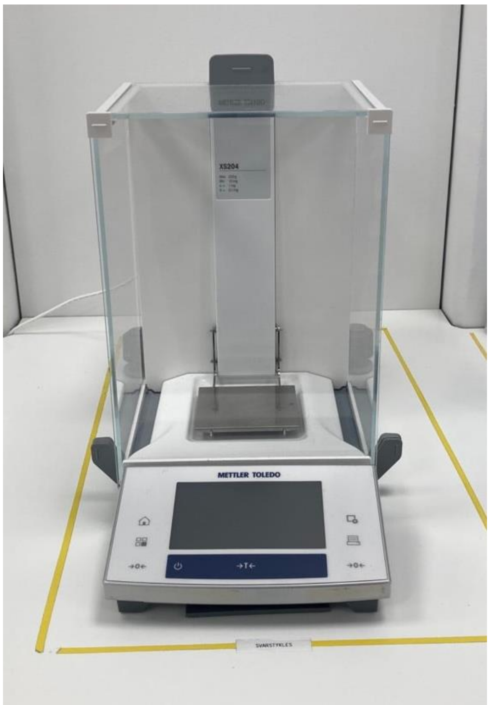


Fig. 17. Used analytical balances

3.3. Starch Based Film Preparation

13 films were made from the components mentioned above. Film 1 was made without essential oils in order to compare the results obtained with films containing essential oils. All the films were made with the same amounts of potato starch, glycerin, and distilled water, only the essential oils and their amount differed. Films were produced based on this methodology [40] with a few modifications. The composition of the films is shown in the Table 16:

Table 16. Films composition

Additive / Film no.	Distilled water, ml	Glycerin, g	Potato starch, g	Essential Oil, ml
1	150	6	6	-
2	150	6	6	Tea Tree EO 0.3
3	150	6	6	Tea Tree EO 0.6
4	150	6	6	Tea Tree EO 0.9
5	150	6	6	Oregano EO 0.3
6	150	6	6	Oregano EO 0.6
7	150	6	6	Oregano EO 0.9
8	150	6	6	Rosemary EO 0.3
9	150	6	6	Rosemary EO 0.6
10	150	6	6	Rosemary EO 0.9
11	150	6	6	Clove EO 0.3
12	150	6	6	Clove EO 0.6
13	150	6	6	Clove EO 0.9

Starch does not dissolve in cold water, so in order for full gelatinization to occur, the solution must be heated to at least 60 °C. To get the films, the following steps were taken:

- Distilled water, starch, and glycerin were added to the beaker and stirred at room temperature until the starch was evenly distributed;
- With constant stirring, the temperature of the mixture was heated to a temperature of 40 ± 2 °C and stirred for 10 minutes until a homogeneous mixture was reached;

– With constant stirring, the temperature was raised to a temperature of 60 ± 2 °C, so that full gelatinization of the starch took place, and it was stirred for 10 minutes while maintaining this temperature;
– The resulting viscous mass was cooled to a temperature of 50 ± 2 °C and the appropriate amount of essential oil was added and stirred for 1 minute.
– The resulting viscous mass is poured onto a plate and left to dry at room temperature for 3 days;
– When the films were dry, they were carefully peeled off the plate.

3.4. Results Obtained from the Experiment

3.4.1. Visual Evaluation

After three days, the films were dry, but some places were a little damp. All films were successfully formed, and no deviations were observed. All the films dried and became smooth, the edges of the films curled a little while drying, but this had no effect on the experiment. The dried films had a distinct odor that reflected the corresponding essential oil. Some essential oils gave a slight color to the film. Visual evaluation is described in the Table 17:

Table 17. Films visuals evaluation

Film no.	Description
1. 	The resulting film is transparent, has a slight blur, soft, smooth with visible air bubbles, odorless, since there are no essential oils.
2. 	The resulting film is transparent, has a very slight bluish tint, which is given by Tea Tree Essential Oil, flexible, soft, smooth with visible air bubbles, has a mild, eucalyptus-like smell, due to the added tea tree essential oil.

Film no.	Description
3. 	The resulting film is flexible, smooth, soft, air bubbles are visible, it has a brighter blue color and a brighter smell, due to the higher amount of added essential oil.
4. 	The resulting film is flexible, smooth, soft, air bubbles are visible, the smell is the strongest and most pronounced compared to film 2 and 3, due to the larger amount of essential oil added, the color is also a little lighter compared to film 1.
5. 	The obtained film has a smooth surface, air bubbles are visible, the film is flexible, soft with a weak oregano smell.
6. 	The resulting film had a wetter area that did not dry in 3 days, there are more air bubbles, but the film is smooth, soft, and flexible. It has a stronger oregano smell and has a slightly browner hue due to the higher amount of oregano essential oil added.

Film no.	Description
7. 	The resulting film is completely dry, without any wetter areas, and a small amount of small air bubbles are visible. Film is soft, flexible and has a distinct smell of oregano essential oil and a stronger brown shade.
8. 	The film is completely dry and does not have any wet areas. The film is soft, flexible, smooth with a small amount of air bubbles. The film is transparent with a slight blur and has a slight rosemary scent due to the addition of a small amount of essential oil, there was no color change.
9. 	The film is completely dry and does not have any wet areas. The film is soft, flexible, smooth with a small amount of air bubbles. The film is transparent with a small amount of blur, has a stronger smell of rosemary, due to the addition of a larger amount of essential oil, no color change was observed.
10. 	The film had a small area of residual moisture, but it had no effect. The film is transparent, with a noticeable blur, has a strong smell of rosemary, because the maximum amount of essential oil has been added. The film is soft, flexible, and smooth and there is no color change.

Film no.	Description
11. 	The film is transparent, with a very small amount of air bubbles, is smooth, flexible, with a faint smell of cloves, because it had the smallest amount of this essential oil. The film dried completely and did not have any remaining moisture and no color change was noticed.
12. 	The film is transparent with noticeable blur, flexible, soft, and smooth. A stronger smell of the film was observed, due to the fact that a larger amount of clove essential oil was added, minimal air bubbles, and no color change was observed.
13. 	The film is transparent with noticeable blur, flexible, soft, and smooth. The film had a strong odor due to the highest added amount of clove essential oil, but no color change was observed.

3.4.2. Thickness Measurements Results

The resulting film thickness is variable between different samples and between thicknesses within the same sample. The thickness variation within the same specimen may be due to the casting method of the films, as they were cast from a container onto the surface and spread with a spatula and this does not ensure uniform distribution over the entire area. From the obtained data, it was observed that the control (film 1) is the thinnest, this may be due to the fact that no essential oil was added to it, and all other samples have a greater thickness compared to the control. The average thickness of the first films was 0.177 mm, but a greater variation was observed from 0.136 to 0.218 mm. From the obtained data, it was observed that the films with the highest amount of essential oils and were characterized by an increased thickness. Films 2-4 contain tea tree essential oil and their average thickness increased from 0.212 to 0.321 mm. Film 5 to 7 contains oregano essential oils and their average width increased from 0.221 to 0.272 mm. Film 8 to 10 contains rosemary essential oil and their average width increased from 0.182 to 0.246 mm. Film from 11-13 contains clove essential oil and their average width increased from 0.211 to 0.270 mm (see Fig. 18).

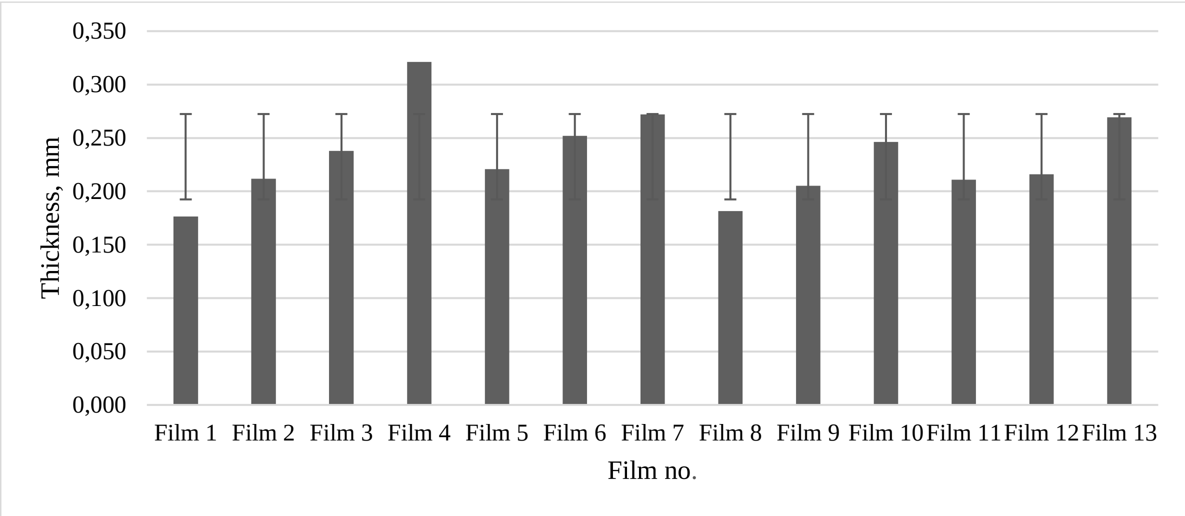


Fig. 18. Thickness measurements results

The thickness of the film affects its mechanical properties, a thicker film can withstand more force, but a thicker film may be less flexible and less adaptable. The film should spread and dry to a uniform width across the entire width, but this requires a different method of casting the films to ensure uniformity.

3.4.3. Film Tension Results

The film no. 1 produced without any essential oil, its maximum yield point was about 0.17 MPa, and the minimum yield point about 0.12 MPa. Such a difference in data may be due to the varying thickness of the film (see Fig. 19). When all the specimens were stretched, it was observed that the specimens tear very easily, and material is considered brittle.

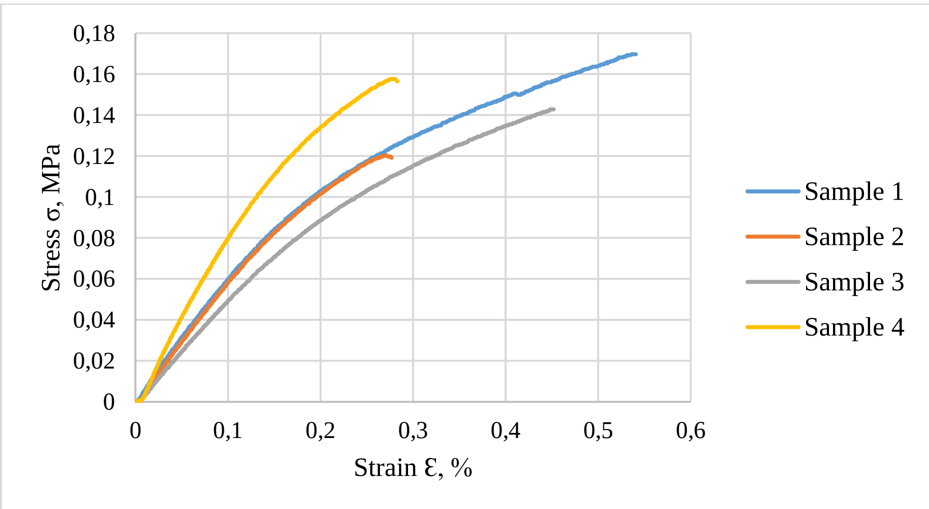


Fig. 19. Film no.1 stress-strain curve

Films from 2-4 contain essential oil in their matrix. From the obtained data, it can be observed that as the amount of tea tree essential oil in the film increases, the stress and strain decrease (see Fig. 20, 21, 22). Film no. 1 with the smallest amount of EO records the maximum yield point of 0.09 MPa, and film no. 3 with the highest amount of EO records the maximum yield point of about 0,05 MPa.

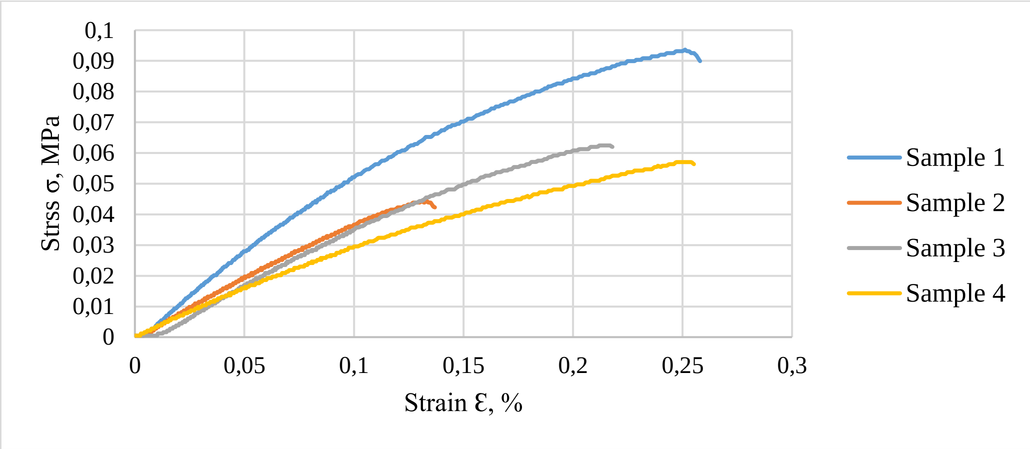


Fig. 20. Film no. 2 stress-strain curve

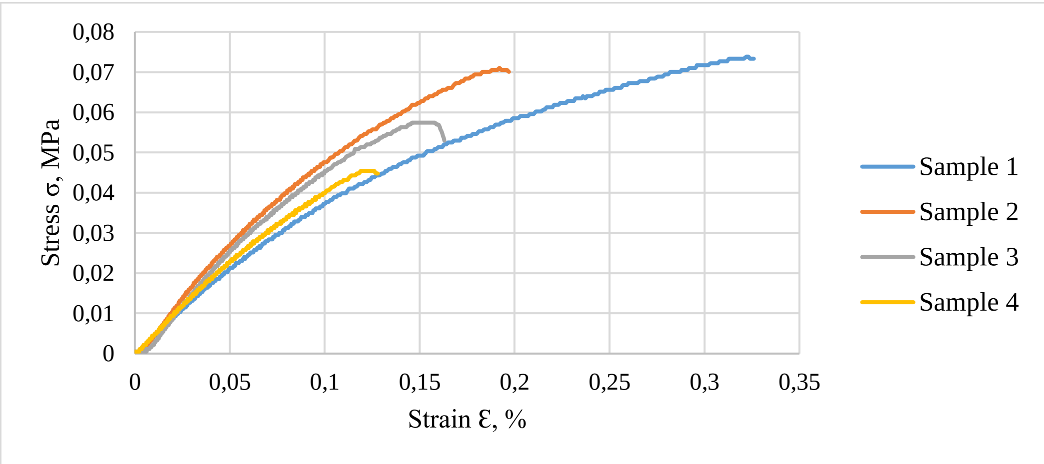


Fig. 21. Film no. 3 stress-strain curve

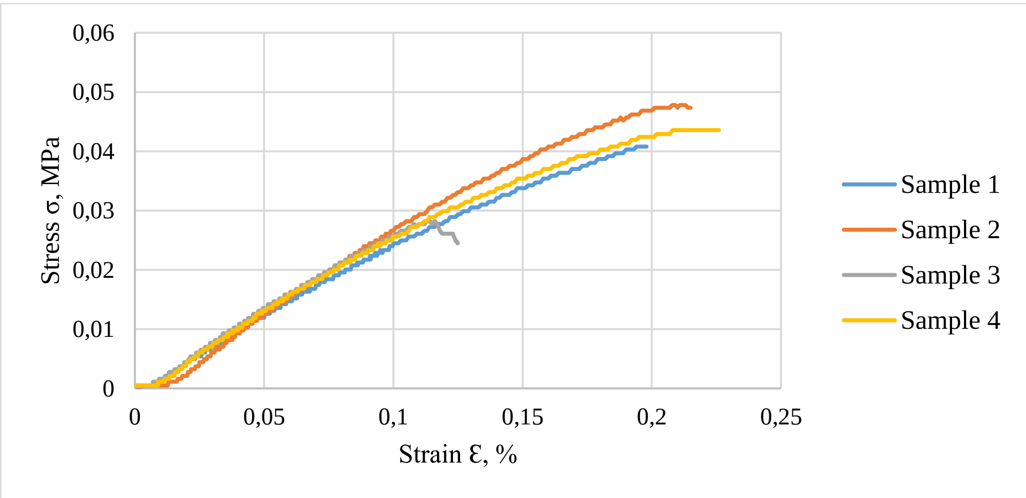


Fig. 22. Film no. 4 stress-strain curve

Films 5-7 were made with oregano essential oil and with correspondingly increasing amounts. As the amount of essential oil in the film increased, the mechanical properties also deteriorated, and the maximum yield point decreased. Maximum yield point decreased from 0.08 MPa (film no. 5) to 0.05 MPa (film no. 7) (see Fig. 23, 24, 25)

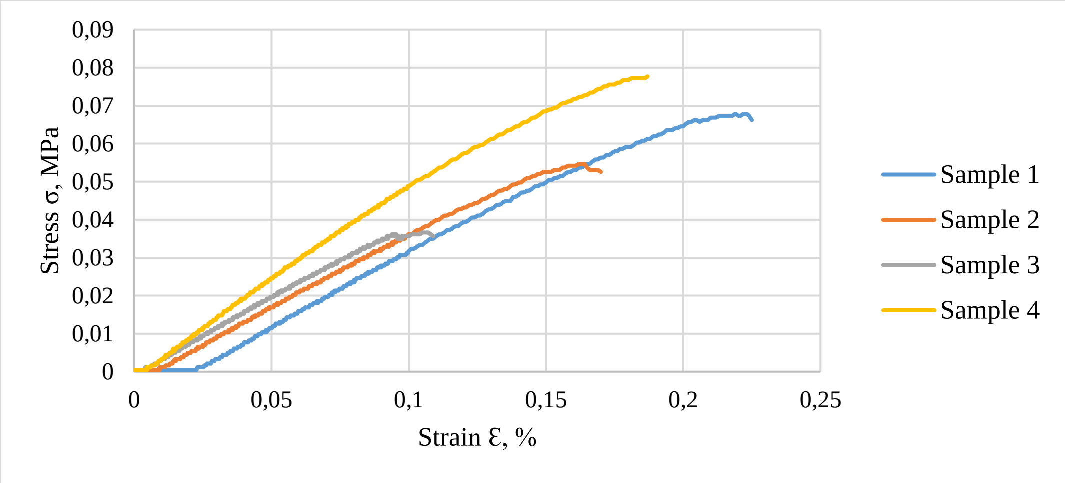


Fig. 23. Film no. 5 stress-strain curve

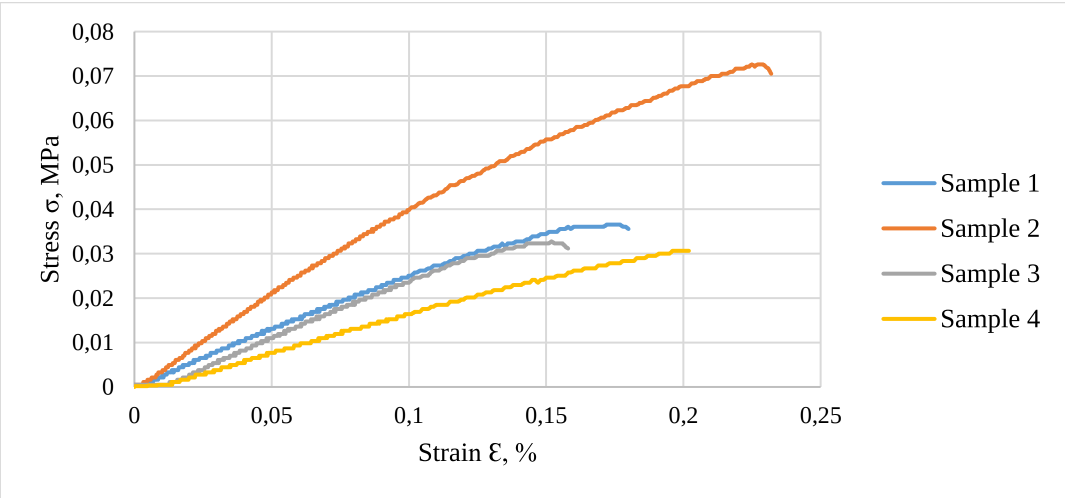


Fig. 24. Film no. 6 stress-strain curve

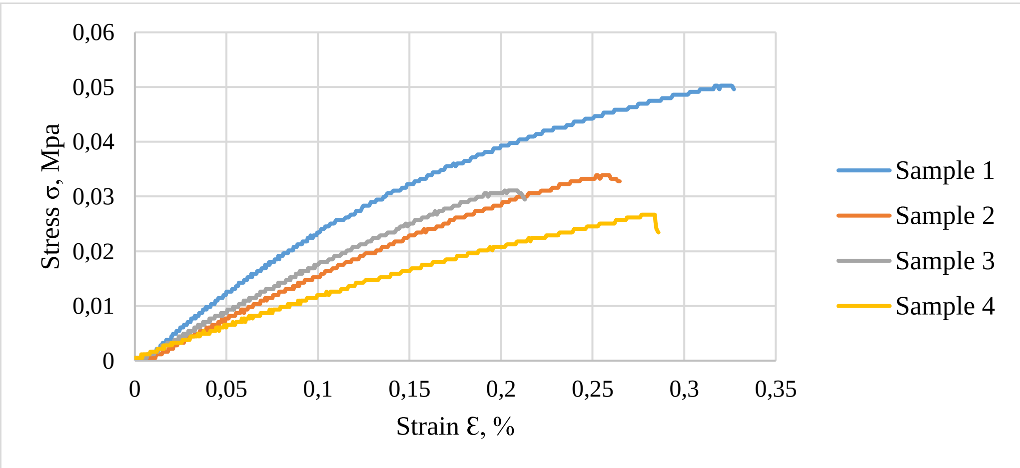


Fig. 25. Film no. 7 stress-strain curve

Films 8-10 were made with rosemary essential oil with correspondingly increasing amounts of essential oil. Although the amount of essential oil in the films increased, the data obtained were quite similar. Maximum yield point values ranged from 0.04 MPa to 0.11 MPa (see Fig. 26, 27, 28).

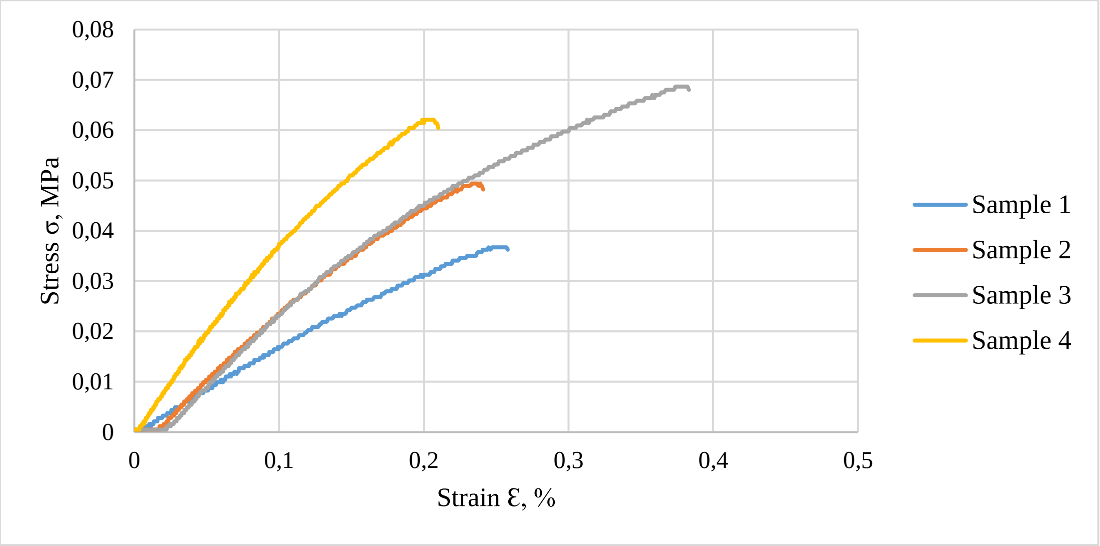


Fig. 26. Film no. 8 stress-strain curve

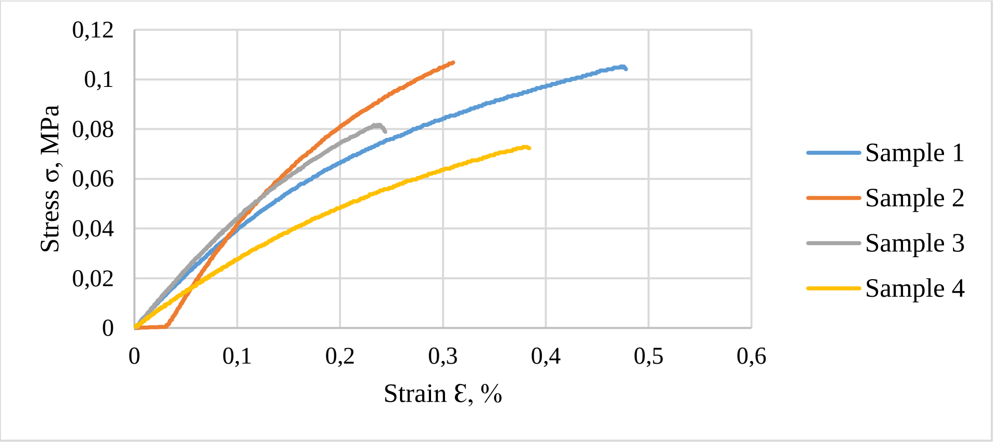


Fig. 27. Film no. 9 stress-strain curve

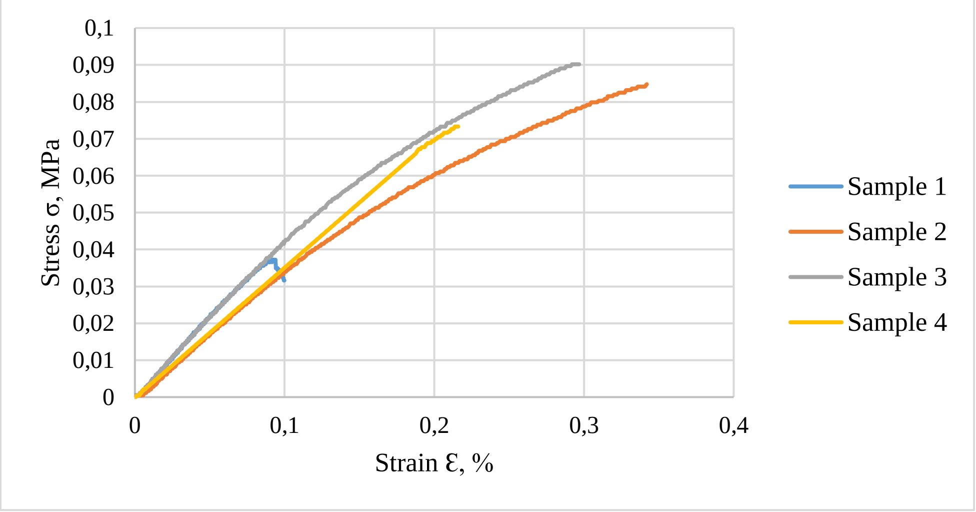


Fig. 28. Film no. 10 stress-strain curve

Films 11-13 were made with clove essential oil and increasing amounts accordingly. The increasing amount of essential oil in the film minimally affected the mechanical properties and the maximum yield point varied from 0.03 to 0.07 MPa (see Fig. 29, 30, 31).

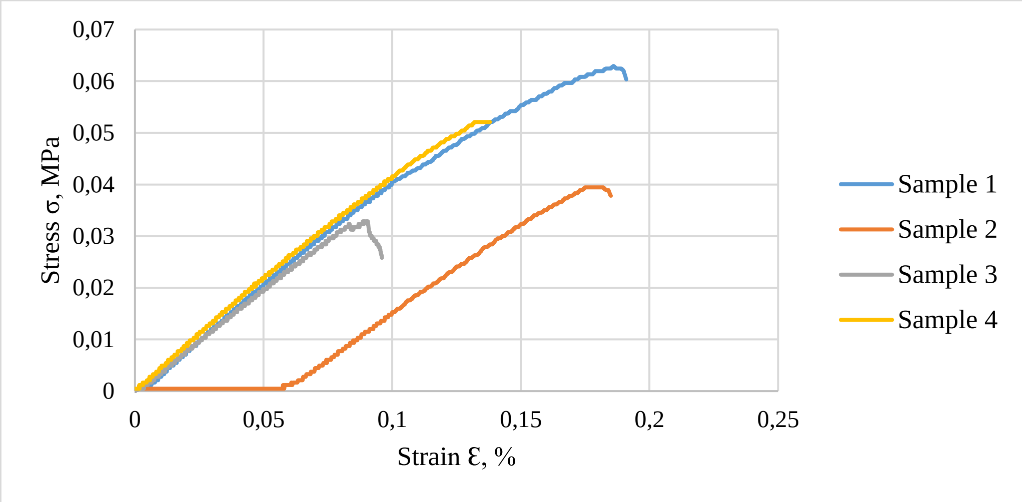


Fig. 29. Film no. 11 stress-strain curve

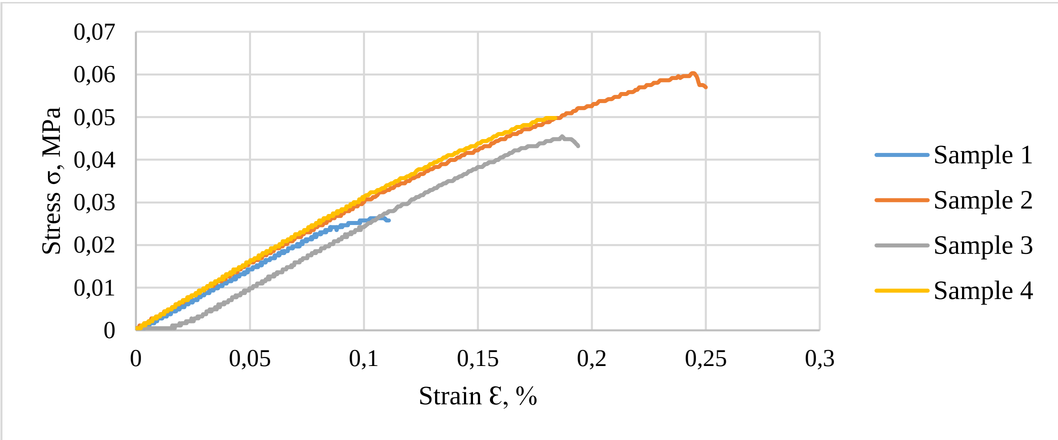


Fig. 30. Film no. 12 stress-strain curve

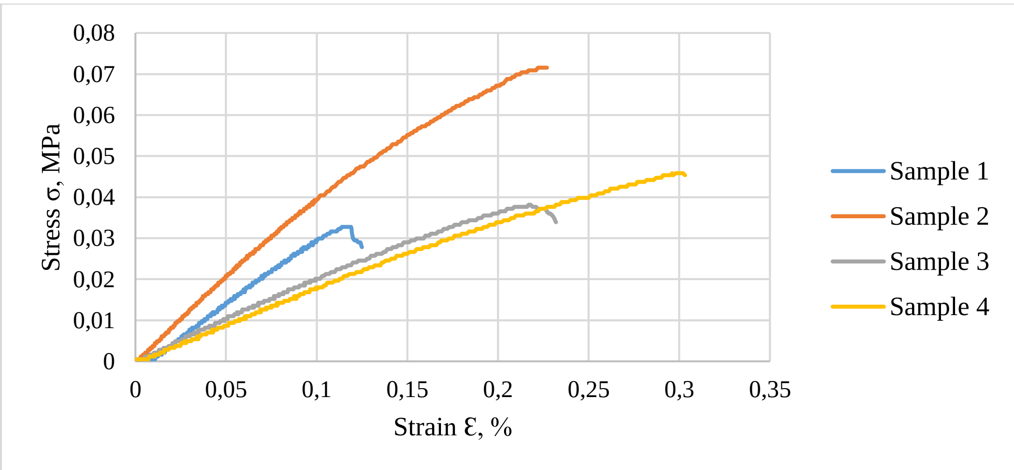


Fig. 31. Film no. 13 stress-strain curve

3.4.4. Film Structure Results

The structure of the film was analyzed with a microscope with direct light. Film no. 1 produced without any essential oil had minimal structure, which is normal for films made from starch and with additives such as plasticizer (see Fig. 32 film no. 1). In all the photos below, you can see the formation of air bubbles, which could have appeared in the structure due to the constant mixing of the mixture, as well as during the pouring of the mixture onto the tray (see Fig. 32, 33). Air bubbles in the structure can be the weakest point and the most easily damaged during packaging or testing. In all the photos below, it can be seen that as the amount of essential oil increases, the structure of the film becomes more and more swollen, and the texture becomes more pronounced than without essential oil. Fig. 33 film no. 13 it is very clear to see the dark spots that are caused by the added essential oil, and it is not distributed over the entire area but distributed on the surface.

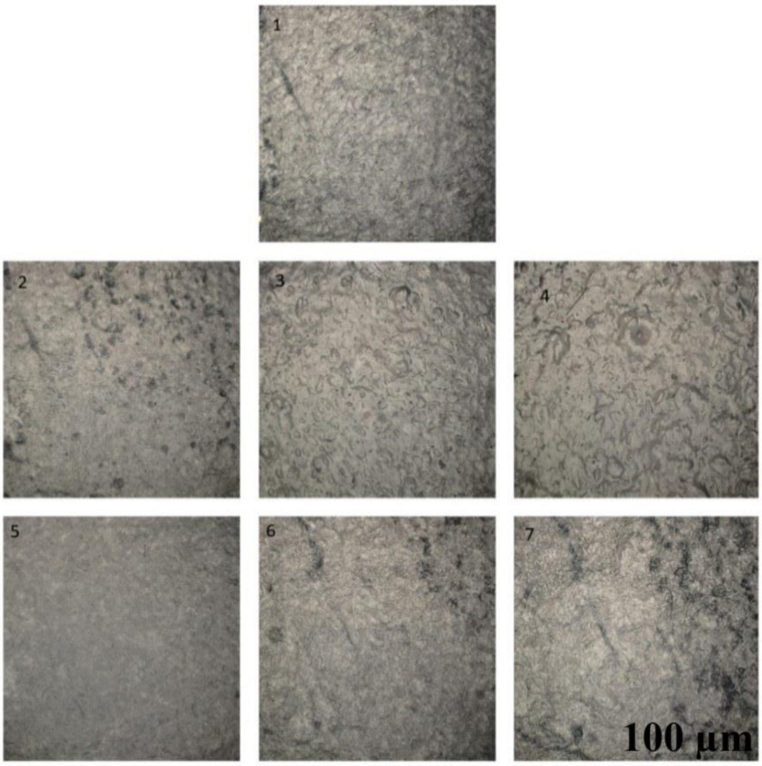


Fig. 32. Film no. 1-7 structure

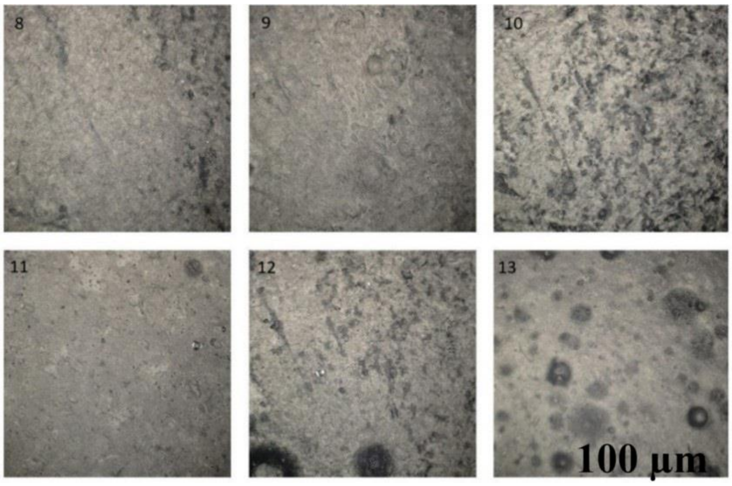


Fig. 33. Film no. 8-13 structure

3.4.5. Colorimeter Results

All tested films were tested by placing them on a white sheet of paper, so measurements were made with a colorimeter and a plain white sheet of paper 5 times at 5 different locations. CIELAB color space is a color model that can accurately define the color coordinates of the material in three-dimensional coordinate system. These coordinates are expressed through 3 main axes – L*, a* and b*:

- L* measure the brightness of the material. A value of 100 means that the material is white (light) and 0 means that the material is black (dark).
- a* can have positive and negative values. A value of -a* means that the material tends to the green color, and a value of +a* means that the material tends to the red color.
- b* can have positive and negative values. A value of -b* means that the material leans towards the blue color, and a value of +b* means that the material leans towards the yellow color [41].

From the obtained data in the Table 18, it can be observed that after measuring the film no. 1 and comparing the data with a sheet of white paper that the lightness of the material has decreased, and this means that the material is darker than the sheet of paper and its color is in the quarter between red and blue.

Table 18. Colorimeter results on white sheet paper and film no. 1

	White paper sheet ± SD	**Film 1 ± SD**
L*	92.482 ± 0.037	89.216 ± 0.107
a*	1.024 ± 0.015	0.724 ± 0.022
b*	2.52 ± 0.020	-0.77 ± 0.052

Films 2-4 are made with tea tree essential oil and also the L* value has decreased which means that the color has become slightly darker than films without any essential oil. When looking at films 2-4 with the naked eye, a bluish tint can be observed, and this is reflected by the data recorded by the colorimeter, that the value of b* is negative. The film that contained the most of this essential oil was characterized by an intense bluish shade (see Table 19).

Table 19. Colorimeter results on film no. 2-4

	Film 2 ± SD	**Film 3 ± SD**	**Film 4 ± SD**
L*	88.942 ± 0.060	88.648 ± 0.157	88.276 ± 0.593
a*	0.708 ± 0.012	0.742 ± .,041	0.682 ± 0.033
b*	-0.73 ± 0.050	-0.68 ± 0.093	-0.668 ± 0.188

Films 5-7 were made with oregano essential oil. Comparing these films with each other, it can be observed that film no. 5, L* value was lower than the other two. Such a difference in brightness can also be seen in the microscope photos above. Film no. 5 also recorded a negative value of b*, and the film no. 6 and no. 7 recorded a positive value of b*. This difference could be due to the added and increasing amount of essential oil and with the addition of this essential amount the color of these films changed, and the change became more pronounced as the amount increased (see Table 20).

Table 20. Colorimeter results on film no. 5-7

	Film 5 ± SD	Film 6 ± SD	Film 7 ± SD
L*	81.772 ± 0.072	88.704 ± 0.353	88.644 ± 0.288
a*	21.466 ± 0.016	0.682 ± 0.010	0.676 ± 0.033
b*	-10.886 ± 0.105	0.372 ± 0.136	0.426 ± 0.126

Film no. 8-10 was made with rosemary essential oil. Comparing the films with each other, it can be observed that as the amount of this essential oil increased, the values of a* and b* also increased. The L* value also varied with the amount of essential oil and the darkest film was film no. 10, as it contained the most essential oil, and this is reflected in the microscope images above (see Table 21).

Table 21. Colorimeter results on film no. 8-10

	Film 8 ± SD	Film 9 ± SD	Film 10 ± SD
L*	88.778 ± 0.277	90.056 ± 0.233	80.98 ± 0.161
a*	0.708 ± 0.019	1.054 ± 0.059	20.674 ± 0.041
b*	-0.664 ± 0.115	-2.202 ± 0.088	-10.608 ± 0.132

Films no. 11-13 was made with clove essential oil. Although the amount of this essential oil in the films increased, the L* value of all samples remained quite similar, but with a tendency to decrease in value. A more pronounced change was recorded in the value of b*, when the values changed from negative to positive, which means that the color of the film changed from bluish to yellowish as the amount of this essential oil increased (see Table 22).

Table 22. Colorimeter results on film no. 11-13

	Film 11 ± SD	Film 12 ± SD	Film 13 ± SD
L*	88.832 ± 0,323	88.846 ± 0.275	88.404 ± 0.472
a*	0.644 ± 0,026	0.638 ± 0.037	0.608 ± 0.045
b*	-0.308 ± 0,145	-0.194 ± 0.178	0.066 ± 0.276

3.4.6. Essential Oils Impact on Fresh Food Evaluation

Strawberries wrapped in film with the respective essential oils and their amount were weighed for 14 consecutive days to evaluate the change in weight and their appearance to determine the effect of the essential oils on fresh food. From the data obtained, it is possible to see which essential oils gave a positive effect and which essential oils did not showed a positive effect. Strawberries are susceptible to losing their weight over time, due to their area of skin, so moisture is easily transferred to the environment, and this leads to changes in their appearance and a noticeable change in weight.

The control film lost 17.99 g of weight in 14 days and averaged 1.38 g of weight loss per day. Comparing the films with each other, it can be seen that the samples that were wrapped in films made of tea tree oil 0.3, oregano 0.9, rosemary 0.6 and clove 0.3 had the greatest change in weight. From the obtained data, it can be observed that some essential oils reduced the weight change of the tested strawberries (see Table 23).

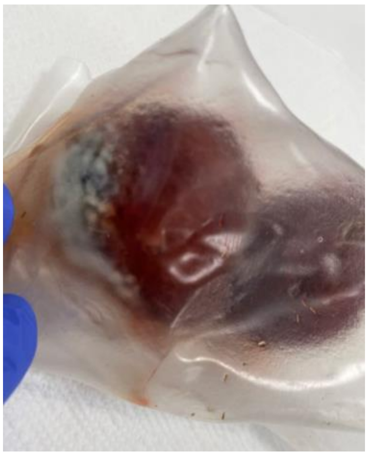
Table 23. Obtained weight results of specimens

Film no.	EO, ml	Mass day 1, g	Mass day 14, g	Mass lost, g	Average mass loss, g	Maximum mass lost per day, g	Minimum mass lost per day, g
1	Control	55.01	37.02	17.99	1.38	3.38	0.01
2	Tea Tree 0,3	68.65	50.19	18.46	1.42	2.41	0.57
3	Tea Tree 0,6	63.53	45.93	17.60	1.35	2.31	0.60
4	Tea Tree 0,9	53.61	36.95	16.66	1.28	2.08	0.61
5	Oregano 0,3	60.50	41.65	18.85	1.45	3.44	0.08
6	Oregano 0,6	54.59	41.84	12.75	0.98	1.47	0.22
7	Oregano 0,9	71.63	52.13	19.50	1.50	2.35	0.52
8	Rosemary 0,3	61.83	50.17	11.66	0.90	1.77	0.19
9	Rosemary 0,6	55.34	42.05	13.29	1.02	1.47	0.3
10	Rosemary 0,9	59.42	46.17	13.25	1.02	1.46	0.17
11	Clove 0,3	94.27	66.61	27.66	2.13	4.33	0.43
12	Clove 0,6	80.97	61.84	19.13	1.47	2.69	0.58
13	Clove 0,9	68.67	50.44	18.23	1.40	3.03	0.47

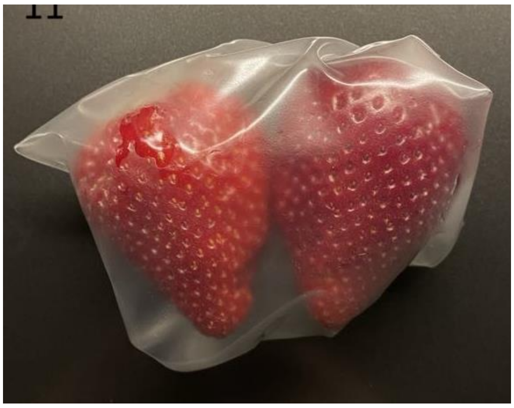
Appearance is the first criterion that the consumer evaluates and evaluates whether it is worth buying fresh products. Visual inspection is a good way to check and identify possible defects. Strawberries wrapped in manufactured films, kept in a controlled environment for 14 days. Photos of day 1 and day 14 are presented in Table 24. More photos are presented in Appendix 1.

These fruits are sensitive to various spoilage processes and this spoilage process is expressed by a change in their appearance such as shrinkage, appearance of mold, change of color. After the initial observation, it is possible to evaluate how different essential oils effectively delayed the decay processes of these fruits. On the surface of the control sample, a color change appeared in one place on the 4th day and the rotting process accelerated until the 14th day. Tea tree 0.9 and clove 0.3 samples showed initial discoloration on day 4 and the rotting process accelerated to day 14, which may have been due to undetected damage before the strawberries were picked (see Appendix 1). Samples wrapped in films made with oregano and rosemary essential oils did not show any color change that would indicate the beginning of the decay process. Strawberries wrapped in tea tree oil films 0.3 and 0.6 developed mold on day 9, while strawberries wrapped in clove films 0.6 and 0.9 changed boldly in appearance as they lost a lot of weight, indicating that a lot of water was lost, and they became soft. All strawberries became darker in color within 14 days. Also, the samples that did not show signs of mold and rot lost weight, which means that the strawberries lost their water, and the loss of water means that they shrunk and became less firm.

Table 24. Visual results on day 1 and on day 14

Days / EO	Day 1	Day 14
Control		
Tea Tree 0.3		
Tea Tree 0.6		
Tea Tree 0.9		

Days / EO	Day 1	Day 14
Oregano 0.3		
Oregano 0.6		
Oregano 0.9		
Rosemary 0.3		

Days / EO	Day 1	Day 14
Rosemary 0.6		
Rosemary 0.9		
Clove 0.3		
Clove 0.6		

EO \ Days	Day 1	Day 14
Clove 0.9		

Wrapping strawberries in films made with respective essential oils and their different amounts showed a positive effect and prevented them from decaying for a longer time compared to the control sample. Strawberries are a very perishable food product and are susceptible to fungi. The white mold seen on strawberries is caused by the fungus *Botrytis Cinerea* and they quickly begin to rot. Because essential oils have an anti-fungal effect, they are being studied for their effects on preserving and extending the expiration date of fresh food.

3.5. Chapter Summary

In this experiment, films were made from starch and essential oils such as tea tree, oregano, clove, and rosemary were used as additives and the effect of essential oils on fresh food was investigated. Starch was used as the base material to make the films together with distilled water to dissolve the starch, glycerin as a plasticizer and essential oils as additives. Essential oils have antimicrobial, antifungal and anti-inflammatory properties. These produced films were analyzed using visual evaluation, their thickness was measured, tensile tests were performed, structure was evaluated using a microscope, and color change was evaluated using a colorimeter. The effects of the essential oils were studied by wrapping selected strawberries in manufactured films and storing them in a controlled environment for 14 days and weighing them daily and performing a visual inspection to assess changes in color or texture. Starch-based films made with essential oils of oregano and rosemary showed the best results, as the samples changed the least, and no mold formation was recorded. Films made with essential oils had a greater thickness compared to the control film, which was made without essential oil, and as the amount of essential oil in the film increased, its thickness also increased. The addition of essential oils also degraded the mechanical properties of the films, as the tensile strength and/or elongation decreased with increasing essential oil content.

4. Evaluation of Economic Value of Starch-Based Film with Essential Oils as Additives

It is very important to evaluate all aspects that affect the profit. Every change in production plans, production processes, transportation, price can positively affect the company's profit. The financial growth of the company allows money to be invested in innovations and improvements that will also have a positive effect on the company. Improving processes, introducing innovations can attract not only buyers, but also investors who can invest even more money in the company.

4.1. Price of Raw Materials

In order to assess the finances and how much you need to invest; you need to know how much each raw material costs to produce the final product. The price may vary due to the supplier, supplier country, raw material price etc. The prices of the raw materials from which the films were made in this work are presented in Table 25:

Table 25. Price of raw materials used in this work

Material	Price	Price in bunk
Potato starch	0,85 Eur/400 g	53 Eur/25 kg
Glycerin	4,19 Eur/60 ml	173 300 Eur/25 kg
Distilled water	1,59 Eur/5 L	318 Eur/1000 L
Tea Tree Essential Oil	5,80 Eur/5 ml	4 408 Eur/3.8 L
Oregano Essential Oil	7,00 Eur/5 ml	5 320 Eur/3.8 L
Rosemary Essential Oil	6,50 Eur/5 ml	4 940 Eur/3.8 L
Clove Essential Oil	6,70 Eur/5 ml	5 092 Eur/3.8 L

However, the cost of producing such a film from such materials in industry can vary significantly. Because these quantities are suitable for laboratory experiments and not for mass production. Bulk production involves supplying raw materials in large quantities to produce large quantities of material. Supplying raw materials in bulk is usually cheaper than buying in small quantities. The prices of raw materials suitable for industrial production in larger quantities that are already in the market are presented in Table 26:

Table 26. Prices of raw materials supplied in bulk

Material	Price
Potato starch	10,25 Eur/25 kg [42]
Glycerin	661 Eur/259 kg [43]
Distilled water	320 Eur/1000 L [44]
Tea Tree Essential Oil	149 Eur/3.8 L [45]
Oregano Essential Oil	418 Eur/3.8 L [46]
Rosemary Essential Oil	186 Eur/3.8 L [47]
Clove Essential Oil	144 Eur/3.8 L [48]

When evaluating prices, if you buy goods not in large quantities, but in small quantities, there is a big difference in terms of money. If you recalculate the prices of laboratory quantities to the prices of raw materials, which are already supplied in large quantities, a huge difference is noticeable. The price differences are presented in Table 27:

Table 27. Recalculated prices

Material	Recalculated prices of used raw material prices	Prices of raw materials already in the market
Potato starch	53 Eur/25 kg	10,25 Eur/25 kg
Glycerin	173 300 Eur/25 kg	661 Eur/259 kg
Distilled water	318 Eur/1000 L	320 Eur/1000 L
Tea Tree Essential Oil	4 408 Eur/3.8 L	149 Eur/3.8 L
Oregano Essential Oil	5 320 Eur/3.8 L	418 Eur/3.8 L
Rosemary Essential Oil	4 940 Eur/3.8 L	186 Eur/3.8 L
Clove Essential Oil	5 092 Eur/3.8 L	144 Eur/3.8 L

4.2. Evaluation Of Food Waste Reduction

Every day, hundreds of kilograms of food are thrown away in stores due to expired or spoiled food. Fruits and vegetables are one of the most perishable foods in our stores, this is because fruits/vegetables are sensitive to temperature, pH changes, environmental humidity and bacteria and fungi can quickly grow in them with favorable conditions.

Thrown food means huge losses for stores, but the environmental aspect is also affected. Food waste means that resources, such as water, raw materials, energy and human labor, transportation costs, were improperly used. Every year, $940 billion worth of food is wasted that could be saved and used more efficiently. The Sustainable Development Goals have set a target until 2030, which, if achieved, would reduce food waste per person by half, but to achieve this, it is necessary to actively change the world and offer environmentally friendly proposals [49].

Improving food packaging can help keep food fresher for longer. In the experimental part, during the study, it was observed that adding oregano and rosemary essential oils to the film increased the shelf life of strawberries compared to other tested oils and the control film. Based on the data obtained from the experiment, on the 4th day, the control sample showed the first color changes and the beginning of rotting, we can say that the shelf life of strawberries is 4 days, while they are still suitable for consumption. If these fruits are not consumed/purchased within this period, they will be discarded. The amount of waste produced is indicated in Table 28:

Table 28. Waste generated by period

Period	Amount, kg
Per day	30
Per week	210
Per month	930
Per year	10 950

The average price of strawberries for 250 g is 4 euros. Thus, it is possible to calculate how many financial losses are experienced during the corresponding period of time (see Table 29).

$$\frac{4\ Eur}{X} = \frac{250\ g}{1000\ g} \quad (3)$$

$$X = 16\ Eur\ for\ 1\ kg$$

$$Per\ period = \frac{Price\ per\ kg\ \times waste\ per\ period}{1\ kg} \quad (4)$$

$$Per\ day = \frac{16\ eur/kg \times 30\ kg}{1\ kg} = 480\ Eur \quad (5)$$

$$Per\ week = \frac{16\ eur/kg \times 210\ kg}{1\ kg} = 3\ 360\ Eur \quad (6)$$

$$Per\ month = \frac{16\ eur/kg \times 930\ kg}{1\ kg} = 14\ 880\ Eur \quad (7)$$

$$Per\ year = \frac{16\ eur/kg \times 10\ 950\ kg}{1\ kg} = 175\ 200\ Eur \quad (8)$$

Table 29. Financial loss per period

Period	**Sum, Eur**
Per day	480
Per week	3 360
Per month	14 880
Per year	175 200

Using modified film, the shelf life of strawberries can be extended up to 14 days, which is 3.5 times longer compared to the control sample. Extending the shelf life of strawberries can save money because the fruit will not be thrown away as often the amount of waste will be reduced by 3.5 times. Based on the experimental data, it was calculated how much waste would be generated if the shelf life of strawberries were longer.

Table 30. Reduced waste generated by period

Period	**Amount, kg**
Per day	8.6
Per week	60.2
Per month	266.6
Per year	3 139

It was calculated how much financial loss would be incurred using modified films using Equation 4. The data are presented in the Table 31.

Table 31. Recalculated financial loss

Period	Sum, Eur
Per day	137.6
Per week	963.2
Per month	4 265.6
Per year	50 224

Having the data, it is possible to calculate how much money can be saved by reducing the amount of waste. The data are presented in Table 32.

Table 32. Financial loss differences

Period	Financial loss when shelf life 4 days, Eur	Financial loss when shelf life 14 days, Eur	Difference, Eur
Per day	480	137.6	342.4
Per week	3 360	963.2	2396.8
Per month	14 880	4 265.6	10 614.4
Per year	175 200	50 224	124 976

4.3. Chapter Summary

Assessing all aspects of the company that affect profits is important. Breakthroughs in production, processes, and transportation costs can have a positive financial impact. All updates and improvements are a good attraction for buyers and investors. When producing starch-based film and using such raw materials as starch, essential oils, glycerin and distilled water, prices may differ when buying in small quantities or supplying large quantities immediately, and the price also depends on the supplier, the supplier's country, and quality. Buying materials in bulk can save you a lot of money. Food waste is also an important topic. The European Union and its sustainability goals aim to reduce food waste per person by half. By using films with essential oils, the shelf life of strawberries can be extended 3.5 times, thus saving 124 thousand per year.

Conclusions

1. The films were made with different essential oils: tea tree, oregano, rosemary and clove and in different amounts: 0.3 ml, 0.6 ml and 0.9 ml. Films made with different oils varied in color. Tea tree husks had a bluish hue, while oregano husks had a yellowish hue. The film made from rosemary oil showed no difference with the naked eye, but according to the colorimeter, it was in the color quarter between red and blue. Films made with clove also changed color to yellowish. The thickness of the films with the highest amount of essential oil increased: tea tree average thickness increased from 0.212 to 0.321 mm, film with oregano essential oils average thickness increased from 0.221 to 0.272 mm, films with rosemary essential oil average thickness increased from 0.182 to 0.246 mm., films with clove essential oil and their average thickness increased from 0.211 to 0.270 mm.
2. After tensile tests, it was observed that essential oils worsen the mechanical properties of the films. The maximum force withstood by the control film was 2.6 N, and the minimum was 1.7 N, the elongation value varied from 27 to 54 mm. As the amount of tea tree essential oil in the film increased, the tensile strength decreased to 0.75 N, while the extension remained similar between 13 and 26 N, and one specimen had the highest extension value of 32 mm. As the content of oregano essential oil in the films increased, the maximum force resistance value was 1.2 and decreased to 0.79N, and the elongation value was very similar between 11 and 27 mm, and one specimen had the extension value of 33 mm. Films that contained rosemary essential oil recorded the highest sustained force of 1.6 N and the lowest of 0.6 N, and the elongation value of the samples was similar from 11 mm to 23 mm and the value of extension was similar for all samples from 20 mm to 39 mm, however, one had the value of extension – 9 mm and another 49 mm. Films made from clove essential oils exhibited tensile strength ranging from 1.1 N to 0.4 N, while elongation remained similar from 11 mm to 30 mm.
3. Starch-based films with essential oils can reduce the mass loss of strawberries and extend their shelf life and maintain their appearance throughout the entire storage period. Strawberries that were wrapped in films made with oregano and rosemary essential oils showed the least changes and mold did not form on the surface of the strawberries. Strawberries wrapped in films made with rosemary essential oil lost the least mass of all samples. This shows that these essential oils showed a positive effect on the freshness of strawberries. However, strawberry wrapped films made with tea tree essential oil and clove essential oil did not show a positive effect on the freshness of strawberries, as they formed mold on the samples or lost a lot of their mass.
4. By applying films made with essential oils, the shelf life of strawberries can be extended from 4 days to 14 days, thus reducing food waste from 30 kg per day to 8.6 kg per day and saving from 480 euros per day to 137.6 euros per day and to save 124 thousand per year.

List of References

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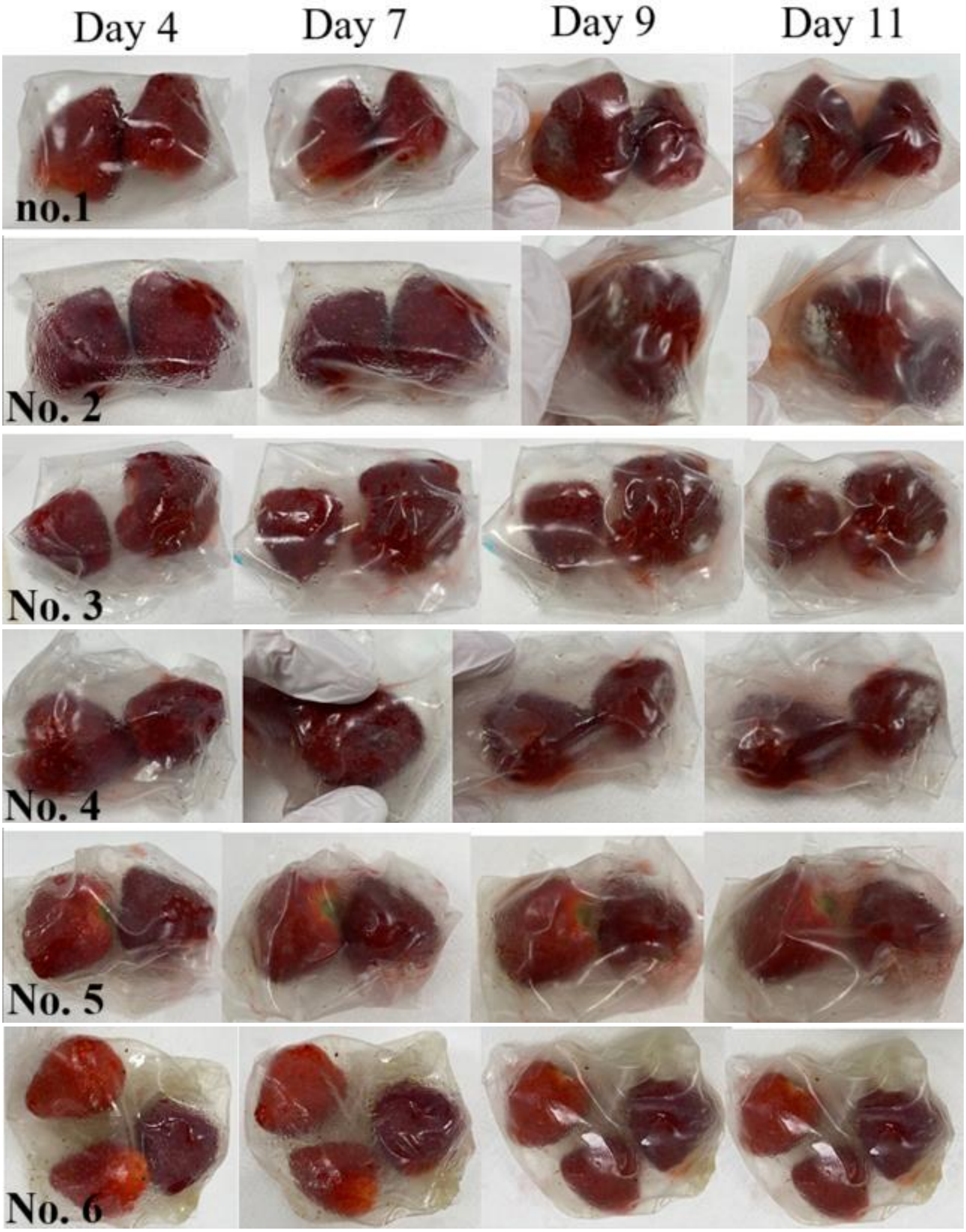
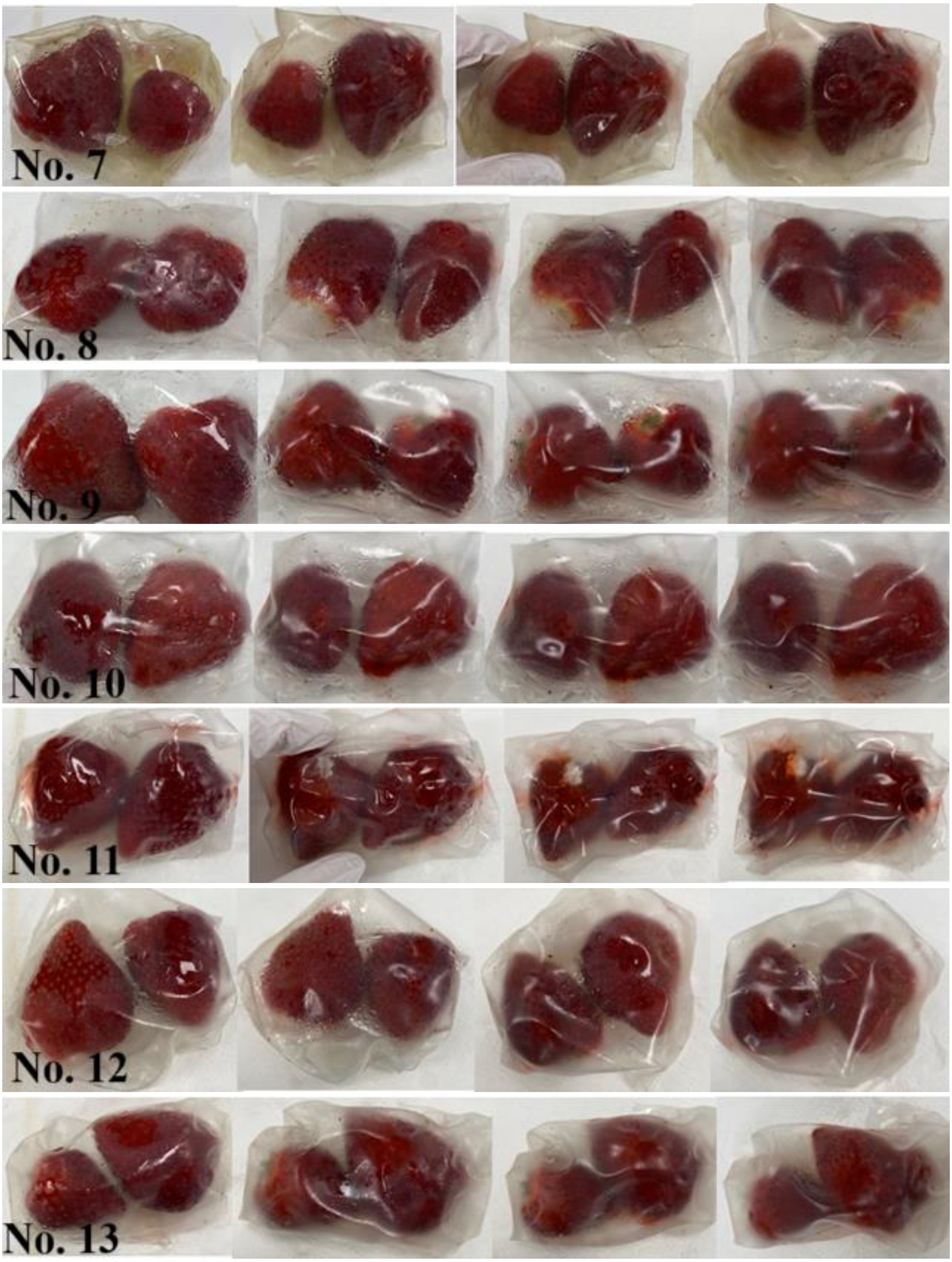
Appendix 1

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