FRUIT WASTES TO BIOMATERIALS

Veronika Bátori

Development of biofilms and 3D objects in a circular economy system
Fruit wastes to biomaterials:
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Digital version: http://urn.kb.se/resolve?urn=urn:nbn:se:hb:diva-15463

ISBN 978-91-88838-21-6 (printed)


ISSN 0280-381X, Skrifter från Högskolan i Borås, nr. 93

Cover photo: materials made in the thesis

Borås, 2019
Abstract

To address the current plastic pollution problem, the replacement of conventional plastics with bioplastics can be considered. Although the land use of crop cultivation for bioplastics is still negligible, there is an increasing interest in the utilisation of lignocellulosic waste products for the production of bioplastics. A latest trend in researching sources for bioplastic production focuses on the use of fruit and vegetable wastes because of their versatile polysaccharides. Among different fruit wastes, orange waste and apple pomace have been evaluated as raw materials in this thesis.

The development of biofilms and 3D objects from the above-mentioned raw materials via the solution casting and compression moulding methods was investigated. Biocomposites are generally made from a bioplastic matrix and reinforcement, or a plastic reinforced with natural fibres. In the present study, pectin was used as a matrix, and cellulosic fibres were used as reinforcement. Orange waste films had an opaque appearance with a yellowish colour and were very flexible, while the 3D objects had brown colour. The films had mechanical properties comparable with those of commodity plastics, such as 32 to 36 MPa tensile strength. The films were biodegradable under anaerobic conditions, and 3D objects showed good biodegradability in soil. Grafting of orange waste with maleic anhydride was performed in order to improve its properties, e.g. the hydrophilicity of the polysaccharides-based materials. Grafting reduced the density by 40 % and increased the hydrophobicity compared with unmodified orange waste. Further improvements included upgrading the film casting method and incorporating maleic anhydride in the recipe. The lowest amount of necessary maleic anhydride was determined (0.4 %), and the resulting films had a smoother and more uniform surface. The original methods were also applied to apple pomace in order to produce films and 3D objects. Films from apple pomace had an elongation of 55 %, a twofold increase compared to that of orange waste films containing maleic anhydride (28 %). Orange waste and apple pomace were also mixed for 3D object fabrication, achieving the highest strength of 5.8 MPa (ratio of 75 to 25, respectively) a threefold increase compared to that achieved with only orange waste alone (1.8 MPa).

The results are promising, but further improvements, e.g. in respect to hydrophilicity and upscaling, are needed for orange waste and apple pomace to develop into raw materials for next-generation bioplastics.

Keywords: apple pomace, biodegradable, bioplastics, circular economy, orange waste, resource recovery
List of publications

This thesis is based on the results presented in the following publications:

Paper I


Paper II


Paper III


Paper IV

V. Bátori, M. Lundin, D. Åkesson, P. R. Lennartsson, M. J. Taherzadeh, A. Zamani The effect of glycerol, sugar and maleic anhydride on the mechanical properties of pectin-cellulose based biofilms produced from orange waste (Submitted)

Paper V

J. Gustafsson, M. Landberg, V. Bátori, D. Åkesson, M. J. Taherzadeh, A. Zamani Investigation of apple pomace for the production of biofilms and 3D shaped biomaterials (Submitted)
Statement of contribution

My contributions to the above publications were:

Paper I. Responsibility for the idea and for the experimental work. Together with the co-authors, I was responsible for the data analysis and writing of the manuscript.

Paper II. Development of the concept was performed together with the co-authors; the experimental work and a major part of the data analysis and processing was performed by me.

Paper III. Responsibility for the idea and writing of the manuscript.

Paper IV. Design and execution of the experimental work. Statistical analyses were performed together with the co-authors. Data processing and writing of the manuscript were performed by me.

Paper V. Supervision of the experimental work and responsibility for data processing and part of the writing of the manuscript.

Publication that is not included in this thesis

Research journey

‘It’s not the destination, it’s the journey’ - Ralph Waldo Emerson

I remember stitching clothes for my first – and only – Barbie doll in Hungary when I was a little girl. Later, I became a product designer (light industry engineer) and ended up working eight years for different clothing companies. It was fun, until the point I realised that it was not making me happy any more. One reason is that I became aware of the negative impacts of the fashion industry on the environment and society. So, when I started working for my last employer, a jeans company, I felt that I needed a change. I had no idea, though, what to do next. Since I was also interested in environmental studies, and already practised an environment-conscious lifestyle, I decided to do a master’s degree, and studied environmental engineering, part time. Those two years indeed were hard. But I was determined, as I wished to make myself useful for the planet and for society. Therefore, I decided to connect the two fields as my thesis subject and investigate recycling of textiles. But there was only one company in Hungary at the time, and it was doing some kind of mechanical recycling to produce filling material for automotive applications, if I remember correctly. That did not interest me too much. So, I continued to look around for opportunities. Suddenly my attention was caught by an advertisement with the slogan ‘Run your car on your old jeans’, which sounded very appealing to me. Thus, I found myself in the laboratories of the University of Borås, producing ethanol from whole stillage, for six months. At first, I had no idea about laboratories and whole stillage, except for ethanol. But my interest in microorganisms and in the precise work in the lab rose quickly, so I did not hesitate to return for the PhD position after I graduated, though my PhD subject turned into something different from what I had researched before. I started to study the development of bioplastic materials from orange waste, which made me happy, because plastic bags have never been my favourites.

The original idea for this thesis was based on a biorefinery approach, which involved the extraction of pectin from orange waste and the cultivation of fungal biomass on the soluble sugars in the orange waste for their chitosan content. Pectin and chitosan would then be used for the production of biofilms. Therefore, the first task I started experimenting with was the production of biofilms with the solution casting method, using commercial pectin and chitosan. In the meantime, the fermentation processes of the soluble sugars by the fungus *Rhizopus oryzae* for growing biomass were investigated. Trials were designed to set up a fed-batch bubble column system for the continuous consumption of sugars to support maximal
biomass growth. Microwave-assisted pectin extraction from orange waste was also performed.

Luckily – or unluckily – none of those methods worked out, except for the pectin extraction. Finally, a decision was made to use the entire orange waste and to follow the previous film forming recipes. Paper I describes the development of the orange waste biofilms, and also presents their properties and antimicrobial activity, and anaerobic degradation studies.

Orange waste films had good mechanical properties, though holes were present in the structure and the hydrophilicity of the films was one negative characteristic, usually polysaccharides-based bioplastics have. Therefore, investigations on the use of a compatibilizer to enable better adhesion between polymers began, and maleic anhydride was used to chemically modify the orange waste powder (Paper II). The modified orange waste powder had interesting characteristics from the standpoint of use as a filler in biocomposites; however, films, in the same way as before, were unable to form.

As it was established that orange waste films are degradable anaerobically within approximately two weeks, a proposed application for biofilms was collecting bags for the organic fraction of municipal solid waste. Therefore, the topic was further investigated, and a study reviewed the anaerobic biodegradability of various bioplastics (Paper III).

To eliminate holes in the biofilms, maleic anhydride was directly incorporated in the orange waste films. Paper IV describes the improvement steps and the optimisation experiments of orange waste films.

Another approach to biomaterial fabrication is the technique of compression moulding, which can reduce the number of chemicals, energy use, time, and water consumption. Biomaterials from orange waste were shaped using only glycerol and orange waste powder. Mechanical characterisation and aerobic degradation studies were also performed in soil.

In the meantime, solid state fermentation of wet and ground orange waste was also performed using the fungi Neurospora intermedia and Aspergillus oryzae, with the intention of using the mycelia as a reinforcing agent. The dried cakes were then pressed. However, the idea seemed promising, practically it did not work out within the applied conditions.
Last, the developed methods were applied to apple pomace. As a consequence of the different compositional structure, biofilms and biomaterials made from apple pomace had different properties, some better than their orange waste precursors (Paper V).
Acknowledgements

I would like to thank everyone who supported, motivated, and believed in me during the past four years and thus helped to bring this thesis to a successful conclusion.

First, I would like to express my gratitude to my main supervisor, Professor Mohammad Taherzadeh, for his guidance and scientific advice through the insightful discussions we had. I am extremely grateful to my co-supervisors, Akram Zamani and Patrik Lennartsson, who supported me with their knowledge, time, understanding, and friendship even in the hardest times. Although Dan Åkesson was not an official supervisor in this thesis, I am also very grateful to him for his support and the numerous discussions we had. I could not have wished for a better ‘crew’ for this thesis.

I would like to thank my examiner, Professor Kim Bolton; the director of studies, Tomas Wahnström; and the head of the department, Peter Therning, for their time and support over these years. I am grateful for the laboratory managers: Marlén Kilberg, Kristina Laurila, Sofie Svensson, Haike Hilke, and Thomas Södergren for providing an excellent working environment in the laboratories. I am also very grateful to the university staff, who helped me with administrative matters: Louise Holmgren, Jonas Edberg, Susanne Borg, Irene Lammassaari, and special thanks to Sari Sarhamo for her enthusiasm.

Special thanks to Magnus Lundin for the keen and patient discussions about statistics, to Ilona Sárvári Horváth for the discussions about anaerobic digestion, and to Professor Staffan Svensson for teaching me about picking the right mushrooms.

I would like to thank my students for teaching me how to feel more confident about something I was not always entirely sure about, for the opportunities you gave me to evaluate the thesis, and for your help and work we did together. Thank you, Zhino, Jennie, Malin, Danh, Jesper, and Mikael.

I would like to express my gratitude to the current and past fellow PhD and visiting PhD students and other colleagues in Resource Recovery and in Swedish School of Textiles, and especially for those we became friends with. Thank you, Miss Rebecca, for your support and great friendship. Thank you, Sunil and Jorge, for ‘making it strong’ in Canada. Thanks, Ram, big Pedro and small Pedro, Johanni, Mosi, Madu, Steve, Sina, Katarina, Swarnima, and Aomi for being here and for the time we spent together also outside the university; it has been great fun. Thanks, Päivi, Luki, Amir, Supriyanto, Kostas, Andreas, Gülru, Anette, Sabina, Kamran,
Francis, Adib, Sajjad, Sherry, Tugba, Taner, Mohsen, Mina, Coralie, Amir, Hanieh, Azam, Anjum, Babak, Zohre, Forough, Foluke, Fatimat, Abas, Maryam, Julius, Karthik, Behnaz, Jhosané, Kehinde, Farzad, Regina, Alex, and Ulla for your presence and smiles, which I am grateful for.

I would like to thank my friends, Soli, Ranjitha, Linda, Kisskati, and Hugi, for their support and love, especially when times were hard.

I am grateful for Ilona and István for being my Swedish family and for everything we have shared together.

I am extremely thankful for the love and support of my mother, sisters, and grandmothers, and I wish I could share this moment with my father and grandfathers… I am very grateful to my partner, Peder, who has been the best support to me since 2018 January, so far. I love you all.

Last, but not least I would like to thank Mano, my friend, who helped me begin this journey…

*I dedicate this thesis to those ones who I love and in memoriam to my father, my grandfathers, and Mano.*
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### Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>EU-28</td>
<td>The 28-member states of the EU (Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherland, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom*)</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>G7</td>
<td>Group of Seven (consisting of Canada, France, Germany, Italy, Japan, the United Kingdom, and the United States)</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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* The United Kingdom is leaving the EU on 29 March 2019, after its citizens voted for this decision in June 2016.
Chapter 1

Introduction

1.1 Overview

Our society today is facing many challenges. One of them is related to the extensive and reckless usage of single-use conventional plastics, which creates a shortage in the supply of valuable oil, kills numbers of species, contaminates our oceans, increases greenhouse gas emissions, is responsible for aesthetic nuisances, and pollutes the food chain; plastics are considered the major toxic pollutants of the present time [1].

Plastics are, however, too useful to be phased out; they have many advantageous properties, which resulted in the acceleration of plastics production to 335 million tonnes in 2016 within the last 70 years. They are durable, lightweight, easy to process, and cheap to mass-produce. Plastics are also easy to recycle, and various separation systems are available today; however, often they are not recycled. Most of the plastic waste still ends up in landfills, and if the current trend continues until 2050, we will have more plastics than fish in our oceans [2]. Due to the durability of plastics – they require hundreds of years to degrade – they inevitably accumulate in the ecosystems. Ocean clean-up is, however, a developing concept, and the first operation has just begun to remove the Pacific Garbage Patch [3], the largest patch among the five vast and dispersed garbage patches. Clean-up alone will not, however, ensure a plastic-free ocean by 2050, without source reduction of pollution. A restructuring and re-understanding of industries and resources; a global shift in thinking; and the development of new technologies is necessary to reduce the use and misuse of fossil-based plastics. Following the waste hierarchy ranks, the most favoured options focus on the prevention and reduction of plastics waste, followed by reuse, recycling, and energy recovery, with disposal being the least preferred option [4]. The inclusion of the management of biodegradable plastics in these strategies could open up end-of-life waste management options for bioplastics, such as composting and anaerobic digestion [5]. These strategies would also address the issue of food contamination in the recycling of fossil-based plastics, meaning no harm for biodegradable plastics if they are biodegraded as an end-of-life option.
Careful consideration needs to be given to the evaluation of what uses and types of plastics can be replaced by bioplastics; that is, oil should be used for purposes that at the moment cannot be replaced by bio-based materials. The bioplastics industry using renewable feedstock is a fast-developing and sustainable sector, yet it does not compete for land use with food cultivation. In today’s society, however, where global food waste and world hunger are two major and sensitive topics, the future lies in the use and recovery of lignocellulosic residues and by-products of food processing and agroindustry.

1.2 Thesis structure

The thesis is divided into the following six chapters:

Chapter 1 introduces the global challenges related to plastics faced by the society today and possible solutions.

Chapter 2 navigates the reader through the holistic issues related to plastics: the global threats of conventional plastics; definition, advantages, and challenges associated with bioplastics; today’s bioplastics market; the position of bioplastics in a circular economy; the global incentives of making the world a plastic-free place; and finally, the social and ethical aspects of the bioplastics industry and the thesis subject.

In Chapter 3, fruit processing industry residues are briefly reviewed, focusing on orange waste and apple pomace as case studies.

Chapter 4 discusses the scientific theory of film formation, with an emphasis on polysaccharide-based films. It also introduces the challenges related to such types of products and possible solutions. In the method development section, a detailed description of the steps of biofilm development is provided. Later, the key results, degradation studies, and proposed application of the developed biofilms are discussed.

Chapter 5 is built up the same way as Chapter 4; however, it focuses on compression-moulded biomaterials.

Chapter 6 summarises the thesis in the form of a conclusion and presents proposals for future work.
2.1 Drawbacks of conventional plastics

Life without plastics today seems inconceivable; however, most plastics date back to only the 1950s. Since then, in the span of approximately two generations, the global production of plastics reached 335 million tonnes in 2016 (Figure 2.1) [6], and this number is expected to double in the next 20 years (Figure 2.2). One of the world’s largest producers is China, accounting for approximately one fourth of the global production [6], and the country is continuously developing its plastic industry, with more efficient companies and high-quality plastics. The largest market for plastics is packaging, which imperceptibly shifted from reusable to single-use items, worldwide. In Europe (EU-28, Norway, and Switzerland), this share is almost 40%, and packaging waste accounts for 59% of all plastic waste [7]. The plastics share in municipal solid waste increased from less than 1% to more than 10% from 1960 until 2005, in middle- and high-income countries [8]. From 1950 until 2017, the total plastics production was 8300 million tonnes; of that, 6400 million tonnes outlived usefulness and became waste, of which only 9% was recycled, 12% was incinerated, and 79% accumulated in landfills [9]. Geyer et al. [10] predict approximately 12,000 million tonnes of plastic waste on landfills or in the environment until 2050 if the current trend continues (Figure 2.2). In accordance with the calculations of Jambeck et al. [8], in 2010, 4.8 to 12.7 million tonnes of plastics – almost 4% of the global plastics production – entered the oceans, from 192 coastal countries. They also predict that without waste management infrastructure improvements, this number will increase by order of magnitude by 2025. Conventional plastics are usually unable to biodegrade, instead they will slowly fragment and accumulate. Therefore, they remain in nature for a very long time (depending on the type, most plastics can last for 200-600 years) in the form of waste, endangering human health and the environment. If the long carbon chains are broken into small pieces, microorganisms can degrade them; however, that process takes too long, so that new plastic waste enters the ecosystem, creating a circle of complications and challenges that need to be resolved [1]. On the other hand, a few spices have been identified, for their plastic degrading ability. The
ability of a mealworm (the larvae of *Tenebrio molitor* Linnaeus) degrading polystyrene [11, 12] within 24 hours, was discovered by Chinese researchers in 2015. In 2017, Bombelli et al. [13] discovered that the caterpillar of the wax moth (*Galleria mellonella*) is able to degrade polyethylene bags. A newly discovered bacterium (*Ideonella sakaiensis*) was isolated for its polyethylene terephthalate degrading enzyme, the PETase [14], by Japanese scientists in 2018.

Figure 2.1 Global plastics production between 1950 and 2016. Adapted from [6]. Hollow dots represent the annual plastic production in Europe, and filled dots show the annual global plastic production.

Figure 2.2 Past and projected cumulative plastic waste generation and disposal. Adapted from [10]. In accordance with the projection, by 2050, plastic recycling will increase, that is, one third of the plastics waste will be recycled, and the rest will be incinerated.
2.1.1 Environmental risks

Debris, especially plastic debris, is the world’s most omnipresent form of pollution affecting our oceans and inland waterways [15]. Plastic debris appears on the surface of oceans, on coastlines, on the sea floor, and in the ice of the Arctic sea [16-18]. As much as 80% [1, 8] of plastics in the oceans originates from land and is carried by rivers [19]. Europe’s second largest river, the River Danube, alone is estimated to carry 4.2 tonnes plastics per day in accordance with a study performed between 2010 and 2012 [20]. Lower-density plastics can also be carried by the wind, and plastic waste is also released by ocean-based sources, such as aquaculture and shipping [19]. The phenomenon called weathering – the breaking down of natural (rocks, soil, minerals) and artificial materials into smaller fragments through contact with the Earth’s atmosphere – causes plastic debris fragmentation into smaller particles. On the basis of the size of plastics, we distinguish macroplastics (≥25 mm), mesoplastics (<25mm-5mm), microplastics (5 mm-1 mm), mini-microplastics (1 mm-1 μm), and nanoplastics (<1 μm) [21]. Primary microplastics (also called microbeads) have a regular spherical shape and are often released by cosmetics and detergents, or they are mistreated ‘virgin’ plastic pellets [17, 21, 22]. During the use of such cosmetics and detergents, these microbeads are washed down in the drains and enter municipal treatment plants via sewage systems, and because wastewater effluents are often discharged into rivers or seawaters, these microplastics enter the aquatic system [21]. Secondary microplastics consist of fibres released from washing machines, degraded larger plastic items, abrasion of tyres, fishing ropes, and nets [17, 21]. Unlike primary microplastics, secondary microplastics often have irregular shapes. These microplastics cause two major problems: first, they are ingested by biota and therefore accumulate in the food chain, and second, they carry toxic compounds [22-25]. Microplastics attract chemical contaminants, such as persistent organic pollutants [1]. Persistent organic pollutants are adsorbed onto the surface of plastic carriers, concentrating the chemical pollution of the surrounding water [21]. Weathering of microplastics increases their surface-area-to-volume ratio [21], allowing the sorption of even higher concentrations of persistent organic pollutants, up to one million times greater than the surrounding water [26]. When microplastics are ingested, species suffer from high exposure to persistent organic pollutants, with plastic acting as a carrier of toxic compounds into the tissues of organisms [26]. Besides ingestion of microplastics, entanglements into larger macroplastics is another threat for animals. However, ingestion is more common than entanglements [1].
2.1.2 (Human) health-related risks

Microplastics not only carry persistent organic pollutants into the tissues of organisms, but they may also transport bacteria and viruses to unpolluted waters [15, 26]. Further, various chemicals, such as phthalates and fire retardants are added to plastics as additives to enhance the performance of the material [27]. Adverse human and wildlife health issues are related to these chemicals. Extensive studies report that these chemicals act as endocrine disrupters, are linked to reproductive difficulties, or are considered to be carcinogens [28-31]. Crawford & Quinn [21] also mentioned that in some urban areas, domestic tap water is sourced from wastewater treatment plants. And because microplastics, especially nanoplastics, are able to escape filtration systems, there is a chance that they could end up in drinking water [21, 32].

Even though actions to reduce plastic bag pollution have long been established, many countries lack implementation strategies; similarly, actions taken to mitigate microbead pollution are restricted to a few countries [33]. However, bans and restrictions on the use of single-use plastic items are not enough; an integrated reformation of the plastics industry is needed with thorough consideration of materials, methods, application, and recovery. One possible solution is the use of biodegradable plastics for some of the single-use items.

2.2 Definitions of bioplastics and biocomposites

The term bioplastics is a broad concept; it is not only a material but a family of materials that encompasses different properties and applications [34]. A plastic material is usually specified as a bioplastic if it is bio-based (derived from renewable feedstock, e.g. corn, sugarcane and beet, potato, wheat, and cellulose), or biodegradable (decomposed by microorganisms, under specified conditions), or fulfils both criteria. The main groups of bioplastics are (1) bio-based and non-biodegradable plastics, (2) plastics that are both bio-based and biodegradable, and (3) plastics that are fossil-based but can biodegrade. Figure 2.3 shows the classification of available bioplastics according to their origin and biodegradability.

The term biocomposites is used for a composite material made of a bioplastic and reinforced by a synthetic material; for a material made of a synthetic plastic and natural reinforcement; or for a bioplastic reinforced with natural fibres or fillers. The latter is also called green composites.
2.3 Positive effects and challenges associated with bioplastics

The benefits of bioplastics and natural reinforcements are broad. The use of renewable resources enables bioplastics to support sustainable production and consumption by (1) increasing resource efficiency (cultivation on an annual basis, and the use of biomass first for materials and then for energy recovery); (2) reducing the carbon footprint of the materials; and (3) reducing the use of fossil fuels [35]. The use of natural fillers is considered beneficial because of their ‘renewable nature, low cost, low density, low energy consumption, high specific strength and stiffness, CO₂ sequestration, biodegradability, and less wear on the machinery’ [36]. An interesting approach for the production of high-performance biocomposites include cost-efficient modification of fibres, and modification of the polymer matrix by e.g. functionalising [37]. Bioplastics, if they are biodegradable, may be recovered biologically by composting or by anaerobic digestion. They can also be recycled via mechanical and chemical treatments [38]. Polylactic acid, for example, can be chemically recovered to its monomer, lactide, to further produce polylactic acid [39]. Repeated
processing via mechanical recycling would, however, degrade and weaken the properties of bioplastics. Compostable bioplastics have been claimed to affect the quality of recyclates in existing recycling systems of conventional plastics; as a response, it has been shown that the impurifying effect of a compostable plastic is less than that of polyethylene terephthalate on polyethylene [40]. Today’s major segment of bioplastics is the bio-based, non-biodegradable plastics, the so-called ‘drop-in’ solutions of plastics. However, they are sourced from renewable materials, are not biodegradable and therefore not the best solutions to address ocean pollution.

2.4 Today’s available bioplastics and bioplastics market

The bioplastics industry is a new and growing sector with an excellent economic and ecologic potential for a low-carbon, circular bioeconomy that uses resources more efficiently [41]. With financial support from the European Union for a bio-based economy, research and development of bioplastics is set to increase. The global production capacity of bioplastics is predicted to grow from 2.05 to 2.44 million tonnes from 2017 to 2022 (Figure 2.4) [42].

![Figure 2.4 Global production capacities of bioplastics 2017-2022. Bio-based, non-biodegradable (bottom bars) plastics account for a bigger share compared to biodegradable (top bars) plastics. Adapted from [42].](image)

The production of bio-based, non-biodegradable plastics, such as bio-based polyethylene and bio-based polyethylene terephthalate, is still ahead of the biodegradable versions (Figure 2.5); however, the production of polylactic acid (PLA) and polyhydroxyalkanoates (PHAs) will grow among biodegradable plastics [43, 44].
Commodity plastics, such as polyethylene, polypropylene, and polyvinyl chloride, can also be made from renewable resources, for example, bioethanol [35]. These bio-based, non-biodegradable polyolefins (such as bio-polyethylene and bio-polypropylene), bio-polyvinyl chloride, and bio-polyethylene terephthalate are also called ‘drop-in’ bioplastics. ‘Drop-in’ bioplastics have chemically identical structures as fossil-based commodity plastics, and therefore there is no difference in the end-of-life solutions of the two groups. There are two major differences, though, between ‘drop-in’ bioplastics and commodity plastics: the price and the environmental footprint [45]. The production of commodity plastics is cheaper, because of the presence of an older, more deeply researched, and mature industry with a higher production capacity; also, transportation of oil through pipelines is better established than transportation of biomass in trucks [45]. ‘Drop-ins’, on the other hand, do not release additional CO$_2$ into the atmosphere during incineration, for example, because the excess CO$_2$ has already been used during plant growth [45]. Bio-polyethylene is already being produced on a large scale by Braskem (Brazil) from sugarcane bioethanol, with an annual production of 200,000 tonnes [46]. Bio-polyethylene terephthalate, the partially bio-based polyethylene terephthalate, is used for packaging; for example, PlantBottle™ was introduced in 2009 by The Coca-Cola Company [47]. The company’s PlantBottle™ contains up to 30 % plant-based materials, and in 2017, 10.5 billion PlantBottle™ packages were sold. To date, the company has distributed 60 billion plant-based packages in 44 markets of 36 brands. Bio-polypropylene and bio-polyvinyl chloride are expected to follow the same trend.

Another group of bio-based, non-biodegradable plastics is referred to as durables, being performance or technical polymers, for example, bio-based polyamide, polyesters (e.g. polytrimethylene terephthalate, polybutylene terephthalate), polyurethane, and polyepoxides. The applications of such polymers, such as textile fibres and various automotive applications, suggest that biodegradability is not a preferred property [35].
Figure 2.5 Global and predicted production of bioplastics in 2017-2022 by material type. Adapted from [43, 44].
The biggest group of biodegradable and bio-based polymers is the starch blends, followed by other polyesters, such as polylactic acid and polyhydroxyalkanoates. These materials have so far been used for packaging or short-lived purposes, unlike cellulose plastics, i.e. regenerated cellulose, which have long been used as a fibre (viscose) and cellulose acetate. Thermoplastic starch can be manufactured via conventional plastic technologies, such as blow moulding, extrusion, and injection moulding; polymer casting can also be used. Starch blends (as the name suggests) usually contain 5 to 90 wt % modified starch and the remaining content is other polymeric material to enhance properties such as hydrophilicity or strength. Novamont (Italy), the world leader of bio-based and biodegradable plastic production, however, pledged at the G7 summit in Bologna (2017) to increase bio-based content in some of its MaterBi™ products to 100 % from 40 % by the end of 2017 [48]. Ingeo™ polylactic acid is manufactured by NatureWorks (USA) entirely from plant-based resources. However, polylactic acid is biodegradable, and it can also be recycled to its monomers; thus, the recovery of polylactic acid is blazing a new path marked by great interest and technical developments [35]. Nodax™, a medium-chain-length branched polyhydroxyalkanoate, is now solely marketed by DaniMer Scientific (USA) but was originally developed by Procter & Gamble (USA) [49]. It is a versatile polymer, blend-able with, e.g. polylactic acid, and exhibits similar mechanical properties as polyethylene; therefore, polyhydroxyalkanoate is considered to be a very promising biopolymer [50]. The introduction of new bio-based monomers, such as succinic acid, butanediol, propanediol, and fatty acid derivatives [35], is stimulating the bioplastic industry to grow.

Biodegradable, fossil-based plastics are a relatively small group of bioplastics. Polyesters, such as polyglycolic acid, polycaprolactone, polybutylene succinate, polybutylene adipate-co-terephthalate, and polyvinyl alcohol belong to this group. These polymers are mostly blended together with starch or other biopolymers to improve the application-specific properties of the latter, e.g. mechanical properties [35].

2.4.2 Natural fibres and fillers for reinforcing biocomposites

Amongst natural fibres and fillers, cellulosic plant fibres, have enjoyed great success as reinforcement in polymer composites, mainly because of their favourable mechanical properties and their light weight [51-53]. Plant fibres are also considered as a substitute for the more commonly used glass fibres [54]. Furthermore, these lignocellulose fibres have a low carbon footprint, because they originate from a renewable resource and are biodegradable. Natural fibres and fillers are summarised in Table 2.1.
### Table 2.1 Natural fibres and fillers classified by type with examples. Adapted from [55].

<table>
<thead>
<tr>
<th>FIBRES</th>
<th>FILLERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>type</td>
<td>example</td>
</tr>
<tr>
<td>MINERAL</td>
<td></td>
</tr>
<tr>
<td>(Strunz</td>
<td>MINERALS</td>
</tr>
<tr>
<td>classification)</td>
<td>(1) native elements</td>
</tr>
<tr>
<td></td>
<td>(2) sulfides</td>
</tr>
<tr>
<td>ANIMAL</td>
<td>HAIR</td>
</tr>
<tr>
<td>HAIR</td>
<td>wool</td>
</tr>
<tr>
<td>(protein)</td>
<td>SILK</td>
</tr>
<tr>
<td></td>
<td>flax, hemp, jute,</td>
</tr>
<tr>
<td>PLANT (cellulosic)</td>
<td>kenaf, rami</td>
</tr>
<tr>
<td>LEAF</td>
<td>abaca, sisal</td>
</tr>
<tr>
<td>SEED</td>
<td>(3) halides</td>
</tr>
<tr>
<td>corn, cotton, kapok</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4) oxides &amp;</td>
</tr>
<tr>
<td></td>
<td>hydroxides</td>
</tr>
<tr>
<td></td>
<td>(5) carbonates and</td>
</tr>
<tr>
<td></td>
<td>nitrates</td>
</tr>
<tr>
<td></td>
<td>calcium carbonate</td>
</tr>
<tr>
<td>STRAW</td>
<td>bagasse, bamboo</td>
</tr>
<tr>
<td></td>
<td>(6) borates</td>
</tr>
<tr>
<td></td>
<td>calcium phosphate,</td>
</tr>
<tr>
<td></td>
<td>hydroxyapatite,</td>
</tr>
<tr>
<td></td>
<td>zirconium silica</td>
</tr>
<tr>
<td>GRASS</td>
<td>soft &amp; hard wood</td>
</tr>
<tr>
<td></td>
<td>(7) sulfates</td>
</tr>
<tr>
<td></td>
<td>halloysite, silica</td>
</tr>
<tr>
<td>WOOD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(8) phosphates</td>
</tr>
<tr>
<td></td>
<td>phosphate</td>
</tr>
<tr>
<td></td>
<td>montmorillonite,</td>
</tr>
<tr>
<td></td>
<td>(9) silicates</td>
</tr>
<tr>
<td></td>
<td>halloysite, silica</td>
</tr>
<tr>
<td></td>
<td>(10) organic minerals</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>collagen</td>
</tr>
<tr>
<td>POLYSACCHARIDES</td>
<td>alginate, starch,</td>
</tr>
<tr>
<td></td>
<td>carrageenan, chitin</td>
</tr>
<tr>
<td>BENZOATES</td>
<td></td>
</tr>
</tbody>
</table>

### 2.5 Position of bioplastics in the circular economy

The dynamic development of the bioplastics industry demonstrates the potential to shape the plastics industry by completing a natural cycle that can help eliminate the fossil-based short-lived plastics. Therefore, in 2015, the European Commission adopted an EU Action Plan for a circular economy, where it identified plastics as a key priority and committed itself to ‘prepare a strategy addressing the challenges posed by plastics throughout the value chain and considering their entire life-cycle’ [7]. In 2017, the Commission confirmed that it would ensure that all plastic packaging will be reusable or recyclable by 2030 [7].
2.5.1 The circular economy

In a biological system, materials flow: a plant is food for an animal, the animal dies, and its nutrients then return to the soil to help grow new plants, while energy is supplied by the sun. The circular economy concept aims to merge the working biological system with the current technological system, which is best described by the linear economy model, which focuses on exploitation of resources, production, and waste disposal after consumption. By combining the biological and technological systems into a sustainable and resource-efficient model, the aim is to value waste as capital, for example, by the use of compostable packaging, and to increase the actions of reducing, reusing, and renewing products, and at the end of their lifespan recycling them into new ones, with transportation and manufacture being provided by renewable energy. In this context, the production of bioenergy and biomaterials have to meet the increasing demands, and a biorefinery concept is needed that visualises the once-negatively-valued waste as a renewable feedstock [56]. This model requires collaboration between all interconnecting companies, with sustainable benefits being created by sustainable technologies, reduction of waste, and improved social performance of companies [57]. Frameworks have started to emerge to support the implementation of technological and non-technological innovations within organisations [58].

2.5.2 The new plastics economy – re-thinking the future of plastics

The European Commission has created a strategy [7] which describes the position of plastics in a circular economy, highlighting the responsibility of the member states to turn plastics-caused challenges into opportunities. The most important EU measures are (1) improving the economics and quality of plastics recycling by better product design, boosting recycled content, and enhancing separate collection of plastic waste; (2) restricting plastic waste and littering by reducing the amount of single-use plastics, controlling marine litter, promoting compostable and biodegradable plastics, and reducing microplastics pollution; (3) driving investments and innovations (e.g. Horizon 2020); and (4) harnessing global actions such as focusing on key regions, multilateral initiatives on plastics, bilateral cooperation with non-EU countries, and international trade [59]. From this thesis’s point of view, the most important measures are the ones performed for compostable and biodegradable plastics. Here, the European Commission proposes a harmonised definition and labelling of compostable and biodegradable plastics, performing a life-cycle assessment to identify conditions when their use is beneficial and the criteria for such applications, and restricting the use of oxodegradable plastics. The strategy, by presenting key commitments to EU countries, calls for
the mobilisation of the private sector together with national and regional authorities and is intended as an example to follow on a global level for an international commitment [7].

The new plastics economy, defined by the Ellen MacArthur Foundation [60], demands a new approach towards re-thinking the future of plastics. To achieve this, the new plastics economy has three main objectives (Figure 2.6). The cornerstone of the new plastics economy is (1) creating an effective after-use plastics economy by improving the economics and uptake of recycling, reuse, and controlled biodegradation that would help achieve the following two objectives: (2) drastically reducing the leakage of plastics into natural systems and (3) decoupling plastics from fossil feedstocks by the use of renewable feedstocks [60].

In January 2018, there were 11 leading brands, retailers, and packaging companies that had started working towards using 100% reusable, recyclable, or compostable packaging by 2025. These companies, such as Amcor, Ecover, Evian, L’Oréal, Mars, M&S, PepsiCo, The Coca-Cola Company, Unilever, Walmart, and Werner & Mertz, are responsible for more than 6 million tonnes of plastic packaging per year [61]. On 29 October 2018, at the Our Ocean Conference (Bali), The Ellen MacArthur Foundation in collaboration with the United Nations Environment Programme announced a Global Commitment to eliminate plastic waste [2]. More than 290 of the world’s leading packaging brands have committed to ensure that 100 %
of plastic packaging can be reused, recycled, or composted by 2025 [2]. According to the Global Commitment, three actions are required to achieve this vision and create a circular economy for plastics: (1) eliminate all problematic and unnecessary plastic items; (2) innovate to ensure that the plastics we do need are reusable, recyclable, or compostable; and (3) circulate all the plastic items we use to keep them in the economy and out of the environment [62].

A potential response to the three main ambitions of the new plastics economy and the goals of the Global Commitment is the use of bioplastics made from renewable feedstock. However, the land use of crops for bioplastics production is still negligible [41]; the use of food industry residues opens new doors towards a more sustainable bioplastics production. This thesis proposes a system where valuable residues of certain fruit-juice-processing industries are used as substrates for biodegradable plastic production, which at the end of their lifetime can serve as energy or nutrition for other organisms (Figure 2.7).

![Figure 2.7 Position of fruit-residue-based bioplastics in a circular economy system.](image)

**2.6 Social & ethical reflections**

The circular economy clearly seems to prioritise the economic systems with primary benefits for the environment, and visualises only implicit gains for social aspects, unlike the holistic view of all three dimensions of sustainability [63]. Eco-efficiency is mainly a business concept that focuses on the economic and environmental dimensions of sustainability, while the resource efficiency concept implies resource reduction and enhanced economic and social well-being at the same time [64].
Establishing plastics-related policies needs a deep understanding of the structure of the problems, and cannot rely on scientific research alone; instead, policymakers need to consider the economic and social benefits of plastics as well as their hazards to human and environmental health [65]. However, the implementation of the new plastics economy in our everyday life requires not only organisational involvement but also social awareness, which plays a key role. A shift in the behaviour of society as a consumer is a vital prerequisite, for which education on an institutional and personal level is essential.

The social and economic development of the bioplastics industry is best described by the growing number of materials, applications, and products. The bioplastics industry has great potential to make an extensive impact on the economy in the coming years. In accordance with the European Bioplastics market report [41] there were 23,000 jobs in the industry in 2013, which can increase more than tenfold until 2030, which implies the creation of 300,000 high-skilled jobs in the European bioplastics sector.

This thesis unfolds the opportunities of using the by-products orange waste and apple pomace from juice industries as raw materials for bioplastics production. In the case of citrus processing plants, which are usually located in citrus-fruit-growing developing countries, establishing a small hands-on business on the site of the processing plants would be beneficial for the companies. In a low-resource country, a simple, effective waste treatment strategy is proposed which even generates additional profit and jobs for the companies via sales of the fruit waste-bioplastic products to higher-income countries. Moreover, there is growing interest in society in the use of bio-based and biodegradable products.
Residues of the fruit processing industry as potential source for bioplastics

3.1 Overview

The circular economy applied to the food system means a reduction in the amount of food waste, the reuse of food, the utilisation of food industry by-products and residues, the recycling of nutrients, and changes in the diet towards more efficient food patterns [66]. Food waste, or the residues of food processing industries, is increasingly viewed as a resource to be diverted from landfilling. In a circular economy, where food waste management is developed sustainably, food waste has great potential for recovery into energy, fuel, natural nutrients, or biomaterials through a set of technologies [4]. This means that approximately one third of the global food production for human consumption, i.e. an annual 1.3 billion tonnes, could theoretically be available as resource, but it is lost or wasted in the steps of the supply chain [67-69]. The EU produces 89 million tonnes of food waste, which is expected to rise to 126 million tonnes by 2020 if no prevention policy is put in place [70].

Developing bioplastics using secondary feedstock (biowaste) instead of dedicated crops has potential in the development of the bioplastics industry [71] and many advantages, for example, reduction of land use, feedstock greenhouse gas emissions, and other feedstock-related impacts such as fertiliser and water use [72]. The term biowaste is a broad concept and several types exist, such as food waste from households, which is mixed, or the unmixed types from certain industries. The latter could be by-products or residues from various food producing industries, agricultural waste, and forest residues. Lignocellulosic wastes, e.g. forest residues and industrial food waste, have the potential to replace conventional plastics by the production of, for example, polylactic acid, polyhydroxyalkanoates, bio-polyethylene, and platform molecules such as hydroxymethylfurfural for the production of bio-polyethylene terephthalate, bio-polyamide, and bio-polycaprolactone; however, the technology is still expensive and challenging [73, 74]. These products are the results of different bio-conversion or bio-synthetisation processes and are not produced by direct conversion of residues to bioplastics.
An emerging trend in bioplastics research is transformation of the residues of industrially processed vegetables and fruits directly into bioplastic films via various processes, without separation of the residue. Examples of various vegetable by-products and processing conditions are shown in Table 3.1. These materials are commonly rich in cellulose and/or lignin and have properties that are comparable with those of commodity plastics.

Table 3.1 Different types of industrial by-products of vegetables used directly for the formation of bioplastics, and their processing conditions.

<table>
<thead>
<tr>
<th>material</th>
<th>used chemicals</th>
<th>method</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>red seaweed</td>
<td>trifluoroacetic acid</td>
<td>selective dissolution, solution casting</td>
<td>[75]</td>
</tr>
<tr>
<td>green seaweed</td>
<td>trifluoroacetic acid</td>
<td>selective dissolution, solution casting</td>
<td>&quot;</td>
</tr>
<tr>
<td>brown seaweed</td>
<td>trifluoroacetic acid</td>
<td>selective dissolution, solution casting</td>
<td>&quot;</td>
</tr>
<tr>
<td>cocoa pod husk</td>
<td>trifluoroacetic acid</td>
<td>selective dissolution, solution casting</td>
<td>[76]</td>
</tr>
<tr>
<td>parsley stem</td>
<td>trifluoroacetic acid</td>
<td>selective dissolution, solution casting</td>
<td>&quot;</td>
</tr>
<tr>
<td>spinach stem</td>
<td>trifluoroacetic acid</td>
<td>selective dissolution, solution casting</td>
<td>&quot;</td>
</tr>
<tr>
<td>rice hulls</td>
<td>trifluoroacetic acid</td>
<td>selective dissolution, solution casting</td>
<td>&quot;</td>
</tr>
<tr>
<td>cocoa shell waste</td>
<td>heptane, silicon</td>
<td>solution casting</td>
<td>[77]</td>
</tr>
<tr>
<td>carrot waste</td>
<td>hydrochloric acid</td>
<td>dialysis, solution casting</td>
<td>[78]</td>
</tr>
<tr>
<td>parsley waste</td>
<td>hydrochloric acid</td>
<td>dialysis, solution casting</td>
<td>&quot;</td>
</tr>
<tr>
<td>radicchio waste</td>
<td>hydrochloric acid</td>
<td>dialysis, solution casting</td>
<td>&quot;</td>
</tr>
<tr>
<td>cauliflower waste</td>
<td>hydrochloric acid</td>
<td>dialysis, solution casting</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

3.2 Case studies

Pectin is an interesting material for its gelling ability and thus for the formation of bioplastics. It is mainly industrially extracted from orange waste and apple pomace; therefore, these two by-products seemed interesting and were used for the production of biomaterials via the transformation of the entire fraction of residues, introducing new methods of waste recovery. On the other hand, both residues could be environmentally harmful if their disposal is not properly carried out.

3.2.1 Orange waste

An annual worldwide production of almost 70 million tonnes of oranges is reported by FAO [79], and approximately 40-60 % of the oranges are processed for juice production [80]. The juice industry generates 50-60 % residue of the original mass [81]. The remaining fraction, after juice pressing, mainly contains peels, pulp, seeds, and membrane residues [80]. This mixture of orange residues also contains valuable polysaccharides, such as pectin, hemicelluloses, and cellulose in the peels and membranes, and di- and monosaccharides in the form of soluble sugars (glucose, fructose, and sucrose) originating from the pulp.
Despite its valuable content, orange waste, if not treated properly can, however, cause environmental damage due to its high content of organic matter and water and relatively low pH [81]. Figure 3.1 demonstrates the different treatments and possible recovery applications of orange waste. Pectin extraction is one of the many major uses of orange waste, accounting for 85.5 % of pectin produced globally [82]; however, a large quantity still ends up in landfills as it is not processed industrially [80].

The orange waste used for this thesis was kindly provided by a former local juice industry, Brämhults Juice AB (Borås, Sweden), and stored at -20 °C until further used. Soluble sugars were extracted from the orange waste in accordance with a previous study [83], which was followed by two further washing steps (Paper I) in order to ensure that no sugar was left in the waste mixture. Orange waste contained 9.48 ± 1.21 % dry matter, 29.83 ± 0.29 % pectin, 20.89 ± 0.89 % hemicelluloses, and 18.66 ± 0.48 % cellulose. The removed soluble sugar content was 11.1 ± 0.5 g/L glucose, 10.6 ± 0.5 g/L fructose, and 3.4 ± 0.6 g/L sucrose [83].

3.2.2 Apple pomace

Apple, one of the most produced and consumed fruits worldwide, is also the main commercial fruit produced in Sweden, accounting for 25.3 million tonnes in 2015 [84]. USDA reported 77.3 million tonnes of global apple production for 2017-2018 [85] and approximately 25-30 % of the apples are further processed [86]. Apple pomace is the processing waste generated after apple juice manufacturing and represents up to 25-30% of the original fruit [87, 88]. Apple pomace mostly consists of skin and flesh (95%), seeds (2% to 4%), and stems (1%), and it is a rich source of digestible fibre, pectin, and phenolic compounds [89].

Because apple pomace is rich in nutrients such as calcium, potassium, and magnesium [89], discarding apple pomace in today’s apple processing industry is a waste of resources. Apple juice by-products are, for example, responsible for 14 % of global pectin production [82]. Other possible applications of apple pomace are shown in Figure 3.2. But just as with orange waste, due to its high organic and moisture content, apple pomace can cause environmental pollution if discarded on landfills [87-89]. Thus, the recovery of such types of residues is vital in order to reduce environmental damage and to exploit the nutrients present in them. Shalini et al. [88] points to the large quantity of generated apple pomace, which suggests that preparation of single products would not be economically feasible; therefore, the production of alternative products would need to be explored.
The apple pomace used in this thesis was kindly provided by Lyckans Äpple (Bredared, Sweden) and was stored at -20 °C until further use. The apple pomace contained 17.27 ± 0.07 dry matter and 55.47 % soluble sugars of the dry matter. The sugar-free dry matter further contained 8.94 ± 1.20 % pectin, 38.99 ± 0.42 % hemicellulose, 29.42 ± 0.44 % cellulose, 22.94 ± 0.12 total lignin, and 2.91 ± 0.00 % starch (Paper V).

Figure 3.1 Possible existing applications of orange waste recovery. Adapted from [81, 90].
Figure 3.2 Possible existing applications of apple pomace recovery. Adapted from [88, 89].
Biofilms from fruit residues

4.1 Biofilms

The production of biofilms from polysaccharide-based residues of fruit and vegetable industries has become the latest trend in the research on bioplastics production from secondary feedstock [75-78, 91-94]. The mixture of components of the various sources of fruit and vegetable industrial by-products, such as pectin, starch, lining, cellulose, and hemicelluloses, make these lignocellulosic feedstocks interesting and promising for the production of bioplastic films.

One preferred method for the preparation of such bioproducts is the film casting method. In this method, the polymer solution is cast onto a non-sticky surface or mould, and a thin film is formed after solvent evaporation. The conversion of a latex (colloidal dispersion of polymeric particles in a liquid [95]) to a coherent film via several different mechanisms, such as capillarity, wet sintering, deformation, compaction, and diffusion, was described by Sheetz [96] as follows. Water evaporates from the air-water interface, and the system becomes so compact that the repulsive energy of the particles is overcome, and flocculation occurs. The compaction force, which causes the polymer particles to pack closely to each other, is normal to the surface and is exerted by water diffusion to the surface as a consequence of evaporation. In response to the compaction stress, the interior particles undergo a deformation. The deformation and compaction squeeze water to the surface. As compaction proceeds, the area of the air-water interface decreases, and the vapour pressure of the liquid water in the surface holes will decrease as the radius of the holes decreases. As a consequence of the capillary forces, wet sintering, and diffusion force, the surface holes initially disappear, and the water leaves the surface by diffusion. The generated compression pressure completes the compaction process, forming a polymer film with no voids present. Although the polymers are now completely compressed together, the interface between them remains intact. Over a period of time, the adsorbed surfactants gradually congregate, and the particle-to-particle contact becomes more intimate, resulting in increased tensile strength and improved water resistance. Routh [97] also explains the so-called coffee-ring effect, which occurs
during drying of a film, in which the particles consolidate at the edge of a drying droplet, and a flow is observed from the centre of a droplet towards the edge, with close-packed particles passing horizontally across the film. Behind the drying front, the solidified film often exhibits cracks as a result of the capillary pressure. The answer to why cracks form lies in the substrate constraint: as the capillary pressure builds up, the film tends to contract. It can do this normal to the surface, but it is constrained horizontally by shear stresses from the particles. The only way the in-plane stress can relax is through the generation of cracks. The temperature, film thickness, induction of particle aggregation, particle blends, and supercritical drying (elimination of capillary stresses) are examples of factors for controlling cracking.

By choosing the right additives and solvent, biopolymers of the mixed substrates can be dissolved, plasticized, or left undissolved in order to perform the required function in the biofilm, such as a matrix, blend, or reinforcement. In general, solvents used for edible film production include water, ethanol, or a combination of both [98], water being the solvent primarily used in the production of pectin-based edible films [99]. Because pectin is present in one third of the cell wall of vascular plants and serves as the cementing material for the cellulosic network, behaving as a stabilised gel, it is evident that pectin is the core component of mixed polysaccharide-based biofilms. Pectin has been reported to be a diverse matrix in biomaterials [55] thanks to its abilities to immobilise cells, genes, and proteins to produce a gel structure for cementation between different reinforcement materials. Its biocompatibility makes it possible for it to be mixed with organic and inorganic substances in order to mimic naturally occurring composites. Biomimicry actually is one main aspect of sustainable innovations and the development of bio-based materials [100].

Food-grade plasticizers include glycerol and sorbitol, with glycerol being the most popular plasticizer used in film-making techniques, thanks to its stability and compatibility with hydrophilic biopolymeric chains [101].

4.2 A challenge associated with polysaccharide-based bioplastics

In bioplastics made from polysaccharides the introduction of intra- and intermolecular bonding is often necessary [102]. Interfacial adhesion and good interaction between the components play a crucial role in achieving adequate physico-mechanical features [103-105]. To obtain composites with excellent mechanical properties, the load must be transferred effectively from the matrix to the fibres, as a result of good adhesion [106]. In such cases, when interfacial tension is low, compatibilizers and coupling agents are often used to enhance the adhesion and compatibility of the often-immiscible substances [106-108].
4.2.1 Possible solution: Use of compatibilizer and coupling agent

Compatibilizers and coupling agents have a similar function: they are used to promote good dispersion and interaction between the polymeric matrices and the dispersed phase. The term *compatibilizer*, however, is mostly used when two polymeric blends are brought together in a physical mixture, while the term *coupling agent* is mostly used in the case of composites, in which it acts as a bridge between fillers and the polymeric matrix; i.e. it brings dissimilar materials close to each other via chemical bonding [109]. The nature of the bonding between the fibre and matrix depends on the atomic arrangement, chemical properties of the fibre, and chemical constitution of the polymeric matrix [106].

Coupling agents can be organic, inorganic, or organic-inorganic, and those that are organic are favoured as they produce a stronger interfacial adhesion [108]. The most popular coupling agents used today include isocyanates, anhydrides, silanes, and anhydride-modified copolymers, and they are most usually applied via the three basic processes that are suitable for coupling treatment: direct coating, mixing, and fully or partly pre-treating before mixing [108].

4.2.2 Maleic anhydride as compatibilizer and coupling agent

Most effective chemical modifications involve coupling agents containing chemical groups that are able to react with the fibre and the polymer [106]. A low-hazard-profile organic compound and coupling agent is maleic anhydride [108] (Figure 4.1). It is the acid anhydride of maleic acid. Maleic anhydride is a bifunctional molecule, forming maleic acid if hydrolysed and generating the half-ester with alcohols.

![Figure 4.1 Chemical structure of maleic anhydride.](image)

Graft copolymers, such as those modified with maleic anhydride, have been proved to be suitable additives that improve the fibre-matrix interfacial adhesion for cellulosic fibre-reinforced matrices that form covalent and hydrogen bonds [106].

In one study, for example, polylactic acid was grafted with maleic anhydride in order to make it compatible with ramie fibres [104]. The results showed increased adhesion between matrix and fibres and also showed a reduced glass-transition temperature compared to the neat polymer. The latter can be associated with an increase in the chain mobility of the polylactic acid molecule chain as a result of grafting with maleic anhydride. Maleic anhydride
has also been used as a good compatibilizer in a starch/polylactic acid polymeric blend [105], where it significantly improved the interfacial adhesion between the two polymers. Maleic anhydride is produced industrially on a large scale for applications in coatings and polymers [110].

A couple of treatment methods with maleic anhydride, such as pre-treating and mixing, have been applied on orange waste and during film formation as a compatibilizer.

4.3 Development of biofilms

The idea was based on the drying of a colloidal dispersion of polymeric particles, that is, the suspension containing the dissolved and undissolved components of orange waste, used for the solution casting method in order to form a film.

4.3.1 Original method for film production

Pectin films have been reported via the solution casting method reinforced with cellulosic fibres [111]. On the basis of that study, the hypothesis was that orange waste, on account of its cellulose and high pectin content, can be used as a substrate without separating the components for the production of biofilms. Therefore, powdered orange waste was suspended in a citric acid solution to dissolve and induce pectin gelling in order to form a matrix in which the undissolved components were trapped as reinforcements. Glycerol was used as a plasticizer to increase the predominantly amorphous character of biofilms by decreasing the intermolecular attraction between polymers [112], and organic antifoam was used to reduce foam formation of pectin. The steps of film casting are shown in Figure 4.2. Films were usually dried in a laboratory oven at 40 °C.

The first specimens of orange waste film had a yellowish colour; they were opaque and flexible, with holes present in the structure (Figure 4.3). The next task, the improvement in morphology, that is, the elimination of holes, was carried out via the establishment of better adhesion between the polymeric chains in different ways.

4.3.2 Comparison of drying methods

To answer the question of whether holes can be eliminated by reducing the arising stresses, another drying method was compared with the laboratory oven: a shaking incubator was used to provide continuous rotary movement, to interfere with the migration of particles and the stresses arising during evaporation (Paper I). The rotary movement improved the film structure and eliminated the holes, although it resulted in uneven thicknesses (Figure 4.2). As it is important for a material to have a consistent thickness, investigations on improving the adhesion between polymeric chains continued.
Figure 4.2 Casted films made from orange waste. The final product in the picture was dried in a shaking incubator (see chapter 4.3.2). The unevenness of the thickness is visible through the changes in the intensity of the colour and the ruffled film edge.

Figure 4.3 Orange waste powder and biofilm prepared from orange waste via the original method. Holes can be seen in the structure.

4.3.3 Pre-treatment of orange waste with maleic anhydride

In a study [103], corn starch was esterified with a dry method using maleic anhydride to replace the hydrophilic hydroxyl groups with hydrophobic ester groups [113] in order to induce some hydrophobic characteristics in the starch. In another study, pectin was grafted
with maleic anhydride using a wet method, to enable chemical cross-linking between polymer chains via the presence of vinyl sites for use in hydrogels and scaffolds [114]. There, maleic anhydride was chosen for its biocompatibility and high reactivity compared to other methacrylate derivatives.

Following this idea, orange waste was modified with maleic anhydride by the wet method in accordance with Almeida et al. [114] in order to change its chemical structure and thereby induce better adhesion between polymeric chains when used in a biofilm. Grafting of orange waste with maleic anhydride was successful (Paper II) via esterification and resulted in several major chemical and physical changes. The new material, maleic anhydride-grafted orange waste had almost 40 % of its hydroxyl groups replaced by ester-bonded maleic anhydride side groups, had a low specific surface area of 2.18 m²/g, and a reduced density (lower by approximately 40 %) that would be preferred for biocomposite production as a filler material (Figure 4.4).

Maleic anhydride-grafted orange waste was then used for film casting by applying the same method as for orange waste; smooth film production was expected, but it was less successful (Figure 4.5).

Figure 4.4 Maleic anhydride-grafted orange waste powder has a medium brown colour.
4.3.4 Upgrading film forming to a sol-gel method

The improvement in the adhesion between the polymeric pectin chains was tested by first solubilising pectin and then gelling it [55]. The original film casting method was then further developed to a ‘sol-gel’ method, where conditions for dissolving pectin and conditions for pectin gelling were applied sequentially (Paper IV) to enhance pectin bonding within the gel, which can trap other compounds. This step is meant to exclude organic antifoam and reduce the number of chemicals used, but glucose was added to enhance pectin gelling [55]. This step was expected to reduce the number of holes in the structure. The sol-gel method improved pectin binding and reduced the number and size of the holes, although some were still present.

4.3.5 Mixing maleic anhydride as a film component in orange waste biofilms

To further improve their morphology, maleic anhydride was mixed as a component in biofilms, on the basis of the previous discussion. The addition of maleic anhydride was expected to both facilitate the adhesion between polymeric compounds for structural development and reduce the hydroxyl groups available to interact with water molecules, so that the hydrophilicity of the films would improve. The addition of maleic anhydride to the films using the sol-gel method was the last step in the biofilm development process described in this thesis (Paper IV).

When maleic anhydride was used as a compatibilizer in high concentrations (100, 50, 25, 12.5, 6.25, and 3.13 %), the films became whiter, less ductile, and opaquer, and no holes were present. When small ratios were used (≤ 1.56 %), the features of the sol-gel method were
typical, but holes were not present (Figure 4.6). The improved films looked homogeneous and flexible, and low concentrations of maleic anhydride had no adverse effect on the colour of the orange waste and previous films.

![Image](image_url)

Figure 4.6 Orange waste powder and biofilms prepared with the addition of low-concentration maleic anhydride in the sol-gel method.

4.3.6 Effect of maleic anhydride, glycerol, and sugar on film properties

Lastly, the effects of and interactions between maleic anhydride, glycerol, and sugar were analysed through a 3-2-2 factor experiment, where the concentration of maleic anhydride was reduced to as low as 0.39%, and the effects of doubling the concentrations of sugar and glycerol were also studied (Paper IV).

The main results of that study and a comparison of the major results of films made via different methods and from different materials are presented in Table 4.1 (see chapter 4.4).

4.3.7 Biofilms from apple pomace

Apple pomace was treated in accordance with the original method for film production (Paper V), and apple pomace films with and without the removal of soluble sugars and without the use of glycerol were also made.

Films made from apple pomace regardless of the preparation parameters exhibited a brownish colour with a slightly more transparent appearance than orange waste films, and holes were not present (Figure 4.7). The smoother surface and structural properties of apple pomace films are a result of the difference in the chemical composition of the raw materials. According to the compositional analyses of the raw materials, apple pomace had a higher fraction of cellulose and hemicelluloses, contained less pectin, and had a relatively high
content of lignin, compared to orange waste (lignin content, however, was not analysed for orange waste, as the literature suggested a very low content (less than 1 %) [115], and therefore it was not of interest to us). In this case, lignin could have the main effect on the apple pomace film structure. It has been reported [116] that the presence of lignin in polylactic acid blend, for example, was responsible for the strong intermolecular interactions between the two polymers.

![Figure 4.7 Biofilm prepared from non-washed apple pomace with the addition of glycerol, as plasticizer. The films have a smooth surface and resemble the colour of milled apple pomace.](image)

### 4.4 Key results

A comparison and summary of the processing parameters/materials/major mechanical results of films is presented in Table 4.1.

The difference due to the use of the sol-gel method compared to the original method was mainly the improved film structure in that there were fewer holes; the values for strength and elongation, however, remained in the same range. The addition of maleic anhydride in high concentrations (100 – 3.13 %) resulted in reduced strength compared to the previous two methods, and as expected, the opposite was observed for the elongation. Because the presence of maleic anhydride increased the elongation, it could be concluded that it acted as a plasticizer. Lower concentrations of maleic anhydride and the effects of different components were then further studied, and it was also proved that glycerol affected the tensile strength. Neither maleic anhydride nor sugar had any significant effect on the strength. The elongation showed somewhat more complicated results, as all three components had a significant effect on it. Glycerol and sugar had positive effects, whereas maleic anhydride had both positive and
negative effects. High glycerol and high sugar produced the highest elongation, while the same result was observed for 0.78 % maleic anhydride. A positive interaction between glycerol and sugar as well as an interaction between maleic anhydride and sugar were also observed. A definite conclusion can then be drawn; the presence of maleic anhydride in orange waste films was positively affecting the elongation, whereas it had no effect on the tensile strength.

Table 4.1 Comparison of tensile strength (TS) and elongation at max. (E) of biofilms made from orange waste (OW) and apple pomace (AP) containing maleic anhydride (MA), sugar (S), and glycerol (G) in different concentrations is represented with the standard deviations (st. dev.) and preparation conditions. Apple pomace was either washed (WAP) to remove impurities and the soluble sugars or was used non-washed (NWAP).

<table>
<thead>
<tr>
<th>Material</th>
<th>Method</th>
<th>MA (%)</th>
<th>CA (%)</th>
<th>S (%)</th>
<th>G (%)</th>
<th>TS (MPa)</th>
<th>St. Dev.</th>
<th>E (%)</th>
<th>St. Dev.</th>
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<td>2.35</td>
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<td>0.70</td>
<td>37.39</td>
<td>10.38</td>
</tr>
</tbody>
</table>

1These results are from a previous experiment

2Dried in a rotary incubator

Morphological studies (Figure 4.8) reveal that the addition of maleic anhydride (Figure 4.8 c, d) in the film structure did produce a more compact and smoother material, compared to the material produced without such addition (Figure 4.8 a, b). Washed apple pomace films with the addition of glycerol (Figure 4.8 g, h) have a very similar structure as the ones made orange waste by the sol-gel method and the addition of 0.39 % maleic anhydride (Figure 4.8
e, f), except for the impurities or other components that are visible in the case of the apple pomace films.

Figure 4.8 Surface and cross-sectional micrographs of films made from orange waste with the sol-gel method (a, b, respectively), using 25 % maleic anhydride (c, d, respectively), using 0.39 % maleic anhydride and low concentrations of glycerol and sugar (e, f, respectively) and films made of washed apple pomace with glycerol (g, h, respectively), showing the more compact structure when high-concentration (25 %) maleic anhydride was used (c, d) compared to the others and apple pomace films containing impurities or other visible substances (g, h) compared to orange waste films.
4.5 Degradation of biofilms

The biodegradability of the next generation plastics in the new plastics economy is a key feature. The biodegradability of biofilms was measured via anaerobic digestion. The bacterial inoculum was obtained from a large-scale thermophilic (55 °C) biogas plant (Borås Energi och Miljö, Borås, Sweden), and tests were performed in batch reactors in accordance with a previous study [117]. The degradation results of the different biofilms are summarised in Table 4.2.

<table>
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<td>95</td>
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<td>59</td>
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<td>59</td>
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The Table shows that incubator-dried and oven-dried films biodegraded to 90 % within approximately 15 days, and the degradation of the other two specimens was somewhat slower, with a maximum of 63 % during the same period of time. One reason for the slower biodegradation could be the difference in the quality of the inoculum that was used for studying the biodegradation of the sol-gel and sol-gel-ma films (the studies were performed at different times). The characteristics of a microbial community in a digester depend on the actual composition of the food waste mixture, and therefore it is not guaranteed that the same microorganisms with the same enzyme activity would be available at different times. The different microbial community can result in different degradation rates, which makes it difficult to compare degradation at different times.

4.6 Proposed applications for biofilms

Anaerobic digestion is the preferred method for the treatment of the organic fraction of municipal solid waste, resulting in a form of renewable energy. The process is as follows: the food waste together with the collecting bags go through a pre-treatment step, in which the whole content is pressed through a start press. During this step, the bags are broken apart to smaller fractions, and the food waste is turned to a slurry, which is then sent to the digester. Bigger plastic parts are removed with this step (that fraction is called the ‘reject’), but smaller ones can escape and end up in the slurry. There are two aspects to consider: (1) the reject can contain valuable organic matter that otherwise could be used for anaerobic digestion, and (2)
the escaped plastic particles will end up in the environment. After anaerobic digestion, the remaining digestate may undergo post-composting before it ends up on the fields in the form of a fertiliser. Even in countries with advanced technologies, complications arise around the collecting bags for the organic fraction of municipal solid waste. In Sweden, for example, the most commonly used collecting bags are paper bags. Paper bags work well for anaerobic digestion; however, due to their hydrophilic nature, they often leak prior to reaching the biogas plants (Paper III). There are two other types that do not leak: (1) fossil-based bags, which are strong and suit optical selecting technologies, and (2) compostable bioplastic bags. Pieces of fossil-based bags will eventually end up in fields, and potentially in the natural water systems. But the challenge among these three alternatives is the biggest with the compostable bags. First, compostable bags create a technical issue, by becoming stuck in pumps and screws, and second, they are also pollutants in the digestate. If post-composting is not performed after the anaerobic digestion treatment, pieces of compostable bags will also end up in fields, where conditions will not meet the industrial composting conditions. Even though bags that are not removed during the pre-treatment step or if post-composting is not performed comply with the EN 13432 standard the contamination is present.

On the basis of these experiences, the professional community would greatly appreciate a new type of material that would biodegrade anaerobically during the hydraulic retention time of a biogas plant [118-120]. Orange waste biofilms developed in this thesis could fulfil the criteria of anaerobic degradation within the operating conditions of a biogas plant; therefore, one of their main uses could be as collecting bags for the organic fraction of municipal solid waste, or as food packaging. However, the water-sensitivity of the material would need to be improved.

An edible film is defined as a packaging material which is a thin layer of edible material placed on or between food components [121]; because apple pomace is entirely edible, it has good potential to be further developed for the purpose of edible food packaging.
Chapter 5

3D objects from fruit residues

5.1 Fibreboards

The solution casting method for biofilm preparation shows easy laboratory-scale processability, although it involves a relatively large amount of water and long evaporation time. Solution casting is applied in a few commercial processes, mostly in the medical industry; however, it is a slower and more energy-consuming method compared to extrusion [122]. In general, compression moulding (placing a mouldable material in a shaping mould, which is then exposed to high temperature and pressure) of sheets is studied as a precursor to extrusion, in order to demonstrate material flowability and fusion and identify conditions suitable for extrusion [123].

From the economic and environmental viewpoints, compression moulding is an interesting way of producing rigid materials because it is fast and requires no solvent [124]. Plant fibres, also having a lower carbon footprint than polymers or synthetic fibres [125] without the addition of a polymer, resin, or a binder, would make the ideal eco-composite [126, 127]. The elimination of synthetic polymer matrices would also mean that no coupling agents or fibre treatments would be needed, resulting in more environmentally friendly materials. An attractive route towards binder-less high-performance cellulosic materials is the application of the self-binding ability of cellulose networks, exploiting the enhanced hydrogen-bonded network present in the cellulose, resulting in good mechanical properties [126]. Binder-less fibreboards have earlier been reported using low-pressure moulding (around 10 MPa) and the thermo-triggered self-binding ability of natural fibres [128]. In accordance with Mobarak et al. [128], the ability of the particles to pack up closely is critical in self-bonding. If the starting material consists of various components, such as cellulose, hemicelluloses, lignin, and proteins or short polysaccharides, the contribution of each component to the bonding remains somewhat unclear [127]. It has been reported, though, that the self-binding ability of polymers is mostly dependent on the melting or glass-transition and degradation reactions of lignin and hemicelluloses, and the presence of sugars and proteins can also play a major role [127]. The general trend in developing self-binding fibreboards has
been the use of lignocellulosic raw materials, such as softwood, where the lignin content is as high as at least 20% and the pressing temperatures are high, approximately 200 °C, to enable in situ plasticization and lignin flow in order to completely bind cellulose fibres together [129-131].

To control the brittleness of biopolymers and to lower the shaping temperature, the addition of a plasticizer is usually necessary [124]. Plasticizers are low-molecular-weight components, usually liquids. The small plasticizer molecules allow chain mobility while occupying positions between the large polymer chains, effectively increasing the interchain distance with a reduction in the secondary intermolecular bonding [132]. The presence of plasticizers improves flexibility, ductility, dispensability, and extensibility while at the same time, it reduces cohesion, rigidity, stiffness, and hardness of a material [99, 132].

5.2 Development of 3D objects

Orange waste has previously been reported to enhance the mechanical properties of petrochemical- and natural-plastics-based composites as a filler material [133-135]. Pectin with the addition of glycerol has also been reported for film formation via compression moulding [136] and extrusion [122]. On the basis of those studies and the self-binding ability of polymers, the hypothesis that orange waste and apple pomace would form binder-less fibreboards and cup shaped 3D objects was tested via pressure moulding (Paper V).

5.2.1 Compression moulding of fibreboards and cups

The processing parameters were adapted from a previous study [136], where the optimal polysaccharide-to-glycerol ratio was 70:30; however, specimens were also made without the addition of glycerol. The best resulting cases (Figure 5.1) of the mixtures were subjected to a pressure of 8 MPa for 20 min at 100 °C.

A comparison of fibreboards made from orange waste with the ones made from apple pomace (Paper V) (Figure 5.1), showed that the latter resulted in firmer and more compact sheets. The higher fraction of cellulose and hemicelluloses, and a relatively high lignin content (>20%), in all likelihood ensured more effective binding of the cellulose fractions based on hydrogen bonding and the in situ bonding of lignin [126], however the authors reported higher temperatures than 100 °C. Fibreboards made from apple pomace containing glycerol had an unexpected very dark brown colour, while the ones that contained no glycerol retained the colour of the milled powder. The darkening could be the effect of the reaction between glycerol and lignin under the applied conditions. Firm fibreboards could not be formed from orange waste without the addition of glycerol or when the size of the particles was 1 mm.
5.3 Key results

A comparison and summary of materials and major mechanical results of fibreboards are presented in Table 5.1.

Table 5.1 Comparison of tensile strength (TS) and elongation at max. (E) of fibreboards made from pure pectin, orange waste (OW), washed apple pomace (WAP), and non-washed apple pomace (NWAP) is shown with standard deviation (st. dev.) and preparation conditions. Processing parameters for the above sheets were the same (8 MPa pressure, 20 min, and 100 °C).

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<th>Particle size (mm)</th>
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<th>OW (%)</th>
<th>NWAP (%)</th>
<th>G (%)</th>
<th>TS (MPa)</th>
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<th>E (%)</th>
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</tbody>
</table>
To summarise the results, the use of smaller particle sizes resulted in a somewhat higher strength. This could be explained by the evidence of fillers of smaller sizes and more regular shapes having the capability to pack up closer, improving the ability to transfer the stresses in a composite [128]. Both, apple pomace and orange waste were able to form a binder-less fibreboard. The combination of the materials increased tensile strength compared to apple pomace and orange waste only; and the highest strength and lowest elongation was achieved when apple pomace was present at 25%. The trend observed for mixed biomaterials is in correlation with the plasticizing effect of the naturally occurring sugars in apple pomace. The reason why the biomaterial prepared from apple pomace only, had less strength compared to the mixed biomaterials, could be because of a possibility of an optimum concentration of sugars necessary for gaining the highest strength. The mechanical properties of the produced biomaterials can be compared with a commercial product, Biotrem™ (Warsaw, Poland), a 100% compostable and edible plate made of wheat bran (having a tensile strength of 3.22 ± 0.63 MPa and an elongation of 0.18 ± 0.08%).

Morphological studies performed on orange waste biomaterials with 30% glycerol content (Figure 5.3 a, b) and non-washed apple pomace binder-less fibreboards (Figure 5.3 c, d) support the assumption of better adhesion when a higher content of cellulose, hemicellulose, and lignin is present in the structure.

![Figure 5.3 Surface and cross-sectional micrographs of fibreboards made of orange waste containing 30% glycerol (a, b, respectively), and a more compact structure of non-washed apple pomace without addition of glycerol (c, d, respectively) can be seen.](image-url)
5.4 Degradation of biomaterials

To study biodegradation of orange waste biomaterials sheets under aerobic conditions, they were buried in soil in accordance with a previous study [137]. To summarise, specified sizes of sheets were buried in soils obtained from the forest and nutrient-enriched soil purchased from the supermarket. Both soil experiments were kept indoors as well as outdoors under ambient conditions. Because the experiment was begun in February, when temperatures were below zero and snow was present, the degradation was expected to progress more slowly for specimens kept in ambient conditions, compared to the ones that were kept indoors. The degradation was monitored as long as the specimens disintegrated, for a maximum of 70 days. The results (Figure 5.4) are indicating that orange waste biomaterials disintegrated after 14 days, in ambient conditions and the degradation was much slower in laboratory conditions, regardless of soil quality.

Another study on the degradation of orange waste biomaterial cups was performed by planting *Epipremnum aureum*, commonly known as the money plant or devil’s ivy, which requires little care, into cups; then together they were planted into a pot of soil in order to study the behaviour of the plant while the material degrades. Reference plants were planted in soil, without the orange waste cups; three plants were used in each case. The plants were photographed once a week, for four weeks. The last photograph was taken eight months later. Figure 5.6 shows the photographs of certain stages of the degradation and plant growth.
Figure 5.6 Effect of the degradation of orange waste biomaterials on the growth of *Epipremnum aureum*. The pots containing the orange waste cups are numbered 1, 2, and 3. Week 1: Orange marks of the disintegrated cups. Week 3-5: Plants need to adjust changed soil conditions. Month 8: plants have longer stems and more leaves.
The disintegration of orange waste cups happened within the first week of the experiment. Earlier stages (until week 5) showed that the presence of orange waste in soil affected plant growth negatively, and extensive fungal growth (best seen on image ‘week 3’, no. 2: white spots) was present around the particles. However, as time passed, the plants recovered, and those that had the orange waste biomaterial present in the soil finally had more leaves and longer stems. Probably after the first ‘shock’, the plants adjusted to the soil conditions, and as additional nutrition was available, the plants showed more growth.

Both degradation studies showed that orange waste biomaterials are able to degrade in soil, and after two weeks the specimens that were subjected to water could not be re-collected.

5.5 Proposed applications for biomaterials

Despite the negative aspects of exposing orange waste to the environment, a study reported enhanced flora and fauna growth after orange waste was deposited on landfill years earlier [138]. This fact has sparked the idea of a potential application of orange waste biomaterials as degradable pots that can be planted in soil together with a plant. Soil-degradation studies showed good degradability of orange waste biomaterials, but because they were too sensitive to water, they might not be the best option for plant-able pots, unless the structure can be further improved.

Biomaterials produced from apple pomace showed easier processability and better stability than those from orange waste, just by observation; because of their nice flavour, they could be potentially used as edible tableware, for instance. The Biotrem™ wheat-bran-based edible plate, which is available on the market, has very similar mechanical properties as the biomaterials produced in this thesis, and is equally sensitive to water. Consequently, cups or plates made from apple pomace biomaterials could be a gluten-free option as a post-meal snack.
Conclusion and future suggestions

6.1 Conclusions

This thesis proved the hypothesis that biofilms can be fabricated from orange waste and apple pomace via the solution casting method; it was also proved that a binder-less particleboard can be formed from orange waste and apple pomace using low-pressure moulding. The following major conclusions can be stated:

• The environmental impact of orange and apple juice industries could be reduced by using their by-products in new applications, however a detailed study would be necessary (e.g. Life Cycle Assessment) in order to know how much the impact can be reduced.

• Production of biofilms with the solution casting method using suspensions of dried orange waste and apple pomace powders is possible, with the properties obtained being comparable to those of certain commodity plastics.

• Orange waste film morphology can be influenced by the type of drying and the addition of other substances, such as sugar and maleic anhydride, which exhibits good behaviour as a coupling agent or compatibilizer.

• As little as 0.4 weight % of maleic anhydride seemed beneficial in eliminating holes, producing a smooth and uniform orange waste film structure.

• The tensile strength of orange waste films was clearly influenced by the concentration of glycerol, whereas the elongation showed somewhat more complicated results, as all three – sugar, glycerol, and maleic anhydride – had a significant effect on it.

• Apple pomace films had a uniform structure (holes were not present) and however the tensile strength was half or even less than that of orange waste films, the elongation values were ten-fifteen times higher than orange waste without maleic anhydride.
• Anaerobic digestion, a preferred method for the treatment of food waste, is an effective method for the biodegradation of certain bioplastics, including orange waste films; however, it is difficult to draw clear conclusions because of the possible differences in the actual microbial community structure. Therefore, more research is needed to study the anaerobic digestion of bioplastics (aiming among others, the future standardisation of the process).

• Furthermore, pre-treatment of dried orange waste powder with maleic anhydride made the material behave in a less hydrophilic manner under certain conditions and reduced its density by approximately 40%, resulting in a material that is suitable as a filler in biocomposites.

• Compression moulding is an energy-efficient method for the preparation of fibreboards from orange waste and apple pomace, compared to the solution casting method.

• The properties of the produced fibreboards are within the range of a commercial bioproduct.

• The biodegradation of the orange waste biomaterial was faster in ambient compared to laboratory conditions, and eventually had a positive effect on plant growth.

• Apple pomace showed easier processability, whether for film or for fibreboard production.

• The hydrophilicity of both films and fibreboards is, however, a negative property that needs further research.

6.2 Future suggestions

To develop fully degradable bioplastics with the potential for use as a bioplastics bag that would disintegrate within the hydraulic retention time used for an anaerobic digestion process, some future developments are essential:

• A major issue is that the hydrophilicity of the films should be improved, e.g. with the use of biodegradable coatings, for example polyhydroxyalkanoates.

• Scaling up of film casting production, e.g. in the way hand-made paper bags are made and the feasibility and/or life-cycle analysis of such a process.

• The percentage and the measuring method (carbon or biomass content) should be clearly stated and calculated when making a ‘bio-based claim’.

• More studies on apple pomace films are required, e.g. gas barrier properties, degradation, food migration.
Researchers may move away from the solution casting method and evaluate the potential of solubilising the polymers with green chemistry methods, suitable for e.g. extrusion.

In the case of the use of fibreboards as potential edible plates or cups, the following further developments are needed:

- Further characterisation of apple pomace fibreboards, e.g. contact with water, food migration, antimicrobial resistance.
- Improving the structure and resistance by applying, e.g. an industrial pressing machine (at high pressure).
- A hydrophobic, edible coating if necessary, e.g. natural waxes of plants.
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Paper 1
Research Article

Production of Pectin-Cellulose Biofilms: A New Approach for Citrus Waste Recycling

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Received 24 July 2017; Revised 23 September 2017; Accepted 28 September 2017; Published 29 October 2017

Academic Editor: Arthur J. Ragauskas

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While citrus waste is abundantly generated, the disposal methods used today remain unsatisfactory: they can be deleterious for ruminants, can cause soil salinity, or are not economically feasible; yet citrus waste consists of various valuable polymers. This paper introduces a novel environmentally safe approach that utilizes citrus waste polymers as a biobased and biodegradable film, for example, for food packaging. Orange waste has been investigated for biofilm production, using the gelling ability of pectin and the strength of cellulosic fibres. A casting method was used to form a film from the previously washed, dried, and milled orange waste. Two film-drying methods, a laboratory oven and an incubator shaker, were compared. FE-SEM images confirmed a smoother film morphology when the incubator shaker was used for drying. The tensile strength of the films was 31.67 ± 4.21 and 34.76 ± 2.64 MPa, respectively, for the oven-dried and incubator-dried films, which is within the range of different commodity plastics. Additionally, biodegradability of the films was confirmed under anaerobic conditions. Films showed an opaque appearance with yellowish colour.

1. Introduction

Plastics production has increased enormously in the past 100 years, and a global production of 322 million tons was reported by Statista [1] for 2015. This vast number of plastic products caused severe plastic pollution by now and they are typically made from nonrenewable sources. On the contrary, bioplastics are made from renewable sources or they are biodegradable; in the best-case scenario, they are both. Today, biopolymers are produced from cultivated crops; however, the land used for bioplastic is still negligible [2].

An example for a biobased and biodegradable material that is built up of different biopolymers, with no land use, is citrus waste. Citrus waste is a globally abundant and environmentally challenging waste that is underutilized [3]. Among citrus fruits, sweet oranges are the most commonly grown tropical fruits worldwide [4]. USDA [5] forecasted 45.8 million tons of sweet orange production for 2015/16. Industrial orange processing, for example, orange juice production generates about 50–60% residue of the original mass of the orange [6]. This vast quantity of waste is high in organic matter content (approx. 95% of total solids) and water (approx. 80–90%) and has a low pH (3-4) and inappropriate handling could cause severe damage to the environment [6]. Orange waste also contains pectin, soluble sugars, hemicelluloses, cellulose, starch, protein, lignin, ash, fat, and flavonoids [7, 8], which have been shown to be beneficial to many yet imperfect disposal and recovery applications [6]. These compounds on the other hand could be interesting for bioplastics applications.

Orange waste has already been applied as a reinforcement in petrochemical or biobased matrices [9–11]. In all of these cases, the authors reported increased mechanical properties of the products compared to the neat polymer. The increased mechanical properties are most probably the direct effect of the present cellulosic fibres. However, pectin, the major component of orange peel, seems to have no significant effect in the above-mentioned composites. Nevertheless, pectin-based composites have been prepared with different reinforcing substances [12] and cellulosic plant fibres have
certainly been of great interest because of their favourable mechanical properties as a potential substitute for glass fibres [13] in biocomposites. Cellulose reinforced pectin composites have been developed, for example, for tissue engineering applications [14] and for food packaging applications [15] from commercial sources. However, there is no study on directly using cellulosic fibres and pectin obtained from citrus waste to prepare a biofilm.

This study investigated transforming orange waste into a biobased film as well as evaluating its properties. As a result of our study, a new function was given to orange waste and biofilms were developed without prior chemical modification of the raw material. Films were prepared with a film-casting method. The orange waste film (OWF) is plant-based and biodegradable and represents competitive mechanical properties with some of the commodity plastics. Therefore, with further improvements, OWF could be potentially used for nonstructural applications, for example, as a sustainable packaging material for the food industry.

2. Materials and Methods

2.1. Materials. Orange waste (OW) was obtained from Bråmhults Juice AB (Borås, Sweden). Until further processing, the OW was stored at ~20°C. Citric acid (monohydrate, >99.5%, Duchefa Biochemie, Netherlands), glycerol (>99%, ARCOS Organics, Belgium), organic antifoam 204 (Sigma-Aldrich, USA), and pectins (P9311, P9436, and P9561, Sigma-Aldrich, USA) were other materials used in this study.

2.2. Pretreatment of Orange Waste for Film Preparation. OW was washed with water to extract soluble sugars. Firstly, the material was soaked in tap water overnight; then, two further washing steps followed. The water (L) to OW (kg) ratio for all steps was 1.5 : 1. Each washing included shaking the OW in a water bath at 115 rpm for 20 min at 35°C. After each washing, the OW was collected from the flask using a metal sieve and rinsed under tap water. Size of the washed OW particles was reduced with a knife and they were dried for 16 h at 40°C. Dried OW was milled to a fine powder using a ball mill (Retsch MM 400, Germany) at a frequency of 30 Hz, for a total of 40 min, allowing the equipment to cool down after each 10 min milling (preliminary tests were also performed for 10, 20, and 30 min).

2.3. Formation of Orange Waste Films. A mixture of 2% (w/v) of OW powder was prepared in 1% (w/v) citric acid solution under constant magnetic stirring while heating up to 70°C. The acid solution also contained 7% (w/v) glycerol and 1 drop of organic antifoam/100 mL solution. The suspension was sieved through a metal sieve to eliminate nascent air bubbles before it was poured onto PTFE plates and dried at 40°C. Each plate contained 30 g of suspension and the diameter of the plates was 100 mm. The drying process was performed in either a laboratory drying oven (Termaks TS9026, Norway) or an incubator shaker (New Brunswick™ Scientific Excella® E24, USA) rotating at 50 rpm. In the following, films dried in the oven are referred to as oven-dried (OD) and in the incubator as incubator-dried (ID). Properties of OD and ID films were compared in the study.

2.4. Characterization of Orange Waste and Biofilms. Soluble sugars were removed from the orange waste before any compositional analyses.

2.4.1. Dry and Moisture Contents. Dry content of wet OW and moisture content of dry OW powder were determined by drying the samples at 105°C until constant weight. Tests were performed in duplicate.

2.4.2. Carbohydrate Content. Structural carbohydrate content of the OW powder was determined according to NREL/TP-510-42618 [17] via hydrolysis with hot sulphuric acid. The different carbohydrate fractions were identified and quantified by high-performance liquid chromatography (HPLC) (Waters 2695, Waters, Milford, USA), using a lead(I)-based column (HPX-87P, BioRad) with two MicroGuard Deashing (Bio-Rad) precolumns operated at 85°C with 0.6 mL/min ultrapure water as eluent.

2.4.3. Pectin Content. Pectin content of the OW was measured by extraction. Pectin was extracted using a laboratory scale high-performance microwave digestion system (Milestone Ethos UP MA182, Italy). Size-reduced OW (20 g) was mixed with 45 mL of acidified water (25 mL of distilled water and 25 mL of 0.1 M HNO₃) to gain pH 2 [4]. The mixture was transferred into a PTFE vial with a total volume of 100 mL and was treated at 120°C for 10 min at a maximum power of 500 W. The treatment followed a 10 min ramping while the mixture was continuously stirred with a magnet stirrer. After the extraction, the mixture was cooled to room temperature and the solids were separated using vacuum filtration through a grade 3 filter paper. Pectin precipitation was performed according to Srivastava and Malviya [18] with slight modifications. The filtrate was adjusted to pH 4.5 [4] with 2 M of NaOH and cooled to 4°C before it was precipitated with ethanol under continuous stirring for 15 min. Ethanol to filtrate ratio was 2 : 1. The mixture was kept at 4°C for another 2 hours to allow pectin floatation, before being filtered through a cheesecloth to collect the extracted pectin. The pectin was then washed with absolute ethanol and dried to constant weight at 40°C.

2.4.4. Degree of Esterification of Pectin. A Fourier transform infrared (FTIR) spectrometer (Thermo Scientific, Nicolet iS10, USA) was used to measure the degree of esterification (DE) of the isolated pectin. Nicolet OMNIC 4.1 software was used to generate spectral data, which were analysed by Essential FTIR® (eFTIR, USA) software. The analysis was performed according to Oliveira et al. [19] with some modifications. Pectins with known values of DE (27 ± 7%, 62.5 ± 7.5%, and ≥85%) were used as standard. Determination of DE was based on the band areas at 1700–1750 cm⁻¹, corresponding to methyl esterified galacturonic acids (EGA), and at 1600–1630 cm⁻¹, corresponding to the free galacturonic acids.
(FGA), and calculated according to the equation obtained from the calibration curve after plotting the standard values:

\[ \text{DE} = 75.308R_A + 29.923, \]

\[ R^2 = 0.9588, \]

\[ R_A = \frac{A_{\text{EGA}}}{A_{\text{FGA}}}, \]  

(1)

2.4.5. Morphology. The morphology of film surface and transversal sections was investigated by FE-SEM imaging (Zeiss, Sigma, Germany). For surface visualisation, films were attached to a carbon tape and covered with gold. For transverse visualisation, images of cross section of the films were also taken. Each film was immersed in liquid nitrogen for one minute; then it was broken and immediately attached to a carbon tape on a stub. Following that, the samples were coated with gold and images were taken. Photomicrographs were taken at 1000 magnifications, using an accelerating voltage of 10 and 25 kV.

2.4.6. Mechanical Testing. A tensile test was performed according to ISO 527, using Tinius Olsen H10KT universal tester and QMat software package. A moving cross-head was used to pull the dumbbell shaped specimens apart with a load cell of 250 N and a test speed of 10 mm/min. Specimens with a gauge length of 22 mm and a width of 4 mm were tested in triplicate and the averages are reported as tensile strength (MPa), elongation at break (%), and elongation at maximum tensile strength (%).

2.4.7. Thermal Analyses. Thermogravimetric analysis (TGA) (Q500 TA instruments, Waters LLC, USA) was performed to determine chemical film properties, such as decomposition. Approximately 6 to 8 mg of each sample was heated from room temperature to 700 °C at a rate of 10 °C/min. The analysis was performed under nitrogen atmosphere. Tests were done in triplicate, and an average is reported.

A differential scanning calorimetry (DSC) analysis (Q2000 TA Instruments, Waters LLC, USA) was performed to determine thermal properties. Approximately 6 to 8 mg of the biofilm sample was heated in an aluminum pan from −20 to 200 °C at a rate of 10 °C/min in the first scan and in the rescanning. The analysis was done under nitrogen atmosphere. Tests were done in triplicate and an average is reported.

A dynamic mechanical thermal analysis (DMTA) (Q800, TA Instruments, Waters LLC, USA) was performed to determine dynamic mechanical properties, operated in a multifrequency strain mode, using a tension film clamp. The samples were cut in a typical width of 5.3 mm by a length of 8 to 9 mm and examined at a heat rating of 3 °C/min from 0 to 140 °C, at a strain frequency of 1 Hz, and at amplitude of 15 μm. Tests were done in triplicate.

2.4.8. Antimicrobial Test. The antimicrobial activity of the films was investigated by cultivating Escherichia coli (ATTC 25922, received from Södra Alvsborgs Sjukhus Borås, Sweden), a food-related and easily growing bacteria, and Aspergillus oryzae (CBS 819.72, Centraalbureau voor Schimmelcultures, Netherlands), a type of mould, in 250 mL Erlenmeyer-flasks. The cultivation flasks contained 30 mL LB (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) and YPD (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose) broth, as well as 5 mL phosphate buffer (pH 7) to maintain the optimum pH for the mentioned microorganisms, respectively. A sample of 0.50 g of the film was added to each flask, except for the reference flask that contained only the microorganism. The test was carried out in duplicate for 24 and 36 hours, respectively, in an incubator shaker at 125 rpm and 30 °C. LB broth was inoculated with one colony, using an inoculation loop obtained from previously grown agar plates. YPD broth was inoculated with 3 mL spore solution of 5.41 × 10⁵ spores/mL obtained from previously grown agar plates. Bacterial growth was determined by measuring the optical density value of the broth with a spectrophotometer (Biochrom, WPA Spectrawave Si1200 Diode Array Spectrophotometer, UK) at a wavelength of 600 nm. Fungal growth was determined by measuring the sugar consumption in the broth with HPLC.

2.4.9. Biodegradability Test. Anaerobic digestion was performed in order to test the biodegradation of the films. Digestion was carried out in batch reactors according to a previous study [21]. Bacterial inoculum was obtained from a large-scale thermophilic biogas plant (Borås Energi & Miljö AB, Borås, Sweden). Total solids (TS) content and volatile solids (VS) content of the films were determined using a gravimetric method. Each 120 mL glass bottle of a working volume of 50 mL contained 35.5 mL inoculum, and the rest was water. Each bottle contained 0.15 g sample VS. The reactors were then flushed with a gas mixture consisting of 80% N₂ and 20% CO₂ for 2 min. The reactors were incubated for 30 days at 55°C. Reagents containing only water and inoculum were used as blanks, and pure cellulose (Avicel®, Sigma-Aldrich) was also used as a reference. The experiment was performed in triplicate. 0.2 mL gas samples were taken with a 0.25 mL syringe (VICI, USA). The gas composition was analysed using a gas chromatograph (Clarus 500, Perkin-Elmer, USA) equipped with a packed column (Perkin-Elmer, 6’ × 1.8’’ OD, 80/100, Mesh, USA) and a thermal conductivity detector (Perkin-Elmer, USA) with an injection temperature of 150°C. The carrier gas was nitrogen, and the equipment was operated at a 20 mL/min flow rate at 60°C. At the end of the digestion, the pH of the digestates was also measured.

2.4.10. Statistical Analysis. Statistical analysis of the data obtained from mechanical and thermal tests was performed using MINITAB 17 Statistical Software. Results were compared by obtaining a p value via t-test, with a 95% confidence interval.

3. Results and Discussion

The results of the compositional analyses of orange waste are summarized in Table 1. Although values depend on the source and on the conditions of treatment and isolation methods, the major component of OW is pectin, followed by
Table 1: Chemical composition of OW according to the literature [20] and the results obtained from material characterization (% (w/dry w)).

<table>
<thead>
<tr>
<th>Compound/content</th>
<th>[20] (%)</th>
<th>Characterization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin</td>
<td>42.5</td>
<td>29.84 ± 0.29</td>
</tr>
<tr>
<td>Cellulose</td>
<td>9.2</td>
<td>18.66 ± 0.48</td>
</tr>
<tr>
<td>Hemicelluloses</td>
<td>10.5</td>
<td>20.89 ± 0.89</td>
</tr>
<tr>
<td>Soluble sugars</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Lignin</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>4.4</td>
<td>about 30</td>
</tr>
<tr>
<td>DE of pectin</td>
<td>42.72 ± 1.10</td>
<td></td>
</tr>
<tr>
<td>Dry matter of OW</td>
<td>9.84 ± 1.21</td>
<td></td>
</tr>
<tr>
<td>Moisture content of OW powder</td>
<td>9.58 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

Hemicellulose and cellulose. In this study, pectin, cellulose, and hemicellulose fibres gained main focus for biofilm preparation. Biofilms from OW were produced by the direct conversion of orange waste (Figure 1) with no prior chemical treatment of the waste. The physical and thermal properties of the films are influenced by both the cellulose and hemicelluloses fractions and the surrounding pectin phase. This new approach could be a solution to the problems associated with orange waste handling.

3.1. Details of the Method for Production of Biofilms from Orange Waste. Soluble sugars were removed from the OW during the pretreatment steps because preliminary experiments showed that when the sugars were not removed, the film surface was heterogeneous and cracked. To obtain a homogeneous OW powder to result in the most compatible film structure, different milling times (10, 20, 30, and 40 min) were tested. 40 min milling showed satisfactory results based on visual observation. Particle sizes were mostly between 125 and 75 μm. Since particle size could not be controlled with ball milling, a variety of the sizes were present in the OW powder.

Films were prepared by a casting method in which cellulose and hemicelluloses fibres were suspended in the pectin solution and further dried to a film. The orientation of the cellulosic fibres in the films could not be aligned and therefore, the particles were randomly distributed. Pectin film formation as well as previous studies on OWF formation showed that pectin forms foam on the surface of the mixture (data not shown); therefore, organic antifoam was added to the solvent liquid. Initial investigations on drying at 30, 40, and 50°C as well as at room temperature were performed. Drying temperature for solvent evaporation seemed not to be sufficient when films were dried at room temperature or at 30°C. When 50°C was applied, cracks were formed in the films. The higher temperature resulted in faster evaporation of the solvent at the edges of the film, thus creating a presumably more intensive replacement of liquid from the centre towards the edges, as explained by Routh [22]. In order to reduce the liquid flow in which the dispersed particles could be carried towards the edges (coffee-ring effect) [22], the temperature was reduced to obtain a slower evaporation of the solvent. Oven drying at 40°C resulted in no cracks, only a few small holes in the films were visible, and about 7 hours were enough for complete drying. Once the temperature was optimized, another drying method was also evaluated to eliminate the holes. An incubator shaker was then used to provide continuously rotating movements. The continuous movement of liquid and particles avoided the so-called coffee-ring effect as explained above. As a result, ID films without holes were produced, though the thickness of those films was uneven. The thickness of OD films was 0.09–0.10 mm, while ID films were more irregular. For mechanical characterization, the most uniform part of the ID films was chosen (0.08–0.10 mm), and the average thickness was used for analysis. By appearances, the biofilms are opaque and have a yellowish colour. The method for production of biofilms from orange waste is schematically presented in Figure 1.

3.2. Morphology of Orange Waste Films. The FE-SEM images shown on Figure 2 reveal that a “second layer” (highlighted...
on the image) was formed on the surface of OD films. In drying films, where there are more than one type of particles, a common phenomenon is self-stratification, which means that one particle ends on top of another [22]. As OWFs are made of a complex mixture of dissolved and undissolved polymers this phenomenon under the “normal” drying conditions seems to occur. When the “normal” drying conditions were disturbed with continuous rotation, this phenomenon was not visible; instead it resulted in a smooth and uniform surface of the ID films. The transversal sections confirm that continuous rotation resulted in a more homogenous material: pores are visible in the structure of OD films, while there are no clear pores in ID films. The few voids that can be seen could be the result of pulling the cellulosic fibres out during fractioning. Since there was no alignment of the fibres, the magnitude of these voids is low.

3.3. Mechanical Analysis of Orange Waste Films. Tensile strengths of the OWFs produced in this study were measured between 28 and 36 MPa (Figure 3). Yu et al. [15] varied the ratio between LM pectin and CMC to prepare nanocomposites by adding up to 8% w/w of montmorillonite reinforcement to the solutions. The tensile strengths of the unreinforced polymers were measured from approximately 16 to 32 MPa, and with reinforcement the tensile strength increased. The tensile strengths of the OWFs are also in the same range for some of the most commonly used polymers, such as low density polyethylene (LDPE), high density polyethylene (HDPE), polytetrafluoroethylene (PTFE), polypropylene (PP), and polystyrene (PS) [16]. A comparison is shown in Figure 3. Elongation however of the same commodity plastics (LDPE 100–650%; HDPE 10–1200%; PTFE 200–400%; PP 100–600%) is much higher than of OWF, except for PS (1.2–2.5%) [16].

3.4. Thermal Characteristics of Orange Waste Films. The results of the thermogravimetric analysis are represented as Table 2 and Figure 4. In both drying cases, the trend was similar, and thermal degradation consisted of three stages. The first thermal event was a weight loss up to 3.79–3.94% at an average temperature of 80.38–80.61°C due to the evaporation of water in the samples. This could be explained by biopolymers and glycerol being hygroscopic substances, and moisture content is dependent on the ambient conditions. The second thermal event occurred at a maximum temperature of 251.89–253.20°C, and the decomposition ratio was 28.54–29.28%, which was related to the depolymerisation of the pectin present in the films [23]. The third thermal event was observed at a maximum temperature of 351.89–354.34°C; the decomposition of the films showed a further 24.10–25.15% that could be referred to the decomposition of the cellulose [24]. The remaining 21.97–23.20% of the films after thermal treatment is ash.
Table 2: TGA thermogram showing decomposition rate of the OWFs: moisture loss, decomposition ratio 1, decomposition ratio 2 and residue and the maximum temperatures related to the thermal events.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moist. loss (%)</th>
<th>MLT (°C)</th>
<th>D1 ratio (%)</th>
<th>DT 1 (°C)</th>
<th>DT 2 (°C)</th>
<th>Residue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD 1</td>
<td>4.07</td>
<td>81.44</td>
<td>27.65</td>
<td>252.58</td>
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<td>26.77</td>
<td>252.6</td>
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<td>81.49</td>
<td>33.43</td>
<td>250.5</td>
<td>23.12</td>
<td>353.91</td>
</tr>
<tr>
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<td>3.94</td>
<td>80.61</td>
<td>29.28</td>
<td>251.89</td>
<td>25.15</td>
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<td>0.86</td>
<td>1.24</td>
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Figure 3: Left axis: tensile strength of different commodity plastics [16] and tensile strength at maximum load of OD and ID films are shown; right axis: elongation at maximum tensile strength (white bars) and elongation at break (grey bars) belonging to OD and ID films are shown (elongation at break of commodity plastics is not shown in the image due to the great difference of values).

Figure 4: TGA thermogram showing weight loss curves and derivative weight curves of OD and ID films.

The films were also analysed using differential scanning calorimetry (Figure 5) to determine glass transition temperature (Tg). An endothermic transition was detected around 83.29 and 88.12°C, with an onset temperature of 61.21 and 66.22°C during the first scanning of the ID and OD samples, respectively. After the rescanning of the samples, the endothermic transition disappeared. Aguilera et al. [25] also reported that endothermic curves of biopolymers had disappeared after rescanning the samples and thus there was no Tg observed. As there was no Tg observed in the thermograms of OWFs, there was no melting point either. It is then assumed that the OWFs have less crystals present and they are mostly amorphous.

DMTA characterization was carried out in order to compensate DSC analysis and to monitor the temperature dependence of the storage modulus, tanδ, and the loss modulus. The results are shown in Figures 6 and 7. The storage modulus (Figure 6) is a measurement of the stiffness of the material, and both ID and OD films showed similar behaviours. The sample OD had a storage modulus of 1.4 GPa at 10°C. As the temperature rose, there was a clear decrease of the storage modulus. At 70°C, the storage modulus decreased to 0.6 GPa. In other words, the sample showed a more elastic behaviour at higher temperatures and a more plastic behaviour (storing energy rather than returning it) at low temperatures, which is a typical characteristic of polymers. The other sample, ID, had a storage modulus of 1.0 GPa at 10°C and 0.4 GPa at 70°C. While OD generally had a somewhat higher storage modulus, the difference between the two samples was not significant. The temperature dependence of tan δ can be seen in the same figure. Under the chosen test conditions, tanδ continuously increased with the increasing temperature. For the sample OD, a weak transition could be seen at 98°C. For ID, no clear transition was observed.
3.5. Antimicrobial Activity of Orange Waste Films. D-limonene, the major component of orange oil, is in turn well-known for its protecting behaviour against pathogens [26]; thus it would indicate an antimicrobial property of the films. However, the results obtained from the optical density measurements and glucose consumption, showed that films did not have antimicrobial property. In both cases, the length of the lag phase and the exponential phase of the microorganisms were the same for blanks and for the samples containing the films. One reason could be that orange oils were removed during the sugar removal pretreatment step, which was similarly performed as the cold pressing method, a preferred method for extracting essential oils from citrus fruits [27], and is usually done by soaking the citrus fruit in warm water and pressing the rind with a sponge to collect the oil.

3.6. Biodegradability of Orange Waste Films. Biodegradability is a key feature of next generation plastics and the European Committee for Standardization specifies a 90% degradation within six months [28]. In the anaerobic degradation study, films reached 90% degradation in about 15 days (Table 3). Figure 8 shows the methane accumulation based on the theoretical maximum (350 NmL/g COD) of a substance by measuring the COD.
Table 3: Degradation (%) of ID and OD films (results based on average) by days.

<table>
<thead>
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<th>Deg. time (d)</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
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<th>25</th>
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<tr>
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<td>95</td>
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</tr>
<tr>
<td>OD</td>
<td>20</td>
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<td>81</td>
<td>89</td>
<td>95</td>
<td>95</td>
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</table>

4. Conclusions

The biofilms made from orange waste show similar physical properties as do some of the commodity plastics. It was observed that the drying method did not make a difference for mechanical and thermal properties of the films but resulted in a more uniform surface. The films were also biodegradable under anaerobic conditions. The main constituents of the biofilms, pectin and cellulose fibres, suggest an application where biodegradability is as important as strength, such as a short-lived packaging material. Generally, the properties are promising, although further characterization and improvements are necessary in order to achieve the desired features, less hygroscopic characteristics, for example. Finally, production of biofilms from orange waste not only opens up opportunities for the production of environmentally friendly biomaterials but also could be a solution for the challenges arisen upon disposal of orange waste.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this article.

Acknowledgments

The authors would like to express their gratitude to ÅForsk Foundation for financial support and to Brämhults Juice AB for the raw material. The authors are also grateful for the technical help received from Lukitawesa, Ilona Sárvari Horváth, Ramkumar B. Nair, and Jorge A. Ferreira and from Christine Räisänen for feedback on the structure and language.

References


Paper 2
Synthesis and Characterization of Maleic Anhydride-grafted Orange Waste for Potential Use in Biocomposites

Veronika Bátori,a,* Mostafa Jabbari,a Rajiv K. Srivastava,b Dan Åkesson,a Patrik R. Lennartsson,a Akram Zamani,a and Mohammad J. Taherzadeh a

The purpose of the study was to develop a less hydrophilic, and therefore more useful, material from orange waste produced in large quantities by the food industry. A new derivative of industrial orange waste was synthesized via esterification with maleic anhydride. The reaction was confirmed via Fourier transform infrared spectroscopy (FTIR), and the degree of substitution of the hydroxyl groups was 0.39 ± 0.01, as determined by a back-titration method. A major change in physical structure was confirmed by scanning electron microscopy (SEM). The flake-like structure of orange waste changed to a sponge-like structure after the reaction, which involved an increased volume and a reduced density by approximately 40%. The sponge-like structure was represented as an agglomeration of particles with a low specific surface area of 2.18 m²/g and a mean pore diameter of 10.7 nm. Interestingly, the grafted orange waste seemed to become more hydrophobic, which was confirmed by contact angle and water dissolution tests; however, the material absorbed more water vapor. Thermogravimetric analysis (TGA) confirmed a thermally more uniform, though, less heat-resistant material. This work suggests a possible way of utilizing orange waste via synthesizing a renewable material with possible applications as a filler in biocomposites.

Keywords: Biopolymers; Esterification; Grafting; Maleic anhydride; Orange waste; Pectin

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INTRODUCTION

In a zero-waste approach, the valorization of the abundantly available lignocellulosic materials has been gaining elevated attention for the production of a wide range of applications (Arevalo-Gallegos et al. 2017; Bilal et al. 2017; Iqbal et al. 2017). Such products include bio-chemicals, bio-fuels, animal feed, enzymes, and biocomposites (Asgher et al. 2017; Ahmad et al. 2017). The use of natural fibres and biopolymers has been of great research interest for the production of biocomposites and bioplastics. While commodity plastics are hydrophobic substances, most of the natural fibres bear hydrophilic properties. Biopolymers are also hygroscopic substances, and when used they result in thermoplastics sensitive to water (Zuo et al. 2013). In general, these hydrophilic plastics have low mechanical properties, and blending is necessary with water-resistant polymers to obtain good mechanical properties (Zuo et al. 2013). In polymeric blends and composites, interfacial adhesion between the components plays a crucial role in achieving adequate physico-mechanical features (Zhang and Sun 2004; Zuo et al. 2013; Yu et al. 2014). Establishing the necessary adhesion between the hydrophilic biopolymers or natural fibres and the hydrophobic commodity plastics is a major challenge. In such cases when interfacial tension is low, compatibilizers are often used to enhance the interfacial adhesion of the often immiscible substances to overcome the weaknesses (Khalid et al. 2008; Arias et al. 2013).

Maleic anhydride has been used as a compatibilizer to modify polysaccharides (Zhang and Sun 2004; Zuo et al. 2013; Yu et al. 2014). In one study, the less polar poly-lactic acid was
grafted with maleic anhydride in order to make it compatible with the polar ramie fibres (Yu et al. 2014). In another study, corn starch was esterified with maleic anhydride to replace the hydrophilic hydroxyl groups with hydrophobic ester groups (Samain et al. 2011) for developing some hydrophobic characteristics in the starch (Zuo et al. 2013). The modification of biopolymers with maleic anhydride also enables chemical cross-linking between polymer chains through vinyl sites (Almeida et al. 2015). Maleic anhydride is an industrially available and bio-based material that is also used in the cosmetic and food industry (Fischer Scientific 2018; PubChem 2018). Therefore, it is a safe material for the fabrication of environmentally friendly biomaterials.

An example for a lignocellulosic raw material for the fabrication of biomaterials is orange waste. The global orange production in 2017/18 was forecast to tumble to 49.3 million tons from the previous year (USDA 2018) and e.g. juice production produces at least 50% waste of the initial mass. Orange waste is a by-product with low use; however, it contains interesting biopolymers, such as pectin, cellulose, and hemicellulose. Therefore it can potentially be used for producing bio-based materials in the bioplastic industry (Rezzadori et al. 2012; Lopez-Velazquez et al. 2013). A drawback of orange waste is the hygroscopic nature of pectin and cellulose, which makes it difficult to blend with any other hydrophobic polymer, whether natural or synthetic.

The goal of this study was to reduce the hydrophilicity of orange waste by grafting with maleic anhydride, thereby making it suitable for applications, e.g., in biocomposites and polymer blends. By grafting of maleic anhydride into the orange waste structure, several major chemical and physical changes occurred. The results were analysed by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC), and tests were performed to examine the density, water affinity, specific surface area, degree of substitution, and contact angle.

The prepared maleic anhydride-grafted orange (OW-MA) could potentially be used as a filler for a biopolymer such as polylactic acid (PLA). PLA is still an expensive polymer and, since orange waste is cheap, renewable and available in huge quantities, adding it to a biopolymer would reduce the cost. Furthermore, for many biopolymers, there is still a need to improve the technical properties. The addition of a filler could modify some of the technical properties. As an example, PLA has been reinforced with various renewable materials such as wood flour (Zhang et al. 2018). The feasibility of OW-MA as a filler merits future study.

EXPERIMENTAL

Materials

Orange waste (OW), consisting of peels, pulp, and seeds was obtained from Brämhults Juice AB (Borås, Sweden). According to a previous analysis (Bátori et al. 2017), OW contained 29.84 ± 0.29% pectin, 18.66 ± 0.48% cellulose, and 20.89 ± 0.89% hemicelluloses. Until further use, the OW was stored at -20 °C.

Pretreatment of Orange Waste for Esterification

Soluble sugars in the OW were removed prior to use by dissolution in water, where the ratio of OW to water was 1:1.5 (kg/L) in all washing steps. First, the OW was soaked in tap water overnight at room temperature. Two further washing steps were conducted at 35 °C for
20 min. The OW was collected using a metal sieve and rinsed under tap water after each washing step. The OW was cut to small pieces with a knife and further dried at 40 °C for 16 h according to a previous study (Bátori et al. 2017). The dried OW was milled to a fine powder using a variable speed rotor mill (Pulverisette 14, Fritsch, Idar-Oberstein, Germany) with a sequence of sieve sizes of 1 and 0.2 at 10,000 rpm, for a maximum of 1 min each.

**Synthesis of Maleic Anhydride-grafted Orange Waste**

Orange waste and maleic anhydride were pre-dried in a vacuum oven (Vacucell, Buch & Holm, Herlev, Denmark) (at less than 0.05 bars) at 40 °C for 12 h prior to use. The synthesis was performed as described by Almeida et al. (2015), with two major differences. N,N-dimethylformamide (DMF) was replaced with dimethyl sulfoxide (DMSO), a safer and less toxic solvent, and the dialysis step was not performed. Briefly, the dried OW (1 g) and MA (3 g) were dissolved in 10 mL and 15 mL of DMSO solvent, respectively, and continuously stirred at room temperature for 12 h. The MA-solution was added to the OW-solution dropwise under continuous stirring at room temperature. The mixture was maintained for 24 h at 70 °C. The grafted OW was then precipitated in acetone (200 mL) under continuous stirring and separated by vacuum filtration. Instead of the dialysis step, the filtered maleic anhydride-grafted orange waste (OW-MA) was washed with acetone three times, after filtration. The material was vacuum-dried at 20 °C under 0.5 bars pressure for 12 h. The pressure was then reduced to less than 0.05 bars and drying was continued for 24 h. The same reaction was also performed on pure pectin (PEC), as a reference material, and some comparative analyses were carried out on maleic anhydride-grafted pectin (PEC-MA).

**Characterization of Maleic Anhydride-grafted Orange Waste**

**Determination of degree of substitution of hydroxyl groups**

The degree of substitution (DS) was determined by titration according to Zou et al. (2013), with slight modifications. 0.5 g of dried OW-MA was weighed accurately and placed in a 100-mL bottle. Then, 10 mL of 75% ethanol solution and 10 mL of 0.5 M NaOH (aq) solution were added. The closed bottle then was stirred and kept at 30 °C for 30 min. The excess alkali was then back-titrated with 0.5 M standard solution of HCl (aq), and a blank titration was carried out using unmodified OW. The DS was calculated according to the following equations,

\[
W_{MA} = \frac{M_{MA} \times C \times (V_0 - V_{sample})}{1000 \times 2 \times W_{sample}} \times 100\% \\
DS = \frac{M_{OW} \times W_{MA}}{M_{MA} \times (100 - W_{MA})}
\]

where \( W_{MA} \) is the content of MA substituted (%), \( M_{MA} \) is the molecular weight of MA (98 g/mol), \( C \) is the concentration of HCl (aq) solution, \( V_0 \) is the volume of HCl (aq) consumed by the blank sample (mL), \( V_{sample} \) is the volume of HCl (aq) consumed by the esterified sample (mL), and \( M_{OW} \) is the theoretical molecular weight of OW (166 g/mol) based on a previous component analysis (Bátori et al. 2017).

**Fourier transform infrared measurements**

An FTIR spectrometer (Nicolet iS10, Thermo Fisher Scientific, Waltham, USA) was used to collect spectra of the modified OW. Spectra were recorded by Nicolet OMNIC 4.1 software from 500 to 4,000 cm\(^{-1}\), and 64 scans were averaged. Essential FTIR® (eFTIR, Madison, WI, USA) software was then used to analyse the data.

**Specific surface area by Brunauer-Emmett-Teller analysis**

Gas sorption measurements were carried out using a Belsorp (Bel Japan, Inc., Osaka, Japan) apparatus with N\(_2\) gas at 77 K. The Brunauer-Emmett-Teller (BET) equation was used...
for calculating the specific surface area with WIBU-KEY software (Wibu Systems, Karlsruhe, Germany).

**Scanning electron microscopy**

Scanning electron microscopy of the samples was performed with an FEI Quanta 200F with Oxford-EDS system IE 250 X Max 80 equipped with a field emission gun (FEG) electron source (Thermo Fisher Scientific, Waltham, MA, USA). The samples were pre-coated with gold before SEM imaging.

**Thermogravimetric analysis**

Thermogravimetric analysis (Q500 TA instruments, Waters LLC, New Castle, DE, USA) was performed to determine the thermal properties of the substances. Approximately 5 mg of each sample was heated from room temperature up to 700 °C at a rate of 20 °C/min. The analysis was performed under nitrogen atmosphere and in triplicates.

**Differential scanning calorimetry**

Differential scanning calorimetry analysis was performed on a TA Instruments Q2000 apparatus (Waters LLC, New Castle, DE, USA). Approximately 6 mg of the modified OW sample was heated in an enclosed aluminium pan from -20 °C to 200 °C at a rate of 20 °C/min in the first scan and in the re-scanning under nitrogen atmosphere.

**Measuring water uptake capacity**

The water uptake of the samples was measured at 20 °C and at 30 °C by keeping 1 g of each sample in a climate chamber (TK 120 Test Cabinet, Nüve, Ankara, Turkey) with 85% humidity for 24 h. The water content of the samples was determined with a gravimetric method after 2, 4, 6, 8, 12, and 24 h.

**Contact angle**

Contact angle measurements were performed using an Attension Theta optical tensiometer (Biolin Scientific, Espoo, Finland). OneAttension software (Biolin Scientific, Espoo, Finland) was used for data collection and analysis. The sessile drop method was used to apply the droplet on the substrate surface. A 4 µL drop was dispensed on the surface, and the images were captured. Approximately 0.35 g of orange waste or grafted orange waste powder was weighed and then placed between two insulating polyester sheets. The sandwich was then pressed (78 MPa) for approximately 5 min to make a compressed film. The thickness of the obtained samples became less than 1 mm. The samples then were placed in the equipment for contact angle measurement.

**RESULTS AND DISCUSSION**

Industrial orange waste (OW), which is difficult to handle, was used in this study to develop a useful and renewable material that can potentially be used for the fabrication of biocomposites and biopolymer blends. For this matter, orange waste was modified with maleic anhydride. By grafting of maleic anhydride into the OW structure, several major chemical and physical changes occurred. First, a number of the hydrophilic hydroxyl groups attached to the OW were converted to the less polar ester groups, which are represented in the maleic anhydride side groups. Maleic anhydride is a bifunctional molecule in the structure, with one side connected to OW and the other side available to connect with another polymer chain, enabling it to crosslink polymer chains and to increase the dimensional stability. The less polar nature of the modified polysaccharide could trigger enhanced adhesion with a less polar or more
hydrophobic natural or synthetic resin. The results of the modification are interesting and, in certain cases, contradictory due to the complexity of the starting material.

Chemical Reaction of Maleic Anhydride with Orange Waste

The reaction of OW was performed with a degree of substitution of 0.39 ± 0.01, meaning that almost 40% of all possible hydroxyl groups in the substance had reacted with maleic anhydride. For the pectin, the degree of substitution was similar at 0.38 ± 0.03. In a study by Almeida et al. (2015), a similar method was used, except that the solvent was dimethylformamide, and a degree of substitution of 0.24 was obtained. The differences between the results might be because of the choice of solvent, which warrants further investigation. The reaction is demonstrated in Fig. 1.

![Fig. 1.](image)

Characterization of the Maleic Anhydride-grafted Orange Waste

Chemical structure

Maleic anhydride is prone to nucleophilic attack by nucleophiles, such as hydroxyl or amino groups, whereas hydroxyl groups that are commonly present in polysaccharides are prone to esterification reaction (Almeida et al. 2015). Esterification of OW was confirmed by FTIR spectroscopy (Fig. 2). As the largest fraction of OW is pectin (approximately 30%), the peaks describing the degree of esterification of pectin were also analysed. Changes of the broadband from 1750 cm⁻¹ to 1700 cm⁻¹ and from 1630 cm⁻¹ to 1600 cm⁻¹ were observed for esterified and free carboxyl groups, respectively (Putiev et al. 1964; Tsaryuk and Frantsesson 1991). The incorporation of maleic anhydride into OW and pectin increased the intensity of the ester carbonyl (C=O) stretching peak. Consequently, the increase of the area related to the peak at 1737 cm⁻¹ and 1734 cm⁻¹, respectively, was noticeable. Both peaks shifted slightly to the right and resulted in a higher peak at 1723 cm⁻¹ in both maleic anhydride-grafted pectin (PEC-MA) and maleic anhydride-grafted orange waste (OW-MA), thus representing the ester linkages between maleic anhydride and the polysaccharides. A study claimed that the peak at 1632 cm⁻¹ was related to the vibration of vinyl groups (C=C) in the modified substance (Li et al. 2014). It is clearly visible in the spectra of OW-MA and PEC-MA that there is a peak at 1638 cm⁻¹, very possibly representing the vinyl groups; however, the peak could also be a modified version of the peaks referring to the free carboxyl groups at 1630 cm⁻¹ to 1600 cm⁻¹. The first case is the more probable one, as the peak shifted to the left (1638 cm⁻¹), and the carboxyl peak is exactly the same for PEC-MA and OW-MA, regardless of the initial composition of the carbohydrates. The potential masking effect caused by water absorption at 1631 cm⁻¹ has been neglected in this analysis as only dry powders were subjected to FTIR
testing. The broadband ranging from 3550 cm\(^{-1}\) to 3200 cm\(^{-1}\) corresponds to the hydroxyl groups (O-H) and for OW-MA and PEC-MA, a reduction of the peaks can be seen, providing further demonstration of esterification. The hydroxyl groups in the polysaccharides can form hydrogen bonds with the carboxyl groups arising from the hydrolysed anhydride (Zhang and Sun 2004), and could result in the shift of the carbonyl (C=O) peak. A double band (one stronger than the other) also appears at 1300 cm\(^{-1}\) to 1000 cm\(^{-1}\) in the modified spectra, representing C-O in the esters (Putiev et al. 1964). Two other smaller peaks show up at 3008 cm\(^{-1}\) and 3063 cm\(^{-1}\), referring to the C-H stretch of sp\(^2\)-hybridized carbons in maleic anhydride, which usually occurs at 3000 cm\(^{-1}\) to 3100 cm\(^{-1}\) (Wade 2014). The increased bands at 821 cm\(^{-1}\) are related to the out of plane deformation of carboxyl (-COOH) groups (Almeida et al. 2015), which arises in the maleic anhydride side groups. Changes in the FTIR spectra were remarkable, confirming the replacement of O-H groups with the maleic anhydride groups via ester linkages, which could demonstrate the reaction between maleic anhydride and the carbohydrates, as described.

![Fig. 2. Changes in FTIR spectra of orange waste (OW), OW-MA, pectin (PEC), and PEC-MA](image)

**Density and microstructure**

When determining filling material for composites, density must be considered. It is important to make the lightest possible material. During milling and weighing processes, the density of OW-MA was less than that of unmodified OW; therefore, its density was measured. The same mass (2.5004 g) of samples was placed in a measuring cylinder, and the filling volume was read at ~5.6 mL and at ~9.5 mL for orange waste powder and OW-MA, respectively. Therefore, the density of the sample was decreased by approximately 40% after the reaction with maleic anhydride.

The lower density could suggest an increased specific surface area. Compared with superabsorbent materials, the specific surface area of modified OW was rather low at 2.18 m\(^2\)/g. The mean pore diameter of OW-MA was 10.7 nm. According to Li et al. (2008), dried orange peel powder (with a particle size between 0.1 mm and 0.2 mm) has a specific surface area of 128.7 m\(^2\)/g and an average pore diameter of 3.05 nm. The explanation for the decrease in
specific surface area might be that the particles agglomerated during the reaction, which resulted in the increase of pore sizes, as well as the decrease of accessible surface area.

The proposal of the agglomeration of the particles is supported by SEM images, which was used to study the morphology of the grafted OW. The sizes of the OW-MA particles were generally greater than those of the OW particles, which can clearly be seen under the same magnification in Fig. 3 (A, B, C, and D). The structural change of OW-MA is also confirmed by Fig 3. During the esterification reaction, the structure of OW was changed from flake-like to a hollow and porous, sponge-like structure. The possible explanation for this change might be that at the molecular level, the previously packed polymer chains are no longer sitting close to each other because the incorporated maleic anhydride side chains are longer than the OH side groups. These peripheral chains provide a distance between the polymer chains and are responsible for opening up the structure. The same change in the structure can be seen in Fig. 3 E and F images taken on pure and modified pectin. The changes in the surface due to grafting of maleic anhydride are also reflected in a decreased specific surface area for the sample.

Fig. 3. (A and B) SEM (scanning electron microscopy) images of unmodified orange waste, (C and D) OW-MA (maleic anhydride-grafted orange waste) sample, (E) unmodified pectin, and (F) PEC-MA (maleic anhydride-grafted pectin) sample. By grafting orange waste with maleic anhydride, the chance of particle agglomeration increases. Scale bar values represent 25 µm. A: Mag=1.00 KX WD=6.5 mm EHT=20.00 kV; B: Mag=2.00 KX WD=6.5 mm EHT=20.00 kV; C: Mag=1.00 KX WD=6.5 mm EHT=20.00 kV; D: Mag=2.00 KX WD=6.5 mm EHT=20.00 kV; E: Mag=500 KX WD=7.5 mm EHT=20.00 kV; F: Mag=500 KX WD=11 mm EHT=20.00 kV.
Thermal properties

Because there was no major difference observed in the thermogram of grafted pectin and grafted OW, only one curve is shown. The degradation of the modified polysaccharides starts at a lower temperature than of the unmodified polysaccharides (Fig. 4). Degradation for both OW-MA and PEC-MA started with an onset decomposition temperature just above 100 °C; otherwise, it was above 200 °C for the unmodified samples. The possible explanation for this could be the same reason as discussed above: the introduction of peripheral chains broke the packed polymer structure and reduced the crystallinity of the material. Heat transfers easier in the modified structure, because there is more space between the chains due to the newly incorporated maleic anhydride side groups, which are longer in length than the short hydroxyl groups, and thus less energy is needed to decompose the polymers. Additionally, the oxygen present in maleic anhydride might catalyse the decomposition reaction (Jabbari et al. 2015).

A new thermal event is visible in both modified samples at approximately 150 °C. This first thermal event is attributed to the decomposition of the new, maleic anhydride side chains (decomposition pioneers), which counts for only approximately 30% of the decomposition in the first step.

In the thermal degradation curve of pure pectin, two major decomposition stages are observed that are not related to the release of water molecules (Aburto et al. 2015). However, the temperature ranges related to these two stages are slightly higher than what is discussed in the literature (Aburto et al. 2015). A possible explanation could be differences in the degree of polymerization of the samples. The second major thermal event occurred at approximately 240 °C and 260 °C for pectin and PEC-MA sample. Thus, the modified pectin required a slightly higher temperature to decompose than the unmodified pectin, but decomposition occurred at a lower rate (Fig. 5A). The higher thermal stability for PEC-MA than for pectin could be attributed to the modification, which was also concluded by Almeida et al. (2015).

In the unmodified OW sample, the first thermal event is related to the decomposition of pectin in orange waste at approximately 250 °C, counting for a slightly higher decomposition rate. The second event is attributed to the decomposition of cellulose in OW at approximately 355 °C with a residue approximately 30% of the sample. In the OW-MA sample, decomposition of pectin occurred as the second thermal event at approximately 280 °C, and in the third thermal event, the decomposition of cellulose occurred at approximately 304 °C. These events were shifted compared with the unmodified sample. The decomposition of pectin in OW-MA occurred at a higher temperature (shifted to the right, same as for pectin and PEC-MA), while cellulose decomposed at a lower temperature (shifted to the left), compared with the unmodified samples, but at a lower rate (Fig. 4B). A similar tendency of maleic anhydride treated cellulose can also be seen in the study performed by Li et al. (2014). One possible explanation for the behaviour of cellulose and hemicelluloses in OW-MA could be the reduced crystallinity in the modified structure that allows a better heat transfer. The complexity of orange waste compared with pure pectin would require further investigations in order to better understand the processes.

The low thermal stability of OW-MA would not be suitable for applications where withstanding high temperatures is a requirement. In contrast, OW-MA could be suitable for applications that require degradation in a certain time interval, i.e., a biodegradable material.

DSC analysis indicated endothermic transitions during the first scan of the OW-MA sample. As it was also reported for orange waste films (Bátori et al. 2017), the endothermic transition disappeared during the re-scanning (data not shown). Similar to the OW films (Bátori et al. 2017), it was concluded that OW-MA does not bear a glass transition temperature or a melting point under the observed conditions, and it is more likely to be amorphous than crystalline. The DSC diagram has been left out for the sake of brevity.
Fig. 4. (A) TGA thermogram of pectin (PEC) and PEC-MA, (B) and of orange waste (OW) and OW-MA. Diagrams are showing the weight % (left axis) and the derivatives of the weight % with respect to the temperature (right axis).

Affinity for water vapor and wettability

At relative humidity of 85%, the modified OW samples absorbed a higher amount of water vapour than the unmodified orange waste samples (Fig. 5). The higher water affinity could possibly be explained either by the changed structure or by the increased volume of the modified OW, facilitating the absorption of water vapour. Thus, in the modified OW, the water vapour molecules have a better access to interact with the carboxyl groups on the maleic anhydride and the remaining OH groups on the polymers, even though their number has been decreased. Contrary to these results, the contact angle test (Fig. 6) showed that the modified OW behaved more as a hydrophobic substance than the unmodified OW. The results of the
Affinity for water vapor and wettability

At relative humidity of 85%, the modified OW samples absorbed a higher amount of water vapor than the unmodified orange waste samples (Fig. 5). The higher water affinity could possibly be explained either by the changed structure or by the increased volume of the modified OW, facilitating the absorption of water vapor. Thus, in the modified OW, the water vapor molecules have a better access to interact with the carboxyl groups on the maleic anhydride and the remaining OH groups on the polymers, even though their number has been decreased. Contrary to these results, the contact angle test (Fig. 6) showed that the modified OW behaved more as a hydrophobic substance than the unmodified OW. The results of the
contact angle test, which is performed in a short time (a maximum of 10s), was significantly dependent on the surface properties of the materials. Replacement of the OH groups with maleic anhydride groups, in the modified orange waste, resulted in a more hydrophobic surface. However, during the long-time exposure (in the affinity test), the water vapor molecule can more easily penetrate through the more porous structure of the modified material and therefore, this material exhibited a higher water affinity compared to the original orange waste.

**Fig. 5.** Water uptake test was performed at 85 % relative humidity at 20 °C and 30 °C. The results are based on the average of triplicate samples of orange waste (OW) at 20 °C (solid line), maleic anhydride-grafted orange (OW-MA) at 20 °C (solid line with circles), orange waste (OW) at 30 °C (dashed line) and maleic anhydride-grafted orange (OW-MA) at 30 °C (dashed line with circles).

**Fig. 6.** (A) Water contact angle measurement of orange waste (OW) powder, and (B) maleic anhydride-grafted orange (OW-MA) powder

**CONCLUSIONS**

1. This study presents a new approach towards the valorisation of the lignocellulosic orange waste, which is produced abundantly during orange juice production. Therefore, grafting of orange waste was performed by replacing approximately 40% of the hydroxyl groups with maleic anhydride side groups via ester bonding.

2. The grafted orange waste behaved in a contradictory manner when interacted with water/water vapour: it had a higher affinity for water vapour (as a result of its high porosity),
but at the same time it showed more hydrophobic behaviour when the surface was directly exposed to water, compared to unmodified orange waste.

3. Esterification reaction made maleic anhydride-grafted orange waste to become lighter than orange waste. The change in density could be favorable for, e.g., composite fabrication.

4. The porous structure allowed a better heat transfer and created a thermally more uniform material. However, the maleic anhydride-grafted orange waste degraded at a lower temperature than orange waste; it could be more suitable for composites that are meant to biodegrade within a certain time interval.

ACKNOWLEDGMENTS

The authors are grateful to ÅForsk Foundation for financial support and to Brämhults Juice AB for providing the raw material. The authors also thank Jennie Nordin, Malin Jessen, and Zhino Muhammed for their practical assistance.

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Article submitted: March 5, 2018; Peer review completed: April 22, 2018; Revised version received and accepted: May 10, 2018; Published: May 15, 2018. DOI: 10.15376/biores.13.3.4986-4997
Paper 3
Anaerobic degradation of bioplastics: A review

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Article info
Article history:
Received 3 July 2018
Revised 19 September 2018
Accepted 24 September 2018

Keywords:
Anaerobic digestion
Biodegradation
Bioplastics
Food waste
Methane
Plastic bags

Abstract
Anaerobic digestion (AD) of the organic fraction of municipal solid waste (OFMSW), leading to renewable energy production in the form of methane, is a preferable method for dealing with the increasing amount of waste. Food waste is separated at the source in many countries for anaerobic digestion. However, the presence of plastic bags is a major challenge for such processes. This study investigated the anaerobic degradability of different bioplastics, aiming at potential use as collecting bags for the OFMSW. The chemical composition of the bioplastics and the microbial community structure in the AD process affected the biodegradation of the bioplastics. Some biopolymers can be degraded at hydraulic retention times usually applied at the biogas plants, such as poly(hydroxyalkanoate)s, starch, cellulose and pectin, so no possible contamination would occur. In the future, updated standardization of collecting bags for the OFMSW will be required to meet the requirements of effective operation of a biogas plant.

1. Introduction
Anaerobic digestion is a method for the treatment of the organic fraction of municipal solid waste (OFMSW) supported by legislation and principally used worldwide for its ability to produce renewable energy in the form of methane. Moreover, the nutrient-rich digestate residue can be utilized as a bio-fertilizer (Albanna, 2013). However, plastic bags, such as those used for OFMSW collection, are not separated properly and are a serious challenge for digesters, even in regions where advanced waste management technologies are used.

The OFMSW is mostly composed of food waste, accounting for up to approximately 45% of municipal solid waste (MSW) in Europe (Cerda et al., 2018) and 46% globally (Hoornweg and Bhada-Tata, 2012). Because of its high moisture content (approximately 80% or some reports as high as 85–90% (Albanna, 2013)) and organic matter content, the OFMSW is commonly treated via composting or anaerobic digestion in many countries, such as Sweden, Germany, and the Netherlands. While composting produces good quality soil fertilizer and heat that are difficult to collect (Smith et al., 2017), anaerobic digestion results in the production of biogas containing mostly methane and carbon dioxide that is easy to collect and use for renewable energy production. Therefore, from an energy perspective, anaerobic digestion is a preferable method for treating the OFMSW. If the OFMSW is separated with source sorted collection, a practice already applied in several countries, such as the Nordic countries, Germany and Austria, it is then usually collected in plastic or paper bags. However, the use of plastic bags can lead to plastic contamination in the biogas plants. A pretreatment method that is often applied in Sweden is pressing the OFMSW together with the collection bags through a star press, by which an easily degradable fraction, called a slurry, is formed, which is sent to the anaerobic digestion plant. The remaining part that does not go through the press is called “reject” and contains, among other components, most of the plastics, but it also contains a large amount of remaining organic matter that otherwise could be utilized for the production of methane. An alternative collection method for the OFMSW, which is applied in certain regions of Sweden, is the use of paper bags. However, some problems still can occur because paper bags can easily be damaged, and acidic leakage that causes corrosion problems in the collection trucks is common. Hence, biodegradable carrier bags, namely, bioplastic bags, could solve the problems mentioned above.

The use of bioplastics has been increasing worldwide for numerous applications, but since various bioplastics are relatively new products on the market, the disposal of bioplastics has been intensively investigated for the most appropriate implementation in existing disposal systems. Studies have thoroughly evaluated the different end-of-life options (Endres and Siebert-Raths, 2011) and the biodegradation and composting processes (Ahmed et al., 2018; Kale et al., 2007; Shah et al., 2008; Song et al., 2009) for bioplastics. A recent review (Ahmed et al., 2018) also highlighted the microbial strains involved in the degradation processes and the
application of certain types of non-biodegradable and biodegrad-
able polymers. However, to the best of our knowledge, there is a
lack of comprehensive reviews on the anaerobic digestion of bio-
plastics. Therefore, this review focuses on the degradation of bio-
plastics in anaerobic conditions while also considering the
requirements for smooth operation at biogas plants.

2. What are bioplastics?

Within the term bioplastics, we distinguish (a) bio-based and
(b) biodegradable plastics, but a bioplastic can also fulfill both of
these criteria. Bio-based plastics are typically made from renew-
able sources by the action of living organisms. They can be polysac-
charides (e.g., starches, such as thermoplastic starch (TPS);
cellulose, such as regenerated cellulose; pectin, and chitin), pro-
teins (e.g., wheat gluten, wool, silk, casein and gelatine), lipids
(e.g., animal fats, plant oils), or products of microorganisms (e.g.,
poly(hydroxyalkanoate)s (PHAs) such as poly(hydroxybutyrate)
(PHB)). Furthermore, bio-based plastics can also be chemically syn-
thesized from bio-derived products (e.g., poly(lactic acid) (PLA),
poly(butylene succinate) (PBS), and poly(trimethylene terephthal-
ate) (PTT)) (IJB, 2017; Song et al., 2009). Moreover, there is
another fraction of plastics that is bio-based but not biodegradable
that is called “drop-ins” (e.g., bio-PET, bio-PE, bio-PP) with identi-
cal features as their petrochemical ancestors. Moreover, biodegrad-
able plastics, with a certain degree of biodegradability, can also be
synthesized from petrochemical origins, such as poly(glycolic acid)
(PGA), poly(caprolactone) (PCL), poly(butylene succinate-co-
terephthalate) (PBST), poly(butylene adipate-co-terephthalate)
(PBAT) and poly(vinyl alcohol) (PVA). Some of these biodegradable
bioplastics are already on the market and produced by different
companies under different trade names. One example for PBAT is
Ecoflex®, Wango, Ecoworld®m, etc. The classification of different bi-
oplastics is illustrated in Fig. 1.

3. Degradation of bioplastics

3.1. Biodegradable plastics

Different polymer degradation pathways, such as photodegra-
dation, thermo-oxidative degradation and biodegradation, are well
discussed in the literature (Amass et al., 1998; Hawkins, 1984;
Shah et al., 2008). During biodegradation, the organic substances
are broken down by means of microorganisms. Several biodegrad-
ability tests exist. The so called “screening tests” are performed in
enzymatic or aquatic conditions, and the latter can be anaerobic or
aerobic. The “real-life tests” distinguish composting, soil burial and
field testing (Itävaara and Vikman, 1996; Lucas et al., 2008).
However, in many cases, the biodegradability of a bioplastic is highly
dependent on the properties of the plastic (Tokiwa et al., 2009)
because both the chemical and physical properties of plastics affect
biodegradation. These properties are the surface characteristics
(hydrophobic or hydrophilic, surface area), the first-order struc-
tures (molecular weight, molecular weight distribution, chemical
structure) and the higher order structures (crystallinity, crystal
structure, modulus of elasticity, glass transition temperature, melt-
ing temperature) of polymers. (Tokiwa et al., 2009).

Biopolymers are long-chain molecules, and in order to mineral-
ize the biopolymers (converting organic matter to minerals or
plant available compounds), an important, often abiotic chain scis-
sion has to occur prior to biodegradation (Fig. 2). During that pro-
cess, the long polymeric chains are broken down due to the effects
of temperature, water and sunlight (i.e., photodegradation) to
shorter oligomers, dimers or monomers. These shorter units are
small enough to pass through the cell walls of microorganisms
and be used as substrates for their biochemical processes and thus
can be degraded (Shah et al., 2008) by microbial enzymes (Fig. 2).
Two main types of enzymes are involved in microbial depolymer-
ization processes: extracellular and intracellular depolymerases
(Shah et al., 2008). As the term suggests, extracellular enzymes
act outside the cells in order to break the longer units down into
shorter molecules, preparing them for further degradation by
intracellular enzymes. As biodegradation can occur in two ways,
aerobically and anaerobically, it offers two types of biological
waste treatment. While the aerobic degradation of biopolymers
has been studied in detail with extended reviews written
(Briassoulis et al., 2010; Calmon-Decrèuil et al., 1998; Grima
et al., 2000; Kale et al., 2007; Meeks et al., 2015), the research on
anaerobic degradation of biopolymers is still in its infancy. This
review, therefore, aims to survey the anaerobic digestion of bio-
plastics. According to the ISO 17088:2012 standards, a plastic can
be considered biodegradable if a significant change in the chemical
structure, i.e., degradation, occurs in the exposed material resulting
in carbon dioxide, water, inorganic compounds, and biomass (new
microbial cell constituents) but no visible or toxic residues
(Standardization, 2012) under composting conditions. The CEN
standard, EN 13432:2000 (Standardization, 2000), for biodegrad-
able polymeric materials also requires that a substance is 90% con-
verted to carbon dioxide within six months as a condition for
composting in the presence of oxygen. For anaerobic degradation,
it specifies a minimum of 50% conversion of the substance to bio-
gas based on the theoretical value in a maximum of two months’
time (Standardization, 2000).

3.2. Aerobic and anaerobic degradation

Aerobic biodegradation usually means composting within
industrial composting conditions. In a high-oxygen environment
(not less than 6%) (Kale et al., 2007), microorganisms utilize the
polymer as a carbon and energy source and produce carbon dioxide
and water as the main degradation by-products in addition to the
remaining part, which is called compost (Eq. (1)). Industrially com-
posting is performed in a warm (approximately 60–70 °C) and
moist (approximately 60%) environment under controlled condi-
tions (pH 8.5) (Mohee et al., 2008):

\[
\text{Organic matter + S + O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{N}_2 + \text{SO}_2 + \text{Heat} + \text{Compost}
\]  

(1)

Anaerobic biodegradation usually means anaerobic digestion in
oxygen-free conditions in mesophilic (37 °C) or thermophilic
(55 °C) biogas plants. In the absence of oxygen, the organic matter
is converted to methane gas, carbon dioxide, water, hydrogen sul-
phide, ammonia and hydrogen, which results in a sequence of
metabolic interactions by different groups of microorganisms
(Mohee et al., 2008). The remaining part is called the digestion resi-
due (Eq. (2)):

\[
\text{Organic matter + H}_2\text{O + Nutrients} \rightarrow \text{Digesterate + CO}_2 + \text{CH}_4 + \text{NH}_3 + \text{H}_2\text{S} + \text{Less heat}
\]  

(2)

The energy stored in organic matter is released in the form of
heat during aerobic degradation, and it requires continuous turn-
ing of the biomass to release some of this heat for a healthy micro-
bial community. This heat is lost and cannot be captured. On the
contrary, in anaerobic degradation, the energy stored in organic
matter is mainly released as methane, and due to the lack of oxy-
gen in the process, less heat and less microbial biomass are pro-
duced (Fig. 2).

4. Biodegradation of bioplastics under anaerobic conditions

Extensive reviews of the various microbial communities are
mostly focused on aerobic degradation of plastics and are available
in the literature (Shah et al., 2008; Shimao, 2001). In addition to the nature of the polymeric substance, the biological degradation of polymers is usually influenced by the kind of microorganism involved in the biodegradation process. If the biodegradation process aims to produce methane gas, a mixture of cultures obtained from active biogas plants are used for the degradation tests. A summary of anaerobic degradation of various bioplastics is presented here.

4.1. Poly(hydroxyalkanoate)s

Poly(hydroxyalkanoate)s (PHAs) are a class of polyesters that accumulate in bacterial and archaean (Koller et al., 2017; Kourmentza et al., 2017) cells through fermentation of sugars and lipids as energy and carbon storage compounds (Lu et al., 2009). Poly(3-hydroxybutyrate) (P3HB) or commonly known as poly(hydroxybutyrate) (PHB) belongs to the PHA class. Other polymers that belong to the PHA class, e.g., poly(4-hydroxybutyrate) (P4HB), poly(3-hydroxyvalerate) (PHV), poly(3-hydroxyhexanoate) (PHH), poly(3-hydroxyoctanoate) (PHO) and their co-polymers, are produced by several types of microorganisms. PHAs that accumulate intracellularly can be degraded by intracellular depolymerase enzymes (Lee and Choi, 1999). However, in environmental conditions, extracellular enzymes are involved in the degradation process which can be produced and excreted by various microorganisms, such as the soil bacteria *Pseudomonas stutzeri* (Shimao, 2001) and *Alcaligenes faecalis* (Reddy et al., 2003; Shimao, 2001), a bacterium that also requires oxygen. *Rhodospirillum rubrum, Bacillus megaterium, Acinetobacter beijerinckii,* and *Pseudomonas lemoignei* are other organisms in which PHB depolymerases have been identified (Reddy et al., 2003). The primary enzyme for the degradation of PHB and its oligomers is PHB depolymerase, an extracellular endo-type hydrolase of *A. faecalis* (Reddy et al., 2003). While studying the anaerobic degradation of natural and synthetic polymers, Abou-Zeid et al. (2001) identified two poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)-degrading strains that showed strain resemblance to *Clostridium botulinum* (with approximately 95% 16s rRNA sequence homology).

According to Siracusa et al. (2008), PHAs are able to degrade to carbon dioxide and water in 5–6 weeks in aerobic conditions, but in anaerobic environments, the degradation is faster, and methane production is advantageous (Siracusa et al., 2008). In a study performed by Shin et al. (1997), PHBV (8% 3HV) reached approximately 85% degradation within 20 days in anaerobic sludge. In another study (Noda et al., 2009), degradation tests performed aerobically and anaerobically showed rapid biodegradation of 14C labelled poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) (PHBO) under both conditions, up to approximately 80% after 25 days under anaerobic conditions. A 16-day fermentation of PHB and PHBV (13 and 20% 3HV) was performed for methane and carbon dioxide formation using anaerobic sewage sludge by Budwill et al. (1992). The results showed that between 83 and 96% of the substrate carbon was transformed to methane and carbon dioxide (Budwill et al., 1992). According to Verlinden et al. (2007) a PHB sample can require a few months to degrade in anaerobic sewage and can require years in seawater. UV light, however, can acceler-
ate the degradation (Verlinden et al., 2007). Nodax™, a Procter & Gamble product that is used for manufacturing biodegradable agricultural films, can be degraded anaerobically, and consequently, it can be used as urea fertilizer (Philip et al., 2007). Nodax™ class PHA co-polymers are a family of promising PHA polyesters consisting of (R)-3-hydroxyalkanoate co-monomer units with medium-chain-length side groups (mcl-3HA) and (R)-3-hydroxybutyrate (3HB) (Noda et al., 2009). PHA co-polymers comprising mcl-3HA have lower crystallinity than PHB homopolymers of PHBV co-polymer, and thus, faster degradation can occur.

4.2. Starch blends

Starch is rarely used on its own for bioplastics production due to its hydrophilic nature and its poor mechanical properties. It is often modified chemically, physically or mechanically and/or blended with other polymeric compounds or plasticizers (Davis and Song, 2006). The starch content of a typical starch blend can vary from 5 to 90 wt% (Davis and Song, 2006). A Novamont product, Mater-Bi™ (MB), composed of a minimum of 60% starch and starch derivatives and of approximately 40% synthetic resin that is hydrophilic and biodegradable, was examined for anaerobic degradation in a study by Mohee et al. (2008). The authors measured the cumulative methane production over 32 days of batch digestion assays and compared it to that of the reference cellulose material. The results showed methane productions of 245 mL and 246.8 mL for MB and cellulose, respectively. In another study (Bastioli, 1998), a Y class (injection moulded rigid items made of thermoplastic starch and cellulose derivatives) MB product showed approximately 90% anaerobic degradability within 30 days. Russo et al. (2009) reported anaerobic degradation of thermoplastic starch (TPS) - PVA blended films in mesophilic conditions. The blends of TPS-PVA were varied at 90:10, 75:25, 50:50, and 0:100 (w/w%). The authors concluded that a higher quantity of starch in the blend resulted in increased biogas production, and starch degraded faster than PVA. They also noted that PVA affected the rate of starch degradation to a lesser extent.

4.3. Pectin-cellulose biofilms

Pectin-cellulose biofilms were produced from orange waste via a solution casting method, and the anaerobic degradation of biofilms was studied in thermophilic conditions (Bátori et al., 2017). Because orange waste consists of pectin (30%) and cellulose and hemicelluloses (approximately 40%), microcrystalline cellulose (Avicel™), was used as the reference for the degradation test. The study showed that 90% of the orange waste films were degraded in approximately 15 days, with an average corresponding methane production of over 300 NmL/g COD (there COD stands for chemical oxygen demand).

4.4. Poly(lactic acid)

Poly(lactic acid) (PLA) is a bio-based and biodegradable thermoplastic polyester, and the monomer, lactic acid, used in the polymerization processes, is commonly produced by fermentation. Despite its biocompatible nature, the degradation of PLA in the environment is not easy because, under ambient conditions, PLA in soil or in sewage was found to be resistant to microbial attacks (Hamad et al., 2015). As Hamad et al. (2015) explains, PLA must be hydrolysed first at elevated temperature (approximately 58 °C) to reduce molecular weight before biodegradation can start. The Amy-
Microorganisms could not consume the polymer after 28 days of digestion according to ISO 14853, and the authors concluded that the PLA did not degrade under anaerobic conditions at all during the 100-day-long investigation. Massardier-Nageotte et al. (2006) examined PLA degradation anaerobically at all during the 100-day-long investigation. According to Shin et al. (1997), PLA did not degrade under anaerobic conditions. According to Shimao et al. (1984; Suzuki et al., 1973) and anaerobic (Matsumura et al., 1986; Shimao et al., 1984; Suzuki et al., 1981; Watanabe et al., 1975) conditions (Matsumura et al., 1999). However, its biodegradability in diluted conditions is low (Pšeja et al., 2006). PVA-degrading microorganisms are omnipresent in nature (Shimao, 2001). Aerobic degradation of Poly(caprolactone) (PCL) is a synthetic fossil-based polyester that can smoothly be degraded by microorganisms. Just as PHA and PHB-degrading bacteria, PCL-degrading bacteria can be found in nature. However, while PHAs are degraded by depolymerases, PCL is degraded by lipases and esterases (Ruiz and Flotats, 2014). Murphy et al. (1996) found that the PCL depolymerase is in fact a cutinase produced by the fungal pathogen Fusarium solani. Cutinase degrades cutin, the waxy polymer that is known as the structural polymer of the plant cuticle. However, PCL degradation is controversial, as some authors did not find anaerobic biodegradability of the material (Abou- Zeid et al., 2001). Kale et al. (2007) reported that PCL did not biodegrade under anaerobic conditions when using ASTM D5511 and ISO 14,853 test methods. Contrarily, Abou-Zeid et al. (2001) made an interesting comparison between PHB, PHBV, and PCL degradation. The authors found that PCL was more resistant to an anaerobic attack than the natural polymers, but the anaerobic degradability of PCL in mesophilic conditions was clearly shown with two different types of anaerobic sludge. In their study, they also isolated four PHB- and PCL-degrading strains belonging to the genus Clostridium. The two PCL-degrading strains showed almost 92% strain resemblance with Clostridium acetobutylicum (16s rRNA sequence homology). Cho et al. (2011) studied the anaerobic degradation of PCL-starch blends according to ISO 14,853 and determined a biodegradability of 83% within 139 days. In comparison, as mentioned above, 92% degradation of PCL powder was achieved by Yagi et al. (2009a) at thermophilic temperatures in 47 days using the adapted sludge from a mesophilic biogas plant.

4.5. Poly(caprolactone)

Poly(caprolactone) (PCL) is a synthetic fossil-based polyester that can smoothly be degraded by microorganisms. Just as PHA and PHB-degrading bacteria, PCL-degrading bacteria can be found in nature. However, while PHAs are degraded by depolymerases, PCL is degraded by lipases and esterases (Ruiz and Flotats, 2014). Murphy et al. (1996) found that the PCL depolymerase is in fact a cutinase produced by the fungal pathogen Fusarium solani. Cutinase degrades cutin, the waxy polymer that is known as the structural polymer of the plant cuticle. However, PCL degradation is controversial, as some authors did not find anaerobic biodegradability of the material (Abou-Zeid et al., 2001). Kale et al. (2007) reported that PCL did not biodegrade under anaerobic conditions when using ASTM D5511 and ISO 14,853 test methods. Contrarily, Abou-Zeid et al. (2001) made an interesting comparison between PHB, PHBV, and PCL degradation. The authors found that PCL was more resistant to an anaerobic attack than the natural polymers, but the anaerobic degradability of PCL in mesophilic conditions was clearly shown with two different types of anaerobic sludge. In their study, they also isolated four PHB- and PCL-degrading strains belonging to the genus Clostridium. The two PCL-degrading strains showed almost 92% strain resemblance with Clostridium acetobutylicum (16s rRNA sequence homology). Cho et al. (2011) studied the anaerobic degradation of PCL-starch blends according to ISO 14,853 and determined a biodegradability of 83% within 139 days. In comparison, as mentioned above, 92% degradation of PCL powder was achieved by Yagi et al. (2009a) at thermophilic temperatures in 47 days using the adapted sludge from a mesophilic biogas plant.

4.6. Poly(butylene succinate)

Poly(butylene succinate) (PBS) is a synthetically produced thermoplastic and biodegradable aliphatic polyester. It is claimed to be biodegradable in certain environments, such as sea, river, soil, activated sludge and compost (Xu and Guo, 2010), but PBS does not seem to be biodegradable under anaerobic conditions. In a study, Cho et al. (2011) showed only 2% biodegradability of PBS under anaerobic conditions in 100 days according to the ISO 14,853 test method. In another study, PBS did not biodegrade at all using the same conditions (i.e., at thermophilic conditions using a sludge adapted from 37°C, where PBS showed 90% biodegradability in 14 days, and PCL and PLA showed 80% and 75% biodegradability in 50 and 75 days, respectively (Yagi et al., 2013).

4.7. Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is a synthetic vinyl polymer that is water soluble and biodegradable. It is the only polymer with a carbon – carbon backbone that can be degraded biologically under both aerobic (Mori et al., 1996; Sakazawa et al., 1981; Shimao et al., 1986; Shimao et al., 1983; Shimao et al., 1984; Suzuki et al., 1973; Watanabe et al., 1975) and anaerobic (Matsumura et al., 1993) conditions (Matsumura et al., 1999). However, its degradation rate, particularly the anaerobic degradation of PVA, is low (Pšeja et al., 2006). PVA-degrading microorganisms are not omnipresent in nature (Shimao, 2001). Aerobic degradation of PVA has been performed using mostly Pseudomonas strains (Sakazawa et al., 1981; Shimao et al., 1986; Shimao et al., 1983; Shimao et al., 1984; Suzuki et al., 1973) and Alcaligenes faecalis

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**Table 1**

Biodegradation rate of different sizes of PLA films (Yagi et al., 2012).

<table>
<thead>
<tr>
<th>PLA Film Size</th>
<th>20 days</th>
<th>30 days</th>
<th>40 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;500 µm</td>
<td>17%</td>
<td>50%</td>
<td>75%</td>
</tr>
<tr>
<td>1 x 1 cm</td>
<td>25%</td>
<td>59%</td>
<td>80-81%</td>
</tr>
<tr>
<td>15 x 34 cm and 39 x 82 cm PLA film</td>
<td>35-40%</td>
<td>70-75%</td>
<td>84%</td>
</tr>
</tbody>
</table>
Anaerobic degradation of bioplastics in biogas plants: challenges, standards, and suggestions

As shown in previous sections, different microorganisms degrade different biopolymers (Shah et al., 2008; Yagi et al., 2013). Thus, not all types of bioplastics are suitable for anaerobic digestion (Endres and Siebert-Raths, 2011; Kale et al., 2007). To determine which kinds of bioplastics are suitable for OFMSW collection, we need to keep in mind that the anaerobic digestion process goes through four typical degradation steps (i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis). These stages have been thoroughly discussed in the literature and are not the main focus of this review. This study focused on conditions needed for effective anaerobic degradation of bioplastics, such as degradation time, temperature, and microbial strains involved in the process. In other words, this study is meant to answer the question of whether a bioplastic bag is suitable for collecting the OFMSW with the aim of reducing the contamination caused by conventional plastic bags in biogas processes. A typical biogas plant treating OFMSW operates with a hydraulic retention time (HRT) of 15–30 days under thermophilic or mesophilic conditions. Therefore, a bioplastic bag suitable for collecting food waste should be able to degrade within these conditions. Some of the biopolymers discussed above degrade within the HRT applied for a biogas plant, such as PHB, starch, cellulose, and pectin. A general order of increasing biodegradation rate of bioplastics is as follows: PHB > PCL > PLA > PVA. However, PCL, PLA and PVA take longer than the usual HRTs used in biogas plants treating OFMSW. We can also conclude that PBS is not suitable for collection bags because it does not degrade under the conditions used at biogas plants.

Standards for bioplastic bags suitable for the collection of the OFMSW have not yet been established thoroughly due to the novelty of the process. First, standardization for biodegradable bags suitable for OFMSW collection should include some mechanical requirements. Tensile strength and water resistance are important aspects because a material that is too strong could be misused for other purposes, and a bag should withstand moisture until it reaches the fermenters but should undergo biodegradation at elevated temperatures. These two qualities are important in all plastics, if not the most important ones. Most conventional, petroleum-based plastics, such as low-density poly(ethylene) (LDPE) and high-density poly(ethylene) (HDPE) are water-resistant, and have enough appropriate mechanical properties to be used as plastic bags. Bioplastics, on the other hand, are more likely to be less water resistant because of the hydrophobic nature of the biopolymers used for their fabrication. Some bioplastics, such as PHA, PLA and PCL, are water resistant and can be used as coatings for other bioplastics, such as those made from starch or agar. Bioplastics are not yet versatile, and further research is needed for the functionalized production of bioplastics. Since the bioplastics industry is in its beginning phase, it is difficult to define how strong and how water-resistant a bioplastic bag has to be in order to be suitable as a food waste collection bag.

In regard to the biogas plants, according to the best of our knowledge, there are few published data available on bioplastic conversion or optimum treatment conditions for the AD process. Nevertheless, there are methods for determining the anaerobic biodegradation of plastic materials, such as ISO 14853:2016 (Standardization, 2016), ISO 15985:2014 (Standardization, 2014) and ASTM D5511 (International, 2018). In the Swedish OFMSW treatment plants, the bags suitable for collection must comply with standards for biodegradation according to SS EN 13,432 (Packaging - Requirements for packaging recoverable through composting and biodegradation) (Standardization, 2000) if the bags are not sorted out in a pretreatment plant prior to the anaerobic digestion process. The standard specifies the criteria for the anaerobic biodegradation of plastics as a minimum of 50% degradation within a maximum of 2 months. However, plastic contamination can still exist in biogas plants since the HRT (i.e., the average period of time a given quantity of substance stays in the digester) is usually less than 2 months. Accordingly, to avoid contamination, a plastic bag ought to be degraded during the HRT used. Therefore, one suggestion is that the standard requirements for the bioplastics bags used for collection should be revised. The requirement should rather specify a maximum of 1-month degradation time for the collection bags. The % degradation of the bags could also be revised to be in accordance with the CEN specifications for biodegradation or at least increased from the actual 50%. If the bags are sorted out first in a pretreatment plant, the requirement for visible contamination in biogas plants in Sweden meets the SPCR 120 regulation (Sverige, 2013) as an average of 20 cm² plastics/kg digestate residue in a sample taken once a month under a period of 12 months. Consequently, there is a need to gain more knowledge about the nature and growth features of microbial strains capable of biopolymer degradation in order to achieve an efficient anaerobic biodegradation process and to understand the mechanisms involved. The microbial strains that are currently isolated or yet to be isolated can then be used as bioactive agents in the anaerobic degradation process. With these suggestions, hopefully, bioplastic bags used for OFMSW collection would avoid plastic contamination and loss of valuable organic matter and improve performance and biological processes.

6. Conclusions

Biodegradation of bioplastics is dependent on the physical and chemical properties of the polymers. Different biopolymers are degraded by different microorganisms, and different microbial communities are available in the same digester depending on the composition of the waste available. The complexity of the biodegradation processes for biopolymers in a digester, challenges regulations. However, standards for the biodegradability of bioplastics exist, although standardization of collecting bags for the OFMSW needs more research to meet the conditions of an active biogas plant and improve the biological processes in the digester, such as the hydraulic retention time and the average composition of the waste.

Acknowledgements:

This study was funded by University of Borås and Åforsk foundation (project ID 14–474). The authors are grateful to Anders Hedenstedt (RISE, Lund, Sweden) for personal conversation conducted about standards applied and requirements fulfilled at the local biogas plant and to Ramkumar B. Nair for his contribution to microbial concerns.


Paper 4
The effect of glycerol, sugar and maleic anhydride on pectin-cellulose biofilms prepared from orange waste

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Abstract: This study was conducted to improve properties of biofilms prepared from orange waste by solution casting method. The main focus was the elimination of holes in the film structure by establishing a better cohesion between the major cellulosic and pectin fractions. For this, the original method was improved first by the addition of sugar to promote pectin gelling, followed by the addition of maleic anhydride. Principally, maleic anhydride was introduced to the films to induce cross-linking within the film structure. The effects of concentrations of sugar, glycerol, as plasticizers, and maleic anhydride as cross-linking agent on the film characteristics were studied. Maleic anhydride improved the structure resulting in a uniform film and morphology studies showed better adhesion between components. However, it was not acting as a cross-linking agent, rather as a compatibilizer. The middle level (0.78%) of maleic anhydride content resulted in the highest tensile strength (26.65 ± 3.20 MPa) at low (7 %) glycerol and high (14%) sugar levels, and the highest elongation (28.48 ± 4.34 %) at high sugar and glycerol levels. To achieve a uniform film surface with no holes present, only the lowest (0.39 %) level of maleic anhydride was necessary.

Keywords: biofilm, glycerol, maleic anhydride, orange waste, sugar

1. Introduction

The post plastic era induces several industries, such as, biomedical, building and packaging industries, to move towards the use of biofilms. Biofilms prepared from biopolymers and containing reinforcing lignocellulosic particles not only have a big potential to replace petroleum-based films, but also form an essential part of the bioeconomy [1]. Cellulose, in particular is abundant and a favorable reinforcement material because of its features, such as, its crystalline structure and good mechanical properties [2].

An attractive research area is the production of biofilms from fruit, - and vegetable residues often by the film-casting method [3-8]. During film-casting, the suspension of a colloidal dispersion of polymers is poured on a non-sticky surface and dried to a film. Various fruit and vegetable residuals are rich sources of different biopolymers, such as pectin, starch, cellulose, hemicellulose, lignin and proteins that can be used to form firm films with competitive properties of commodity plastics. By choosing the right additives, biopolymers can be dissolved, plasticized or kept undissolved in order to perform the required function in the biofilm, i.e. turning them to a matrix, blend or use as reinforcement. One study [6] for example discussed that in biofilms made from carrot powders, the insoluble, mostly crystalline cellulose fractions were the responsible for the film structure, while the soluble, mostly pectin and sugar phase had a plasticizing effect.

When fabricating bioplastics from polysaccharides, one has to bear in mind that those are hydrophobic substances that have low water vapor barrier properties [9], and the mechanical and barrier properties are affected by moisture content. Several methods exist to enhance barrier and mechanical properties of hydrocolloid films; one promising alternative is the modification of physical properties by inducing intra-, and intermolecular bonding by chemical, enzymatic or physical cross-linking [10]. Formaldehyde, glutaraldehyde and glyoxal are potent cross-linking agents that enhanced water barrier properties and increased strength of edible protein-based films [11]. But due to the toxicity, their use is limited for environmentally friendly applications. A low hazard profile
compound, and cross-linking agent is maleic anhydride [12, 13]. Maleic anhydride has also been used as a compatibilizer between natural fibers and poly(lactic acid) matrix [14, 15] to improve adhesion.

Biofilms from orange waste have previously been developed with suitable mechanical properties, such as tensile strength ranging between 27.3 and 36.7 MPa [8]. Briefly, citric acid solution, containing orange waste (composed mainly of pectin and cellulosic fibers) powder and glycerol was casted for biofilm production. But due to the mechanism of drying of colloidal films, a number of holes were present in the structure. In the same study, the use of a rotary movement during drying showed positive effects, but film thicknesses were uneven plus the continuous rotary movement had higher energy consumption. To deal with this negative aspect, further improvement in the preparation conditions and structure of the films was necessary.

This study, therefore, was dedicated first to investigate the enhancement of pectin gelling establishing a three-dimensional network, in which other components are trapped [16]. To achieve this goal, first, conditions for solubilizing pectin had to be provided, which was followed by conditions that allow pectin gelling. The reason for this was the fact, that within conditions for gelling, solubilization would not happen, and vice versa. This meant that the addition of citric acid (being acid one of the triggers for pectin gelling) took place in a later stage together with sugar to promote pectin gelling [16]. This step was aimed to reduce the holes by establishing an improved structure of pectin. This study was also aimed to investigate the use of maleic anhydride to improve the properties of orange waste pectin-cellulose biofilms. Maleic anhydride has been used previously to develop maleic anhydride grafted orange waste [17], therefore it was expected that it would have a cross-linking effect on the films. Mechanical tests and morphological studies were performed to investigate the effect of each component and optimize the preparation conditions.

2. Materials and Methods

2.1 Materials

Orange waste was kindly provided by Brämhults AB (Borås, Sweden) before the headquarters changed location, and was kept under -20 °C until further use. Maleic anhydride (≥99 % Sigma Aldrich, St. Louise, USA), citric acid (monohydrate, >99.5 %, Duchefa Biochemie, the Netherlands), D-(+)-glucose (≥99.5% Sigma Aldrich, St. Louise, USA) further referred as sugar, glycerol (>99 % ARCOS Organics, Belgium) were other materials used in the experiments.

2.2 Pretreatment of orange waste

Pretreatment of orange waste was performed according to a previous study [17] in which soluble sugars were removed prior to size reduction, drying and milling the material to a fine powder. In the first step of milling, a variable speed rotor mill (Pulverisette 14, Fritsch, Germany) was used with a sequence of sieve sizes of 1 and 0.2 mm at a revolution of 10,000 rpm for a maximum of 1 min each. The powder was further milled with a ball-mill (MM 400, Retsch, Germany) at frequency of 30 Hz for 10 min. The second step of milling was necessary to perform in order to gain the same range of particles (mostly between 125 and 75 µm) as was used in a previous study [8].

2.3 Film formation

Film formation of orange waste was further developed from the method applied in our previous study [8]. The first step of the modification was done by applying a “sol-gel method”. In the sol-gel method, first, conditions for solubilizing the pectin was provided, then conditions for gelling the pectin followed [16]. By this method, 2% (w/v) orange waste was introduced to 100 mL vigorously stirred distilled water that already contained 7 % glycerol (w/w of orange waste powder) and 1 drop of organic antifoam, at 40-50 °C. The mixture was then heated up to 70 °C and cooled to about 60 °C. At that point, citric acid (to gain 1% (w/v) solution) and 7 % sugar (w/w of orange waste powder) were added to the mixture to initiate the gelling and rotation was reduced to 200 rpm to support pectin gelling. Then, 30 g of the suspension was poured through a metal sieve, to capture occasionally
formed air bubbles, onto non-sticky PTFE plates and dried in a laboratory oven (Termaks, TS9026, Norway) at 40 °C.

“Sol-gel-ma method” was a step further in which, maleic anhydride, as potential crosslinking agent was also incorporated in the recipe of the sol-gel method; while antifoam was removed to reduce the number of chemicals. In the sol-gel-ma method different concentrations of maleic anhydride (100, 50, 25, 12.5, 6.25, 3.13 and 1.56 (w/w) %) were used and maleic anhydride was added to distilled water as one of the initial ingredients together with glycerol. All other steps and concentrations were the same as in sol-gel method. A schematic image of the different methods is presented in Figure 1.

Figure 1. Schematic image of the different film forming methods discussed in this study. Glycerol (GLY), orange waste (OW), citric acid (CA) and maleic anhydride (MA) were ingredients used in the methods.

A 3-2-2 factorial designed experiment for the sol-gel-ma method with factors maleic anhydride, sugar and glycerol was performed to optimize preparation conditions and to determine the minimal amount of maleic anhydride needed. All the concentrations are shown in Table 1, demonstrating the randomized treatments used. This study is further referred as “optimization” and concentrations of 7 % (as used in former studies) are referred as low, while concentrations of 14 % (doubled amount) are referred as high concentrations. Preliminary experiments showed that only films with presence of citric acid had good appearance, therefore it was concluded that citric acid is necessary for film formation in order to induce pectin gelation.

Table 1. Different concentrations (w/w %) of maleic anhydride, sugar and glycerol in the optimization experiment. Citric acid concentration was constant at 1 % (w/v).

<table>
<thead>
<tr>
<th>Maleic anhydride</th>
<th>Sugar</th>
<th>Glycerol</th>
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<tbody>
<tr>
<td>1.5</td>
<td>7</td>
<td>7</td>
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<tr>
<td>0.39</td>
<td>7</td>
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<tr>
<td>0.78</td>
<td>14</td>
<td>7</td>
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<tr>
<td>1.56</td>
<td>7</td>
<td>14</td>
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<td>0.78</td>
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<td>0.78</td>
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<td>0.39</td>
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<td>0.39</td>
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2.4 Characterization of films

2.4.1 Thickness

The thickness of films was measured by a constant-load micrometer. Two measurements were taken of each specimen and the average was reported (mm).

2.4.2 Mechanical testing

Mechanical testing was performed according to ISO 527 using Tinius Olsen H10KT tensile tester and data was analyzed by QMat software package. A moving cross-head was used to pull the specimens with a load cell of 100 N and a velocity of 10 mm/sec. Tensile strength (MPa) and elongation at maximum tensile strength (%) were measured for 5 specimens and the average was reported.

2.4.3 Water vapor permeability

Water vapor transmission permeability (WVP) was determined according to ASTM E96 [18] with modifications and calculated according to [19]. A pre-dried (at 70 °C) glass container was filled with 20 ml distilled water, covered with the biofilms and sealed with paraffin film. The distance between the film and water was 16 mm. The weight of the whole system was recorded at 0 h and was placed into a desiccator for 5 days. Every 24 h the weight of the systems was recorded to observe the reduction of weight from day 1 to day 5. The mass loss was plotted against time and the slope obtained was used for calculation. WVP of the films was calculated according to the following equation:

\[
WVP = \frac{S \times t}{A \times \Delta P} \tag{1}
\]

where “S” is the slope of the plot (kg/s), “t” is the thickness of the film (m), “A” is the cross-sectional area of the film (m²), and “\(\Delta P\)” is the difference between the vapor pressure of water in the desiccator (assumed to be zero) and inside the container (assumed as the saturation value at room temperature, Pa) [19]. The test was performed in duplicates and averages are reported as water vapor transmission rate (kg/m Pa s).

2.4.4 Interaction with water

Interaction with water-test for selected specimens was performed according to Perotto et al. [6] with slight modifications. A specified size (30*15 mm) of pre-dried film (for 1 h at 70 °C) was immersed in 20 ml distilled water for 30 min, and then dried for 1 h at 70 °C and the weight loss was calculated by gravimetric method. The measurements were performed in duplicates and the results are reported as weight loss (%).

2.4.5 Morphology

Surface and transversal morphology was studied by field emission scanning electron microscopy (FE-SEM) (Zeiss, Sigma, Germany) imaging. For surface visualization films were attached to a carbon tape and covered with gold. For transversal visualization, cross-sectional images of films were taken as follows: films were immersed in liquid nitrogen for one minute before they were broken and immediately attached to a carbon tape and covered with gold. Photomicrographs were taken at 500, 1.00 K, 5.00 K, 10.00 K x magnifications, using an accelerating voltage of 10.00 and 20.00 kV.

2.4.6 Biodegradation test
Biodegradation of the films was measured by anaerobic digestion. The test was performed according to a previous study [20]. In brief, the films were incubated in 120-mL glass bottles, within thermophilic conditions (55 °C) for 30 days. The working volume of the reactors was 50 mL (containing 35.5 mL inoculum and the rest was distilled water) and each bottle contained 0.15 g film volatile solids (VS). The inoculum was obtained from Borås Energi & Miljö AB (Borås, Sweden), a large-scale thermophilic biogas plant. Samples were taken at specified times and analyzed for gas composition with a gas chromatograph (Clarus 500, Perkin-Elmer, USA).

2.5 Statistical analysis

Statistical analyses were performed using MINITAB® software (version 17.1.0, Minitab Inc., State College, PA, USA). Analysis of variance, ANOVA, using the general linear model, was performed to determine the main effects and interaction of different components in the optimization experiment, using 5% significance level.

3. Results and Discussion

Lignocellulosic by-products and waste materials are important participants of the bioeconomy: their valuable biopolymer content makes them attractive for the bioplastic industry which, in the same time, is a useful method to address waste handling issues. An example to such case, is biofilm prepared from orange waste in a previous study [8] which represented some holes in its structure. The goal of this study was to modify the film formation method in order to develop a uniform film-structure with no holes in which the adhesion of polymeric components is promoted.

3.1 The effect of pectin gelling and maleic anhydride on the appearance and the properties of the films

The hypothesis, that a more coherent film-structure would result in the reduction or even elimination of holes in the orange waste film, was tested first by the improvement of pectin gelling, followed by the addition of maleic anhydride into the film.

3.1.1 Appearance

Films prepared by the original method had some holes present in the structure, which could be eliminated by a rotary movement during drying, however that method resulted uneven thicknesses [8] and required more energy. Inducing pectin gelling prior to casting, improved pectin binding and resulted in reduced number of holes which had smaller size. By applying this method, the use of rotary movement was not necessary, and films had even thicknesses. But because some smaller holes were still present, further improvements were necessary and maleic anhydride was used.

When maleic anhydride was used for film preparation, the holes were completely disappeared and films with uniform structure were obtained.

3.1.2 Morphology

On a microstructure level, according to FE-SEM images a more compact film construction was achieved using maleic anhydride compared to the sol-gel method. Both, the surface (Figure 2 a, c, e) and the cross-sectional images (Figure 2 b, d, e) show increasing tendency of denseness and more compact structure as the concentration of maleic anhydride increased (Figure 2). The compact structure is probably the direct effect of the additional ester bonds between grafted maleic anhydride intermediates and cellulose [21], and/or pectin [22], and/or glycerol [23]. Since biofilms prepared in this study represent a complex mixture of biopolymers (e.g. pectin, cellulose, hemicelluloses) and other hygroscopic substances, such as glycerol and sugar that contain OH groups, and can function as plasticizers [24], it is therefore difficult to determine which component reacted most with maleic anhydride.
Figure 2. FE-SEM micrographs of the film surface and cross-sectional images prepared with the sol-gel method (a, b); Sol-gel-ma (1.56 % maleic anhydride) (c, d); and sol-gel-ma (25 % maleic anhydride) (e, f), respectively. The magnification for surface images was 1.00 K x and for cross-sectional images was 500 x.

As the introduction of maleic anhydride improved the appearance and structure of the films in general, the next step was to find the optimal concentration of maleic anhydride and other components in the films. Aiming at reducing the amount of chemicals used, the goal was to find the minimum concentration of maleic anhydride in which a film with improved characteristics is obtained.

3.1.3 Thickness and mechanical properties

The thicknesses of the films prepared by the original and sol-gel method ranged between 0.098 and 0.135 mm with an average value of 0.115 ± 0.010 mm (standard deviation). Mechanical properties of the films made by the original and sol-gel methods were not significantly different, resulting in tensile strength of 21.08 ± 7.78 and 21.69 ± 8.72 MPa; and elongation of 3.67 ± 1.80 and 4.83 ± 1.79 %, respectively. In general, the addition of maleic anhydride into the films resulted in lower tensile strength and higher elongation compared to those of the sol-gel method. At higher concentrations of
maleic anhydride (> 1.56 %) the elongation of the films increased as maleic anhydride content was reduced. This fact is contradictory with the hypothesis that maleic anhydride would act as a cross-linker in the pectin-cellulose system, because cross-linking is generally increasing the strength and stiffness, and reduces elongation. Therefore, instead of a cross-linking agent, maleic anhydride could rather be considered as a compatibilizer that improved film formation and also provided a significant plasticizing effect. The plasticizing effect of maleic anhydride on cellulose diacetate was also confirmed before [23].

3.2 Optimization of film preparation condition

Based on the preliminary experiments, the reduction of maleic anhydride below 1.56 % was also studied at 0.78 and 0.39 % levels. In the same time, the effect of sugar and glycerol at elevated levels (14 %) and the interaction between the components affecting the mechanical properties was also studied.

3.2.1 Morphology

Films containing the highest glycerol, sugar and maleic anhydride with the lowest concentrations of the other two components, respectively, were studied for morphology and are shown in Figure 3, 4 and 5.

The surface images of the films containing the highest amount of glycerol (Figure 3 a, b) show softer, more curvy edges of particles and a fluffier cross-section image can also been seen (figure 3 c, d). The fluffiness of the structure can be explained by the plasticizing effect of the glycerol, in which the small molecular weight compound is pushing the molecules slightly further apart to enhance the mobility and the softness of the structure.

Surface images of films containing the highest concentrations of sugar show elegantly arranged flower-like patterns (Figure 4 a, b). These patterns may be formation of sugar crystals, when sugar is present in a high concentration of the solution. As water evaporates during the drying of films, the solution becomes more saturated with sugar and the molecules will continue to come out to the surface to re-arrange themselves in to crystals. This phenomenon was only observed when sugar was added to the mixture in high concentration that may be the reason of the excess sugar in the system that was not taking place in the film-forming reactions. On the cross-sectional images (Figure 4 c, d) the same could be observed. There is no evidence if this phenomenon is re-crystallization of sugar and how it could have a positive impact on elongation. However, different sugars are known to act as plasticizers in biopolymer films [24].

Films that contain the highest concentration of maleic anhydride have a leather-like and denser look on the surface (Figure 5 a, b), and they represent a more compact structure when the cross-sectional images are observed (Figure 5 c, d). The denseness of the films could be the result of the additional bonds formed between maleic anhydride and other molecules.
Figure 3. FE-SEM micrographs of film surfaces with a magnification of 1.00 K x (a) and 5.00 K x (b) and cross-sectional images with a magnification of 1.00 K x (c) and 10.00 K x (d) containing the highest concentration of glycerol (14 %) prepared according to sol-gel-ma method (maleic anhydride and sugar concentration was 0.39 % and 7 %, respectively).

Figure 4. FE-SEM micrographs of film surfaces with a magnification of 1.00 K x (a) and 5.00 K x (b) and cross-sectional images with a magnification of 1.00 K x (c) and 10.00 K x (d) containing the highest concentration of sugar (14 %) prepared according to sol-gel-ma method (maleic anhydride and glycerol was 0.39 % and 7 %, respectively).
Figure 5. FE-SEM micrographs of film surfaces with a magnification of 1.00 K x (a) and 5.00 K x (b) and cross-sectional images with a magnification of 1.00 K x (c) and 10.00 K x (d) containing the highest concentration of maleic anhydride (1.56 %) prepared according to sol-gel-ma method (both, glycerol and sugar were 7 %).

Attention has also been payed to films that contain high concentrations of both, sugar and glycerol of varied maleic anhydride levels. Specifically, the possible morphological difference between specimens containing the middle level of maleic anhydride and the other two levels has been sought. The same that has been observed for high sugar and high glycerol content can as well be observed on all of these images: floral patterns are formed on a fluffy background, representing the excess sugar and loosening effect of glycerol. The effect of maleic anhydride however is difficult to observe when glycerol and sugar are used in high concentrations. Because of the magnitude of the number of FE-SEM images, these images are available as supplementary material (S1).

3.2.2 Thickness of films

The thicknesses of the films prepared in the optimization experiments ranged between 0.095 and 0.139 mm with an average value of 0.120 ± 0.010 mm (standard deviation), similarly to previous experiments (section 3.1.3). The use of different concentrations of glycerol had a significant positive effect of film thicknesses (p=0.000), while sugar had small but non-significant (p=0.067) and maleic anhydride had no effect (p=0.620). A clear trend however cannot be observed (Figure 6).
Figure 6. Individual value plot of thicknesses of the films prepared in the “optimization” studies. X-axis represents the concentrations (%) (7, 4, 0.39, 0.78 and 1.56) of glycerol (G), sugar (S) and maleic anhydride (MA) used in the films.

3.2.3 Mechanical properties

Looking at the interval plot for tensile strength (Figure 7 a), glycerol shows to have a significant negative effect (coefficient -3.64, p=0.000) when the load is increased from low (7 %) to high (14 %) concentration, as it was expected. While sugar and maleic anhydride (p=0.213 and p=0.231, respectively) does not have any significant effect on the tensile strength.
Figure 7. Mean values of tensile strength (a) and elongation at max (b) of films of the optimization experiments. 95% confidence interval (CI) was used for calculating the mean value. X-axis represents the concentrations (%) (7, 4, 0.39, 0.78 and 1.56) of glycerol (G), sugar (S) and maleic anhydride (MA) used in the films. Individual standard deviations were used to calculate the intervals.

The elongation values are clearly more complicated, as a number of mechanisms may be involved in the plasticization of bioplastics with low molecular weight compounds [25]. Elongation was significantly affected by glycerol (reverse effect as for tensile strength, as expected) and sugar (coefficient 5.52, p=0.000 and coefficient 3.57, p=0.000, respectively), as well as their interaction had a significant positive effect on it (coefficient 2.18, p=0.000). This result, in the observed pattern of high glycerol and high sugar levels, gives the highest elongation for all maleic anhydride levels (Figure 7b). The maleic anhydride main effect and maleic anhydride*sugar interaction are both significant on elongation. When maleic anhydride is used in low (0.39 %) concentration the effect on elongation is negative (coefficient -1.76, p=0.013) and when maleic anhydride is used in 0.78 % the effect of it is positive (coefficient 1.72, p=0.015). The interaction between maleic anhydride*sugar is most noticeable when 0.78 % maleic anhydride is used (coefficient 2.18, p=0.03). Clearly, the highest elongation levels can be reached if the middle level of maleic anhydride (0.78 %) is used.

3.2.4 Water vapor permeability and Interaction with water

In general, all films were flexible and uniform, and among them, four were selected (containing 1.56, 0.78, 0.38 and 1.56 % maleic anhydride, 14, 7, 14, and 14 % sugar, and 14, 14, 7 and 7 % glycerol, respectively) for water vapor permeability and water interaction test, having the absolute most uniform structure based on observation.

Low water vapor permeability is often a requirement for e.g. food packaging materials. The lower the value the higher the resistance is to water vapor. The water vapor permeability of biofilms prepared in the optimization experiment, regardless of preparation method represented a mean value of 1.19 (± 0.08)*10^{-13} kg/m Pa s, which can be compared with WVP of fungal biomass reinforced pectin films of 2.35*10^{-13} kg/m Pa s [26] and edible films from alginate-acerola reinforced with cellulose whiskers of 1.67 (± 0.42)*10^{-13} kg/m Pa s [27]. Both studies reported lower WVP values when the reinforcement load increased. The lowest value was gained when fungal biomass load was 35 wt% [26] and when cotton cellulose whiskers was used at 15 wt% [27].

These results are, however higher than that of polysaccharides-based locust bean gum films prepared with solution casting containing different plasticizers (polyethylene glycol 200, glycerol,
propylene glycol and sorbitol), ranging between 1.2 and 2.6 \(10^{-14} \text{ kg/m Pa s}\) [28], meaning a less resistance toward the passage of water vapor.

All of the specimens used for the test lost more than half of their weight, 56.15 ± 3.37 %. In a study performed by Perotto et al. [6] bioplastic films fabricated from vegetable wastes, however showed approximately 35 % solubility after 108 h of immersion in water. These results are indicating the unfortunate feature of polysaccharides-based bioplastics, their hydrophilic nature.

### 3.3 Biodegradation

1.56 % of maleic anhydride was the highest concentration used in the optimization experiment; therefore it was used for biodegradation studies. The maximal biodegradation rate of orange waste-films made with sol-gel and sol-gel-ma method was 63 % and 52 %, respectively (Table 2). According to a previous study [8] orange waste films produced with the original method showed 90 % biodegradability within approximately 15 days. The reason for a lower degradation rate in this study could be 1) the effect of maleic anhydride present in the structure may make the film more difficult for the microorganisms to degrade and, however sol-gel films did not contain maleic anhydride; 2) the inoculum was not the same as in the previous study. The available microbial community in a digester, used as inoculum, depends on the actual composition of the food waste available, which cannot be guaranteed to be the same. Therefore, in this case, however the same experiment was performed with the same parameters, and with the same source of the inoculum, at a different time, it resulted in lower biodegradation rates.

<table>
<thead>
<tr>
<th>Table 2. Biodegradation rate (%) of biofilms produced by the sol-gel and sol-gel-ma methods, containing 1.56 w/w % maleic anhydride.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
</tr>
<tr>
<td>Sol-gel</td>
</tr>
<tr>
<td>Sol-gel-ma</td>
</tr>
</tbody>
</table>

### 4. Conclusions

The present study recognized the complexity of the different components used in the pectin-cellulose system, and the interpretation of the contrasting behavior can be rather difficult. The enhancement of pectin gelling prior to film casting had positive effect on the film structure, reducing the holes present. The positive effect of maleic anhydride was further proved to eliminate holes in orange waste biofilms via the improved structure with a smooth and uniform surface. For that, maleic anhydride was only necessary at very low concentration (0.39 %). On the other hand, statistical analyses proved that tensile strength of the films depended on glycerol content, and elongation was dependent on both, glycerol and sugar and the interaction between them was also significant. The highest elongation values were obtained when maleic anhydride was used in 0.78 %. This observation rejected the hypothesis that maleic anhydride would act as a cross-linking agent, therefore it was concluded that maleic anhydride in the orange waste-films rather acts as a compatibilizer.

Because orange waste biofilm was biodegradable within anaerobic conditions, it could be suitable as a future material for collecting bag of the organic fraction of municipal solid waste; however the hydrophilicity of the material needs to be improved. The degradation test performed however contained 1.56 % maleic anhydride but results of other analyses showed that as little as 0.39 % is sufficient for a uniform material, the degradation of orange waste film containing 0.39 % would probably be acceptable in a biogas plant, but to state this, exact measurements would be needed.

### Supplementary Materials:

Figure S1: FE-SEM micrographs of film surfaces with a magnification of 1.00 K x (a) and 5.00 K x (b) and cross-sectional images with a magnification of 1.00 K x (c) and 10.00 K x (d) containing the highest concentrations of glycerol and sugar, and the lowest concentration of MA (0.39 %); of film surfaces with a magnification of 1.00 K x (e) and 5.00 K x (f) and cross-sectional images with a magnification of 1.00 K x (g) and 10.00 K x (h) containing the highest concentrations of glycerol and sugar, and the middle level of MA.
concentration (0.78 %); of film surfaces with a magnification of 1.00 K x (i) and 5.00 K x (i) and cross-sectional images with a magnification of 1.00 K x (k) and 10.00 K x (l) containing the highest concentrations of glycerol and sugar, and the highest concentration of MA (1.56 %).

**Author Contributions:** conceptualization, B. V. and M. L., methodology and investigation, B.V.; formal analysis, B. V., software, M. L.; writing—original draft preparation, B. V.; writing—review and editing, A.Z., P. R. L., D. Å., M. L., M. J. T.; supervision, A. Z.; funding acquisition, M.J.T

**Funding:** This research was funded by University of Borås.

**Acknowledgments:** The authors are grateful to Brämhults AB for providing the raw material and to Ilona Sárvári Horváth for the discussions about biodegradation studies.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**


Paper 5
Development of biofilms and 3D objects from apple pomace

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Abstract: Extensive quantities of apple pomace are generated annually but its disposal is still challenging. This study addresses this issue by introducing a new, environmentally friendly approach for the production of sustainable biomaterials from apple pomace, containing 55.47 % free sugars and a water insoluble fraction, containing 29.42 ± 0.44 % hemicelluloses, 38.99 ± 0.42 % cellulose and 22.94 ± 0.12 % lignin. Solution casting and compression molding were applied to form biofilms and 3D objects i.e. fiberboards, respectively. Using glycerol as plasticizer resulted in highly compact films with high tensile strength and low elongation (16.49 ± 2.54 MPa and 10.78 ± 3.19 %). In contrast, naturally occurring sugars in the apple pomace showed stronger plasticizing effect in the films and resulted in fluffier and connected structure with significantly higher elongation (37.39 ± 10.38 % and 55.41 ± 5.38 %). Getting benefit of the self-binding capacity of polysaccharides, fiberboards were prepared by compression molding at 100 °C using glycerol or naturally occurring sugars as plasticizer. The obtained fiberboards exhibited tensile strength of 3.02 – 5.79 MPa and elongation of 0.93 – 1.56 %. Possible applications for apple pomace biomaterials are edible/disposable tableware or food packaging.

Keywords: apple pomace, biofilm, biomaterials, compression molding, fiberboard, solution casting

1. Introduction

The widespread use of synthetic plastics is leading to significant, well-documented impacts on the environment and replacing them with bio-based alternatives may mitigate the effects of pollution as well as greenhouse gas emissions. This has led to the development of a rich and diverse field of research in bioplastic production. Biopolymers from agricultural resources such as starch [1], cellulose [2], proteins [3], and pectin [4] are among the predominant materials used for bioplastic production.

The latest research [5-10] focuses on by-products and waste materials of the fruit and vegetable industries for bioplastic production. An interesting raw material for bioplastics production is apple pomace. Worldwide, approximately 70 million ton apples are produced annually [11]. Apple pomace represents 25-30 % of the original apple weight [12, 13]. Therefore, millions of tons of apple pomace are generated every year in the world as a byproduct of juice, cider, or wine production. The acidic characteristics of apples with their high sugar and low protein content makes pomace unsuitable for landfilling and animal feedstock [11-13]. This residue has a high moisture and biodegradable organic content which can be used for bioplastic production. Apple pomace consists mainly of cellulose (7-44 %), starch (14-17 %), pectin (4-14 %) and insoluble lignin (15-20 %)[14].

Films and 3D objects are among the category of biomaterials with high demands in the market. Solution casting in which biopolymer solutions are applied on a surface and dried is a very effective method for production of thin films. Recently, we have developed pectin-based biofilms from citrus waste using solution casting method [5]. The method was based on pectin dissolution along with dispersion of cellulose fibers in citric acid solution which was then dried to thin films. Compared to other studies [6, 8, 10, 15] in which fruit or vegetable wastes were used for biofilm fabrication with solution casting method, using hydrochloric acid solution [8], trifluoroacetic acid solution [6, 15] or...
heptane [10], the pectin-cellulose biofilm from orange waste have applied so far the less harmful solvent, such as citric acid solution. This method has not been applied on apple pomace before.

Molding usually has lower energy demand compared to casting. For non-thermoplastics biopolymers, such as proteins, heat compression molding has been applied to produce bioplastic items [16]. Lignocellulosic materials have shown interesting self-binding capacity under pressure and heat, which opened up opportunities for preparation of binder-less bio-composites [17-21]. The technique has also been successfully applied for the production of pectin films [22]. Compression molding, unlike casting method, gives the possibility for the formation of 3D objects. This method has not been tested on apple pomace before.

The goal of the current study was to develop and evaluate biofilms and 3D objects from apple pomace using solution casting and compression molding techniques, respectively. The new materials can potentially be used as disposable or edible packaging and tableware.

2. Materials and Methods

2.1 Materials

Apple pomace was kindly provided by Lyckans Äpple (Bredared, Sweden) which was stored at -20 °C until used. Other used materials in this study were glycerol (≥ 99.5 %, Fisher BioReagents, Belgium) and citric acid monohydrate (> 99.5 %, Duchefa Biochemie, Netherlands).

2.2 Pretreatment of apple pomace

Apple pomace was either washed with water to remove free sugars and other soluble nutrients or not washed. The washing was carried out according to a previous report [5] with minor modifications. Cold water was used in the washing process to avoid starch dissolution. Initially, apple pomace was soaked overnight in cold tap water. The apple pomace to water ratio (kg/L) was 1:1.5 throughout the whole washing procedure. The water was then removed by manual pressing. The resulting material was washed two more times. In each washing step, the apple pomace was stirred in cold tap water for 10 minutes. A kitchen sieve was used to collect and rinse the remaining pomace. Both, the washed and the non-washed apple pomace were dried at 40 °C in a laboratory oven (Termaks, Norway).

2.3 Formation of apple pomace powder

A fine powder of the dried washed and non-washed apple pomace was obtained by a sequence of milling to sizes of 1.0 mm and 0.2 mm, using a variable speed rotor mill (Fritsch Pulverisette 14, Germany). To produce powders of even smaller particle sizes, approximately equivalent of using a 0.08 mm sieve, a ball mill was used (Retsch MM 400, Germany) in periods of 10 min at a frequency of 30 Hz.

2.4. Preparation of films and 3D objects from apple pomace

Solution casting and compression molding techniques were employed for production of biofilms and 3D objects from apple pomace. The details of the methods are presented in Figure 1.
2.4.1 Preparation of biofilms from apple pomace by solution casting method

Formation of biofilms was performed by modifying the method Bátori et al. [5] used for the formation of orange waste films. A mixture was prepared containing 2 % (w/v) of washed apple pomace powder (particle size of approx. 0.08 mm) and 7 % glycerol (w/w of apple pomace powder) dissolved in 1 % (w/v) of citric acid solution. The mixture was made while heating to 70 °C under constant magnetic stirring at 560 rpm. By using a metal kitchen sieve, air bubbles were removed before 30 g of mixture was poured onto a non-sticky plate (PTFE, 100 mm in diameter) for casting. Biofilms were made in triplicates. The plates were dried at 40 °C in a laboratory drying oven (Termaks, Norway). The dry films were removed by gently pulling them off with pincers and stored in plastic zip bags until analyses. Additionally, two further mixtures of the non-washed apple pomace were prepared in the same way with or without the use of glycerol.

2.4.2 Preparation of 3D biomaterials from apple pomace by compression molding method

Apple pomace powders of size 1.0 mm or 0.2 mm, either washed or not washed, were used for preparation of 3D objects. The apple pomace powders were either mixed with glycerol (apple pomace to glycerol ratio was 70:30) prior to compression molding according to Gurram et al. [22] or used directly. To fill the mold, 40 g of apple pomace-glycerol mixture or non-washed apple pomace without glycerol was placed into a 100*100 mm square mold. A 10-ton molding press (Rondol C2348, UK) was used. A pressure of 8 MPa was applied for 20 min at 100°C to form the 3D objects according to Gurram et al. [22]. The shaping mold was opened when it cooled to room temperature and the fiberboard was removed and stored in plastic zip bags for further analyses.

2.6 Compositional analyses of apple pomace

For compositional analyses, material recovery was performed as follows: the apple pomace was dried at 40 °C until constant weight. The moisture content of apple pomace was determined by a gravimetric method. Apple pomace was then soaked in distilled water overnight, taking the moisture
content of the dry material into account, with apple pomace to water ratio of 1 to 1.5. Apple pomace was then collected via vacuum filtration using a grade 3 filter paper. This was then washed two more times, keeping the same ratio of apple pomace and water. The recovered solutions, after soaking and the two washing steps, containing soluble sugars and other solutes were analyzed for soluble sugars content as described in section 2.6.3. Pectin extraction was performed directly after the last washing step (using the wet material) according to section 2.6.1. The rest of the apple pomace was then dried at 40 °C and milled to a powder of 0.2 mm particle size. The apple pomace powder was then used for starch (section 2.6.2), carbohydrate, and lignin analyses (section 2.6.4).

2.6.1 Pectin content

High-performance microwave digestion system (Milestone Ethos UP MA182, Italy) was used for pectin extraction according to Bátori et al. with a minor modification, where the acidified water consisted of 7.5 mL of 0.1 M HNO₃ and 37.5 mL of distilled water, in order for the mixture of 20 g apple pomace and 45 mL acidified water to reach pH 2. The rest of the procedure was performed in the same way [5]. An average of 4 measurements is reported as pectin content % (w/w).

2.6.2 Starch content

The total starch content of the apple pomace powder was determined according to Total Starch HK Assay Kit (K-TSHK, Megazyme, Ireland). The test was performed in duplicates and the average is reported.

2.6.3 Sucrose, fructose and glucose content

The soluble sugars i.e., sucrose, fructose and glucose of the apple pomace powder was determined according to Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG, Megazyme, Ireland) and high performance liquid chromatography (HPLC) (Waters 2695, Waters, Milford, USA) was used as control measurement for quantifying glucose and mixture of other sugars using an analytical ion exchange column based on hydrogen ions (Aminex HPX-87H, Bio-Rad, USA) operated at 60°C with 0.6 mL/min of 5 mM H₂SO₄ as eluent.

2.6.4 Carbohydrate and lignin contents

Structural carbohydrates, containing arabinose, glucose, mannose, and xylose, as well as lignin contents of sugar free apple pomace powder was determined according to NREL/TP-510-42618 [23], via two step Sulphur acid hydrolysis. The different fractions of carbohydrates were quantified with HPLC using a lead(II)-based column (HPX-87P, BioRad) with two Micro-Guard Deashing (Bio-Rad) pre-columns operated at 85 °C with 0.6 mL/min ultrapure water as eluent. The measurements were performed in triplicates and the averages are reported. Lignin content was determined following the same protocol in which acid soluble lignin was quantified by absorption (at 240 nm) and acid insoluble lignin was quantified by a gravimetric method.

2.7 Mechanical testing of biofilms and fiberboards

Dog-bone shaped specimens were created by a laser cutting machine (GCC LaserPro Spirit GLS, Taiwan) from the compression molded fiberboards and casted biofilms. The specimens were analyzed for mechanical properties, according to ISO 527-1:1993 using a universal tester (Tinius Olsen H10KT, US) and QMat software package. A moving cross-head was used to pull the specimens with a load cell of 100 N and a velocity of 10 mm/sec. An extensometer was used for the fiberboards to measure strain. The average of 5 specimens is reported as tensile strength (MPa) and elongation at maximum tensile strength (%).

2.8 Morphological analyses of biofilms and biocomposites
The morphology of the biofilms and biocomposites was analyzed by a field emission scanning electron microscopy (FE-SEM) (Zeiss, Sigma, Germany). Surface and cross-sectional images of samples were taken after the samples were coated with gold. Photomicrographs were taken at 250 and 1.00 K x magnifications, using an accelerating voltage of 20.00 kV.

3. Results and Discussion

The need to replace conventional synthetic plastics has led to development of a rich and diverse field of research in bioplastic production. Production of bio-based materials from fruit wastes and fruit residues not only reduces the negative environmental effect of synthetic plastics but also contributes to the waste management issues. A wide variety of fruit waste has been tested for bioplastic production [7, 24-28]. The entire fraction of apple pomace, without separation of seeds and stems or without the extraction of structural components was used in the current study for the production of new biofilms and 3D objects. The compositional analysis of the apple pomace is presented in Table 1.

Table 1. Characterization of apple pomace. By washing of apple pomace, the free sugars and some other water-soluble components were removed, resulting in the sugar free material.

<table>
<thead>
<tr>
<th>Component</th>
<th>Characterization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery of sugar free water insoluble fraction(^2)</td>
<td>39.41</td>
</tr>
<tr>
<td>Water insoluble fraction</td>
<td></td>
</tr>
<tr>
<td>Pectin(^1)</td>
<td>8.94 ± 1.20</td>
</tr>
<tr>
<td>Starch(^1)</td>
<td>2.91 ± 0.00</td>
</tr>
<tr>
<td>Cellulose(^1)</td>
<td>38.99 ± 0.42</td>
</tr>
<tr>
<td>Hemicelluloses(^1)</td>
<td>29.42 ± 0.44</td>
</tr>
<tr>
<td>Acid soluble Lignin(^1)</td>
<td>6.51 ± 0.12</td>
</tr>
<tr>
<td>Acid insoluble Lignin(^1)</td>
<td>16.43 ± 0.12</td>
</tr>
<tr>
<td>Water soluble fraction</td>
<td>55.47(50.39)</td>
</tr>
<tr>
<td>Total free sugars(^3)</td>
<td>17.53</td>
</tr>
<tr>
<td>Sucrose</td>
<td>26.92</td>
</tr>
<tr>
<td>Fructose</td>
<td>11.01(16.63)</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td>Non-determined water-soluble fraction(^3)</td>
<td>5.12(10.2)</td>
</tr>
<tr>
<td>Moisture in wet apple pomace</td>
<td>82.725 ± 0.07</td>
</tr>
</tbody>
</table>

\(^1\) Based on the sugar free dry material
\(^2\) Based on the dry apple pomace
\(^3\) Values in parenthesis were determined by HPLC

Two typical approaches were used for preparation of films and 3D objects from apple pomace. The films were prepared getting benefit of the film forming ability of polysaccharides by solvent evaporation [29] in the casting process. As an alternative to the casting process, the self-binding ability of the biopolymers using low pressure molding at high temperatures [20] was used for the development of apple pomace 3D objects without the use of any solvent or binder.

3.1 Production of thin films from apple pomace by solution casting

Both, washed and non-washed apple pomace were used for film production by casting method according to Bátori et al. [5] (Figure 1). A brownish color with semi-transparent appearance was observed for apple pomace films regardless of preparation conditions. In general, the films were smooth and they did not contain holes as reported for orange waste films [5]. Presence of lignin in apple pomace, which is not present in orange waste, may be reasonable for a better quality of the apple pomace films regarding the absence of holes. The well-known adhesion properties of the lignin [30], is probably responsible for a better adhesion between the cellulose fibers and other
carbohydrates in the apple pomace films. The thickness of the films was around 0.1 mm and was not
affected significantly by the preparation conditions (Table 2).

3.1.1 Morphology of apple pomace films

In order to study the morphology of the films, FE-SEM images were taken from the surface and
the cross-section of the films (Figure 2). Accordingly, surface of the film made from washed apple
pomace using glycerol as plasticizer was smoother than those prepared from non-washed apple
pomace (Figure 2). This may indicate higher orientation degree of the fibers in the film. In contrast,
the films made of non-washed apple pomace showed a fluffier structure which was also confirmed
by cross-section images. The film made of washed apple pomace had a compact layered structure
(Figure 2 f). In contrast, the films made of non-washed apple pomace exhibited a fluffy structure
where the polysaccharide particles seemed to be more connected to each other, in the cross-sectional
images (Figure 2 d, e). This is probably because of the plasticizing effect of sugars as suggested by
Vieira et al. [31] in starch-, and protein-based biofilms. The film prepared from the non-washed apple
pomace using glycerol as plasticizer, showed a swollen structure compared to the other films (Figure
2 e). This is because of the presence of the glycerol which acts as a secondary plasticizer in the system,
making the movement of the particles much easier. Some studies have reported that in biofilms,
prepared from starch or gluten the effect of the added plasticizer (glycerol) is strongly affected by
naturally occurring water (moisture content) [32, 33]. This supported the assumption of a three-phase
system of polysaccharides-sugar-glycerol that is collectively affecting the film properties. Moreover,
the results indicate a stronger plasticizing effect of the sugars compared to glycerol in the two-phase
systems of polysaccharides-sugars and polysaccharides-glycerol, as the films with glycerol and
without sugar showed a non-connected, layered structure (Figure 2 f).

Figure 2. Biofilms prepared from non-washed apple pomace without (a and d) and with the use of
glycerol (b and e) show a fluffy and more interconnected structure compared to biofilms prepared
from washed apple pomace with the use of glycerol (c and f). Images of (a), (b) and (c) are taken of
the surfaces of films; while (d), (e) and (f) are cross-sectional images. Micrographs were taken
respectively at 250 x and 1.00 K x magnifications using a 20.00 kV accelerating voltage.

3.1.2 Mechanical properties of the apple pomace films

The mechanical properties of the films are presented in Table 2. The film prepared from washed
apple pomace using glycerol as plasticizer exhibited the highest tensile strength at max and lowest
elongation at max. This is also in agreement with the results of scanning electron microscopy where
a layered morphology with higher fiber orientation was observed for this film (Figure 2 f). The
elongation of the non-washed films was significantly increased in the presence of sugars confirming
the plasticizing effect of the sugars. The highest elongation (55%) was obtained for the films prepared
from non-washed apple pomace and glycerol indicating the effect of primary (sugar) and secondary
(glycerol) plasticizers. The stronger plasticizing effect of the sugars as predicted by FE-SEM images
(Figure 2), was also confirmed here as the film prepared from non-washed apple pomace was at least
3 times more flexible than the one prepared from washed apple pomace and glycerol (Table 1).

Higher flexibility (elongation) was accompanied with a significant reduction in the mechanical
strength of the films which is reasonable and has also been also reported before by Cao et al. [34] for
gelatin films and by Bourtoom et al. [35] for rice starch-chitosan films.

Table 2. Different preparation conditions and main properties of biofilms prepared from apple

<table>
<thead>
<tr>
<th>Washing step</th>
<th>Glycerol (%)</th>
<th>Particle size 1 (mm)</th>
<th>Thickness (mm)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>7</td>
<td>0.08</td>
<td>0.11 ± 0.01</td>
<td>16.49 ± 2.54</td>
<td>10.77 ± 3.19</td>
</tr>
<tr>
<td>no</td>
<td>7</td>
<td>-0.08</td>
<td>0.11 ± 0.01</td>
<td>3.27 ± 0.31</td>
<td>55.41 ± 5.38</td>
</tr>
<tr>
<td>no</td>
<td>0</td>
<td>-0.08</td>
<td>0.09 ± 0.00</td>
<td>4.20 ± 0.70</td>
<td>37.39 ± 10.38</td>
</tr>
</tbody>
</table>

1 apple pomace particle size used for preparation of the films

3.2 Production of 3D objects from apple pomace by compression molding

3D square shape objects (fiberboards) were developed from apple pomace using compression
molding technique according to Figure 1. Both washed and non-washed apple pomace were
employed for production of fiberboards using this method.

Thanks to the thermo-triggered self-binding ability of natural fibers, production of binder-less
eco-composites from lignocellulosic materials has been reported in several studies [17-21]. Although
the mechanism of the fiber binding at high temperature under pressure seems to be still unclear, in-
situ plasticization and lignin flow has been reported to be among the factors which facilitate the fiber
binding at very high temperatures (200°C) [17, 19, 21]. Although lignin is present in the apple pomace
(Table 1), plasticizing effect of lignin might not be very significant in the formation of apple pomace
fiberboards as the processing temperature was only 100°C.

On the other hand, addition of plasticizers may lower the shaping temperature by increasing the
mobility of the macromolecules and filling the voids [33] making the binding possible at lowered
temperatures. Two plasticizers were tested in this study i.e., glycerol and free sugars present in apple
pomace. Glycerol has been previously used in several studies [22, 33]. Dark brown sheets (10*10 cm)
were formed from washed apple pomace (0.2 mm particle size) using glycerol as plasticizer and the
thickness of the sheet was 3.2 mm (Table 2). Darkening of gluten films was reported in the presence
of glycerol when pressing temperatures were higher than 100 °C [33] and explained by the possible
effects of higher network density, extensive aggregation, temperature effects of pigments and
Maillard reactions [36, 37]. Apple pomace however does not contain gluten, but glycerol may have
reacted with lignin under the applied conditions. To support this assumption, preliminary
experiments resulted in a dark brown, spilled patch, instead of a firm sheet when lignin was mixed
with glycerol applying the same preparation conditions. There is, however no evidence of lignin
being responsible for the darkening of apple pomace fiberboards when glycerol was added, but it
could be the case.

Sugars have not been examined before for their plasticizing effect in binder-less lignocellulosic
fiberboards. In this study the sugars available in the apple pomace were tested as plasticizer. Non-
washed apple pomace (with particle size of 0.2 mm) was subjected to compression molding and
resulted in light brown sheets with a thickness of 2.2 mm (Table 2). The difference between the
thicknesses of the sheets made of washed and non-washed apple pomace indicate a more compact
structure of the material made of non-washed apple pomace. This confirms the performance of sugars
as plasticizer in formation of binder-less sheets from apple pomace. Moreover, the thickness of the
sheet prepared from non-washed apple pomace with particle size of 1 mm was 2.9 mm indicating a less compact structure due to the presence of bigger particles (Table 3).

3.2.1 Morphology of apple pomace 3D objects

Morphology of the 3D objects prepared from washed and non-washed apple pomace (0.2 mm particle size) was studied using scanning electron microscopy (Figure 3).

Similar to the films, the surface of the material prepared from washed apple pomace was smoother than the non-washed material (Figure 3 a, b). This may indicate a higher orientation of the fibers in washed material and more flexibility of the fibers in non-washed material. However, more cracks are visible in the structure of the film made from the washed material (Figure 3 b), which is indicating a better binding when the non-washed material was used with free sugars as plasticizer (Figure 3 a). The cross-sectional images show somewhat a similar structure for both 3D objects, and some cracks are visible in the washed material (Figure 3 d) compared to the unwashed (Figure 3 c). Therefore, FE-SEM analysis confirms that slightly better binding has been achieved in the presence of sugars as plasticizer compared to glycerol.

Figure 3. Fiberboards prepared from non-washed apple pomace without the addition of glycerol (a and c) show a more connected, smoother structure compared to the ones made from washed apple pomace with the addition of glycerol (b and d) that had cracks in the structure. Images of (a) and (b) are taken of the surfaces of specimens; while (c) and (d) are cross-sectional images. Micrographs were taken respectively, at 250 x and 1.00 K x magnifications using a 20.00 kV accelerating voltage.

3.2.2 Mechanical properties of the apple pomace 3D objects

Fiberboards made of washed apple pomace and glycerol had a slightly higher tensile strength compared to the one made of non-washed, 5.8 and 3.7 MPa, respectively (Table 3). This is probably because of the higher orientation of the fibers in the washed biomaterial, which could be the result of the wetting effect of glycerol. The difference was however not as high as the one observed between different apple pomace films. This is because of the differences in the mechanism of the particle binding in the casting and compression molding methods. The tensile strength of the biomaterials prepared from non-washed apple pomace with 1 mm particle size was slightly higher than the one...
with 0.2 mm particle size which indicates better binding of particles in the case of smaller particle sizes.

The modulus is a measurement of the stiffness and it follows the same pattern as the tensile strengths with somewhat higher modulus for the washed biomaterial. However, specimens prepared of 0.2 mm particle size, without washing had slightly higher strength and lower modulus than the specimens of 1 mm particle size. Considering the standard deviations, the values for tensile strength and for modulus are not significantly different from each other.

Moreover, the elongations at max of the fiberboards were much lower compared to the films. This is due to the weaker binding of the particles in the compression molding compared to the solution casting method.

Table 3. Different preparation conditions and main features of fiberboards prepared from apple pomace applying 8 MPa pressure for 20 min at 100°C.

<table>
<thead>
<tr>
<th>Washing step</th>
<th>Glycerol (%)</th>
<th>Particle size (mm)</th>
<th>Thickness (mm)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation (%)</th>
<th>Young’s modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>30</td>
<td>0.2</td>
<td>3.18 ± 0.07</td>
<td>5.79 ± 0.79</td>
<td>1.54 ± 0.09</td>
<td>633.4 ± 65.6</td>
</tr>
<tr>
<td>no</td>
<td>0</td>
<td>0.2</td>
<td>2.17 ± 0.51</td>
<td>3.71 ± 0.80</td>
<td>1.56 ± 0.13</td>
<td>367.1 ± 82.6</td>
</tr>
<tr>
<td>no</td>
<td>0</td>
<td>1</td>
<td>2.91 ± 0.02</td>
<td>3.02 ± 0.65</td>
<td>0.93 ± 0.21</td>
<td>485.7 ± 94.7</td>
</tr>
</tbody>
</table>

1 apple pomace particle size used for preparation of fiberboards

4. Conclusions

In this study, solution casting and compression molding techniques were successfully employed for preparation of biofilms and 3D objects from apple pomace, respectively. The choice of the plasticizer affected the characteristics of the products. The use of glycerol resulted in films and fiberboards with higher tensile strength. Using natural occurring sugars as plasticizer resulted in products with more connected structure, where in the case of the films; it resulted in much higher elongation at maximum tensile strength. Finally, using apple pomace to produce biofilms and 3D objects paves the way for producing environmentally-friendly materials that could both be a solution to the problem of plastic pollution and apple pomace disposal. The new materials may be suitable for different applications including edible packaging and tableware.


Funding: This research was funded by University of Borås.

Acknowledgments: The authors would like to express their gratitude to Lyckans Äpple who kindly provided the apple pomace for this project. The authors would also like to extend their gratitude to Dahn Hoang for collaboration in the laboratory and to Patrik R. Lennartsson for technical support.

Conflicts of Interest: The authors declare no conflict of interest.

References


The extensive and reckless usage of single-use conventional plastics, creates shortage in the valuable oil supply, kills numbers of species, contaminates our oceans, increases greenhouse gas emissions, leads to aesthetic nuisances, pollutes the food chain and plastics are considered as the major toxic pollutants of present time. The replacement of conventional plastics with biodegradable and bio-based plastics is one possible way to address today’s plastic pollution. The land use for cultivating crops for bio-based plastics is however still negligible, the interest in utilization of secondary feedstock for the production of bioplastics has been emerging. The recovery of lignocellulosic by-products further reduces the arable land used, feedstock greenhouse gas emissions and other feedstock related impacts.

Lignocellulosic by-products such as forest residues and by-products of food processing industries have a good potential in the production of different bio-based plastics through processes that usually involve more than one production stage. An example for that is the production of lactic acid from lignocellulosic waste via fermentation that is then used for the production of the bioplastic polylactic acid. An emerging trend in bioplastics research is however, the transformation of the entire residue of industrially processed vegetables and fruits without separation, directly into biomaterials. In this thesis, the production of flexible biofilms and solid biomaterials were carried out from orange waste and apple pomace. Both residues are sources of industrial pectin production and have relatively high cellulose content which materials have been intensively researched for the production of bio-based materials.

Biofilms and 3D biomaterials fabricated in this thesis had mechanical properties comparable with commodity plastics, but further improvements e.g. on the water resistance and upscaling are needed for orange waste and apple pomace to become the raw materials of the next generation bioplastics.