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Sheet extrusion and plasticisation of Novatein thermoplastic protein

A thesis

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Abstract

The purpose of this study was to develop a relationship between protein structure, rheological properties and plasticization of Novatein. Novatein is a biomass-based thermoplastic in which the main constituent is blood meal, a highly aggregated protein-rich biopolymer, which is a by-product from the meat industry. It can be processed with industrially scalable thermo-mechanical processing methods such as extrusion and injection molding. However, its properties makes more challenging processing methods such as sheet extrusion limited. This is considered a rheology problem and is related to the protein's structural characteristics that can be modified by using polyol plasticizers such as ethylene glycol (EG), glycerol (GLY), propylene glycol (PG) and triethylene glycol (TEG).

Rheological characterisation revealed the apparent shear viscosity of highly plasticized Novatein and polypropylene (PP) to be very similar even though only PP can be sheet extruded. Novatein's extensional viscosity was significantly higher and its entrance pressure accounted for up to 80% of the total pressure drop with no-polyol plasticized Novatein. Increasing polyol content and temperature were found to decrease the extensional viscosity but simultaneously increased the shear viscosity due to better flow development in the capillary. In other words, poor elongational properties of the no-polyol composition led to flow behavior closer to plug flow. Thus, with longer capillaries apparent shear viscosity of polyol plasticized samples (at the same water content) could become even higher but also raises the question whether fully developed flow is a desired property for processing Novatein.

Synchrotron-based FT-IR measurements with support of XRD explained the difference in the rheological performance further. Blood meal's protein structure was highly aggregated, consisting of up to 50% β -sheets that do not melt into a fully amorphous state at suitable processing temperatures. The behavior of Novatein was considered to be closer to filled polymers, consisting of nano-crystallite aggregates, rather than a semi-crystalline polymer that has reached a molten state. This made sufficient plasticization of the amorphous fraction crucial for processability.

Plasticization was classified into primary and secondary plasticization. In primary plasticization, the plasticizer interacts directly with the protein network by replacing the protein's hydrogen bonding sites

with water. In secondary plasticization, the polymer network becomes saturated, leading to phase separation and increases hygroscopicity. The effect is substrate and plasticizer dependant and secondary plasticization was dominant with Novatein due to its aggregated structure. The plasticizer content at which the equilibrated moisture content (EMC) became equal to that of no-plasticizer compositions was called the point of equivalence (POE). This is also the point at which primary plasticization turns into secondary plasticization.

The POE is unique for all plasticizers and dependant on molecular characteristics such as size and theoretical hydrogen bonding sites. The EMC is a result of these changes (including secondary structure) and strongly correlates with mechanical properties and the brittle-to-ductile transformation. Water provided the ability to form ideally mixed phases, explaining the applicability of the free volume-based plasticizer theory. The Novatein network consisted of protein-rich, plasticizer-rich and an intermediate phase, and the fractional composition and the relative magnitude of each phase was determined by using the Couchman-Karasz model. The role of the intermediate phase was found to make the biggest difference in plasticizer performance and behaved in accordance to the observed secondary structure changes.

Of the selected plasticizers, GLY clearly showed the highest tendency to phase separate followed by TEG, PG, and EG. Of the tested plasticizers the POE values varied between 20 pph_{BM} to 29 pph_{BM} in the order of GLY, TEG, PG, and EG. However, TEG had the lowest POE on a molar basis and nearly three times the molar amount of EG was required to reach the POE in comparison to TEG, whereas for GLY it was only 1.3 times. TEG's plasticization is based on its ability to interact efficiently with the protein network and modify the secondary structure sufficiently. GLY, instead, showed a strong tendency to phase separate leading to the highest hydrogen bonding potential above the POE, and therefore also raised the EMC above TEG level. Despite the increase in ordered secondary structures, GLY led to the highest strain at break, which was attributed to phase separation. EG and PG as smaller sized molecules were able to diffuse into polymer network more efficiently.

Even though the plasticization mechanism varied significantly, EMC was the dominant factor determining mechanical properties with a brittle-to-ductile transformation observed at 8%. However, it is important to understand that an EMC of 8% required different amounts of plasticizer for each polyol. In accordance to the constraint theory, the amount of theoretical hydrogen bonding sites of plasticizer was most appropriate to predict changes EMC. The role of water was significant in forming a ternary system that behaves in accordance to the free volume theory despite the protein's heterogeneous structure. For dried samples, TEG and GLY formed clusters in the polymer network in the absence of water, whereas for hydrated samples the plasticizer was well distributed through the polymer network, albeit in micro-separated regions.

The concept of primary and secondary plasticization provided a better understanding of rheological behavior as well. Elongational flow was dominated by primary plasticization of the protein-rich and intermediate phases whereas secondary plasticization played a significant role in the reduction of the shear viscosity. Flow without secondary plasticization was characterised as a plug flow. PG showed the most efficient plasticization in both shear and elongational viscosity, which was attributed to the combination of its small molecular size and ability to enhance both primary and secondary plasticization. GLY acted mostly as a secondary plasticizer and had the higher elongational and the lowest shear viscosity. TEG as an efficient primary plasticizer with an ability to modify secondary structure performed exceptionally well in terms of extensional viscosity considering its high molecular weight. Higher shear viscosity levels were comparable to EG that was shown to diffuse very efficiently in the polymer network.

With a fundamental understanding of plasticization and rheology, significant process improvements were made by increasing temperature and combining PG and TEG leading to efficient sheet extrusion.

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1

Introduction

Introduction

The implementation of a circular economy is considered one of the required changes considering best practice to overcome climate change [1,2]. It considers utilization of waste and by-products of industries in accordance to a waste minimization initiative, diminishing oil-dependency in the best case. Increased understanding of ecological problems has led to stricter legislation and shifted consumers' demand towards more sustainable alternatives. This has led to a growing demand for bio-based and biodegradable materials [3].

New Zealand has a large agricultural industry which produces a large amount of biomass as by-products [4]. The low-value by-products are often utilised as fertilisers and animal food. However, these often have a great potential to be converted into value-added products [5]. Biomass-based plastics is one potential application and has been shown to be a sustainable alternative to conventional plastics. In addition to renewable resources, they are considered one of the rare polymers that actually degrades in the undesired event of uncontrolled waste management [6]. Starch and protein-based plastics are examples of biomass-based polymers that can be converted into thermoplastic materials with the potential to have similar properties compared to conventional plastics.

Novatein is a protein-based thermoplastic material patented and commercialized by Aduro Biopolymers LP [7,8]. Novatein is produced from blood meal which is a protein-rich polymer by-product from the meat industry. It is a highly aggregated polymer network held together through hydrogen bonding, hydrophobic and ionic interactions as well as covalent crosslinks [9]. Due to this, suitable modification is required to obtain sufficient chain mobility to form a thermoplastic. Similar to conventional polymers, Novatein can be reshaped in thermo-mechanical processing methods such as extrusion and injection molding.

The processing behavior of Novatein is different from conventional polymers. Proteins have a semi-crystalline structure like conventional polymers, however, the ordered structures do not unfold into a fully amorphous state in the process [9]. Relaxation mechanisms of amorphous regions relating to the glass transition temperature (T_g) play a significant role in terms of achieving sufficient chain mobility.

The T_g of blood meal is above its degradation temperature due to excessive heat treatment during steam-coagulation. This requires sufficient plasticization to allow thermo-mechanical processing well below the polymer's degradation temperature.

Currently, Novatein's processing properties are insufficient for more demanding methods such as sheet extrusion. Attempts to sheet extrude compositions that are suitable for the injection molding were unsuccessful as the extrudate was unable to fill the width of the sheet die. The process is also very pressure sensitive and stoppages can easily lead to extruder blockages; in other words, the process as a whole is not understood well enough.

The problem described is rheology related. A better understanding of the flow properties and process requirements are required to expand production capabilities into sheet and film forming, and ultimately blow molding. Limited deformation properties refer to limitations in the extensional viscosity properties and the inability of the chains to rearrange under the strain. This has been strongly linked with the polymer's molecular weight (M_w) and molecular weight distribution (MWD) which are furthermore related to the protein's structure.

A general strategy to improve rheological properties is plasticization. Conventional polymer plasticization theories are also applicable here and somewhat describe the experienced process behaviour. However, with Novatein, the plasticization potential is much more limited in comparison to other studies in the field [10]. Comparable plasticizer addition to other studies leads to a slurry-like substance that cannot be processed. It is therefore necessary to understand the plasticization mechanisms relevant to Novatein and tailor it for the more demanding processes accordingly.

Water is a very efficient plasticizer due to its small molecular size and is a critical component for plasticizing Novatein and many other bio-based polymers. From previous work, tri(ethylene glycol) (TEG) has been shown to be a very effective plasticizer to modify tensile strength and ductility [11]. It also has beneficial effects in terms of secondary structure changes by creating more ordered regions [12]. Despite a large number of studies relating to plasticization effects, the latest reviews have concluded plasticization mechanisms are still not understood well enough [13,14].

The objective of this research is to understand the relationship between protein structure, its modification via plasticisation and rheological behaviour. Plasticization mechanisms are considered a crucial precondition in terms of achieving better process control and predictability in terms of material properties. The study addresses the interaction between the protein network, polyol plasticizers and water, which creates a ternary system in its simplest form. Plasticizers are known to affect a protein's secondary structure, which is further studied in this study in light of plasticization.

In the thesis, the technical objectives were addressed in four journal papers, tied together in an overall discussion. These were preceded by a literature review in the form of a book chapter. Dynamic mechanical thermal analysis (DMA) and X-ray diffraction (XRD) were used to investigate thermal transitions and chain relaxation while Synchrotron-based Fourier transform infrared spectroscopy (FT-IR) was used to investigate chain architecture and structural changes. Extrusion, injection moulding, screw driven capillary rheometer and mechanical testing were used to investigate macroscopic properties.

A general lack of understanding about bioplastics has created confusion and misinterpretation among consumers and industry. Chapter 2 ties together the current state of the bioplastic market providing an up-to-date definition of bioplastics, technologies involved, their environmental profile and contribution to the global economy as well as future trends. This chapter, together with the literature presented with each additional chapter, forms the scientific background and foundation of this study.

Chapter 3 examines the effect of different polyol plasticizers on protein plasticization. The focus is in assessing the change in water adsorption of Novatein and how plasticization affects this in terms of hydrogen bonding with the protein (primary plasticization) and phase separation (secondary plasticization). These are considered in the context of structural changes to the protein and mechanical properties, in particular, the brittle-to-ductile transition.

Chapter 4 expands on concepts presented in Chapter 3 by assessing the plasticizers effect on sorption isotherms and the role of water in plasticization. The study focuses on understanding how the plasticizer and water interact in a competitive hydrogen bonding environment and how phase separation is

dependent on the environment in which the material is altered. Conventional plasticization mechanisms are discussed with particular reference to the free volume and constraint-based theories and how these are applied to predict material properties. The works aim to build on the theories around hydrogen bonding presented in Chapter 2 by also considering plasticizer distribution in the protein network.

The concept of ternary plasticization mechanisms was studied in Chapter 5. Free volume and constraint-based plasticization theories were applied to Novatein using dynamic mechanical analysis (DMA) in which chain relaxation and thermal transitions were related to material behaviour. The aim was to understand the role of phase separation as a part of total plasticization and unify the theories presented in Chapters 3 and 4.

The final chapter deals specifically with the rheology of plasticized Novatein and the possibility to sheet extrude it. Chapter 6 aims to put the rheology of Novatein in the context of conventional polymers, with a focus on extensional viscosity which is rarely characterised with protein-based plastics. The study draws on synchrotron-based FT-IR that is used to assesses the secondary structure of proteins and aims to link this to the material's rheology. The study is the first in the field that brings together rheological characterisation with a practical approach of sheet extrusion.

The technical chapters are followed by a concluding chapter (Chapter 7) that discusses sheet extrusion of protein-based biopolymers in general based on the fundamental understanding of Novatein plasticization and its rheology. The purpose of this chapter is to highlight how all the technical chapters contributed to the overall aim of the work. It also provides recommendations for future work.

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2

Bioplastics

An invited book chapter published in

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By

C.J.R. Verbeek and Jussi M. Uitto

Bioplastics

Chapter 2 evaluated the current state of the bioplastic market including technological, environmental, financial and social aspects. This chapter highlighted the technological potential and limitations of bioplastic production, how they are generally perceived and the biggest barriers for the market entrance.

Although I was not first author of this work, it has been included in Thesis as my contribution considered 85 per cent of the total chapter preparation. As a co-author, I prepared the first draft of the chapter, which was, together with my supervisor, revised and edited into the form submitted for publication.

ABSTRACT

Bioplastic materials have been developed in response to environmental concerns regarding the widespread use of conventional polymers. It is one of the cornerstones in the circular economy approach that is expected to change the way materials and energy are produced and consumed. However, bioplastics still cover less than 1% of total market, and the industry is still in its early stages. A holistic sustainability assessment takes into account the origin of a material, its end-of-life destination, as well as social and economic effects; new materials in the market need to improve sustainability but also be cost- and performance-competitive. Biobased polymers have taken the leading role in the market, largely because of total production of bioplastics is expected to increase almost fivefold over the next 5 years. This article provides an up-to-date definition of bioplastics, their environmental profile, and contribution to the global economy.

BIOPLASTICS

1. Introduction

Over the past 50 years, polymer production has increased more than 20-fold, now exceeding 300 million tonnes/year (1). Polymer materials have revolutionized several industries by making a huge impact on the efficiency and convenience of supply chains, from material processing to packaging. What is more, polymers had an obvious positive economic impact, but also an indirect environmental impact. The availability of polymeric packaging is one of the major factors that led the change in the packaging and transportation industries by reducing the amount of food waste and making transportation systems more efficient (1).

However, polymers are also facing a lot of criticism, ironically due to their effect on the environment. Their superior durability combined with mass consumption and people's poor recycling habits have led to a global waste problem. In Europe alone, annual polymer waste generation amounts to about 25 million tonnes/year, compared to a global recycling rate of only 21 million tonnes (2). Waste is often burned, landfilled, or in the worst case scenario ends up in the ocean. Marine litter has reached a critical level, and by some rough estimate increases by 4.8–12.7 million tonnes/year (3).

Polymer material's impact is not only measured by biodegradability or recyclability; a much more holistic view is required, often captured by a life cycle assessment (LCA), or in a simpler form, its carbon footprint. Considering the size of the polymer industry, striving toward carbon neutrality could potentially make a huge positive environmental contribution (2). This could be achieved by using more renewable raw materials or by using waste and/or by-products of other industries.

In accordance to a general waste minimization initiative, global implementation of a circular economy model, as a replacement for the linear petrochemical economy, is one of the main strategies against global warming, littering, and oil-dependency (2,4). A circular economy is intended to reduce carbon emissions by using renewable resources and/or decreasing waste buildup by using waste products as raw materials, thereby providing an alternative recycling method to the primary product. The change can be seen from supportive regulation and legislation that has encouraged environmentally sustainable business practices and consumer behavior. Fortunately, this is also driven by consumers, evident from the change in behavior seen from the world's biggest companies in relation to their sustainability practices.

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Biopolymers has been widely recognized to fill this demand, but still plays a minor role by covering less than 1% of the polymer industry, but it is forecasted to change significantly (5). In 2012, the petrochemical market information provider ICIS stated,

The emergence of bio-feedstocks and biobased commodity polymers production, in tandem with increasing oil prices, rising consumer consciousness and improving economics, has ushered in a new and exciting era of bioplastics commercialization. However, factors such as economic viability, product quality and scale of operation will still play important roles in determining a bioplastic's place on the commercialization spectrum. (6)

In 2015, more money has been invested in the bioeconomy despite the oil price being at its lowest level in past 20 years (7,8). However, globally, the idea of a bioeconomy has just started; a number of competitive techniques and innovations are piloted in parallel to find the most suitable and sustainable solutions. This industry is extremely dynamic and has great potential to change the way material sourcing is approached in future.

The idea of biomaterials is not that new; in the beginning of the 20th century, most appliances were made from biomaterials such as cellulose acetate, vulcanized rubber and casein, and, for example, Henry Ford had his vision of bringing a "soybean car" to the automotive industry. However, oil-based thermoplastics dominated the market after the 1950s as they provided a better platform for product diversity together with easier processability. It can be stated that back in 1900s, technology and the understanding of polymer physics were not developed enough, and conventional plastics, despite the current criticism, has been a necessary step to learn material behavior to the extent where we can now manipulate the properties of natural materials.

Poor material properties as well as high cost are often considered the major drawbacks of bioplastics. For example, the mechanical properties of biodegradable grocery bags may not be as good as commodity polymers and they may be more expensive as well. However, biobased commodity plastics such as BioPE have the same properties and similar processing characteristics as their petrochemical equivalents. In biomedical applications, bioplastics may offer even greater benefits, providing better biocompatibility or by removing the need for removal after it has performed its function in the body. Another good example is in the agricultural industry where the replacement of mulch film with biodegradable alternatives has been well received, and some examples have been able to provide some functional properties such as extended lifetime of crops (9,10). In the food industry, protein-based films have been reported to provide extended shelf life for cheese products because of their superior oxygen barrier properties in comparison to petrochemical films (11,12). There is also growing potential for edible biobased plastics, for example, silk-fibroin coating of fruits has been reported to also extend shelf life (13).

Historically, the onset of a new era of materials (eg, stone, bronze, iron, and plastics) has been based on the development of improved material properties. Material properties are still the driving force in the market; however, sustainability is gaining importance as a driving force for purchasing decision making and, in

some cases, consumers may even be ready to pay for it (14). Generally, some the following factors should be valid for a bioplastic solution to be considered feasible. The material should have a sustainable origin, or the material should be produced from waste and/or by-products. Alternatively, the proposed material should have the same or better performance in comparison to its petrochemical equivalent. Or lastly, the material should have a controlled end-of-life destination, that is the material's recycling or degradation properties have to be same or better than its petrochemical alternative, for example, being fully biodegradable.

Unfortunately, one factor that has limited market entry has been some confusion around terminology. Generally, bioplastics is associated with more sustainable alternatives to conventional plastics but further subcategories such as the origin of the polymer, biodegradability, or polymer blends has caused some confusion under consumers as well as industry. Consumers often associate bioplastics with biodegradability; however, focus should be more on its complete lifecycle. In contrast, the biomedical industry often associates bioplastics with the requirement of biocompatibility in human body whereas within the polymer industry it could mean anything, as long as it improves the market potential.

This article intends to provide an up-to-date definition of bioplastics, their environmental profile, and contribution to the global economy. It will cover current bioplastics, applications and their markets. This will be supported with case studies that describe the potential but also complexity of the market.

2. The Definition of Bioplastics

The definition of bioplastics is not precise, and combined with a general lack of understanding, has created some confusion and misinterpretation throughout the value chain. The Society of Plastic Industry's (SPI) bioplastics council suggested that confusion around terminology is one of the four major growth challenges in the bioplastic industry (15). The terms bioplastics, biobased origin, and biodegradability are considered being related, but need some further clarification as, for example, some bioplastics can also be petroleum derived but biodegradable, or biobased but not biodegradable.

Probably the most appropriate description for the term "bioplastic" is that it is more often used as a catch phrase; it vaguely represents a larger group of different kinds of polymer products that are more sustainable alternatives to petroleum-based commodity plastics, but not necessarily biobased or biodegradable, as they are commonly perceived. In 2012, The International Union of Pure and Applied Chemistry (IUPAC) discouraged the use of the term bioplastic and recommended to use biobased polymer and classified it only to be derived from biomass (16). However, this can also be problematic because it excludes some petroleum-based biodegradable polymers. The latest industry publications as well as those from the United Nations are supporting a vaguer definition (17,18). European bioplastics defined bioplastics as "biobased, biodegradable or both," whereas the SPI's definition is "partially or fully biobased and/or biodegradable" (19). The overlap between these terms is accurately summarized in Figure 1 and highlights why the term bioplastic could be so confusing without further clarification.

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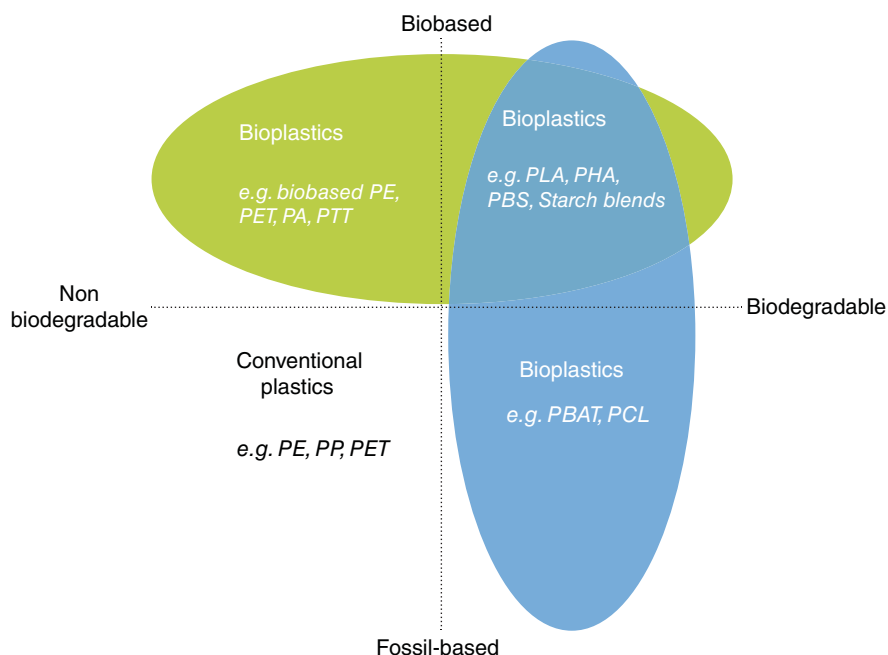


Fig. 1. Definition of 'bioplastic' according to European bioplastics. Reprinted with permission from Ref. 17. Copyright 2015 European Bioplastics.

The basic ideology behind bioplastics is using natural carbon sources instead of petroleum-derived equivalents. Technically, almost all fossil-based materials could be substituted from biobased alternatives (20). The biobased content of bioplastics is defined by its biobased carbon content or simply the mass fraction biobased content. Biobased carbon content is quantified by the material's C-14 content because it is not found in fossil fuels and is described in CEN/TS 16137 as well as ASTM 6866. The mass fraction biobased content is a complementary method that takes into account chemical elements other than biobased carbon, such as oxygen, nitrogen, and hydrogen. Also related certifications by the Belgian certifier Vinçotte or German certifier DIN CERTCO have been created to simplify customer's decision making in the markets. For example, Vinçotte has created a four-star "OK-biobased" classification system in which one star would represent 20% biobased content whereas four stars requires at least 80%.

Biodegradability is often wrongly understood, even misleading, and sometimes used as a marketing tool even if there would be better recycling methods available. Biodegradability does not necessarily refer to environmental degradation and in most instances refers to degradation in industrial composting at elevated temperatures. Most importantly, biodegradability should be seen as complementary and secondary alternative to recycling, rather than a material property that removes the littering problem and the responsibility from individuals to dispose plastics responsibly.

Biodegradability implies that a material can be completely converted into natural substances, including biomass, water, carbon dioxide, and/or methane, via

Table 1. The Most Common Backbone Structures of Polymers

Type of Bond	Natural Example	Synthetic Example
Carbon-carbon (-C-C-)	Polyolefins (eg, rubber)	Polyolefins (eg, PE, PP)
Ester (-O-C=O-)	Nucleic acids (eg, DNA, RNA)	Polyesters (eg, PLA, PET, PCL)
Amide (-C=O-NH-)	Polypeptides (eg, wool, silk, enzymes)	Polyamides (eg, nylon)
ether (-O-)	Polysaccharides (eg, starch, cellulose)	Special plastics

the action of naturally occurring microorganisms such as bacteria and fungi (21). If degradation is incomplete, it may lead to micro- and nanosized fragments as well as other synthetic products that may have harmful environmental and human health effects. All polymers will degrade, but the speed and degree of degradation differs; hence, biodegradability implies degradation within a reasonable timescale (22). High molecular weight, melting point, and crystallinity all reduce the degree to which a polymer is likely to degrade, whereas some of polymer backbone structures, such as ester, ether, or amide bonds, are characteristic of higher biodegradability (Table 1) (18).

Biodegradation is a complex phenomenon and primarily dependent on both the structure of material and the environment. The well-known citation of Louren Baas Becking stated, “Everything is everywhere, but, the environment selects” and is applicable to biodegradation as well (23). Biodegradation is primarily microbiological population, temperature, and time dependent, but also has some indirect and interactive mechanisms; for example, Brodhagen and co-workers discussed the idea that disposing biodegradable plastics into soil would potentially encourage the enlargement of populations of fungi and bacteria capable of degrading them (24).

For the sake of simplicity, biodegradation is usually divided into subcategories (Fig. 2) and standardized mechanisms. For example, EN 13432 distinguishes between fresh and sea water degradability. Mechanisms can be described as abiotic or nonbiological (hydrolysis and photodegradation) and biological or biotic (eg, enzymatic and inside the cell membrane) (19). The most common form of biodegradability is compostability, which is described in standards such as EN 13432 or ISO D6400. In these cases, biodegradation and disintegration take place in an industrial facility in a controlled aerobic environment in which bacteria and fungi are able to break down the polymer at high temperatures (50–60°C). A product is considered biodegradable if 90% biodegradation is reached within 180 days. EN 13432 does not cover home composting, which mimics industrial composting, but at lower temperatures and a less stable environment. This leads to lower degradation rates and also excludes some polymers that require higher temperatures to start biodegradation (eg, poly(lactic acid) (PLA)). Products labeled home compostable need to demonstrate at least 90% biodegradation within a year at temperatures below 30°C and at least 90% disintegration within the first 6 months.

6 BIOPLASTICS

	Anaerobic Bacteria, no fungi	Aerobic Bacteria & fungi
50-60°C	Chemical pulp Starch PLA Starch/PCL PHA	Chemical pulp Mechanical pulp Starch PLA starch/PCL PHA PBAT
≤35°C	Chemical pulp Starch Starch/PCL PHA	Chemical pulp Mechanical pulp Starch starch/PCL PHA PBAT

Fig. 2. Different biodegradation mechanisms based on fermentation type and temperature. Reprinted with permission from Ref. 27. Copyright 2011 Elsevier.

The most difficult condition for biodegradation is the open environment, such as soil, fresh, and sea water (18,25). The report from the United Nations Environment Program (UNEP) entitled “Marine plastic debris and microplastics—Global lessons and research to inspire action and guide policy change” in 2016 underlined society’s responsibility toward waste management and collection in the fight against marine plastic pollution (18). The report concluded that biodegradable plastics are not the solution to the marine litter problem. Sea water, with a low population of micro-organisms, makes it hard for materials to degrade; however, some polymers such as starch and PHA are able to degrade even in the sea (26). Even with these polymers significant differences among polymer types can be seen as the half-life of starch–PHA-blend was 19 and 158 days of starch and PHA, respectively.

One notable subcategory of “biodegradability” is oxo-degradable (or photodegradable) plastics. In this case, prooxidants are added to normally nondegradable polymers to make them disintegrate upon exposure to sunlight and oxygen. The subject of whether mineralization occurs is under intense debate and with concern that undesired microplastic fragments may form under these conditions. For the same reason, blending recyclable, nondegradable polymers with biodegradable polymers may ruin both recycling and biodegradation for the blend.

A polymer’s end-of-life is probably the most crucial question from a sustainability point of view and is also strongly linked to public perception of bioplastics. However, it is also one of the biggest factors causing confusion. Bioplastics are quite often considered a “guilt-free” material choice that takes responsibility away from the individual. However, this is rarely the case. Littering is, in part, an outcome of uncontrolled waste management, but not the plastic material itself. Biodegradability is not the solution for the littering problem, and bioplastics

BIOPLASTICS			
Renewable polymers		Fossil-based biodegradable polymers	
<p>Biomass-based</p> <p>Polysaccharides Starch-based polymers Cellulose Hemi-cellulose Chitosan Lignin</p> <p>Proteins Casein, whey, collagen, bloodmeal, zein, soya, gluten</p> <p>Lipids</p>	Biotechnology		
	<p>Biosynthetic</p> <p>Polyhydroxyalkanoates (PHAs)</p> <p>PHB, PHBV, PHBH</p> <p>Xanthan, Curdlan</p>	<p>Semi-biosynthetic</p> <p>PLA</p> <p>PGA</p> <p>PBS, PBAT</p> <p>BioPE, BioPP, BioPET</p> <p>Polyamids</p>	<p>Polycaprolactone (PCL)</p> <p>Polyvinylalcohols (PVAs)</p> <p>Polyesteramides</p> <p>Aliphatic copolyesters</p> <p>Aromatic copolyesters</p>

Fig. 3. Bioplastic subcategories and examples of the most common polymer types.

may not necessarily biodegrade. However, biodegradability is definitely not an unwanted property and does offer great complimentary end-of-life options, even soil and marine degradability in some cases. One aspect of bioplastics not fully resolved yet, as in contrast to petrochemical plastics, is collection for recycling or collection for reuse.

3. Current Bioplastics

Synthetic polymers are conventionally produced via polymerization of petrochemical derivatives such as ethylene. During the last century, numerous thermoplastics and thermosets have been developed to serve different needs of the market. One of the aims of the bioplastic industry is to offer an environmental alternative to these polymers, replacing as many petrochemical polymers as possible.

Generally, bioplastics can be divided into several different subcategories based on their origin (Fig. 3). At the top level bioplastics is divided into renewable and fossil-based biodegradable polymers. Renewables can be divided further into several different subcategories based on which way polymerization occurred. For example, starch is a very common raw material for all subcategories of renewable polymers. It can be used directly as a material source, but mostly it is fermented into monomers and then polymerized via conventional processing techniques. One drawback of this could be a higher carbon footprint; however, this may be inevitable as material properties of biomass-based plastics are often not good enough to satisfy the market needs.

Replacing petrochemical polymers with bioplastics requires careful consideration of several aspects such as sourcing, processing, application properties, end-of-life destination, and price. In Table 2, the materials are grouped based on their most common characteristics in different steps of the value chain. The

Table 2. Bioplastics and Their Properties

Polymer	Sourcing	Processing	Application Properties	End of Life When Recycled	End of Life When Littered	Price
Biomass-based	++	-	(+)/-	+	++	++
Biosynthetic	+	+/-	+	+	++	--
Semibiosynthetic polyesters	+	+	+	+	(+)/-	-
Semibiosynthetic drop-in polymers	+	++	++	++	--	-
Fossil-based biodegradable	-	+	+	+	+/-	+/-
Fossil-based commodity plastics	-	++	++	++	--	++

Definitions: - -, outcome that should be avoided; -, negative outcome; +/-, negligible outcome; +, positive outcome; ++, desired outcome.

Table 3. Examples of Biomass Sources for Plastic Production (32–34)

Starches	Plant-Based Protein	Animal-Based Protein
Rice	Wheat gluten	Bloodmeal
Maize	Corn zein	Casein
Potato	Soy protein	Whey
Cassava	Rice bran	Keratin
Wheat	Peanut protein	Collagen
		Gelatin

classification is only indicative, but highlights their benefits and drawbacks and how polymer types differ from each other. Ultimately, the most suitable polymer has to be selected based on its application. For example, biodegradability might not be the most important property for car tires, whereas plastic bag production will require very good processability. Furthermore, sourcing and end-of-life properties are strongly dependent on geography; poly(ethylene terephthalate) (PET) bottles often end up in the ocean, whereas in many Western countries the vast majority is collected and recycled. The most suitable materials have to be selected accordingly.

3.1. Biomass-Based Polymers. Industrial and agronomical waste- and by-product streams have the potential to provide a great amount of organic matter that can be used as a raw material for biopolymers (28). This material group is also called agropolymers as they are extracted from plants or animals (Table 3). As opposed to other polymer types, no further fermentation or polymerization is required, as nature’s own building blocks are used as a raw material. Thus, using biomass is often considered the most efficient source for bioplastics (29). Common characteristics of agropolymers are their hydrophilicity, fast degradation rate, and sometimes unsatisfactory mechanical properties, particularly in wet environments (30,31). Biomass-based polymers can be polysaccharides, proteins, lipids, or other greatly abundant natural compounds such as lignin. Similarly, to conventional plastics, all of these polymers consist of a carbon backbone with a variety of different side groups that can form inter- and intramolecular hydrogen bonds. The capability of forming a plastic material comes from the ability to disrupt these hydrogen bonds temporarily under controlled circumstances and cause flow into new material shapes and sizes.

The most widely commercialized biomass-based plastic is thermoplastic starch (TPS). Starch is the world’s second most abundant natural polymer as is the energy-storage mechanism for plants. It can be derived from maize, rice, potatoes, or wheat. Starch is a polymeric carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. It normally consists of 20–25% linear amylose and 75–80% helical amylopectin units (Fig. 4). The mechanical properties of TPS depend on the original sources of starch, additives, glass-transition temperature (T_g), crystallinity, and the ratio of amylose to amylopectin (35). It has been shown that pure amylose films are stronger, whereas pure amylopectin films are more brittle (36). Starch rich in amylose is usually preferred for conversion to TPS as the linearity of amylose improves the processability of starch even

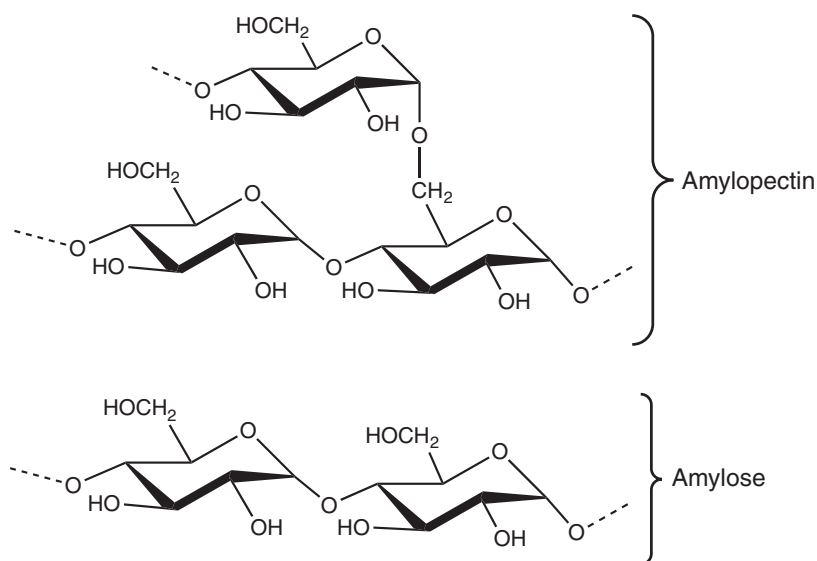


Fig. 4. Amylose and amylopectin units of starch.

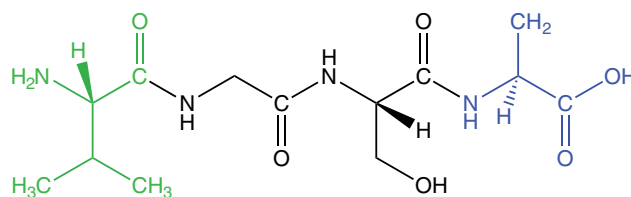


Fig. 5. An example of primary structure of polypeptide chain (protein).

though it is present as a minor component (between 20 and 30 wt%). Amylose has been suggested to lead to higher crystallinity and thus also stronger mechanical properties.

Proteins also have commercial potential, especially when sourced from industrial by-product and waste streams. As opposed to starch, they are complex, heteropolymers consisting of up to 20 different amino acid monomer units (Fig. 5). Each amino acid has its own specific side group with characteristic properties such as polarity, structural complexity, and electric charge. As a result of this broad range of potential functional groups, amino acid residues (the repeat unit in the protein chain) are able to form numerous intermolecular bonds and interactions, leading to significant structural differences (33). Composition of different proteins varies considerably and determines their properties and behavior (37). For example, the average molecular weight of proteins varies between 17 and 300 kDa (38). The amino acid sequence is called the primary structure and in the native conformation is folded further into a secondary (conformation), tertiary (overall folding of the polypeptide chain), and quaternary structure (specific association of multiple polypeptide chains) also responsible for its crystallinity (33).

In terms of processing and applications of biomass-based polymers, their native structure together with plasticization and processing history plays the most significant role (39). The structure of biomass-based polymers varies significantly, but, for example, native starch does not have a thermoplastic character. Also different processing steps in by-product and waste streams may have affected their sensitive structure, and thus reduced processability even more. Plasticization is an efficient way to modify biomass' thermoplastic character (32). Common plasticizers include water or polyols such as glycerol, propane-1,2-diol, and ethylene glycol. Once biomass is plasticized, it has to be processed in the correct thermo-mechanical processes in a way that, for example, starch, is able to undergo gelatinization and denaturation for proteins (40).

In addition to processing, plasticizers also greatly affect mechanical properties. In Table 4, some of the most relevant properties (T_g , ultimate tensile strength, Young's modulus, and elongation at break) of biomass-based materials with different plasticizers and plasticizer contents are compared to some commodity polymers. As a rough generalization, biomass-based plastics are brittle, but strength can be modified to be almost as strong or as ductile as commodity polymers but the outcome is often a compromise between these two properties. Additionally, the amphiphilic nature of biomass influences its mechanical properties, meaning that, similar to wood, it absorbs and desorbs water from the surroundings. Because of their moderate properties, biomass-based materials will probably not replace commodity plastics; however, it is a sensible option for applications where their properties are good enough and their biodegradability is important.

To date, both starch and protein have been commercialized in the field of bioplastics. Applications have focused on markets for which renewability and biodegradation are value-added properties, for example, different packaging and agricultural products. One of the frontiers in markets has been Novamont with starch-based MaterBi[®], which has several different grades (49). Their blend of starch and polycaprolactone (PCL) has shown good performance even in film extrusion applications. Novatein[®] is protein-based by-product of the meat industry and is mostly used in agricultural applications (50). In addition to starch and proteins, chitosan and lipids are of interest. Chitin is already used in biomedical applications because of its biocompatibility and degradability but also for bioplastic applications (51). Different wood components such as cellulose, hemicellulose, and lignin are currently under investigation and are an interesting sourcing option, as the polymer is not coming from industrial agricultural production.

3.2. Biosynthetic Polymers. Many monomers that are normally derived from petrochemical process can be produced via microbiological activity as well. Biosynthetic polymers are produced intercellularly in bacteria from various different carbon sources such as sugars and lipids. Polyhydroxyalkanoates (PHAs) are polyesters, synthesized by bacteria and usually considered as carbon natural energy storage structures of plants (Fig. 6) (52,53). While the production of other biobased polyesters requires fermentation and polymerization of a carbon source, PHAs are polymerized inside the cells of biota, but requires a separation process for recovery. Over 100 different PHA structures with different properties are recognized and can be controlled by bacteria selection. Most often PHA refers to poly-3-hydroxybutyrate (PHB) or poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHVB). PHAs with three to five carbon atoms are

Table 4. Mechanical Properties of the Most Common Synthetic Plastics and Bioplastics

Polymer	T_g	UTS	Modulus	$E\%$	Reference
LDPE	-120	20	50-250	100-600	(41)
PP	-20	30-70	600-1700	400-900	(41)
PS	100	50	3000-3100	3-4	(41)
PVC	82	10-60	3400	20-80	(41)
PET	74-79	80	2000	60-165	(42)
6,6-Nylon	-	83	2800	60	(41)
PLA (Ingeo)	55-60	70	3500	6	(43)
PBS (GSPla)	-38	40	550-700	350	(44)
P(3HB)	4	40	3500-4000	3-8	(41)
P(3HB-co-4HV)	-	24	-	242	(41)
Pea starch	-	55	2210	4.1	(45)
No plasticizer	-	42,2	1770	2.2	(45)
10 Glycerol	-	20	790	4.9	(45)
25 Glycerol	-	11.3	710	28	(46)
protein based	60	35.5	392	813	(47)
Novatein®	90	66.7	2070	4.2	(48)
PCL	-	-	-	-	-
PEF	-	-	-	-	-

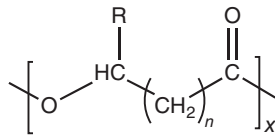


Fig. 6. Structure of polyhydroxyalkanoates.

considered as short chain PHAs (scl-PHAs). Examples of this class include poly(3-hydroxybutyrate), P(3HB) and poly(4-hydroxybutyrate), P(4HB)). Medium chain length PHAs (mcl-PHAs) contain 6–14 carbon atoms. Examples include homopolymers poly(3-hydroxyhexanoate) P(3HHx), poly(3-hydroxyoctanoate) P(3HO), and heteropolymers such as P(3HHx-co-3HO) (54).

It is stated that PHAs are the most versatile fully biodegradable polymer with properties closest to synthetic polymers (54). PHAs can be derived from biowaste, modified into various different structures with different processing and application properties and are also biodegradable even in marine environments (55). However, a drawback is that sourcing is still inefficient, leading to a high price that has been the biggest barrier for market entrance. The most frequently reported factor influencing the price of PHA is the cost of the carbon source and the separation process after polymerization (56). For waste products to be used extensively, the consistency and reliability of the raw material, storage issues, and the correct balance of the ingredients will need to be considered carefully.

PHA represents a wide material family and because of its wide range of material properties can be successfully blow molded, injection molded, or even foamed (56). However, the most common commercial grades are mostly used for injection molding, have good mechanical properties, but are relatively brittle. PHAs are degraded in the environment because many microorganisms present in soil are able to secrete enzymes that hydrolyze the ester bonds into water-soluble monomers and oligomers (55). Microorganisms are then able to metabolize these degradation products into water and carbon dioxide.

Only a few PHA products have been commercialized, for example, Biogreen, Nodal, Biocycle, and Biopol, and their future largely depends on improved production efficiency. It is bioplastic that has been under heavy investigation for long time. Despite the absence of a final breakthrough, research in this field is providing more encouraging results all the time (57,58). A single step biosynthetic route could be attractive for PLA and its copolymers as well, if it eliminates some unit processes (59,60).

3.3. Semibiosynthetic Polymers. Polymers are considered semibiosynthetic when microbiological activity is used for producing simpler monomer compounds that, in plastic's case, would be normally derived from oil. Furthermore, these monomers can be polymerized into the same structures as their petroleum-based equivalents (61). By this processing route, biomass can be used for producing simpler, well-known, and better controlled substrates for plastic production.

A great example of a semibiosynthetic polymer is PLA, which was initially produced as a synthetic polymer via the hydrolysis of lactonitrile (62,63). However, as a petrochemical polymer, production costs were too high to be commercially viable. Nowadays, PLA production is more than 350 kilotonnes/year and more than

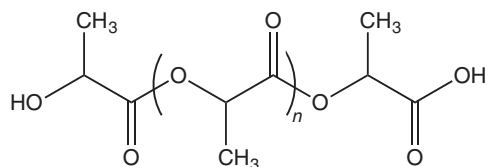


Fig. 7. PLA structure.

90% is done via polymerization of lactic acid fermented from glucose. Semibiosynthetic polymers are currently mostly based on the development of petroleum-based plastics, but the field also has great potential to produce novel green materials that may challenge current market trends.

3.3.1. Polyolefines. In recent years, “drop-in” polymers, or biobased polymers that have the same chemical structure as their fossil-based counterparts, have started to claim a bigger share of the total plastic market and, especially, the bioplastic market. The change has been sudden; in 2010, these materials have been virtually nonexistent, but now represent more than half of the bioplastics market (17). ‘Drop-ins’ mainly refers to commodity plastics, for example, polyolefins and PET; however, it is good to recognize that also many polyesters in the next section have been originally developed as “drop-ins.”

Fermentation processes can be used to produce monomers that are normally used for producing petroleum-based polyethylene (PE) and polypropylene (PP), which are the most produced plastics in the world. Starch is fermented into ethanol, which is processed further into ethylene, which can be further polymerized to PE, PP, or polyvinylchloride (PVC) in a same way they would have from fossil-based monomers. As mentioned earlier, their properties are equivalent to their petroleum-based counterparts and recyclable but not biodegradable.

Brazil-based Braskem is the clear leader in the field, utilizing local sugarcane-derived ethanol/ethylene as feedstock. In September 2010, Braskem started commercial production of biobased HDPE with a capacity of 200,000 tonnes/year (64). They also have a BioPP production plant producing 30,000–50,000 tonnes/year. Another BioPE plant was built in Brazil by Dow Chemical and Mitsui. That plant has a capacity of 350,000 tonnes/year. Biopolyolefins are estimated to have about a 20% costs premium at the moment. However, higher yield in production are predicted to lower this gap in the future.

3.3.2. Polyesters. Polyesters represent a large group of polymers in which also PHAs from the previous section belongs. They can be aliphatic, semiaromatic, and aromatic and are synthesized via step growth polycondensation of diols and diacid/diesters or hydroxyacids/hydroxyesters and ring opening polymerization of cyclic monomers such as lactones, cyclic diesters, and cyclic ketene acetals (65).

The most widely recognized aliphatic polyester is PLA (Fig. 7). It can be considered a bioplastics frontier and has become a widely recognized renewable and biodegradable polymer. Biobased PLA is produced by industrial polycondensation of lactic acid, which can be derived from renewable sources, such as corn sugar, potato, and sugar cane. However, production seems to be moving toward using the second-generation feedstock to make PLA (66). PLA provides comparable optical, mechanical, thermal, and barrier properties when compared to commercially

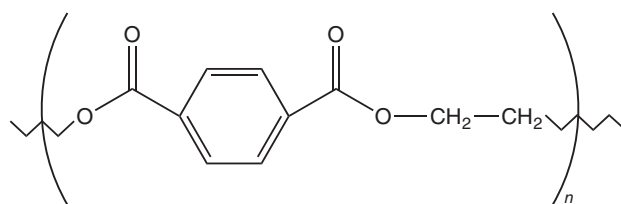


Fig. 8. Chemical structure of PET.

available commodity polymers such as PP, PET, and polystyrene (PS). Despite many desirable material characteristics, PLA is rather brittle which has limited some of its applications. PLA can be blended with petroleum-based polymers or fibers, either synthetic or natural, to improve the heat resistance or durability of the plastic.

Another important class is “drop-in” biobased semiaromatic polyesters, which are synthesized from either isophthalic acid or terephthalic acid. Semi-aromatic polyesters generally possess better thermal and mechanical properties, which can be used as commodity plastics and engineering plastics; however, they are often not biodegradable. PET is clearly the most commonly used semiaromatic polyester and fourth most produced plastic with global supply of more than 19.8 million tonnes in 2012 (Fig. 8); it is mostly used in plastic bottles and textile fibers. PET is conventionally made of terephthalic acid (PTA) and monoethylene glycol (MEG) of which only MEG has been produced from biomass. The production of aromatic compounds, such as the PTA precursor (xylene) using fermentation has been a challenge. However, in June 2015, Coca-Cola company joined forces with Virent Ltd, giving them access to the elusive BioTPA compound and thus the ability to produce 100% BioPET bottles.

2,5-Furancarboxylic acid (FDCA) has been considered having a large potential as a replacement for PTA. Werpy and co-workers in 2004 considered one of the top two bioderived chemicals of the future, which can be also seen from the number of patents and articles has increased exponentially for the past 10 years (67,68). The process uses carbohydrates to produce 5-hydroxymethylfurfural (HMF), which can be further processed into FDCA that is similar molecule to PTA (Fig. 9). Especially, FDCA-derivate polyethylene furanoate (PEF) has risen as a new 100% biobased competitor for petrochemical PET in the field of recyclable plastics, but there are many other end product alternatives as well. PEF can be polymerized from FDCA and biobased ethylene glycol (EG) and is considered to be a competitor for PET in both price and performance, and at the same time having a significantly better environmental profile (69). According to Avantium, PEF has significantly better barrier properties, higher glass transition temperature, lower melting temperature and higher Young’s modulus than PET (70).

3.3.3. Polyamides. Polyamides are polymers in which the monomer units are linked together by amide bonds. This group includes naturally occurring polyamides such as proteins, and synthetic polyamides such as polycaprolactam (nylon 6), poly(hexamethylene adipamide) (PA 6,6), or poly(p-phenylene terephthalamide) (PPTA, Kevlar). Similarly to polyesters, polyamides can be classified as aliphatic, semiaromatic or aromatic. Aliphatic polyamides are

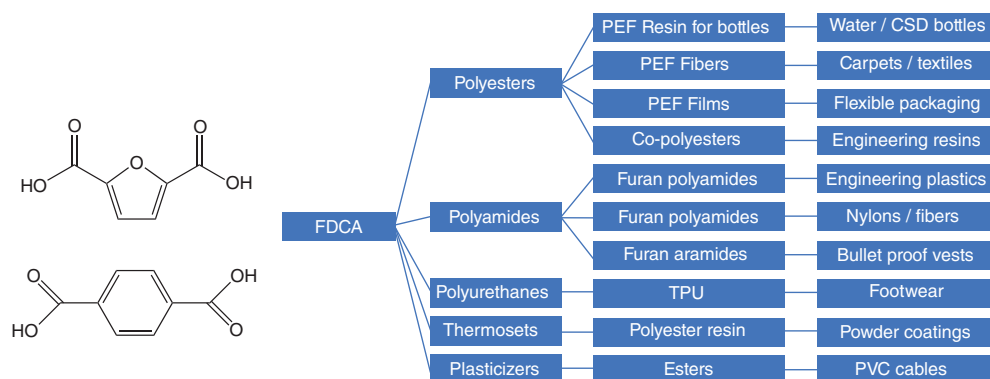


Fig. 9. Chemical structure of furandicarboxylic acid above and terephthalic acid below together with different modification possibilities of FDCA (71).

commercially known as nylons, of which 4.2 and 2.1 million tonnes are produced per annum for nylon 6 and nylon 6,6, respectively. However, none of the monomers required for polymerization are currently bioderived.

Currently, some biobased polyamides are commercially available, including fully biobased nylon 4,10, nylon 10,10, and nylon 11, and partially biobased nylon 6,10, nylon 10,12, and PA 10,T (65). They are made through polycondensation reactions of diamines and dicarboxylic acids. Biobased polyamides are mostly derived from 11-aminoundecanoic acid and sebacic acid which can be synthesized through chemical conversion of ricinoleic acid, which is the major fatty acid component of castor oil, whereas the diamine source varies. Diamines are currently mostly made synthetically, despite various biobased pathways being available; few of these are commercially viable yet. The most common polyamide pathways can be seen in Figure 10.

3.4. Petroleum-Based Biodegradable Polymers. The role of petrochemical biodegradable polymers has been controversial; however, the development of several polyesters and polyamides were developed and the chemistry of these have largely informed that behind bioplastics. For example, poly(butylene succinate) (PBS) is still mainly produced synthetically; however, European Bioplastics classifies it as a biobased, biodegradable plastic seeing that the biobased pathway already exists. At the same time, BioPET30 has been widely recognized as a sustainable alternative for commodity PET even though most of it is still petrochemical. The role of petrochemical biodegradable plastics cannot be underestimated; some may argue that biodegradability takes precedence over the material's origin. MaterBi® is a good example of the benefit of combining the advantages of biobased and petrochemical polymers. Improved processability was achieved by blending starch with PCL without ruining the material's biodegradability. It would therefore be foolish to ignore petrochemical biodegradable plastics from a discussion on bioplastics.

PBS belongs to the poly(alkylene dicarboxylate) family that can be synthesized from aliphatic dicarboxylic acids such as, succinic and adipic acid, and diols such as 1,4-butanediol and ethylene glycol (48). PBS is biodegradable and considered having properties in between of PP and LDPE. It is suitable for

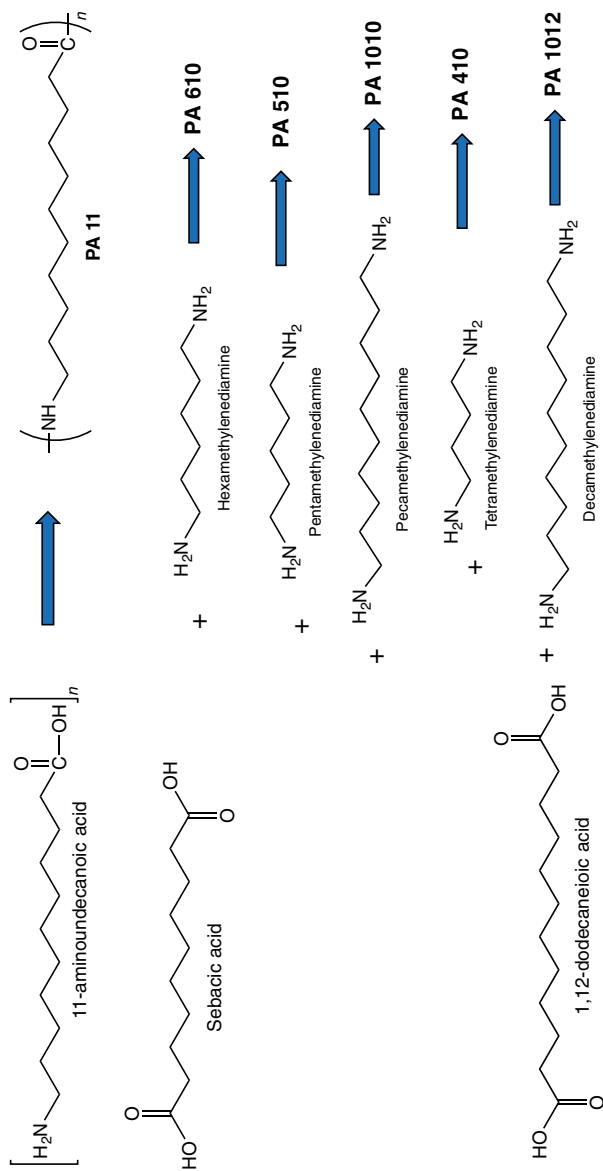


Fig. 10. Monomers required for synthesis of different kinds of polyamides. Reprinted with permission from Ref. 72. Copyright 2013 Inno-Centrix.

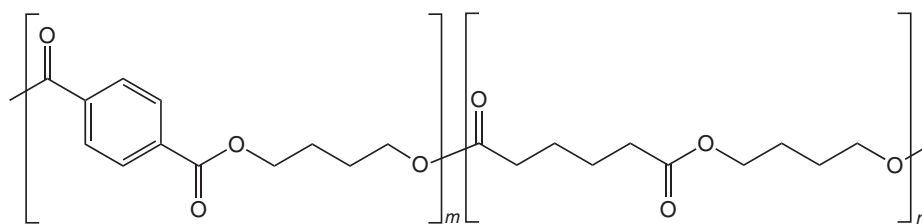


Fig. 11. Structure of PBAT.

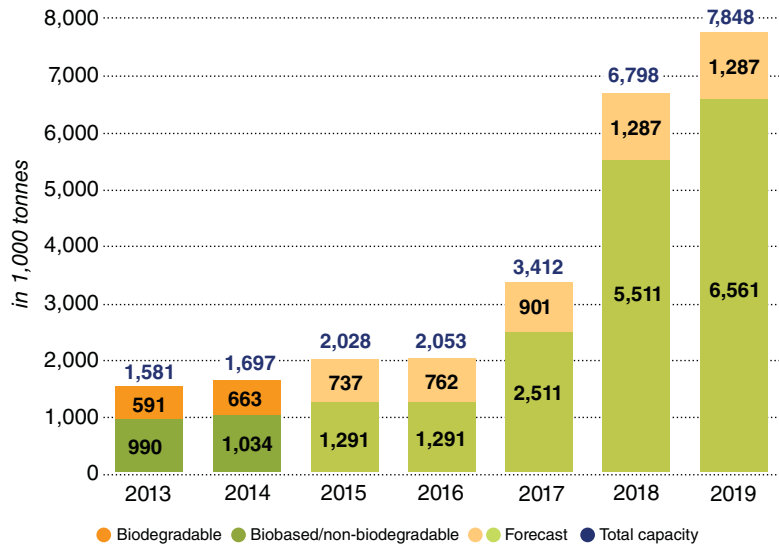
applications such as films, bags, and food packaging and has been commercialized under trademarks such as Natureworks Ingeo, Bionolle, and Enpol. Currently, PBS is mainly produced from petrochemical sources, presumably for economical reasons, but the bioderived pathway exists as well. Furthermore, properties of PBS can also be varied over a wide range via copolymerization with other dicarboxylic acids and diols. For example, with PBSA better elongation at break and impact strength properties are achieved by introducing adipic acid in the polymerization process (48). Also semiaromatic polyesters have been copolymerized to combine the properties of these polymer types, for example, biodegradability of PBS and strength of TPA (73). Poly(butylene adipate-*co*-terephthalate) (PBAT) is a semiaromatic polyester made by copolymerization of adipic acid, 1,4-butanediol, and dimethyl terephthalate (Fig. 11). Ecoflex™ and Eastar Bio™ produced by BASF and Eastman, respectively, are probably the most important commercially available aliphatic-aromatic polyesters.

PCL is one of the completely petrochemical aliphatic polyesters that is commercially available. PCL is usually manufactured via ring-opening polymerization of ϵ -caprolactone, which can be derived from cyclohexane (74). PCL is a tough and flexible polymer with crystallinity around 50%. It has been commercialized as Capa™ by Perstorp and used widely in different applications such as mulch films, food and medical applications.

4. Current Market Size and Statistics

Bioplastics only covers approximately 1% of an annual 300 million tonnes plastic market (5). However, a significant change in the next few years has been predicted. According to European Bioplastics, it will increase from 1.7 to 7.8 million tonnes from 2014 to 2019 (Fig. 12). Future Markets Insights lead-analyst stated, “Increasing consumer awareness regarding benefits of greener products, strict regulations to incorporate biobased products in automotive & packaging, and increasing investments by local companies are expected to fuel the demand for global bioplastics market.” The change is largely led by the growth in drop-in polymers, such as BioPE and BioPET, for which production is predicted to increase sixfold in this period of time. At the same time, production of biodegradable plastics is only expected to increase from 0.7–1.2 million tonnes. According to Bioplastics Europe, more than 60% of bioplastics was biobased in 2014 and is expected to increase to over 80% in 2019 (before 2010 these were practically nonexistent).

Global production capacities of bioplastics



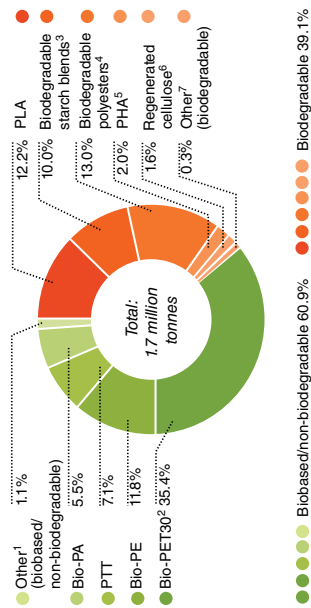
Source: European Bioplastics, Institute for Bioplastics and Biocomposites, nova-Institute (2015).
 More information: www.bio-based.eu/markets and www.downloads.ifbb-hannover.de

Fig. 12. Global production capacities of bioplastics. Reprinted with permission from Ref. 75. Copyright 2015 European Bioplastics.

An indication of the dynamic market is the high variability in production capacity predictions among different sources. Allied Market Research (2015 Nov) reported a 17.5% compound annual growth rate (CAGR) during 2015–2020, IndustryARC (2016 May) 12% from 2016 to 2021, Sandler Research (2016 June) 29.3% over 2016–2020, whereas Futuremarket Prediction (2015 Feb) stated a 28.8% growth (76–78). However, there is a consensus that biocontent is preferred over biodegradability, and consumption of drop-in bioplastics, especially BioPET, will continue to dominate the overall bioplastics market. Global Market Insights predicts the CAGR to be 42% between 2015 and 2023, raising from 496 kilotonnes to 6.67 million tonnes, whereas European bioplastics predicts it to be 6 million tonnes in 2019 (Fig. 13) (79). Distant seconds are biodegradable polyesters such as PBS and PBAT, which are followed by PLA, biobased PE, and starch blends (75).

However, forecasting the future of such a dynamic environment is challenging. For example, feasible production of biobased monoethylene glycol made the introduction of Coca-Cola’s “PlantBottle” possible. The most significant developments happened in the past 5 years, as before 2010 commodity replacements or biopolymer drop-ins were almost nonexistent. Furthermore, similar drastic changes can be expected in the near future, with different market shares for each. Variables, such as legislation changes, breakthroughs in competing techniques, or bioplastic implementation by market leaders, may be the game changers of the

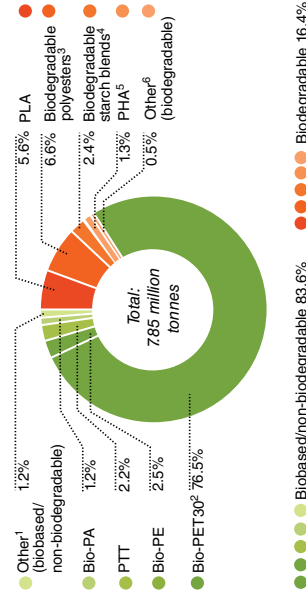
Global production capacities of bioplastics 2014
(by material type)



¹Contains durable starch blends, Bio-PC, Bio-TPE, Bio-PUR (except thermosets), PEF; ²Biobased content amounts to 30%; ³Biobased content amounts to 30%; ⁴Biobased content amounts to 30%; ⁵Biobased content amounts to 30%; ⁶Biobased content amounts to 30%; ⁷Biobased content amounts to 30%; ⁸Biobased content amounts to 30%;

Source: European Bioplastics, Institute for Bioplastics and Biocomposites, nova-Institute (2015). More information: www.bio-based.eu/markets and www.downloads.ifbb-hannover.de

Global production capacities of bioplastics 2019
(by material type)



¹Contains durable starch blends, Bio-PC, Bio-TPE, Bio-PUR (except thermosets), PEF; ²Biobased content amounts to 30%; ³Biobased content amounts to 30%; ⁴Biobased content amounts to 30%; ⁵Biobased content amounts to 30%; ⁶Biobased content amounts to 30%; ⁷Biobased content amounts to 30%; ⁸Biobased content amounts to 30%;

Source: European Bioplastics, Institute for Bioplastics and Biocomposites, nova-Institute (2015). More information: www.bio-based.eu/markets and www.downloads.ifbb-hannover.de

Fig. 13. Production capacities of bioplastics 2014 and forecasts for 2019 by material type. Reprinted with permission from Ref. 75. Copy-right 2015 European Bioplastics.

future. For example, IKEA’s collaboration agreement with Newlight’s PHA production technology can be a big market changer in terms of PHA production (58). Also legislation changes, such as banning plastic bags, can have an impact. Another example is France, which was the first country in the world that endeavored to ban all disposable plastic cups and plates by the year of 2020 (80). These kinds of changes might have a positive impact on the growth of biodegradable plastics.

The market segmentation of bioplastics can be seen from Figure 14. Biobased rigid and flexible packaging covers more than two thirds of the overall market followed by textiles, consumer goods, and agriculture. According to Sandler Research, the packaging and food services segment is expected to account for almost 69% of the overall market and is the dominant shareholder in the bioplastics market globally. The change caused by BioPET can be seen most significantly from rigid packaging, but also from the textile and automotive industry.

Rigid packaging is food-industry driven and largely affected by Plant Bottles™ (BioPET30). Also different kinds of containers and cups are included. Flexible packaging includes shrink-wrap, plastic bags, and many other food-packaging applications. They are mostly made from biodegradable polymers such as PLA and starch blends but also low-density PE, which is used as a film wraps and plastic bags, for example. BioPET can be used as a drop-in in the textile industry, whereas some nylons, PLA, and even cellulose-based fibers have been identified as alternatives. The automotive and transport industry is predicted to increase fourfold over the next 5 years, largely driven by drop-in biopolymers. For example, Ford Motor Company has announced sustainability aims in terms of bioplastics and is currently running a project in which bioplastics are produced from agave waste (82).

5. Sustainability

In the modern market, sustainability is often linked with the demand for bioplastics. Sustainability is maintaining conditions under which humans and nature co-exist harmoniously and where social, economic, and environmental requirements of present and future generations are met. Demand for the change is coming from the fact that global resources cannot sustain the modern world’s mass consumption behavior together with unorganized recycling patterns. These factors have caused concern regarding climate change and littering problems. The world population is expected to reach 9.7 billion by the year 2050 putting even more strain on the environment (83).

Measurement tools such as “12 principles of green chemistry” and “12 principles of green engineering” by the American Chemical Society, as well as those from the International Organization of Standardization (ISO), which include standardized LCA and ecoprofiles (cradle-to-gate) have been developed for more extensive environmental impact measurements (84,85). This section identifies the most important drivers of bioplastics demand in terms of sustainability and discusses factors that normally arise in terms of sustainability. It also provides a brief review of LCA.

5.1. Resourcing. As a consideration of finding sustainable alternatives for fossil-based fuels, first-generation biofuels, derived from agricultural products,

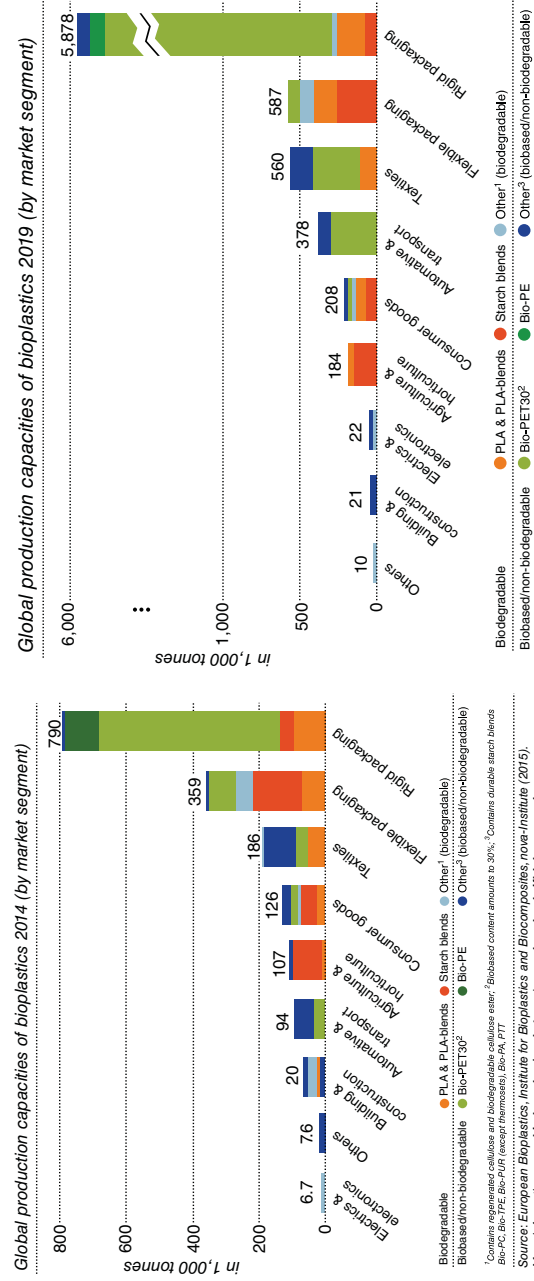


Fig. 14. Market segmentation of bioplastics in 2014 and forecasts for 2019. Reprinted with permission from Ref. 81. Copyright 2015 European Bioplastics.

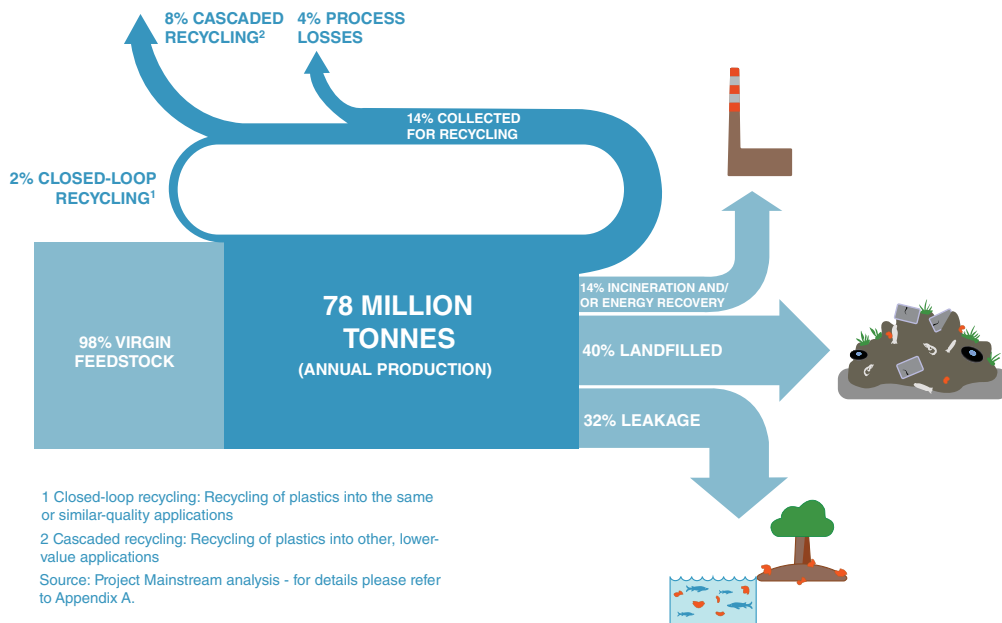


Fig. 15. Global plastic consumption patterns. Reprinted with permission from Ref. 2. Copyright 2016 Ellen MacArthur Foundation.

have produced an impact on grain prices, tightening the supply chain, and availability of land for food production (86). For the same reasons, material production from potential food sources may not be seen ethical either. Production of materials from nonfood sources is called second-generation bioplastics and is generally considered to be a more sustainable option. However, the subject has been under strong debate; from industry’s point of view, food-based plastics or fuel will never require the predicted land-use demand (0.68 million ha) and will, at most, require between 0.01 and 0.02% of the global agricultural area, respectively. It has also been established that the global food shortage is driven by logistical problems rather than a lack of arable land (87,88).

Regardless if utilizing food resources is ethical or not, it is also widely accepted that the carbon source is one of the main factors affecting the cost of biobased plastics. Waste valorization may have significant environmental benefits, especially if its cultivation promotes the primary stream, for example, edible starch. According to the United Nations Environment Programme (UNEP), 5 million tonnes of biowaste is generated every year and could potentially be used for bioplastic production (88). The use of municipal and commercial waste, as well as sludge derived from urban water treatment could cut greenhouse gas emissions by 62 million tonnes of carbon dioxide equivalents by 2020 compared to levels in 2008 (89). Different techniques, such as syngas, are under intense investigation to generate new resources from biowaste as well as plastic waste (90–94).

5.2. End of Life. Waste accumulation is a globally recognized problem; 78 million tonnes of plastic waste is generated each year (2,18). This will be recycled, landfilled, incinerated, or, in worst-case scenario, disposed in nature (Fig. 15). Only

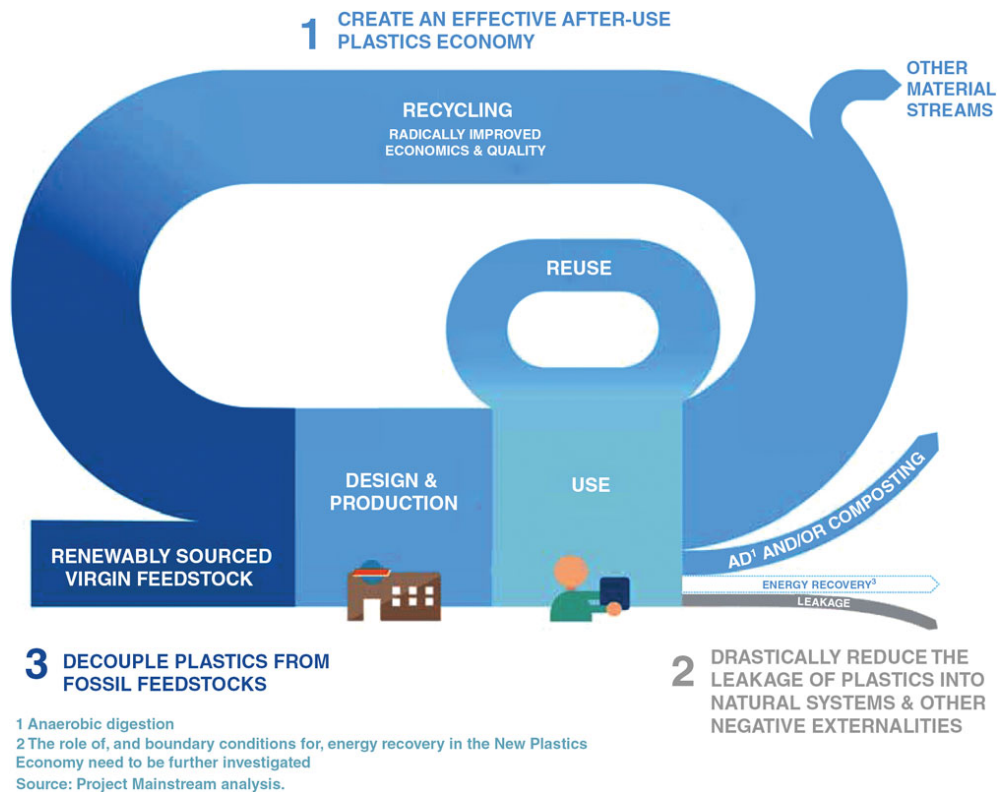


Fig. 16. Ideal plastic economy approach from Ellen McArthur Foundation (2). Reprinted with permission from Ref. 2. Copyright 2016 Ellen McArthur Foundation.

14% is collected for recycling from which only 2% is reused, hardly fulfilling the requirement of a “circular economy.” Ellen MacArthur’s Foundation’s illustration of ideal circular economy model can be seen from Figure 16.

A product’s end-of-life destination is heavily dependent on culture, and thus has lots of variation based on geological locations. Figure 17 shows waste deposition cultures at different countries around the European Union. It can be concluded that even as localized as Europe, differences among countries are significant. Overall, 29.7% is recycled, 39.5% is incinerated, and 30.8% goes to landfill. In comparison to 8 years ago, recycling has increased by 64%, energy recovery by 46%, whereas landfill has decreased by 38%. However, Europe is one of the frontiers when it comes to sustainable recycling patterns, even if it still not sufficient. The top-five waste producers in the world (China, Indonesia, Philippines, Vietnam, and Sri Lanka) are responsible for 54.5% of total uncontrolled plastic waste (17.34 million tonnes/year), which is 25% of world’s total plastic production (3).

Marine debris accumulated from uncontrolled plastic waste disposal has created lots of discussion, and the European Commission has identified it as one of the main threats for the environment. The amount of microplastics in the North Pacific has tripled during the past decade. The UNEP claims that every square mile of ocean contains 46,000 pieces of floating plastics (18). According to a new

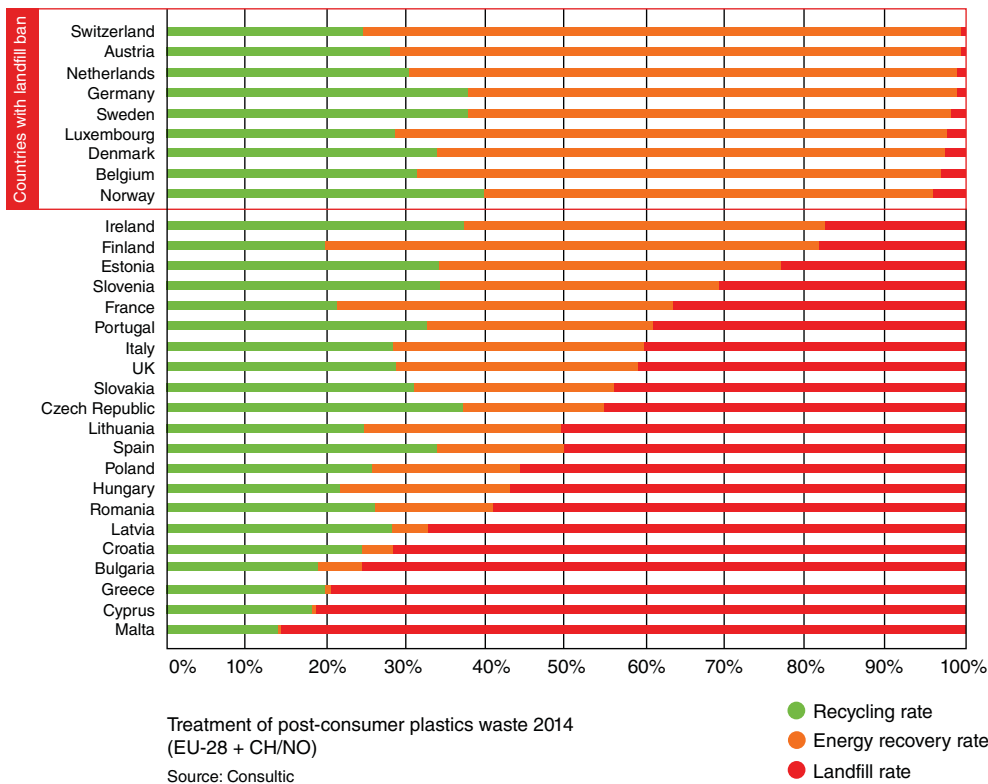


Fig. 17. Plastic waste behavior in different European countries in 2014. Reprinted with permission from Ref. 1. Copyright 2015 Plastics Europe.

Ellen MacArthur Foundation report, 8 million tonnes plastics leaks into the ocean every year and without further action could double by 2030 (2). Geographical differences also play a role here; Jambeck and co-workers concluded that 82% of modern marine litter is coming from Asian countries, 2% of United States and Europe, and 16% from rest of the world (3). Later studies have shown that microplastics is much wider problem than just a result of uncontrolled waste management. Napper and co-workers' latest study showed that more than 700,000 kg polyester, polyester-cotton, or nylon microplastic fibers could be released per 6 kg washing (95,96). A Norwegian group, instead, concluded that about 50% of total microplastics in Norwegian front waters accumulated from transportation by-products such as tire dust and asphalt wear, which have been conveyed into the sea with drain water (97).

Marine debris is extremely detrimental to animals as they either become entangled in it or digest it. Microplastics, in particular, are polymer fragments that cannot be degraded completely. Small plastic particles itself are not extremely unhealthy, but tend to absorb different organic toxins in, and if ingested by animals have a negative effect on animals and humans (98,99). Intuitively, biodegradable polymers would be a perfect solution for this problem. However, the UNEP has the opinion that biodegradability will not significantly decrease the quantity of

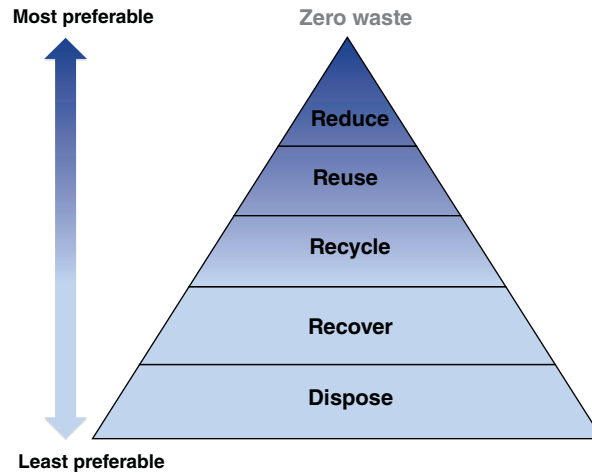


Fig. 18. Waste minimization principle.

plastics entering the ocean. Furthermore, biodegradable plastics could be seen to remove the responsibility from individuals to dispose plastics responsibly (18). Increasing social awareness is probably a priority, changing consumer behavior. Figure 18 illustrates current consumer behavior. In ideal world, the pyramid should be built opposite, such that disposal should be creating the smallest fraction at the top of the pyramid.

5.3. Life Cycle Assessment. Sustainability can be assessed using LCA, or also called an ecobalance or cradle-to-grave analysis (100). It evaluates the environmental impact of a given product or service over its entire existence, considering raw material sourcing, production process, packaging, distribution, usage and waste management, including transport. The LCA methodology has been standardized under the ISO-14040 series, and it distinguishes between four phases: goal and scope definition, inventory analysis, impact assessment, and interpretation (85). However, the approach focuses on the environment, and it has been argued that ecological and social considerations should be as well as the timescales involved (18,75). According to the UNEP, “without such evaluation, decisions made in good faith may result in ineffective mitigation measures, unnecessary or disproportionate costs, or unforeseen negative consequences.” LCA, however, can be a great tool providing understanding of different areas of environmental impact, or at least an understanding of the environmental impact of single product. However, it is good to remember that even the most sustainable material alternatives still have to meet market demands.

A full LCA is called a cradle-to-grave system and contains every step of a product system’s life cycle, and provides opportunity to draw fair and accurate comparisons between specific applications. However, complete LCA analysis has received some criticism of being excessively based on assumptions and having inconsistent results. Each system, based on choice of material, function, and how it is disposed, will contribute uniquely to its environmental impact. Thus, an absolute sustainability comparison between two different systems is difficult if not impossible (75). A cradle-to-gate assessment (ecoprofile) begins with extraction of

materials from the earth but end at the factory gate, and consists of greenhouse gas emissions and nonrenewable energy use. This is useful for the purpose when a material might be used for different applications, and a full LCA is impossible. This method, however, is not comprehensive because it does not take the material's end-of-life into account. Especially in case of biodegradable plastics, using the cradle-to-gate method eliminates at least one of its potential environment impact factors (disposal). However, this can be used to supply inventory data for a full cradle-to-grave assessment of a particular application.

Comparing bioplastics with fossil-based plastics is quite complicated, and there is not conclusive analysis for any of them. Appropriate allocation of impacts from a multifunctional processes is one of the biggest challenges and most talked about methodological issues in LCA (101). LCA applies to a specific product or service, not to bioplastics in general or all products available. For example, using different crops or fermentation technologies will affect the environmental impact for producing a particular bioplastic. One of the challenges has been to integrate new innovative systems, in a fair and comprehensive way. Thus, projections in regard to potential yields should be taken into account, but may not be a realistic approach. Thus, the possibility to make sound substantiated comparisons is limited even though LCA is currently the best tool available for that purpose.

Hottle and colleagues conceded that their review of biobased polymers focused mainly on global warming potential (GWP) and fossil resource depletion, ignoring many of the other potential impacts (102). Tabone and others reviewed several cradle-to-gate assessments of bio- and fossil-based polymers, and presented normalized results based on the highest values of each subfactor (Fig. 19) (84). Biopolymers generally result in a decrease in fossil-fuel use and GWP but increase in other impact categories such as eutrophication, human health impacts, and ecotoxicity. These impacts result from fertilizer use, pesticide use, land use changes required for agricultural activities as well as from the fermentation and other chemical processing steps (103). Biopolymer production resulted in the highest impacts for 5 of the 10 categories listed: ozone depletion, acidification, eutrophication, carcinogens, and ecotoxicity. Similarly, Bier and co-workers collected a series of biopolymer results and found that biobased plastics uses less nonrenewable primary energy (NRPE) but may have higher greenhouse gas emissions impact (29).

Renewable resources are not necessarily better than fossil-based resources from an environmental impact point of view. For example, some fermentation processes are energy intensive, and polymers such as PHA and PLA have similar NRPE impacts than commodity plastics (29). However, using combustion of biomass or wind power drastically improves the greenhouse effect. One example of this is Ingeo® cutting their carbon footprint by using wind power. Biomass-based plastics, such as starch, does not require fermentation, and their energy requirement for producing thermoplastic materials is relatively low (104). However, their ecoprofile is largely dependent on how allocation is done. One may argue that using a by-product and waste materials should not take cultivation effects into account (fertilizer, pesticide, and land use change required) because material production is not their primary purpose, and this results in a completely different outcome.

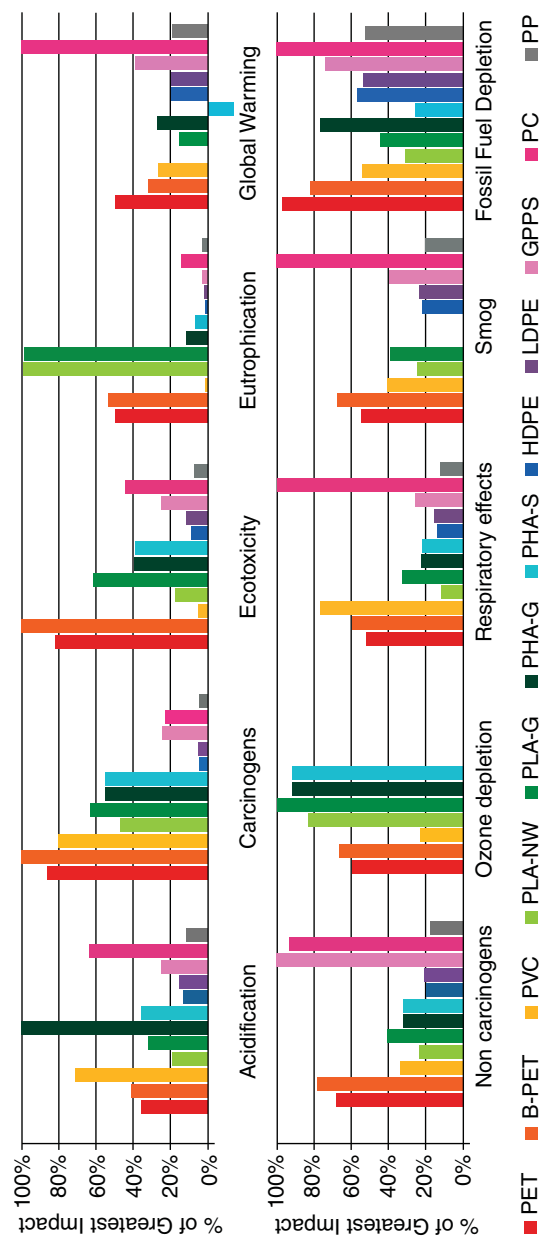


Fig. 19. Environmental impacts of different polymer types. Reprinted with permission from Ref. 84. Copyright 2010 American Chemical Society.

6. Current Success Stories

Despite the market pull for sustainable materials there is still a lack of communication to consumers and industry regarding making responsible choices. In the beginning of the 21st century, bioplastics were just about biodegradability and, together with biocontent, were the leading marketing tools. The Ellen McArthur foundation is an institution whose mission it is to accelerate the transition to a circular economy and to integrate global companies that have the same vision. They were established in 2010 and focuses on four interlinking areas: education, business and government, insight and analysis, as well as communication. Currently, global partners are Cisco, Google, H&M, Intesa Sanpaolo, Nike, Philips, Renault, and Unilever.

One of the frontiers has been PLA which is probably the most widely recognized and commercialized bioplastic. The interest toward this material can also be seen also from an exponential growth of publications during the past 20 years (105). Natureworks IngeoTM, followed by Evonik Industries and Corbion PURAC, were some of the first producers. Other biodegradable polyesters such as PCL and PBS have also been successful, but are not manufactured from renewable resources (106–108). However, an Italian company, Novamont, has been able to turn that around by making a starch-based blend with PCL called MaterBi[®], which has claimed a good market share of biodegradable plastics. The company has also launched an educational Web site called “Discover MaterBi” with the purpose to widen the knowledge of biodegradable materials for customers and as educational tool for children (109).

Another market revolution was the arrival of drop-in polymers. Coca Cola’s implementation of PlantBottleTM in 2009 has been instrumental toward increasing the market share of bioplastics as well as being a big step toward environmental and social sustainability. Coca Cola started their collaboration project with several chemical manufacturers such as Virent Inc. and Avantium with the vision “to maximize game-changing technology, using responsibly sourced plant-based materials to create the globe’s first fully recyclable PET plastic bottle made entirely from renewable materials.” In June 2015, Coca Cola and Virent Inc. announced that they have been able to produce the first 100% plant-based PET bottle with technology that has enabled them to produce bioparaxylene (that can be further purified to PTA) from beet sugar. Furthermore, the technology has been licensed to companies such as H.J. Heinz for use in it ketchup bottles, Ford Motor Company in their fabric interior, Nike, Nestle, Danone, P&G, and Unilever. This has also lead to the establishment of the Bioplastic Feedstock Alliance, which is a strategic work group focused on accelerating the development and use of 100% plant-based PET materials and fibers (110). According to Coca Cola, since the launch in 2009, more than 35 billion PlantBottles has been sold, which has saved the equivalent of more than 315,000 tonnes of carbon dioxide emissions (111).

Some other brands have adopted the use of biobased products as well. Mitsubishi is investing on PBS tailored for their interior design of cars (112). Goodyear and Genencor have produced a renewable car tire using their BioisopreneTM technology (113). BASF, Cargill, and Novozymes have developed a process for the conversion of renewable raw materials into biobased acrylic acid

and has demonstrated pilot-scale production of 3-hydroxypropionic acid (3-HP), a possible precursor for acrylic acid (114,115). IKEA's long-term goal is for all of its plastic materials used in home furnishing to be 100% renewable and/or recyclable. In their 2015 annual report, around 23% of its plastic products are already made from renewable or recycled sources. They are also collaborating with Newlight with the promise to buy 50% of their methane-based PHA (58).

7. Conclusions

The bioplastic sector is a crucial building block for a more sustainable future circular economy. The field is still young and extremely dynamic. However, the core of the industry is getting stronger and confusion around terminology and standardizing systems have decreased. The future of the plastics industry is highly driven by sustainability issues, although, new materials have to be cost- and performance-competitive. Sustainability is not just an environmental impact minimization, but also a social and economic considerations, providing people with information to make sustainable decisions. The bioplastic sector has the potential to simultaneously increase economic input, provide jobs, drive research and innovation, promote the efficient use of resources, contribute to sustainable economic growth, and reverse the depletion and deterioration of our natural capital.

The term "bioplastics" is difficult to define precisely. It can be biobased or biodegradable, or both. Synthesis can be via several process routes, some of them creating materials straight from biomass whereas others require additional processing steps. Each processing step brings additional cost and impact to the environment, and, in the worst-case scenario, leads to a more expensive material with negligible environmental benefits. However, there are helpful tools such as LCAs to provide information on environmental impacts. End-of-life properties have to be considered according to a material's purpose and its end-of-life destination. Biodegradability, as a property, is often overvalued as it might prevent recycling for some applications in which it could be possible. Additionally, it often does not provide significant benefits when discarded in nature.

The industry is going through a major growth phase, expecting a fivefold increase in production capacities over the next 5 years. In the past 5 years, the biggest change has been the appearance of drop-in polymers, such as BioPE and BioPET. According to forecasts, these nondegradable polymers will maintain a bigger market share of the bioplastics market and will be responsible for bioplastic's increased market share in the larger plastics market.

Even today, one of the major disadvantages of bioplastics is cost. Discovery of new carbon sources and finding the most efficient way to polymerize it are hoped to improve yields to mitigate this problem. Second-generation feedstocks are hoped to be cheaper; however, it does include more variability in comparison to, for example, corn starch sourcing. Advances in biotechnology is expected to provide more efficient fermentation processes and without such strong feedstock quality dependence.

Overall, bioplastics have the potential to revolutionize the materials market and to act as a messenger of sustainable values in people's every-day lives. There has been a strong demand for more sustainable materials, but they are not

quite affordable and viable yet. The responsibility for providing material alternatives and bringing information to consumers has become a world-wide initiative by industry and some charity foundations.

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Glossary

ASTM	American Society for Testing and Materials
BioPE	Biobased polyethylene
BioPET30	30% Biobased polyethylene terephthalate
BioPET	Biobased polyethylene terephthalate
BioPP	Biobased polypropylene
CAGR	Compound annual growth rate
CEN	European Committee for Standardization
FDCA	2,5-Furancarboxylic acid
HMF	5-Hydroxymethylfurfural
IUPAC	International Union of Pure and Applied Chemistry
ISO	International Organization of Standardization
LCA	Life cycle assessment
PA	Polyamide
PBAT	Poly(butylene adipate-co-terephthalate)
PBS	Poly(butylene succinate)
PE	Petroleum-based polyethylene
PEF	Polyethylene furanoate
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PLA	Poly(lactic acid)
PCL	Polycaprolactone
PP	Petroleum-based polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
PTA	Polyterephthalic acid
SPI	Society of Plastic Industry
TPS	Thermoplastic starch
UNEP	United Nations Environment Programme

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3

Phase separation of plasticizers in thermally aggregated protein-based thermoplastics

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By

Jussi M. Uitto and C.J.R. Verbeek

Phase separation of plasticizers in thermally aggregated protein-based thermoplastics

Chapter 3 aimed to investigate the effect of different polyol plasticizers (ethylene glycol, glycerol, propylene glycol and triethylene glycol) on Novatein. The focus was to understand how their molecular characteristics affects to their behaviour in the polymer network and furthermore how that relates to the material properties such as equilibrium moisture content, glass transition temperature and mechanical properties.

In regard to main thesis objectives the aim of this study was get a good general understanding of the starting points and variables affecting the plasticization.

As first author of this paper, I prepared the initial draft manuscript, which was refined and edited in consultation with my supervisor, who has been credited as co-author.

RESEARCH ARTICLE

Phase separation of plasticizers in thermally aggregated protein-based thermoplastics

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Abstract

Blood meal-based thermoplastic protein (Novatein) was plasticized with up to 40 parts ethylene glycol, glycerol, propylene glycol, or tri(ethylene glycol) per hundred parts blood meal. The effect of plasticizers was investigated by relating the effect of equilibrium moisture content, phase separation, and protein secondary structure to the glass transition temperature and the mechanical properties. Plasticizers can diffuse through the polymer network and either be part of a protein-rich phase where primary plasticization dominates or a plasticizer-rich phase where secondary plasticization dominates. Equilibrium moisture content and added theoretical hydrogen bonding sites had the strongest correlation with the results. The point at which the equilibrium moisture content reached an equivalent moisture content (POE) to that of compositions without a plasticizer was found to be a critical point at which plasticization changes from primary to secondary, with a corresponding change in mechanical properties from brittle to ductile.

KEYWORDS

biopolymers, extrusion, FT-IR, phase separation, proteins

1 | INTRODUCTION

Polymers from industrial by-products and waste streams show great potential as a low-cost bioresource when looking for sustainable alternatives to petroleum-based plastics. Some biomass-based bioplastics can directly be converted to plastic materials, without energy-intensive fermentation or polymerization steps and have shown great potential from a sustainability point of view.^[1] However, in comparison with conventional polymers, biomass-based materials typically have their challenges regarding processing and mechanical properties due to a lack of chain mobility in the polymer network.^[2]

Many protein-based materials have a high glass transition temperature (T_g), either naturally or as a result of processing history.^[3] Most amino acids have large side chains, hindering bond rotation thereby decreasing chain flexibility. Also, inter- and intramolecular bonds, mainly driven by hydrogen bonding, hydrophobic interactions, and covalent cross-links,

further increase the T_g . Plasticization promotes chain mobility and affects processability by modifying mainly the amorphous regions thereby widening the processing window (i.e., the difference between the onset of rubbery flow and the degradation temperature).

Plasticization is a complex phenomenon and still considered to be unpredictable and case dependent.^[4,5] For example, Pommet et al^[6] showed that, from 23 plasticizers, only five could successfully be used to plasticize wheat gluten. A low melting point, low volatility, and a sufficient amount of hydrophilic groups were the most important factors, suggesting that smaller molecules are a precondition for effective plasticization. However, ethylene glycol (EG) and propylene glycol (PG) with similar molecular characteristics often show opposite behavior.^[7–9]

Barone et al^[10] defined the plasticization efficiency index as the ratio of a protein's hydroxyl and cysteine groups. Higher amounts of hydroxyl groups, playing a major role in the hydrogen bonding environment, create more theoretical

plasticization sites in the polymer network. Cysteine groups instead are responsible for disulfide cross-links preventing chain movement. However, one of the drawbacks of this theory is that the approach assumes that all the hydroxyl groups are equally available, that is, perfect protein unfolding in the plasticization environment. This is a challenge, especially with by-products and waste streams that may have highly aggregated structures. For example, Oliviero et al^[9] observed a significant change in PEG400 performance due to the processing history of zein before plasticization and was also linked to the protein's secondary structure.

Due to their hygroscopic nature, biopolymers naturally have some amount of water in its network. The addition of a plasticizer brings about further plasticization caused by the change in the equilibrium moisture content, being itself hygroscopic.^[11,12] T_g is very sensitive to moisture and may drop to around 10°C for every 1% added water, at low plasticization levels.^[2] Plasticizer plasticizers are larger than water molecules and thus have milder effect on T_g , and the change in equilibrium moisture can be seen as a secondary effect. Godbillot et al^[11] studied the water-binding mechanisms of glycerol-plasticized starch films and found that glycerol tends to replace water from protein–water–protein interactions, whereas plasticizer–water interactions will become more abundant once the protein is saturated with a plasticizer. The equilibrium moisture content of the plasticized protein significantly changes after the saturation point because of the new free –OH groups from the plasticizer. After the saturation point, phase separation into plasticizer-rich and polymer-rich occurs, leading to a wide glass transition temperature range.^[7,13,14]

Blood meal is a high protein content by-product of the meat industry, which has been successfully converted to a thermoplastic material.^[15] It has undergone excessive heat treatment as a part of its manufacturing process, and the

protein is therefore highly aggregated, leading to a T_g that is higher than its degradation temperature. However, the protein structure can be modified in a way that thermoplasticity can be achieved. This method has been patented and recognized as commercially available Novatein® Thermoplastic Protein.^[15]

In this study, four commonly known plasticizers were compared for their plasticization efficiency in Novatein considering the difference between their chemical structure, factors affecting their compatibility, and mechanisms leading to brittle–ductile transformations. The objective was to explain how different plasticizers interact with protein chains and how this is correlated to plasticization. Synchrotron-based FTIR was used in conjunction with dynamic mechanical analysis to determine phase separation and how this affected mechanical properties and moisture absorption.

2 | EXPERIMENTAL

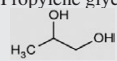
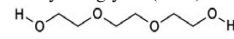

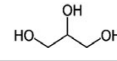
2.1 | Materials

Blood meal was obtained in powder form from Wallace Corporation, Hamilton, New Zealand, and sieved to 700 µm. Technical grade sodium dodecyl sulfate (SDS) was obtained from Biolab NZ and analytical grade sodium sulfite from BDH Lab supplies. Plasticizer plasticizers were obtained from Merck Millipore New Zealand.

2.2 | Sample preparation

Novatein® has been developed and patented by Aduro Biopolymers, New Zealand.^[16] Thermoplasticity is achieved by blending 100 parts by mass blood meal with 3 pph_{BM} sodium dodecyl sulfate (SDS) and sodium sulfite

TABLE 1 Different plasticizer types used with Novatein

Plasticizer	M_w^a	Melting temperature (°C)	N_{H-b}^b	%HG ^c
Water	18	0	4	100
Propylene glycol (PG) 	76	–60	6	44.7
Triethylene glycol (TEG) 	150	–7	10	44
Ethylene glycol (EG) 	62	–13	6	54.8
Glycerol (GLY) 	92	18	9	55.4

^aMolecular weight (g/mol).

^bNumber of theoretical hydrogen bonds.

^cPercentage hydrophilic groups.

(SS), water, and four different levels of four different plasticizers (Table 1). These were selected based on previous scoping experiments and appearance in literature. Plasticizers differ from each other by their molecular mass, hydrophilic character, and molecular structure. Ethylene glycol is a small and symmetric molecule with two carbon atoms with hydroxyl groups at each end. Propylene glycol (1,2-propylene diol) has an additional methyl group making it a bit more hydrophobic in comparison to glycerol (1,2,3-propanetriol); this methyl group has been replaced with more hydrophilic hydroxyl group, whereas triethylene glycol has ethylene glycol groups tied together with two ether groups. The number of theoretical hydrogen bonds (N_{H-b}) is calculated according to the plasticizer's chemical structure where the oxygen atoms are expected to form two hydrogen bonds as acceptors, and an additional hydrogen bond (as donor) is ascribed to of each of the hydrogen atoms in the hydroxyl groups.

A total of 17 different compositions were prepared by dissolving SS and SDS in the appropriate amount of water, followed by blending with 100 parts blood meal (BM) powder in a high-speed mixer, after which 0–40 parts per hundred parts BM ($p_{ph_{BM}}$) of plasticizer was added. The water content of samples was 40 $p_{ph_{BM}}$, although compositions with the lowest plasticizer content (10 $p_{ph_{BM}}$) required 50 $p_{ph_{BM}}$ water content whereas no plasticizer versions required 60 $p_{ph_{BM}}$ for being able to be extruded. The effect of water content was assumed to be minor when the samples were conditioned to their equilibrium moisture content.

Extrusion trials were performed using a Labtech corotating twin-screw extruder at a screw speed of 200 rpm. The temperature profile increased over 11 barrel heating sections, from 100°C at the feed throat and the main barrel, and increasing to 120°C at the die. The extruder had an L/D ratio of 44 and was fitted with a single 10 mm circular die. Mass flow rate, pressure, and torque were recorded. Mass flow rate of all compositions was 100 ± 20 g/min; pressure varied from 20 to 70 bar. Die pressure and torque varied depending on the level of plasticization in a way that higher plasticization led to lower torque and pressure, as expected. The thermomechanical effect for all the compositions was not equal, and for example, structural changes caused by the difference in specific mechanical energy (SME) were considered as a characteristic property of certain plasticizer and plasticizer level. The experiment was designed in a way that all the compositions could be processed and residence time in the extruder would be similar.

The extruded material was granulated using a tri-blade granulator from Castin Machinery Manufacturer Ltd., China. Specimens for the tensile test were produced in a BOY 35A injection molding machine. The shape of the tensile test specimens was by ASTM D638. A temperature profile of barrel

and mold were necessary to be altered between the compositions in a way that injection pressure was aimed to be at $1,000 \pm 200$ bar. Cooling time and temperature varied based on composition in a way that more plasticizer required lower mold temperature and longer cooling time.

2.3 | Analysis

Spatially resolved FTIR experiments of 10 and 30 $p_{ph_{BM}}$ plasticized samples were undertaken on the infrared microspectroscopy beamline at the Australian Synchrotron, Victoria, Australia. Spectra were collected using a Bruker Hyperion 3,000 with an MCT collector and XY stage using Opus 6.5 software (Bruker Optik GmbH 2009). For each sample type, two visually most representative spots of the microtomed sample were selected for measurement of 13×13 sized map with $5 \times 5 \mu\text{m}$ a spot size revealing the structural characteristics from two different $650 \times 650 \mu\text{m}$ areas from the sample. For each point, 32 spectra were collected in transmission mode with a resolution of 4 cm^{-1} between 3,900 and 700 cm^{-1} and averaged using Opus 6.5 software (Bruker Optik GmbH 2009).

The synchrotron data were interpreted in two different ways according to methods developed earlier.^[17,18] The integral of the primary alcohol group ($1,045\text{--}1,090 \text{ cm}^{-1}$) to the amide III ($1,180\text{--}1,330 \text{ cm}^{-1}$) region was considered representative of the ratio between plasticizer and protein in the sample and was used to map the plasticizer distribution (taking a ratio also accounts for thickness variations). Furthermore, the second derivative of the Amide III region was used for calculating the mass fraction of secondary structure components.^[18] The second derivative was inverted by dividing by negative one, and peak height integral above the zero line was compared with each other. Secondary structure for each point of the spatial maps was calculated, and then the average was taken to represent the secondary structure changes. Taking an average of the entire spatial map caused a reasonable standard deviation as a characteristic of the selected method, but also underlines the differences in the structure at small scale.

Wide-angle X-ray scattering (WAXS) was conducted using a PANalytical Empyrean X-ray diffractometer with a generator voltage of 45 kV and a current of 40 mA using $\text{CuK}_{\alpha 1}$ radiation. Tensile bar samples were scanned from $2\theta = 2\text{--}35^\circ$ at 0.0263° steps. A Soller slit of 0.04 rad was used, with a fixed incident beam mask 10 mm and fixed 0.5 divergence slit. For the diffracted beam path, a fixed 7.5 antiscatter slit was used and detected using a PIXcel3D area detector. Data were smoothed using a cubic function with 7-point convolution in the X'Pert HighScore Plus software. A linear baseline was fitted to the minima between 4 and 35° and subtracted and normalized to the peak occurring at 19° in Excel.

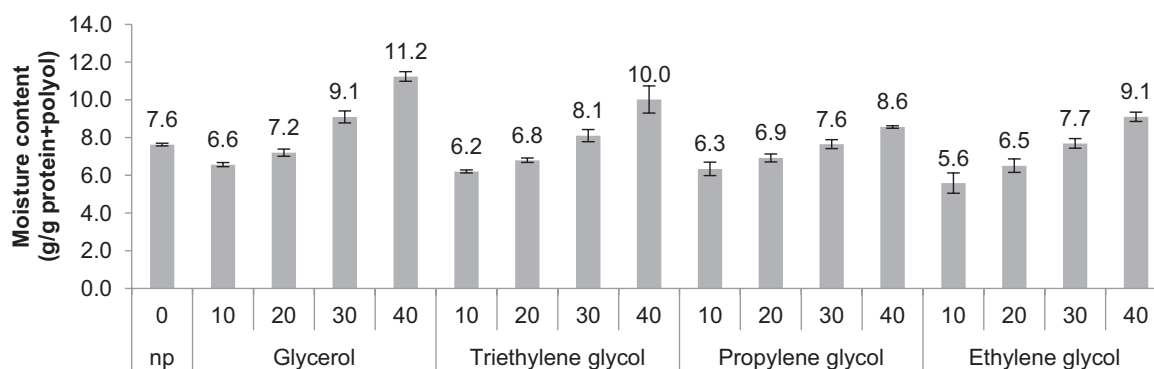


FIGURE 1 Equilibrium moisture content of Novatein with different plasticizer types and amounts

Test specimens were conditioned at 50 RH% and 23°C from 7 up to 14 days. After conditioning, the moisture content of samples was determined by drying at 60°C for seven days. Tensile properties were determined using an Instron model 33R4204. Tensile strength (TS), elongation (ϵ), and modulus of elasticity (E) of each specimen have been determined according to ASTM standard D638-03. An extension rate of 5 mm/min and an extensometer gauge length of 50 mm were used for testing. Samples were tested in replicas of six directly after removal from the humidity chambers. Dynamic mechanical thermal analysis (DMTA) was performed with a Perkin-Elmer dynamic mechanical analyzer (DMA 8000). The temperature sweep was performed at a rate of 2°C/min from -80 to 160°C. During this, the storage and the loss modulus at six different frequencies (0.1, 0.3, 1, 3, 10, and 30 Hz) were recorded and furthermore used for determining $\tan \delta$.

3 | RESULTS AND DISCUSSIONS

3.1 | Plasticization

In addition to a plasticization effect, each plasticizer has its characteristic way of modifying the polymer's ability to adsorb water.^[11,12] All the plasticizers tested here showed the same trend (Figure 1); increasing equilibrium moisture content (EMC) with increasing plasticizer content. The EMC was lower than unplasticized Novatein (np) for all the plasticizers below 30 pph_{BM}. The plasticizer amount at which the moisture content was the same, as compared to nonplasticized Novatein, was called the point of equivalence (POE) (Table 2). EMC reached an equivalent level to nonplasticized Novatein between 20 and 30 pph_{BM}, depending on plasticizer type. TEG had the lowest POE on a molar basis, in other words requiring the least molecules (or a number of hydrogen bonds) to achieve the same EMC as with no plasticizer addition. Nearly three times the molar amount of EG was required to reach the POE in comparison with TEG, whereas, with GLY, it was only 1.3 times.

TABLE 2 The point of equivalence for plasticizers

Polyol	Point of equivalence		N _{H-b} ^a
	pph _{BM}	mol mol ⁻¹ TEG	
PG	28.1	2.25	1.35
TEG	24.6	1	1
EG	29.1	2.85	1.71
GLY	19.9	1.32	1.19

^aTheoretical hydrogen bonding sites from the polyol at the POE normalized to the amount for TEG.

Intermolecular bonding in a protein network includes protein-protein and protein-water-protein interactions and to a certain extent determines the materials' EMC. When a small amount of plasticizer is added, the EMC dropped, indicating that the plasticizer occupied binding sites (on the polymer) that would normally be occupied by water, probably as hydrogen bonding. A plasticizer has several theoretical hydrogen bonding sites (Table 1) and can interact with the protein inter- and intramolecularly, at multiple sites. One plasticizer molecule can replace more than one molecule of water, resulting in a decrease in moisture content (Figure 1). In this region, the plasticization is referred to as primary plasticization.

Above the POE, the EMC is higher than compared to a material without a plasticizer, in which case the plasticizer provides additional hydrogen bonding sites (for water) leading to an increase in the EMC. This further plasticizes the material and is called secondary plasticization.

Primary and secondary plasticization may occur simultaneously, but primary plasticization will most probably dictate until the POE after which phase separation takes place, reported elsewhere as well.^[11,14,19,20] Based on the PEO, TEG, and GLY should be the closest to phase separation.

As each plasticizer is unique, it would be convenient to consider how the EMC changes with plasticizer amount,

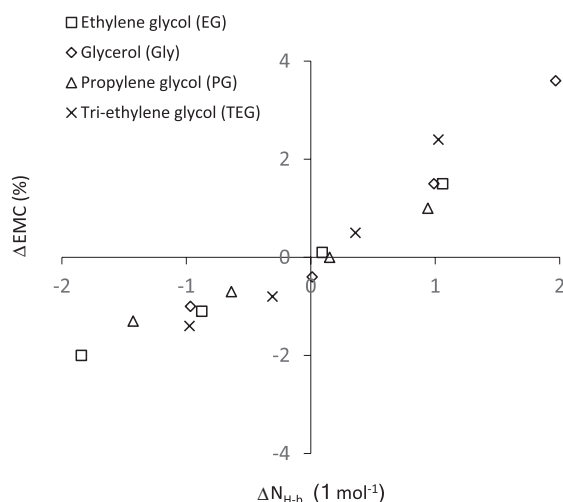


FIGURE 2 The difference between the number of theoretical hydrogen bonding sites at the chosen level of plasticization and that at the POE (ΔN_{H-b}) vs. the difference between the EMC at the chosen level of plasticization and the POE (ΔEMC)

irrespective the plasticizer type. Figure 2 considers the difference between the EMC (at any level of plasticization) and the POE versus the difference between the theoretical hydrogen bonding sites at the POE and the chosen level of plasticizer. Using this format, all the plasticizers behaved similarly; the additional uptake of water (above the POE) is simply related to the difference between the number of additional hydrogen bonding sites introduced above the POE. From this point, further plasticization is driven by more complex protein–plasticizer–plasticizer–protein, protein–plasticizer–water–plasticizer–protein, and protein–plasticizer–water–protein interactions that potentially offer better circumstances for bond slippage and more complex multiphase material properties.^[12]

Phase separation of the plasticizers was evaluated using S-FTIR (Figure 3) by considering the ratio between the primary alcohol groups ($1,045\text{--}1,090\text{ cm}^{-1}$) to the amide III ($1,180\text{--}1,330\text{ cm}^{-1}$) region. Naturally, a higher plasticizer amount led to a higher ratio, but the maps also indicated significant spatial variation. Two maps of each formulation were scanned that also showed some variation, indicative of phase separation on a scale greater than the spot size used. However, within the same plasticizer compositions, only 10TEG showed statistically significant difference between the two measured maps ($P > 0.05$). For an area of $650 \times 650\ \mu\text{m}$, the spatial variation only gives an indication of phase separation and was further explored with DMA (discussed later).

It is difficult to visually quantify the difference between the different plasticizers based on the maps presented. Therefore, histograms for the various maps were compared in Figure 4. From these, three observations were

made. The average ratio was different for each plasticizer, increased with increasing plasticizer and the distribution became wider at higher plasticizer content (even more so between samples of the same formulation). Theoretically, per gram of plasticizer, PG, and EG have more than double the amount of primary alcohol groups compared to TEG, whereas with glycerol this ratio is just under 2. One would, therefore, expect that the peak ratio mentioned above would scale in the same way, but the average ratio was in the order $GLY > TEG > PG > EG$. However, the technique used was bias toward plasticizer molecules, not hydrogen bonded to the protein. One can therefore conclude that, because GLY had the highest average ratio, it was the least hydrogen bonded to the protein, as would have been included at a lower molar amount compared to EG. The amount of hydroxyl groups should therefore not be the only factor used to assess plasticizer efficiency.

For each plasticizer, the maps presented are above and below its POE. For EG and PG, 30 pph_{BM} plasticizer was closer to the POE, and for both, the average ratio at 10 and 30 pph_{BM} was much closer compared to TEG, and more so for GLY. In other words, the further away from the POE, one would expect more severe phase separation, as confirmed by the data presented in Figure 4. Lastly, above the POE, a slightly wider distribution was observed for each plasticizer except for TEG, suggesting more phase separation on a micro scale, compared to below the POE. For TEG, additional hydrogen bonding possibilities, not from hydroxyl groups, but from the ether groups, changed the behavior of TEG compared to the other plasticizers.

Also, DMTA data provided supporting data for the earlier results (Figure 5). Loss modulus graphs revealed two peaks that can be seen at 80 and -5°C with no plasticizer addition. Their relative magnitude reverses and peaks move toward lower temperature with increasing plasticizer content. For glycerol, the intensity of the low-temperature peak is much higher than for the other samples, while for PG, the two peaks are almost the same. Glycerol had the most obvious lower temperature peak, being responsible for normally considered as a β -relaxation, clearly present at 10 pph_{BM}. The same can also be seen with the other plasticizers but not as clearly with the lower amount as it is with GLY.

Tan δ graphs revealed a shift in the shape of the α -relaxation with increasing plasticizer content. The α -relaxation peak intensity of PG and EG was smaller compared to GLY and TEG. Also, a β -relaxation peak was observed for all the plasticizers at 30 pph_{BM} and can correlate to the lower temperature loss modulus peak sizes.

The tan δ peaks of the different compositions, including 40 pph_{BM}, suggested that the α -relaxation consisted of at least two different phases (Figure 6). The main α -relaxation occurred between 50 and 120°C and the second between 130 and 180°C . The smaller peak was the clearest for GLY, seen

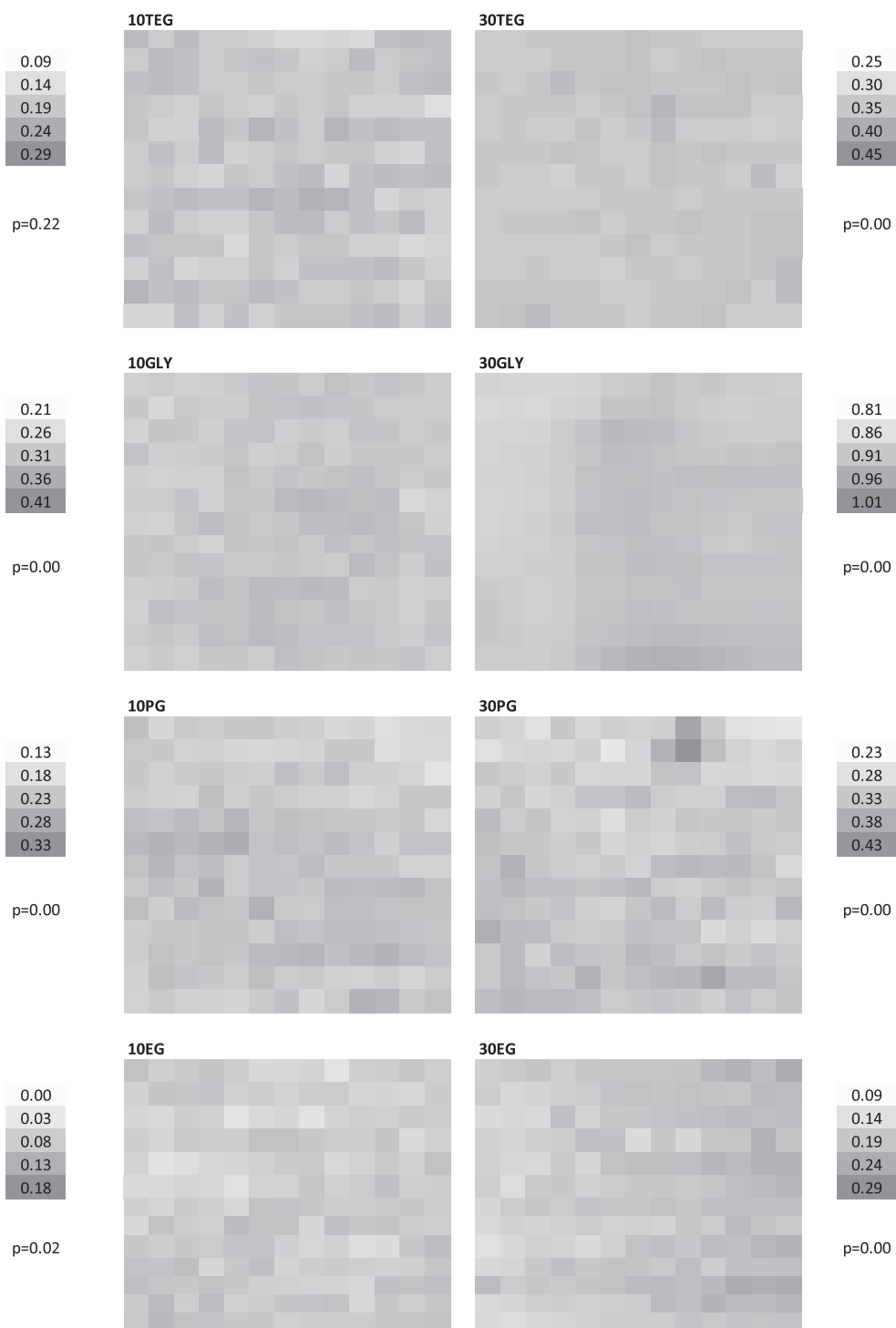


FIGURE 3 Spatial distribution of the primary alcohol group to the amide III ratio resolved with S-FTIR. *P*-value represents the statistical difference between the two maps measured within a sample

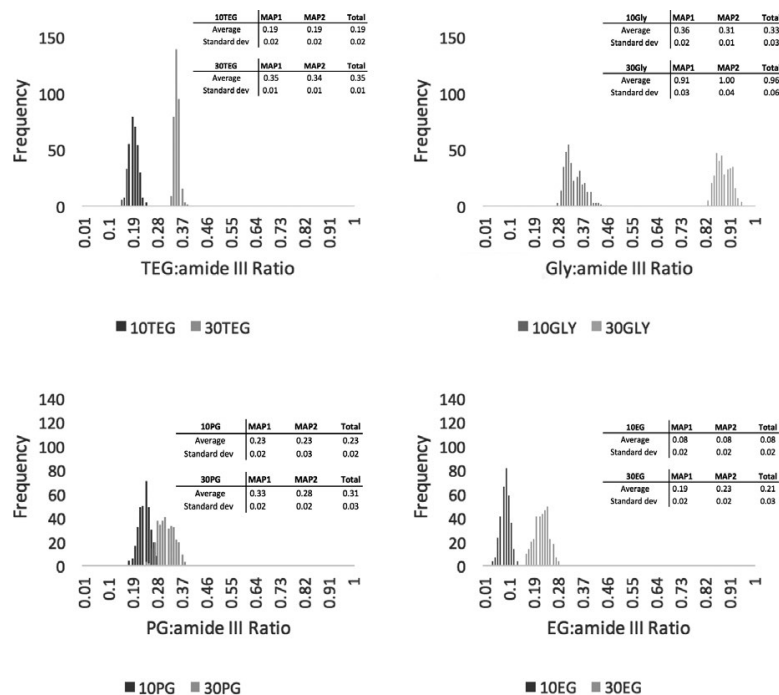


FIGURE 4 Histograms of the primary alcohol group to the amide III ratio resolved with S-FTIR. 10 and 30 pph_{BM} compositions of the four plasticizers selected

from the right-hand side of the main peak with 10 pph_{BM} samples. At 30 pph_{BM}, it gets stronger and merges with the main peak, followed by a left-hand side shoulder forming at 40 pph_{BM}, most clearly seen for PG and GLY.

The main α -relaxation peak is presumably responsible for the protein network plasticized after plasticizer addition. The second peak occurring only after plasticizer addition suggested a phase-separated region. A smaller peak represents fewer chains in the phase, and the transition moves to a lower temperature faster with increasing plasticizer content than the main α -relaxation peak.

At 10 pph_{BM}, the distance between the first and second peaks was indicative of the amount of phase separation. GLY with, presumably, the clearest phase separation was closest to the first peak and had the highest peak, intensity (having more chains involved in the transition). This is followed by TEG, PG, and EG in the same order than FTIR and moisture content results.

At 30 pph_{BM}, phase separation for all the plasticizers was severe enough that the peaks merged. Glycerol, being the most separated, clearly had the broadest transition, indicating that the lowest amount of plasticizer was absorbed into the protein network. This was followed by TEG and EG with similar peaks, and finally PG with the narrowest transition which is also located at the lowest temperature.

With increasing plasticizer content, the peaks continued to widen, and a clear shoulder appeared for GLY and PG at 40 pph_{BM} plasticizers. The left shoulder for these plasticizers is presumably a combination of phase separation, not strong

enough for EG and TEG. Verbeek et al.^[21] studied blood meal's moisture sorption properties with TEG and found that height of $\tan \delta$ peak decreased and widened as the moisture content increased, and samples conditioned at the highest relative humidity showed two different $\tan \delta$ peaks representing protein domains just plasticized by water. Similar behavior has been found with soy and gluten proteins.^[13,22] Even though moisture changes have an effect on peak shape, it does not fully explain these changes; plasticizer type and amount also influence the $\tan \delta$ peak position. Overall, it suggests that the α -relaxation peak consists of at least two different regions; one plasticizer-rich and one protein-rich.

Some studies have also linked changes to the β -relaxation with plasticizer-rich domains and evidence of a low compatibility between the plasticizer and biopolymer.^[22] A β -relaxation was observed between -50 and -20°C , and the intensity increased with increasing plasticizer content. Changes in peak intensity are not only due to relaxations of the polymer but could be relaxation observed for the plasticizer itself, especially when phase separated. For example, the T_g of glycerol is around -60°C , explaining the increased intensity of this low-temperature thermal transition.

3.2 | Structural effects

An oversimplified interpretation regarding the secondary structures of a thermoplastic protein could be that β -sheets acts as an impediment to processing, such as extrusion, while

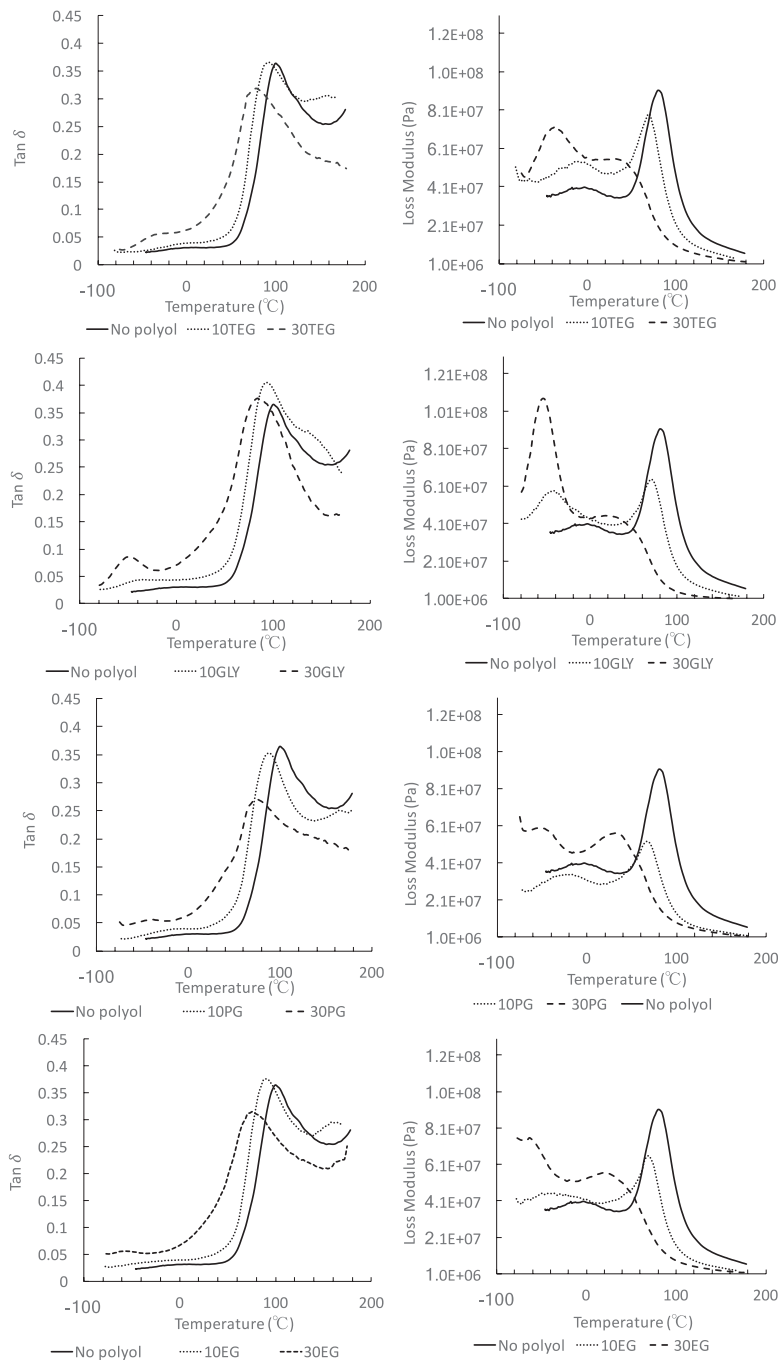


FIGURE 5 Tan δ and loss modulus below and above the POE of all the compositions

α -helices and random structures are neutral.^[2,9] Blood meal consists of mostly albumin and hemoglobin subunits, known for its naturally high helical content.^[23] Thermal processing causes aggregation leading to almost 50% of blood meal's structure to be β -sheets. High β -sheet content has been linked to the insolubility of kafirin which is in agreement with blood

meal's complete insolubility in water.^[24] A thermoplastic polymer is formed by incorporating sodium sulfite (SS) and sodium dodecyl sulfate (SDS) into the polymer network when producing Novatein. SS is known for its ability to break disulfide cross-links, whereas SDS interacts with hydrophobic regions. This causes a change in secondary structure of

the protein, evident from an increase in random coils at the expense of α -helices and β -sheets (in the absence of a plasticizer) (Figure 7). The addition of a plasticizer is optional. However, it is often used to provide more suitable processing conditions and end-product properties.

A high variation in these secondary structures was observed (Table 3), and similar to plasticizer distribution, the average values were calculated from 338 measured points from two $650 \times 650 \mu\text{m}$ areas. Even though the standard deviation is high, and differences between average values for the various samples were relatively small, t test results revealed differences to be statistically significant. However, for

10PG, the average random (random coils and β -turns) and ordered structures (α -helices and β -sheets) were not statistically different to that for samples without plasticizer addition ($P < 0.05$). The most significant change was that of TEG, for which the random structures increased significantly.

For GLY and EG, ordered structures increased slightly, either because of an increase in β -sheets or α -helical content, but rarely both. 30GLY had the largest change, having an increase in the α -helical content at the cost of random structures. Barone et al^[25] also found an increase in ordered structures for egg albumin, lactalbumin, and wheat gluten when they were plasticized with glycerol.

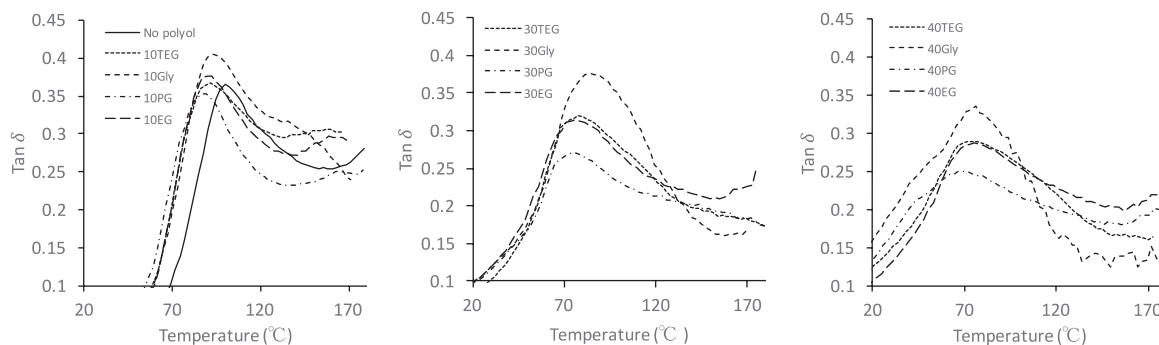


FIGURE 6 A closer look at the shape of Tan δ peaks with different plasticizer content

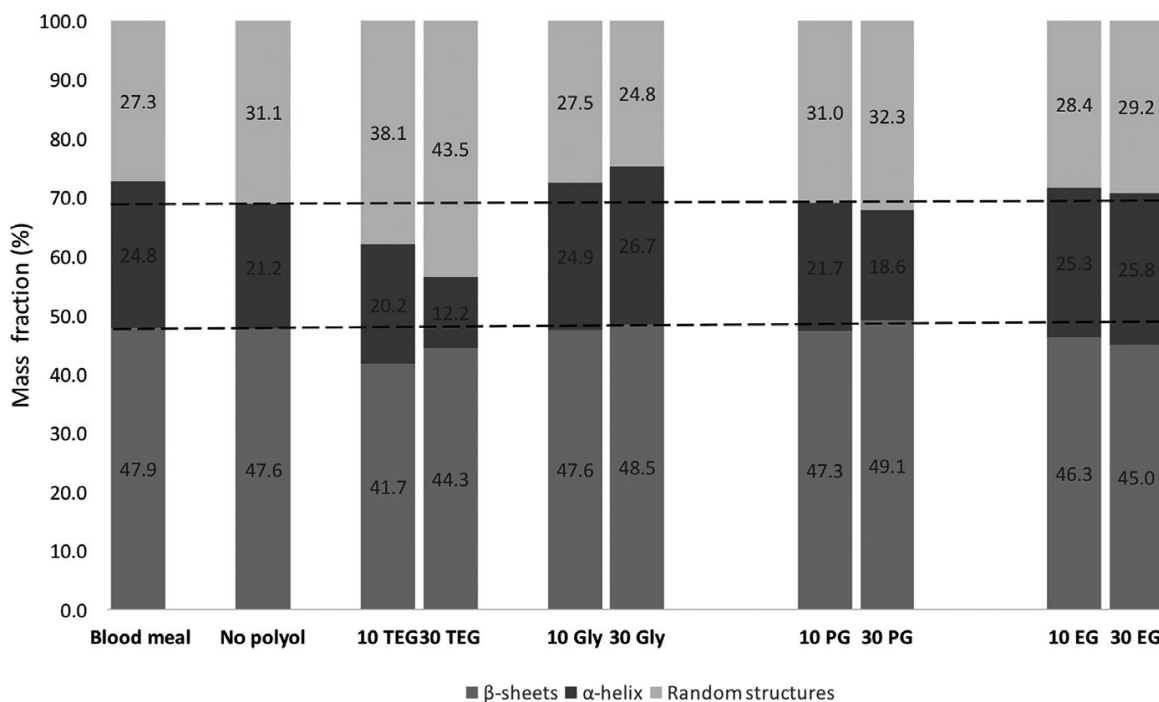


FIGURE 7 Effect of plasticizers on the secondary structure of Novatein. Dotted line added to make a comparison between no plasticizer Novatein easier

TABLE 3 Effect of plasticizers on Novatein's secondary structure

Composition		Ordered structures	α -helix	β -sheets	Random structures	Random coils	β -turns
Pure BM	Mean values	72.70	24.80	47.90	27.30	18.70	8.60
	Standard deviation	4.11	3.25	4.18	4.11	4.70	3.65
	<i>t</i> test vs No polyol	0.00	0.00	0.682*	0.00	0.00	0.00
No polyol	Mean values	68.87	21.24	47.63	31.13	25.51	5.62
	Standard deviation	6.53	4.54	6.50	6.53	5.94	4.74
	<i>t</i> test vs No polyol	0.00	0.00	0.00	0.00	0.00	0.00
10TEG	Mean values	61.92	20.19	41.73	38.10	33.60	4.50
	Standard deviation	5.44	4.08	5.00	5.44	5.15	4.09
	<i>t</i> test vs No polyol	0.00	0.00	0.00	0.00	0.00	0.00
30TEG	Mean values	56.50	12.20	44.30	43.50	39.20	4.30
	Standard deviation	1.85	1.67	1.90	1.85	2.91	2.31
	<i>t</i> test vs No polyol	0.00	0.00	0.00	0.00	0.00	0.00
10Gly	Mean values	72.50	24.90	47.60	27.50	24.40	3.10
	Standard deviation	5.73	3.59	4.98	5.73	5.93	3.14
	<i>t</i> test vs No polyol	0.00	0.00	0.903*	0.00	0.02	0.00
30Gly	Mean values	75.20	26.70	48.50	24.80	18.50	6.30
	Standard deviation	6.59	3.87	5.82	6.59	7.10	4.15
	<i>t</i> test vs No polyol	0.00	0.00	0.07	0.00	0.00	0.05
10PG	Mean values	69.00	21.70	47.30	31.00	26.90	4.10
	Standard deviation	5.19	3.49	4.93	5.19	4.92	3.58
	<i>t</i> test vs No polyol	0.756*	0.12*	0.438*	0.756*	0.00	0.00
30PG	Mean values	67.70	18.60	49.10	32.30	19.70	12.60
	Standard deviation	6.89	4.67	6.94	6.89	6.32	5.24
	<i>t</i> test vs No polyol	0.03	0.00	0.01	0.03	0.00	0.00
10EG	Mean values	71.60	25.30	46.30	28.30	24.90	3.40
	Standard deviation	4.92	3.38	4.61	4.92	4.47	3.06
	<i>t</i> test vs No polyol	0.00	0.00	0.00	0.00	0.165*	0.00
30EG	Mean values	70.76	25.79	44.97	29.24	26.74	2.50
	Standard deviation	5.07	3.58	4.87	5.07	4.69	3.04
	<i>t</i> test vs No polyol	0.00	0.00	0.00	0.00	0.00	0.00

Statistically different vs 'No plasticizer'-composition

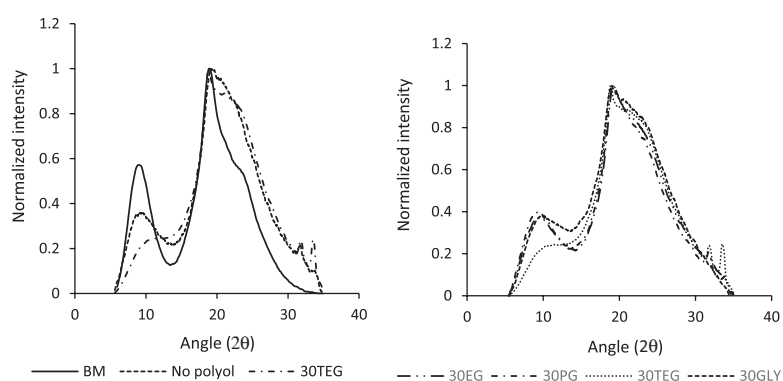


FIGURE 8 XRD spectra for blood meal, Novatein without plasticizer, and Novatein plasticized with 30 pph plasticizer (normalized to the highest peak at 19° 2θ)

This is at odds with the TEG results where plasticization had a clear influence on secondary structures. PG was similar to TEG, but its effect was much less pronounced. It can, therefore, be concluded that, for at least TEG, and to a smaller extent PG, the mechanism of plasticization is not only that of increasing chain mobility in the existing amorphous phase, but also that it increases the amorphous fraction by changing the conformation of protein chains. TEG's effect was also evident from changes in the T_g , presented later.

The XRD results supported the results of FTIR (Figure 8). The Bragg peak at around $9^\circ 2\theta$ has been correlated to both interhelical packing and bigger clusters of β -sheets.^[2] Barone et al^[25] showed that plasticization lowered the peak intensity, especially for proteins with lower aggregation and cross-linking. Plasticization increases the distance between ordered clusters by a dilution effect, but with additional aggregation (thermal processing) would restrict these changes. The same behavior was observed here; the peak intensity lowered when blood meal was turned into Novatein (no plasticizer), with the biggest reduction when TEG was used. Glycerol was the only plasticizer that led to an increase in ordered structures (FTIR) and had a wider peak in this region (Figure 8).

The second peak ($19^\circ 2\theta$) is attributed to repeating distances of hydrogen bonding of α -helices or β -sheets. Le Tien et al^[25] demonstrated that a lower ratio between the intensity and width of the second peak could be correlated to a higher degree of cross-linking in whey protein. Widening of that area could also be seen when blood meal was turned to Novatein with and without plasticizers. The peaks at $31\text{--}33^\circ 2\theta$ are characteristic for SS crystallites.

3.3 | The glass transition temperature

Multiple phases existed (more than one peak in $\tan \delta$) and identification of a single T_g is a challenge; however, at high

frequency, these merge into one and would be more appropriate to assess plasticizer efficiency without bias. As the phase-separated fraction is not accurately defined, both the molar number of plasticizers and plasticizer+water were considered as the most appropriate approach for T_g interpretation (Figure 9).

At 30 pph_{BM}, EG had the highest molar amount of plasticizer ($0.65 \text{ mol } 100^{-1} \text{ g}^{-1} \text{ blood meal}^{-1}$), followed by PG, GLY, and TEG. Instead, when the molar amount of water was included, GLY had almost the same molar amount of total plasticizer as EG. On a total moles plasticizer basis, PG and TEG had the greatest effect on T_g , and at 40 pph_{BM} PG decreased it to 72°C , solely because of the large molar amount of PG. This is followed by EG and glycerol, T_g being 88°C with 40GLY, 16°C higher in comparison with 40PG.

The results regarding changes in T_g highlighted the importance of plasticizer's ability to be absorbed into the polymer network. If not, it will cause phase separation as it was the case with glycerol. The phase-separated fraction will act on its own and thus might not efficiently lower the T_g of the protein network. Furthermore, water absorption above the POE did not play a significant role regarding the T_g , as it is part of the phase-separated fraction.

Interestingly, the total molar amount of plasticizer was not the direct cause of the change in T_g either; rather, the plasticizer type seemed to be more important. TEG, with the highest molecular weight, reduced the T_g just as much as PG, except at very high plasticizer content. This is most likely related to changes in secondary structure, as discussed earlier. However, as can be seen from samples plasticized by EG (also a small molecule), the ability to diffuse into the polymer network is not the only factor. TEG and PG are more hydrophobic plasticizers to EG and GLY and, thus, might be able to interact better with the hydrophobic regions of protein as well (Table 1).

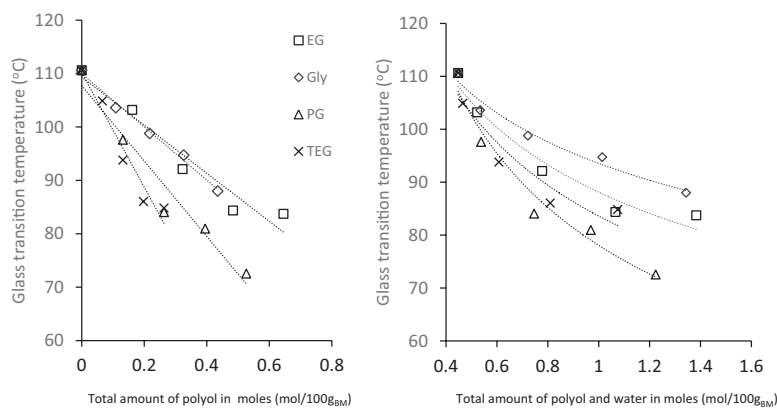


FIGURE 9 T_g based on the molar fraction of plasticizers taken at 30 Hz temperature sweep

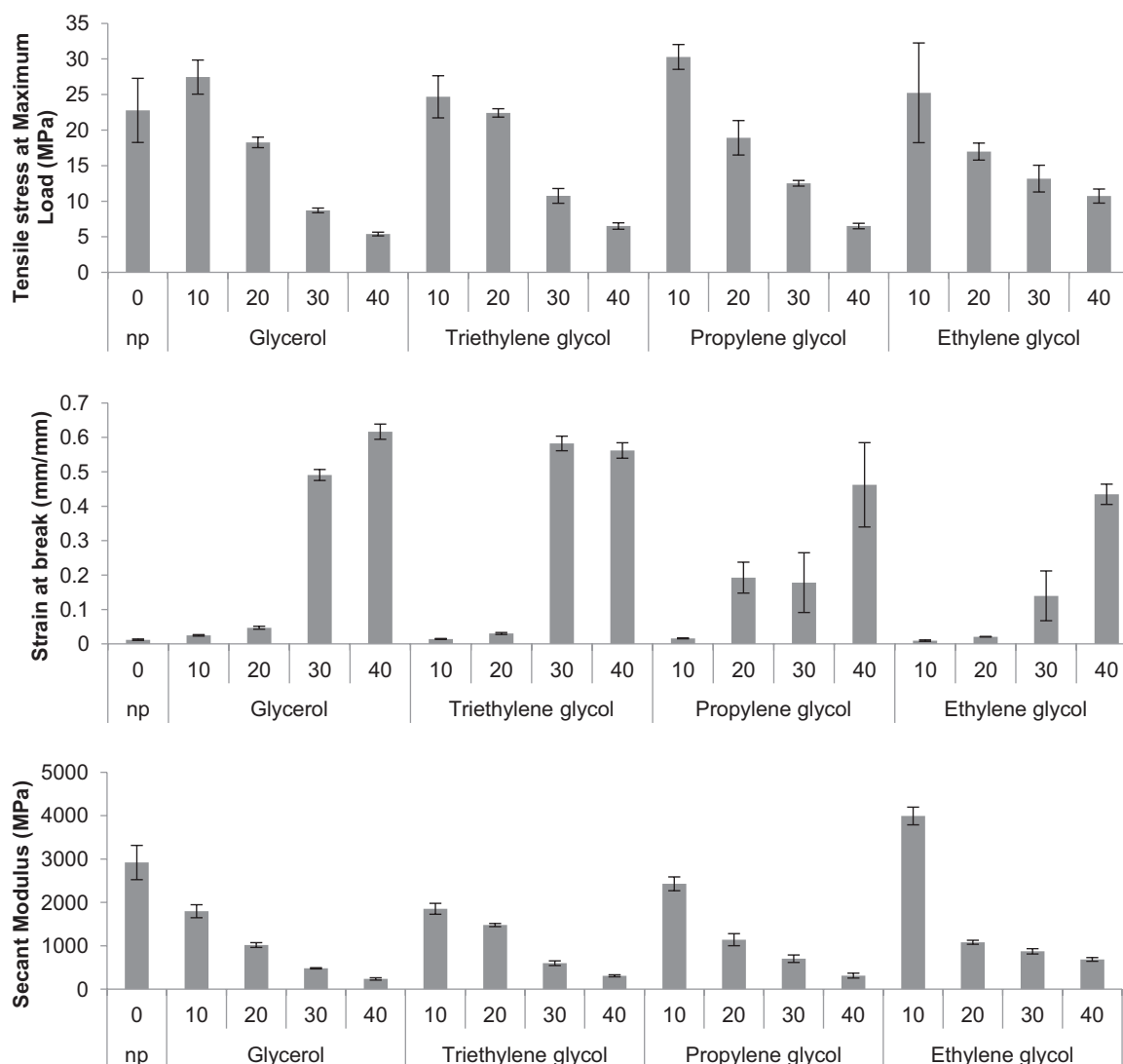


FIGURE 10 Mechanical properties with standard deviation of different Novatein compositions

3.4 | Mechanical properties

All the plasticizers followed the expected trend of decreasing tensile strength and modulus with increasing plasticizer content (Figure 10). Lower plasticizer levels led to brittle and glassy material characteristics, whereas increased plasticization led to more ductile and rubber-like materials. Mechanical properties had a strong correlation with water content and were driven by the distance from the POE, determined by plasticizer type and amount (Figure 11).

The tensile strength was dependent on the distance from the POE and had a much stronger dependence below the POE (Figure 11). Above the POE, the tensile strength did decrease with increasing plasticizer, but slower. As the amount

of plasticizer is approaching the POE, the protein network is saturated with a plasticizer, that is, any added plasticizer has a limited effect on properties, as observed here.

At low plasticizer content (10 pph_{BM}), a strengthening effect was observed for all the plasticizer types and is referred to as antiplasticization. The mechanical strength improved even though the T_g decreased for all the compositions. In general, plasticizers behaved similarly. However, 10GLY and 10PG were able to improve the tensile strength from 22 MPa (no plasticizer) to 27.4 and 30.2 MPa, respectively. PG had the biggest effect even though it had the smallest drop in T_g . Small plasticizers may have an antiplasticization effect due to their high compatibility and ability to penetrate into the polymer network. Smaller

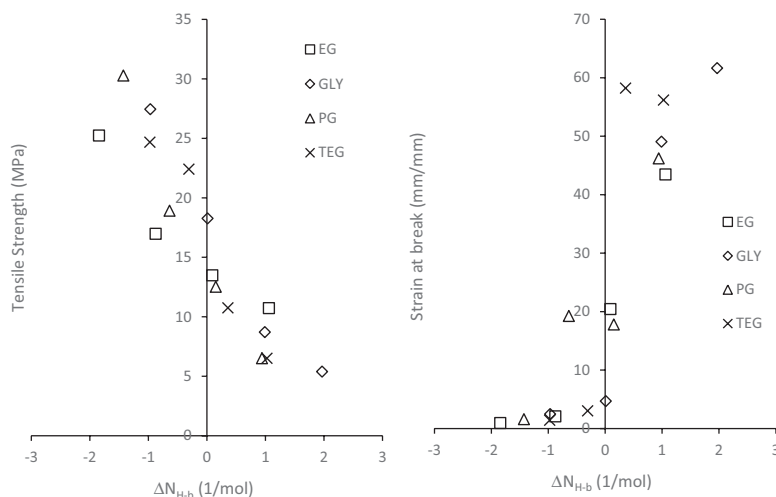


FIGURE 11 Tensile strength and elongation at break in relation to the distance of theoretical hydrogen bonding sites from POE

molecules can diffuse easier into the polymer network and form stronger hydrogen bonds with the protein, leading to physical cross-linking if the same plasticizer is bonded to more than one site.^[7] Plasticizers such as TEG seems to have an optimal size for creating free volume in the polymer network but still small enough to be able to provide efficient plasticization. However, the effect of TEG goes beyond this, despite the larger molecular size, it was seen to create more disorder in the protein's secondary structure, in which chains are not as tightly bound as in structures such as β -sheets.

Also clear from Figure 10 is distinct brittle-to-ductile transformation which can be correlated to the POE (Figure 11). Below the POE, samples were brittle and were strongly influenced by the distance from the POE. Above the POE, the samples were more ductile and less dependent on the distance from the POE. As the absorbed water was found to be mainly bound in the phase-separated fraction, it can also be presumed that transformation to a ductile material is related to the phase separation of Novatein. TEG and GLY with presumably higher phase separation were found also to turn ductile at lower plasticization level. Standard deviation at strain at break results also shows that the material performance was more constant.

The POE is the amount of hydrogen bonding sites associated with each plasticizer such that the moisture content will be the same compared to a sample without plasticizer. At this point, the protein network seems to get saturated with the combination of plasticizer and water, leading to a change from brittle to ductile. After phase separation, the effect of plasticization on the mechanical properties diminishes. As the total theoretical hydrogen bonding sites are related to the equilibrium moisture content, mechanical properties have an obvious correlation to it. However, the moisture content is driven by the plasticizer type, if compared on the basis of the POE

(Figure 11), the materials behaved similarly, regardless the plasticizer type.

4 | CONCLUSIONS

The plasticization of injection-molded Novatein was studied using different plasticizers. Plasticization resulted in the transformation from brittle to ductile properties that were explained by primary and secondary plasticization mechanisms. The equilibrium moisture content (EMC) varied greatly between different plasticizer types as well as the amount added. The difference between plasticizer types was attributed to their ability to hydrogen bond with proteins, as well as their tendency to alter the EMC. The plasticizer's ability to change the EMC is related to the theoretical amount of hydrogen bonding and plasticizer's tendency to phase separate and can be quantified using the POE.

Primary plasticization was thought to dictate up to the POE after which phase separation occurred, and performance was driven by secondary plasticization. The degree of phase separation was different for each plasticizer, increased with increasing plasticizer amount, and the distribution became wider at higher plasticizer content. Two different α -relaxation peaks were representative of two phases, plasticizer-rich and protein-rich. The high-temperature transition was thought to be protein-rich, was smaller, changed faster with increasing plasticizer content, and was more obvious for GLY and TEG.

Of the selected plasticizers, TEG had the most obvious effect on the secondary structure of the polymer; increasing random structures, mainly at the cost of α -helices. β -sheets were only decreased by TEG. The highly aggregated structure of blood meal, shown by the high β -sheet content, is

generally seen as a processing impediment and is why TEG was such an effective plasticizer, not only plasticizing amorphous material, but also increasing the amount.

ACKNOWLEDGMENTS

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4

The role of water in plasticizing thermally aggregated protein-based thermoplastics

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By

Jussi M. Uitto and C.J.R Verbeek

The role of water in plasticizing thermally aggregated protein-based thermoplastics

Chapter 4 expanded on the plasticization study by investigating the role of water as part of a ternary system. The aim was to expand the understanding of plasticization with a focus on primary and secondary plasticization, presented in the previous chapter. Furthermore, different plasticization theories were evaluated for their applicability in a ternary biopolymer system in which phase separation is prominent.

With regards to main thesis objectives, the aim of this study was to understand the difference between water and polyol plasticization, and how their hydrogen bonding environment is affecting plasticization as a whole.

As first author of this paper, I prepared the initial draft manuscript, which was refined and edited in consultation with my supervisor, who has been credited as co-author.

The role of water in plasticizing thermally aggregated protein-based thermoplastics

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ABSTRACT: Blood meal-based thermoplastic protein (Novatein) is made from a highly aggregated protein network, and as a result, water plays a significant role during plasticization. Novatein was plasticized with up to 40 parts tri(ethylene glycol) or glycerol and equilibrated at different relative humidities. The equilibrium moisture content (EMC) was the dominant factor determining mechanical properties, with a brittle to ductile transformation observed at 8% EMC. However, EMC was not sufficient to explain this behavior and the point of equivalence (POE) was introduced to differentiate between primary and secondary plasticization. It was shown that the constraint theory, which relates to hydrogen bonding plasticizers, was more applicable below the POE whereas the free volume theory, and the formation of the microscale phase separation, described material behavior above the POE. Water played a critical role improving ideal mixing conditions in the material and was also related to the brittle-to-ductile transition. © 2018 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2018**, *135*, 46746.

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INTRODUCTION

Plasticized biopolymers are complex ternary systems, typically consisting of a polymer, plasticizer, and water. Plasticization has been extensively studied, however, for biopolymers, the consensus is that the mechanisms are not fully understood.^{1,2} The free volume, gel and lubricity theories are the three most commonly used theories.² The gel and lubricity theories describe the plasticizer's ability to disrupt and replace polymer–polymer interactions, and furthermore, reduce the internal friction and act as a lubricant. The free volume is the most often used and explains molecular mobility with internal space around molecule chains.

In multiphase systems, such as protein-based plastics, the free volume theory is not fundamentally flawless as it is built on the basis of the free volume being the sum of the system's ideally mixed components.³ Protein-based plastics are semicrystalline and consist of a heterogeneous polymer network of hydrophilic and hydrophobic regions and a polymer backbone containing a wide variety of side groups. In terms of plasticization, this does not provide an environment for ideal mixing. For example, dynamic mechanical analysis of protein plastics have shown wide, and in some cases, multiple transition peaks with relatively low intensity, being representative of a rather heterogeneous system under nonideal mixing conditions.^{4,5}

Despite this, free-volume-based models, such as the Gordon–Taylor (GT), Couchman–Karasz (CK), and more detailed models such as the free-volume-Flory–Huggins (FVFH) model have been

able to predict the behavior of ternary systems with moderate success.⁶ The FVFH was introduced to consider nonideal mixing as well as structural changes to the polymer network.

An alternative approach used for explaining the glass transition behavior of materials plasticized with polyhydric alcohols, is a constraint theory-based model introduced by Nakanishi and Nozaki.⁷ Here, the atomic degree of freedom is compared to interatomic force-field constraints; if the number constraints are less than the atomic degree of freedom, the network is considered flexible and vice versa. They proposed, in contrast to the free volume theory, that the number of alcohol groups has a bigger effect on the T_g than the length of the carbon chain. Nakanishi and Nozaki's model has been successfully applied to ternary systems and showed good a correlation between the T_g and the number of hydrogen bonding sites for multiple biopolymer systems.^{3,8}

One advantage of constraint theory-based models is that it does not require consideration of nonideal mixing conditions.^{9,10} On the other hand, it has been shown that the CK and GT models are surprisingly accurate, considering the heterogeneity of biopolymers. Water seems to have a critical role in reducing nonideal mixing effects and brings about compatibility to the system. Van der Sman suggested that the role of water would be filling the holes between different clusters in a microheterogeneous system in a way that it approaches ideal mixing.³

Therefore, the role of water in plasticization of biopolymers is critical as a biopolymer's natural tendency to absorb water is

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modified when plasticizers are introduced.^{11,12} For example, Godbillot *et al.* introduced a model illustrating different ternary interactions in a starch system. The study not only showed that plasticizers occupy some of the water sorption sites, but also provide new binding sites after the saturation point have been reached. This has been linked to the formation of phase-separated systems as well.^{12,13} In respect to the constraint theory, it does make sense that in some cases plasticizers strengthen the biopolymer at low plasticizer content as they replace loosely bound water with stronger plasticizer interactions.^{13,14} Furthermore, plasticizers may affect the biopolymer's structure, for example, a protein's secondary structure, which may further alter the constraints in the polymer network.

In previous work from the authors, a plasticizer's tendency to phase separate was quantified using the point of equivalence (POE), which is the point where the equilibrated moisture content (EMC) equals the EMC of the same polymer in the absence of a plasticizer.¹³ It is also the point at which the brittle to a ductile transition occurred. It is in good agreement with the constraint theory where below the POE the plasticizer strengthened the material, whereas above the POE the degree of freedom is increased significantly due to phase separation. In this article, the effect of water in thermally aggregated protein-based thermoplastics is explored using glycerol (GLY), tri(ethylene glycol) (TEG) as well as compositions without plasticizers, equilibrated at different relative humidities (RH%). The study focuses on how plasticization changes the EMC in relation to its hydrogen bonding environment and extends the general understanding of plasticization to include the POE. This has been done by relating the mechanical properties of different Novatein compositions, conditioned at different RH%, to structural properties determined from synchrotron Fourier transform infrared (FTIR) results.

EXPERIMENTAL

Materials

Blood meal (BM) was obtained in powder form from Wallace Corporation, Hamilton, New Zealand and sieved to 700 μm . Technical grade sodium dodecyl sulfate (SDS) was obtained from Biolab, Auckland, New Zealand and analytical grade sodium sulfite from BDH Lab supplies Auckland, New Zealand. Plasticizers were obtained from Merck Millipore, Auckland, New Zealand.

Sample Preparation

Novatein has been developed and patented by Aduro Biopolymers, Hamilton, New Zealand.¹⁵ Thermoplasticity is achieved by blending 100 parts by mass BM with 3 parts per hundred parts BM (pph_{BM}) SDS and sodium sulfite (SS), water and five different levels of two different plasticizers including a composition without plasticizer. The compositions were selected based on the results of the previously published paper.¹³ In this article, the selected plasticizers (TEG and GLY) were assumed to have the most significant difference in primary plasticization and were expected to have the greatest effect on sorption isotherms. In this article, the term "plasticizer" refers to external additives used as a plasticization enhancer but not water which can be defined as a plasticizer as well.

Total of nine different compositions were prepared by dissolving SS and SDS in the appropriate amount of water, followed by blending

with 100 parts BM powder in a high-speed mixer, after which 0–40 pph_{BM} of plasticizer was added. The processing water content was 40 pph_{BM}, although compositions with the lowest plasticizer content (10 pph_{BM}) required 50 pph_{BM} water, whereas no plasticizer versions required 60 pph_{BM} for being able to be extruded. However, the effect of water when processing was assumed to be minor when the samples were conditioned to their equilibrium moisture content (EMC) which is driven by the polymer network.

The samples were prepared using a Labtech corotating twin-screw extruder at a screw speed of 200 rpm. The temperature profile increased over 11 barrel sections; from 100 °C at the feed throat to 120 °C at the die. The *L/D* ratio of the extruder was 44 with a single 10 mm circular die. The mass flow rate of all compositions was $100 \pm 20 \text{ g min}^{-1}$; pressure varied from 20 to 70 bar, depending on the composition used. Die pressure and torque varied depending on the level of plasticization in a way that higher plasticization led to lower torque and pressure, as expected. The thermomechanical effect for all the compositions was not equal, and for example, structural changes caused by the difference in specific mechanical energy was considered as a characteristic property of certain plasticizer and plasticizer level. The experiment was designed in a way that all the compositions could be processed and residence time in the extruder would be similar.

The extruded material was granulated using a triblade granulator from Castin Machinery Manufacturer Ltd., Hongkong, China. Specimens for the tensile test were produced in a BOY 35A injection molding machine. The shape of the tensile test specimens was by American Society for Testing and Materials (ASTM) D638. A temperature profile of barrel and mold were necessary to be altered between the compositions in a way that injection temperature varied from 140 to 160 °C and pressure was aimed to be at $1000 \pm 200 \text{ bar}$. Cooling time and temperature varied based on composition in a way that more plasticizer required lower mold temperature and longer cooling time.

Tensile test specimens were conditioned at 23 °C in containers with saturated salt solutions to achieve different RH conditions. Saturated salt solutions were prepared according to ASTM E104 standard using P₂O₅, NaBr, NaCl, and KCl (dried, 58RH%, 75RH%, and 85RH%). The samples were kept in a conditioning chamber for 5 weeks after which their mechanical properties were tested, and moisture content measured. For estimating water sorption isotherms, granulated samples were equilibrated at 23 °C in a Binder refrigerated incubator (Germany) at different RH ranging between 30 and 80%. The moisture content of all samples was determined by drying at 60 °C for a 7-day period, to avoid evaporation of plasticizer.

Analysis

Tensile properties were determined using an Instron model 33R4204. Tensile strength (TS) and elongation at break (ϵ) have been determined according to ASTM standard D638-03. An extension rate of 5 mm min⁻¹ and an extensometer gauge length of 50 mm was used for testing. Samples were tested in replicas of six directly after removal from the humidity chambers.

Spatially resolved FTIR experiments were undertaken on the infrared (IR) microspectroscopy beamline at the Australian

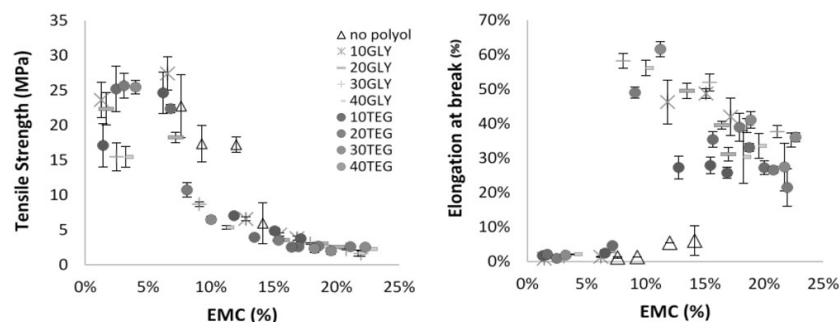


Figure 1. TS and elongation at break of the samples regarding their EMC% with error bars.

Synchrotron, Victoria, Australia. Spectra were collected using a Bruker Hyperion 3000 with microconcentrating collector (MCT) and XY stage using Opus 6.5 software (Bruker Optik GmbH 2009). For each sample, two visually most representative spots of the microtomed sample were selected for measurement of 13×13 sized maps with a $5 \times 5 \mu\text{m}$ spot size revealing the structural characteristics from two different $650 \times 650 \mu\text{m}$ areas of the sample. For each point, 32 spectra were collected in transmission mode with a resolution of 4 cm^{-1} between 3900 and 700 cm^{-1} and averaged using Opus 6.5 software (Bruker Optik GmbH 2009).

Samples containing 10 and 30 pph_{BM} plasticizers were selected for FTIR tests, conditioned at three different RH (dried, 50RH% and 80RH%). These represented samples above, below or nearby their POE. Plasticizer distribution (or level of phase separation) was evaluated by calculating the ratio between the primary alcohol groups ($1045\text{--}1090 \text{ cm}^{-1}$) to the amide III ($1180\text{--}1330 \text{ cm}^{-1}$) region, secondary structure by interpreting the second derivative of the Amide III region based on the method developed earlier, and the hydrogen bonding strength by determining the NH-stretch vibration peak position in the Amide A region ($3300\text{--}3170 \text{ cm}^{-1}$).^{16,17} The collected spatial maps were converted to histograms that can illustrate the distribution of these properties.

RESULTS AND DISCUSSION

Mechanical Properties

Figure 1 shows the variation of TS and elongation at break ($\epsilon\%$) in terms of EMC for different Novatein compositions. As expected, the TS decreased with increasing EMC, while $\epsilon\%$ increased. The TS varied significantly, decreasing from 27.4 MPa for 10GLY@50RH% to 2.2 MPa for 40GLY@85RH%. $\epsilon\%$ increased from 1.3% (10GLY@0RH%) up to 61.6% for 40GLY@50RH%. Most notably, an abrupt change in TS and $\epsilon\%$ was observed in the region of 8% EMC.

The EMC was found to be the dominant factor determining the mechanical properties, regardless of the plasticizer content. The exception was for samples in the absence of a plasticizer for which $\epsilon\%$ only reached 6.1%, even with an EMC of 14.1%. Thus, a plasticizer can be considered as a precondition for higher ductility in the presence of water. However, between GLY and TEG, the variability was much more considerable for GLY plasticized

samples, suggesting a more severe sensitivity to environmental changes.

Despite water being the dominant factor determining mechanical properties, the brittle-to-ductile transformation required both water and plasticizer. According to the gel theory, water has a direct effect on the polymer network, forming protein–water–protein interactions via hydrogen bonding.² In Novatein, plasticizers not only replaced some water–protein interactions with plasticizer–protein interaction but also formed a second phase which allowed additional water–plasticizer interactions, leading to a higher EMC. This would also be in agreement with Van der Sman's suggestion that water would be filling the holes between different chain clusters.³ The occurrence of the second phase was correlated with the brittle-to-ductile behavior of Novatein in previous work from Uitto and Verbeek.¹³ With a heavily aggregated protein system, the addition of a plasticizer would be required to bring about flexibility by modifying the environment for water interaction with the protein. This may be either simple plasticization or direct modification of the protein secondary structure.¹⁸

It is clear from the results that a protein's hygroscopic character affects the material properties and changes in environmental conditions (RH) will change the material's properties significantly. Even though the properties are driven by the EMC, the type of plasticizer also has a significant effect.

The Hygroscopic Character of Novatein

As expected, the EMC for all samples increased with increasing RH% (insert graphs, Figure 2). BM equilibrated to about 10% moisture at high RH%, and processing it into Novatein, without a plasticizer, led to a further increase in EMC. SS and SDS are used to break covalent disulfide crosslinks and to disrupt hydrophobic interactions, revealing new water sorption sites, evident from the increase in EMC.¹⁵

Including a plasticizer increased the EMC further by introducing additional hydrogen bonding sites from the plasticizer itself, or the disruption of intermolecular bonding between protein chains. The behavior of GLY and TEG was similar, however, with 40GLY noticeable macroscopic phase separation occurred (plasticizer visible on sample surface) resulting in an EMC similar to 30GLY, similar to earlier observations.¹⁹

It is interesting to note that at low plasticizer content, a decrease in EMC was observed, underlining the complex hydrogen bonding

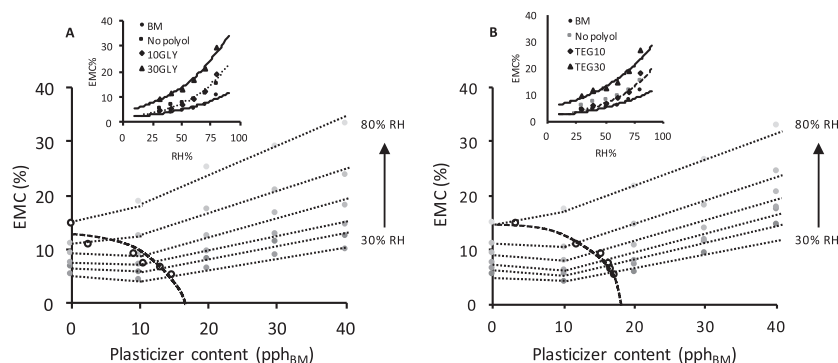


Figure 2. EMC% for various amounts of (A) GLY and (B) TEG showing the point of equivalence (POE) as dashed lines. Insert graphs represent the EMC% isotherms for different compositions.

environment in biopolymer systems, similar to other work.^{11,12} It was previously concluded that phase separation occurred above the POE, accompanied by a brittle-to-ductile transition.¹³ This refers to the point at which the moisture content of a plasticized sample reached an equivalent EMC to a sample in the absence of a plasticizer and is shown as a dashed line in Figure 2. A clear difference between GLY and TEG was observed; a higher amount of TEG is required before the POE is reached, especially at higher RH%. This further highlighted the interactive effects between protein–protein, water–protein, protein–plasticizer, and plasticizer–water bonding, which ultimately determined the material’s mechanical properties, as shown earlier.

Figure 3 shows the change in EMC with respect to the change in theoretical hydrogen bonding sites relative to the POE. A linear increase for both the plasticizers was observed. However, TEG led to a more drastic increase compared to GLY. This would suggest that TEG is more efficient at modifying the hydrogen bonding environment of Novatein, that is, for the same additional hydrogen bonding sites (ΔN_{H-b}), using TEG will result in a more plasticized material. Below the POE, TEG, and GLY essentially behaved the same, with primary plasticization dominating, accompanied with little plasticization. At 40GLY, GLY samples showed macroscopic phase separation, that is, the number of additional hydrogen bonding sites was overestimated in the figure.

It was clear that plasticization at low plasticizer levels (primary plasticization) differs from plasticization after the POE (secondary plasticization), for which an apparent drop in mechanical properties was seen. Additional plasticizer increased hygroscopicity which is driven by the creation of new hydrogen bonding sites in the network. GLY seems to phase separate earlier; however, the slope of the $\Delta EMC\%$ versus ΔN_{H-b} (Figure 3) is lower in comparison to TEG and may be related to structural changes (similar to those caused by SS and SDS).

Structural Effects

The plasticizing effect of water and polyol is discussed in terms of two theories; secondary plasticization or the free volume theory and primary plasticization or the constraint theory including the effect of plasticization on protein chain conformation.

Secondary Plasticization and Free Volume. Figure 4 presents the spatial maps of plasticizer distribution for 10 and 30 pph_{BM} GLY and TEG dried and conditioned at 80% RH. The compositions analyzed represent samples below or near the poly(ethylene oxide) (PEO) and highlight the degree of phase separation. Although microscale phase separation is visible from the maps, it is difficult to quantify the difference between them visually. Therefore, histograms representing the distribution for each map were added.

As expected, a higher plasticizer content led to a higher ratio, but the spatial variation is also evident from the distribution around the mean. The average ratio was lower for 10 pph_{BM} TEG than GLY, and the distribution was much narrower for TEG. Furthermore, for TEG, the distribution was very similar at all RH%, with the broadest distribution of the dried samples, evident of phase separation. GLY instead, showed a much wider distribution than TEG, except at 80% RH. At 80% RH, both TEG and GLY were above their POE, and at 10 pph_{BM} plasticizers, their behavior is very similar. However, at 30 pph plasticizer, TEG samples had much less phase

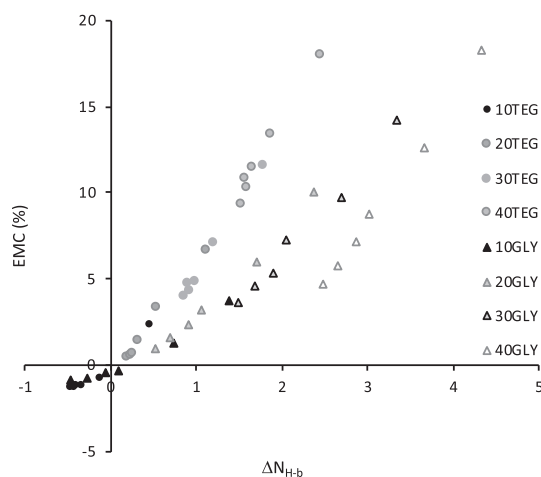


Figure 3. Theoretical hydrogen bonding sites in relation to change of EMC when samples normalized to the POE.

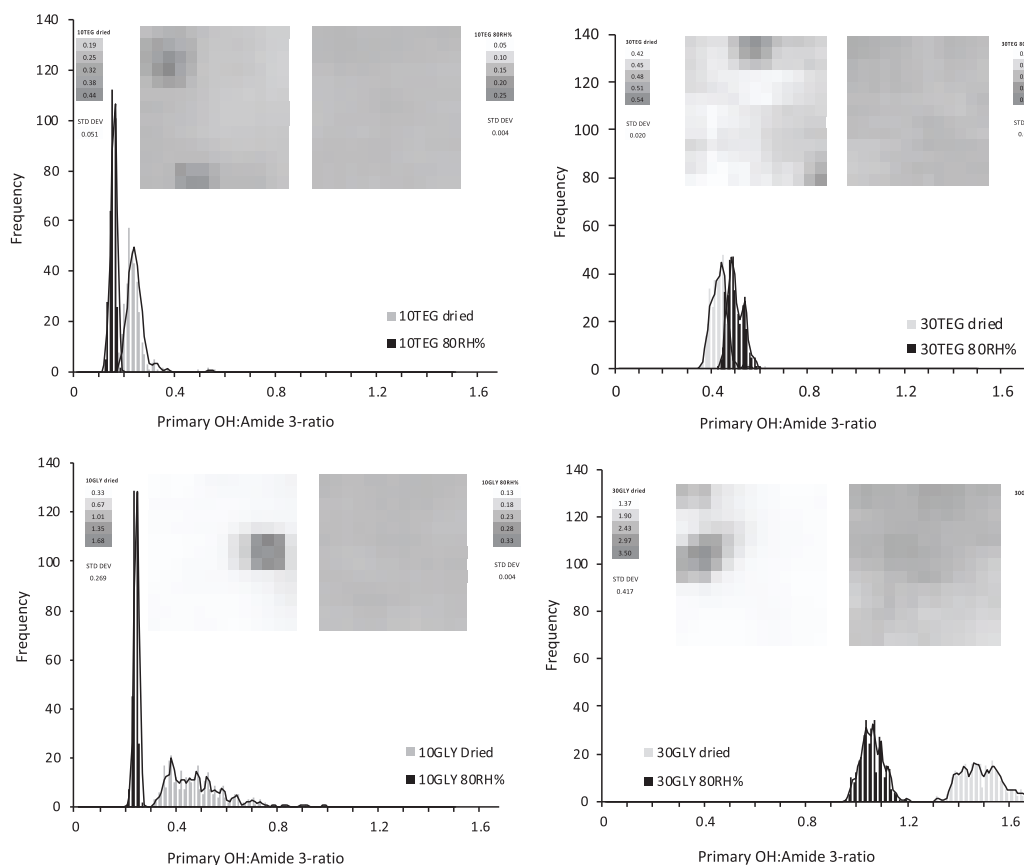


Figure 4. Spatial maps of the plasticizer distribution of 30GLY in different RH.

separation suggesting better absorption into the protein network. 30GLY samples had the broadest and most erratic distribution, indicating GLY's inability to be absorbed efficiently into the protein network compared to TEG. The results showed that water played a significant role in phase separation and compositions above the POE showed a higher level of primary alcohol groups (higher ratio), which has been linked to phase separation in previous work.¹³ At high RH%, water may replace protein-protein hydrogen bonding, also evident from the sorption isotherms.¹¹ However, the same effect does not occur at 10 pph_{BM}, for which samples were below the POE. This is most likely due to the difference between absorbed and phase separated plasticizers.

For both plasticizer types, the plasticizer distribution was narrower at higher water content, supporting the theory that water improved mixing conditions. Phase separation occurred at all RH, but the presence of water homogenized the phase separated fraction (narrower distribution). It is more accurate to consider the free volume theory rather than the constraint theory after phase separation has occurred. In comparison to primary plasticization, phase separation most likely lead to additional plasticization, as shown by others as a discontinuity in the glass-transition temperature.^{4,8} As a very aggregated polymer, phase separation occurs at a much lower plasticizer level for Novatein compared

to, for example, starch.¹¹ Thus, the role of the second phase is rather important, as the primary plasticization may not be enough to provide flexibility to the material.

Primary Plasticization and the Constraint Theory. Changes in the hydrogen-bonding environment are presented in Figure 5, based on -OH stretching (ν OH) and its shift in position (indicating the length and strength hydrogen bonding).²⁰ For samples without plasticizer, an apparent shift to the right was observed with increasing RH% for the average bond length (Figure 5), suggesting a stronger hydrogen bonding environment. However, water also absorbs in the Amide A region, making it difficult to draw definite conclusions, and the shift observed was more likely as a result of increasing moisture content. It was notable that the same behavior was not observed for compositions below their POE (for which phase separation was not expected). Compositions above their PEO showed an apparent shift toward the right with increasing RH%, for which phase separation was observed earlier.

Considering dried materials only, GLY samples showed almost no shift in comparison to no plasticizer compositions. A slightly wider distribution was observed for 30GLY possibly due to either more severe phase separation or increased ordered regions in the protein chains. On the other hand, TEG showed a shift from

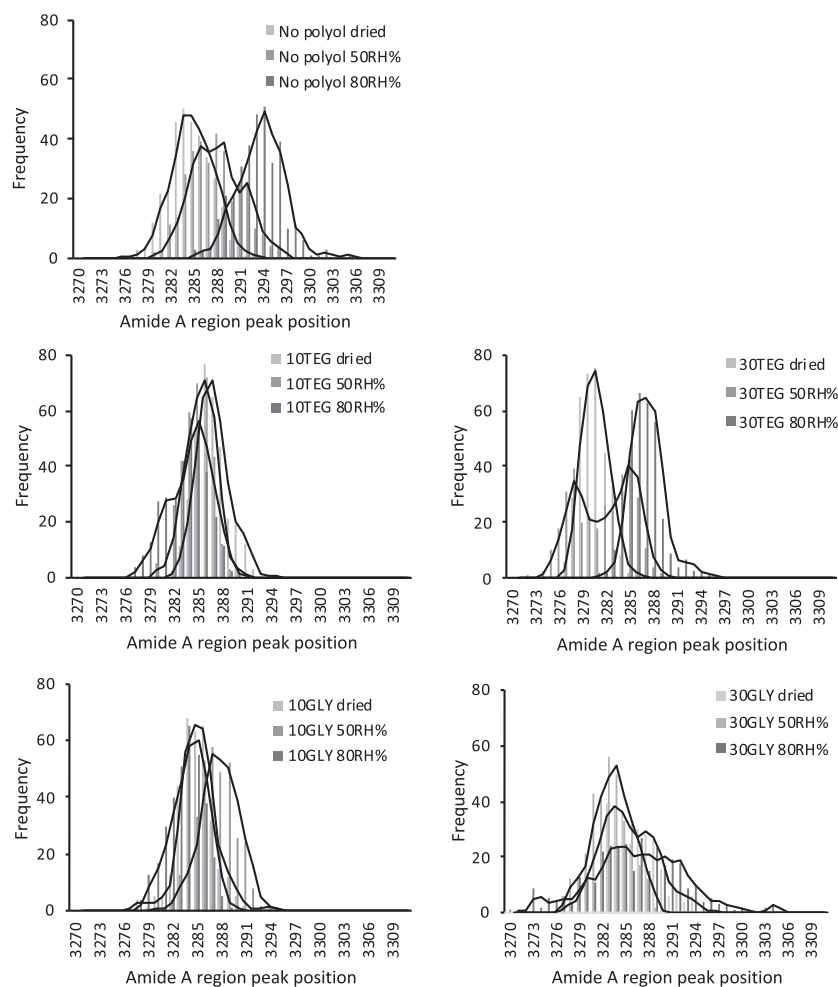


Figure 5. Histograms of Amide A region peak position representing the hydrogen bonding strength.

3284 to 3286 cm^{-1} at 10TEG, indicating a stronger hydrogen bonding environment, consistent with the anti-plasticization effect seen earlier. Increasing the TEG content to 30 reduced the average to 3280 cm^{-1} , consistent with a more plasticized material. The plasticizer's ability to interact with the protein network changes the number of constraints, depending on the plasticizer type and their compatibility with the protein. For example, Ullah and Wu suggested that propylene glycol's brittle mechanical properties be caused by too strong hydrogen bonding interaction with feather-fiber-based thermoplastic.²¹

Secondary structure changes as a result of plasticization and changes in RH% are presented in Figure 6. Plasticizers, similar to SS and SDS, influence the secondary structure of proteins, essentially decreasing a polymer network's constraint factors.¹³ The results showed that GLY and TEG had different effects on the protein's secondary structure, but was mostly independent of RH%. The effect of plasticization is consistent with previous work and concludes that TEG increased the number of random

structures whereas GLY decreases it. However, increased chain mobility brought about by higher moisture contents did not facilitate further structural changes. Water's effect in plasticization is probably limited to secondary plasticization.

Changes in the hydrogen bonding environment and changes in secondary structure would support the observation that TEG formed a more homogenous blend and would explain its more consistent mechanical properties. Contrary to TEG, it would appear that GLY did not have a strong primary plasticization effect; TEG unfolded the protein network whereas GLY made it more aggregated. This is in agreement with the moisture absorption behavior seen in Figure 3. GLY's performance as a plasticizer was mostly driven by phase separation and its somewhat limited, but sufficient, bonding to the protein network. Due to phase separation, it provided sufficient plasticization despite the unfavorable secondary structure changes. Even though the moisture content was found to be driving force for changes in mechanical properties, GLY samples showed greater sensitivity to changes in RH.

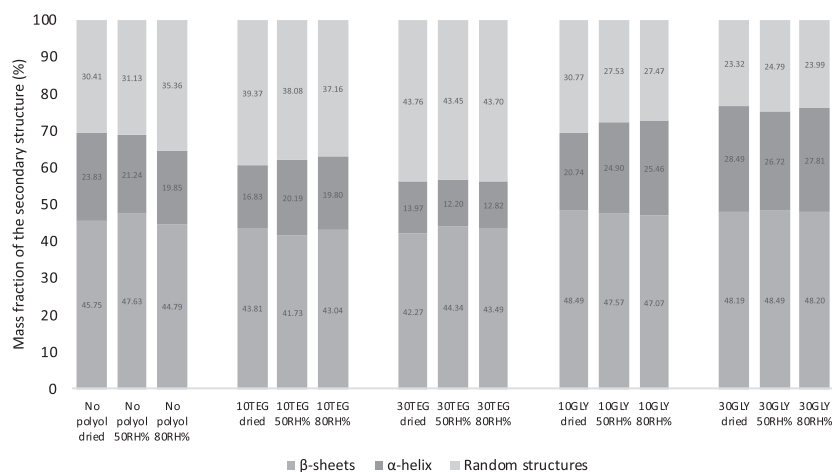


Figure 6. Secondary structure changes of different plasticizer and moisture contents.

The results expand our understanding of the applicability of the free volume and constraint theories applied to biopolymers. The free volume theory does not explain primary plasticization sufficiently and is limited to conditions above the saturation point (or POE) as the water of the second phase seems only to be able to provide homogeneity for the polymer network. This might also be related to the discontinuity behavior of the ternary systems.³

On the other hand, the constraint theory also considers structural effects where functional groups and their interactions dominate. This may explain why many high-molecular-weight plasticizers are ineffective plasticizers for biopolymers and that a low melting point, low volatility, and hydrophilicity are most important when choosing a plasticizer.²² However, this may only be characteristic for more aggregated polymer networks such as Novatein and gluten. Here, long chain plasticizers may not be able to provide sufficient interaction with the polymer network while forming a phase separated fraction. In contrast, zein has been successfully blow molded when plasticized with PEG400, but processability decreased significantly for more aggregated proteins.²³ Considering the constraint theory, the number of functional groups may give a better indication of plasticization; however, it does not explain it exclusively. For example, in the case of the secondary structure changes, the amount of added -OH groups may only play a minor role compared to the change in water sorption behavior.

Modifying a biopolymer ultimately requires consideration of multiple plasticizers targeting primary and secondary plasticization. For proteins such as Novatein, this is further complicated by the presence of water. Water seems to be a precondition for a homogeneously behaving polymer network, and the dominant factor for determining mechanical properties. Using polyols and water as plasticizers, their combined primary and secondary plasticization effects are responsible for changes in material properties.

CONCLUSIONS

Plasticization is better understood when the concept of EMC is extended in terms of the POE, and if it is recognized that

plasticisation mechanisms are significantly different above and below the POE. The free volume theory was considered more applicable above the POE where water has saturated the polymer network, enabling more homogenized plasticization. The constraint theory, instead, was more applicable below the POE, because chain flexibility, at low water content, was more dependent on the hydrogen bonding environment rather than free volume.

The POE was dependent on RH%, implying a competitive hydrogen bonding environment between water and plasticizer. The interaction between the polymer and plasticizer defined the plasticization mechanism; TEG was able to modify and interact with the protein efficiently, whereas GLY was rather loosely bound. Due to phase separation, both can sufficiently plasticize the polymer network above the POE, but GLY was more sensitive to RH% because of its less efficient protein network plasticization and undesired effect on the secondary structure.

The EMC was found to be a dominant factor determining mechanical properties, and the brittle to ductile transformation occurred at 8% EMC. Due to the aggregated nature of Novatein, the presence of water was a precondition for successful plasticization. However, water and a plasticizer were required to provide a ductile polymer. Phase separation occurred at all RH, but the presence of water led to a more homogeneous distribution.

ACKNOWLEDGMENTS

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5

The role of phase separation in determining the glass transition behaviour of thermally aggregated protein-based thermoplastics

A paper submitted in

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By

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The role of phase separation in determining the glass transition behaviour of thermally aggregated protein-based thermoplastics

The Chapter 5 continues the investigation of ternary system plasticization with the phase behaviour of particular interest. The objective was to relate phase behaviour to free volume and constraint based theories for glass transition temperature prediction. This required an understanding of the role of phase separation as a part of total plasticization, unifying the theories presented in previous chapters.

In regards to main thesis objectives, the focus is to understand how chain relaxation and thermal transitions, relevant to extrusion, are affected by plasticization.

As first author of this paper, I prepared the initial draft manuscript, which was refined and edited in consultation with my supervisor, who has been credited as co-author.

Full Paper

The role of phase separation in determining the glass transition behaviour of thermally aggregated protein-based thermoplastics

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ABSTRACT

The glass transition behaviour of a highly aggregated protein-based material (Novatein), plasticized with tri(ethylene glycol) (TEG) and glycerol (GLY), was studied using dynamic mechanical analysis with the aim to investigate the interactive effect of water and polyol on phase separation and how this is used to predict the glass transition temperature (T_g). Understanding the thermo-mechanical properties is important for processing which requires the material's softening point to be lower than the processing and thermal degradation temperatures. Novatein showed a very broad thermal transition, and phase separation was linked to the occurrence of multiple glass transitions. The T_g for each phase varied linearly with the amount of added hydrogen bonding sites, with different slopes depending on primary or secondary plasticization, which occurred below or above the point of equivalence (POE) respectively. With GLY, the intermediate phase formed above the POE, with a similar slope to the plasticizer-rich phase. With TEG instead, the intermediate phase was below the POE, interacting strongly with the protein fraction. In practice, this meant that at the highest plasticization levels, the polyol-rich phase dominated the material properties. For the material as a whole, a single T_g cannot be found from experimental data, however, it is the most accurate way of describing the state of heterogeneous polymer system and can be determined using the Couchman-Karasz model.

Keywords: phase separation; extrusion; thermoplastics; sorption isotherm; proteins

1 Introduction

Thermo-mechanical processing of proteins requires their softening point to be lower than processing and thermal degradation temperatures.^{1,2} Some proteins are like semi-crystalline polymers, and their softening point is related to their glass transition temperature (T_g), however, the crystalline regions typically do not melt during processing.² Novatein is a thermoplastic material made from blood meal and can only be extruded or injection moulded if sufficiently plasticized. It was shown previously that Novatein plasticization occurred as primary and secondary plasticization.^{3,4} In primary plasticization, the plasticizer replaced the protein's hydrogen bonded water with plasticizer. This decreases the equilibrium moisture content (EMC) of the material in comparison a material without a plasticizer.³⁻⁶ In secondary plasticization, the polymer network is saturated, and water will also hydrogen bond with the plasticizer, increasing the EMC.

The glass transition of protein-plasticizer-water systems typically occurs over a broad temperature range and recent studies are in good agreement that multiple glass transitions in biopolymers can be linked to phase separation into polymer and plasticizer rich phases.^{2,4,7-9} Novatein is no exception and phase separation was demonstrated in previous work using synchrotron-FTIR spatial mapping.³ The plasticizer's tendency to phase separate was quantified using the point of equivalence (POE), which is the point where the equilibrated moisture content (EMC) equals the EMC of the same polymer in the absence of a plasticizer.^{3,4} Tri(ethylene glycol) (TEG) and glycerol (GLY) formed clusters in the polymer network below the POE, whereas they were well distributed thorough the polymer network in micro-separated regions above the POE. This approach effectively explained plasticization of Novatein when the volumetric density of hydrogen bonding sites is normalized to the POE at different moisture contents. However, the effect will be dependent on the polymer and plasticizer, as the POE has been found to be higher for glycerol plasticized starch.^{5,8}

The aim of this study is to investigate the interactive effect of water and polyol plasticizers on the phase separation of a thermally aggregated protein network and how this is used to predict the T_g above and below the POE. The Couchman-Karasz model (CK) is one of the most used free volume theory-based models, however, van der Sman pointed some limitations at low moisture content.⁹ In contrast, the

constraint theory is based on volumetric hydrogen bonding density and Djabourov et al. presented a unified phase diagram of gelatin films using this approach.⁷ The model was later confirmed by van der Sman for various other biopolymers.⁹ In this study, dynamic mechanical analysis (DMA) was used to characterize the phase behaviour of Novatein conditioned to different moisture contents, plasticized with TEG and GLY. Using the POE offers a unique approach to explain phase separation and plasticization in terms of models based on the free volume and constraint theories.

2 Method

2.1 Materials

Blood meal was obtained in powder form from Wallace Corporation, Hamilton New Zealand and sieved to 700 μm . Technical grade sodium dodecyl sulphate (SDS) was obtained from Biolab NZ and analytical grade sodium sulphite from BDH Lab supplies. Plasticisers were obtained from Merck Millipore New Zealand.

2.2 Sample Preparation

Novatein has been developed and patented by Aduro Biopolymers, New Zealand.¹⁰ Thermoplasticity is achieved by blending 100 parts by mass blood meal with 3 pph_{BM} sodium dodecyl sulphate (SDS) and 3 pph_{BM} sodium sulphite (SS), water and five different levels of two different plasticisers including a composition without plasticizer (Table 1). All compositions were prepared in a high-speed mixer by blending water with the dry ingredients, after which the plasticiser was added. The water content had to be varied to allow extrusion, but the effect of water when processing was assumed to be minor considering the samples were conditioned to their equilibrium moisture content before testing, which is driven by the protein network. The compositions and plasticizers were selected based on the results of the previously work to allow for the maximum difference in primary and secondary plasticization.⁴ The term ‘plasticizer’ refers to the poyols used, not water which can be defined as a plasticizer as well. Novatein was prepared using a Labtech co-rotating twin-screw extruder at a screw speed of 200 rpm. The temperature profile increased over 11 barrel sections; from 100 °C at the feed throat to 120 °C at the die. The L/D ratio of the extruder was 44 with a single 10 mm circular die. The mass flow rate of all compositions was 100±20 g min⁻¹ and the pressure varied from 20 to 70 bars, depending on the composition. Die pressure and torque varied depending on the level of plasticization in a way that higher plasticization led to lower torque and pressure, as expected. The thermo-mechanical effect for all the compositions was not equal, and for example, structural changes caused by the difference in specific mechanical energy (SME) was considered as a characteristic property of certain plasticiser and

plasticiser level. The experiment was designed in a way that all the compositions could be processed and the residence time in the extruder would be similar.

The extruded material was granulated using a tri-blade granulator from Castin Machinery Manufacturer Ltd., China. Specimens for the dynamic mechanical analysis and tensile testing were produced in a BOY 35A injection moulding machine, according to ASTM D638. The mould temperature, and the temperature profile of the barrel had to be altered between compositions to maintain an injection pressure of 1000 ± 200 bar. Cooling time and temperature varied based on composition in a way that more plasticiser required lower mould temperature and longer cooling time.

Injection moulded specimens were conditioned at $23\text{ }^{\circ}\text{C}$ for 14 days in containers with saturated salt solutions to achieve different relative humidity (%RH) conditions. Saturated salt solutions were prepared according to ASTM E104 using P_2O_5 , NaBr and NaCl (0 RH%, 58 RH% and 75 RH%). In addition, a separate set of samples were conditioned at 50 RH% in a Binder incubator (Germany) for 14 days. Final moisture content of all samples was determined by oven drying at $60\text{ }^{\circ}\text{C}$ for a seven-day period, to avoid evaporation of plasticizer during drying.

2.3 Analysis

Dynamic mechanical thermal analysis (DMA) was performed with a Perkin-Elmer dynamic mechanical analyser (DMA 8000). A temperature sweep was performed for samples with dimensions of $3.5 \times 6.5 \times 30\text{ mm}^3$ at a rate of $2\text{ }^{\circ}\text{C min}^{-1}$ from $-80\text{ }^{\circ}\text{C}$ to $160\text{ }^{\circ}\text{C}$, at 0.1, 0.3, 1, 3, 10 and 30 Hz and a dynamic displacement of 0.05 mm in a single cantilever bending.

The point of equivalence (POE) has been calculated based on previous work, using the sorption isotherms to determine the plasticizer content at which the moisture content becomes equal to that with no plasticizer.^{3,4} This was correlated to the theoretical hydrogen bonding sites of the plasticizer where the number of theoretical hydrogen bonds ($N_{\text{H-b}}$) is calculated according to the plasticizer's chemical structure where the oxygen atoms are expected to form two hydrogen bonds as acceptors and an additional hydrogen bond as donor (for each hydroxyl or ether group) (Equation 1). $\Delta N_{\text{H-b}}$ represents the number of hydrogen bonding groups when $N_{\text{H-b}}$ is normalised to the POE at a particular RH% environment (Equation 2).

$$N_{H-b} = \frac{x_{polyol} m_{TOT}}{M_{polyol}} (3N_{OH} + 2N_{ether}) \quad (1)$$

$$\Delta N_{H-b} = N_{H-b}^{polyol} - N_{H-b}^{POE_{polyol}} \quad (2)$$

In Equation 1, x represents the mass fraction polyol, m_{TOT} is the mass, M_i is the molar mass and N_i the number of OH or ether groups per plasticizer molecule. Theoretical hydrogen bonding considers acceptor and donor sites, in other words, 3 hydrogen bonds per OH-group and 2 for ether groups.¹¹

3 Results

Novatein samples were conditioned at 4 different levels of relative humidity and equilibrated to a moisture content accordingly. As expected higher RH% resulted in higher EMC% and Novatein's behaviour was extensively analysed in previous work.^{3,4}

3.1 The Storage Modulus

Samples with and without polyol plasticizer were analysed after conditioning to investigate the effect of water and plasticizer on the storage modulus, which when using DMA represent the resistance to reversible deformation as a function of temperature. The storage modulus of samples plasticized with TEG and GLY were relatively similar, generally displaying four regions; a glassy region at low temperature, a small transition region and a significant rubbery transition region ultimately ending in the rubbery plateau. To understand the role of polyol and water, the effect of moisture was compared for conditioned samples with and without polyol (**Figure 1**).

In the absence of a plasticizer and at 50 RH% (Figure 1A), the protein network is only slightly hydrated, with a corresponding decrease in the onset of the transition region (over the sample at 0 RH%) and a formation of a rubbery plateau. The glassy modulus was mostly unaffected by moisture content at this stage, despite the drastic change in T_g . This is presumably due to water being diffused in through the polymer network and strongly bound. Increasing the moisture content (75 RH%), a second small transition formed, presumably due to water saturating the protein network, resulting in the formation of a second phase.

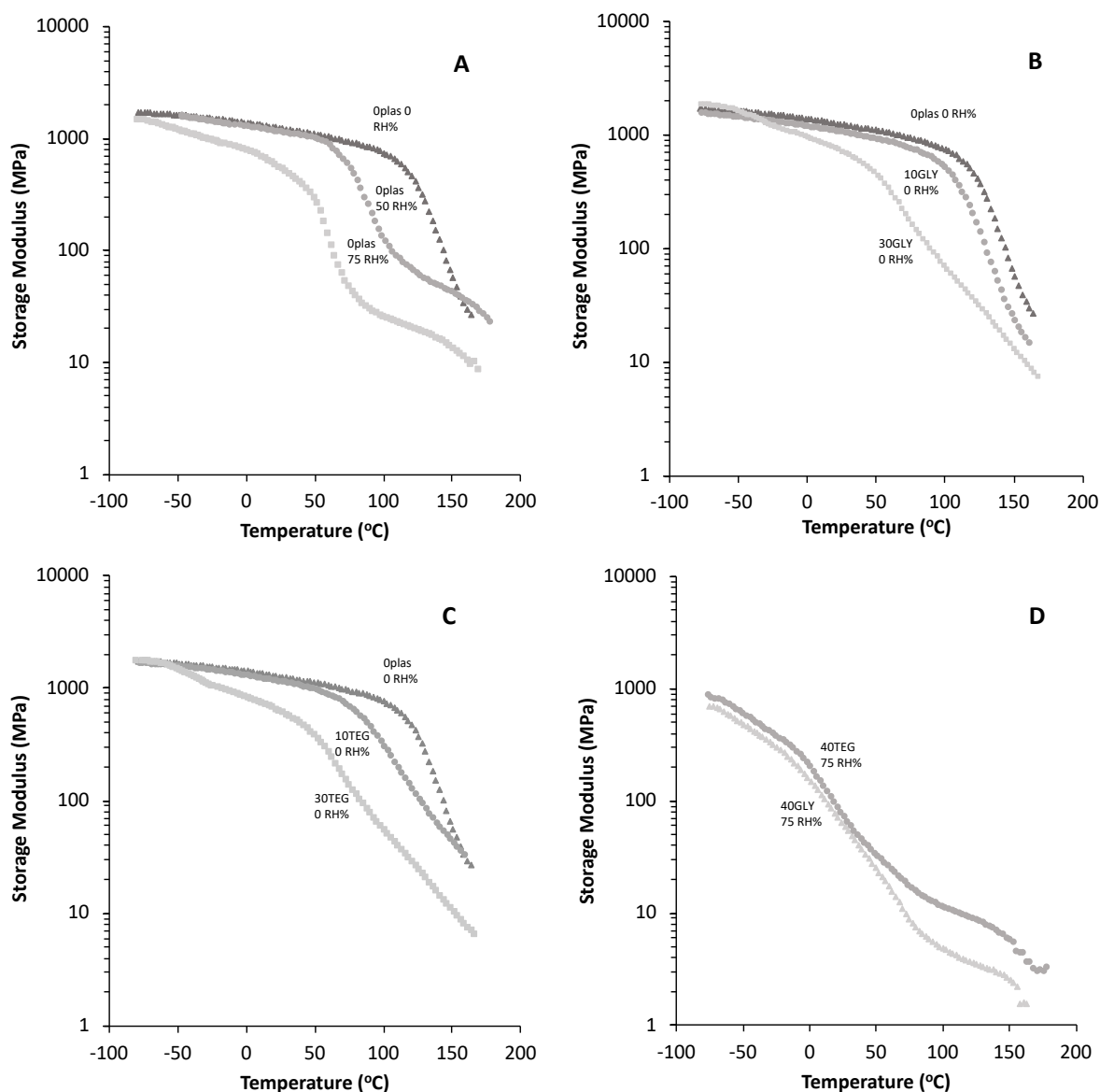


Figure 1. The storage modulus of Novatein plasticized with (A) no polyol, at 0, 50 and 75 RH% (B) 0, 10 and 30 pph_{BM} GLY, conditioned at 0 RH% (C) 0, 10 and 30 pph_{BM} TEG, conditioned at 0 RH% (D) 40 pph_{BM} GLY and TEG conditioned at 75 RH%.

For polyol plasticized samples conditioned at 0 RH%, the same effect was observed, but the effect was less drastic than for changes in EMC%. At 10 pph_{BM}, only a small change in the onset of the transition region was observed, with TEG having the largest effect. This agrees with recent results suggesting stronger interaction between TEG and Novatein whereby the secondary structure of the protein network changed beneficially compared to glycerol which aggregated the polymer network further.³ At high polyol content, the second minor transition region was also observed, but was overshadowed by the

very broad rubbery transition starting above 0 °C. The combined effect of water exaggerated these effects and led a drastic reduction in the transition temperature over a broader range.

The combined effect of water and polyol was further studied by evaluating the storage modulus in the glassy and rubbery regions (-68°C and 150°C respectively, **Figure 2**). For the rubbery modulus, values for the dry samples were excluded as they never reached a rubbery plateau. In the glassy region, TEG and GLY had very similar trends and the four moisture content isotherms showed that the modulus had a much greater dependency on moisture content than on polyol content. The effect of water dominated the rubbery modulus when using either GLY or TEG as plasticizer, while TEG content played almost no role in the glassy modulus.

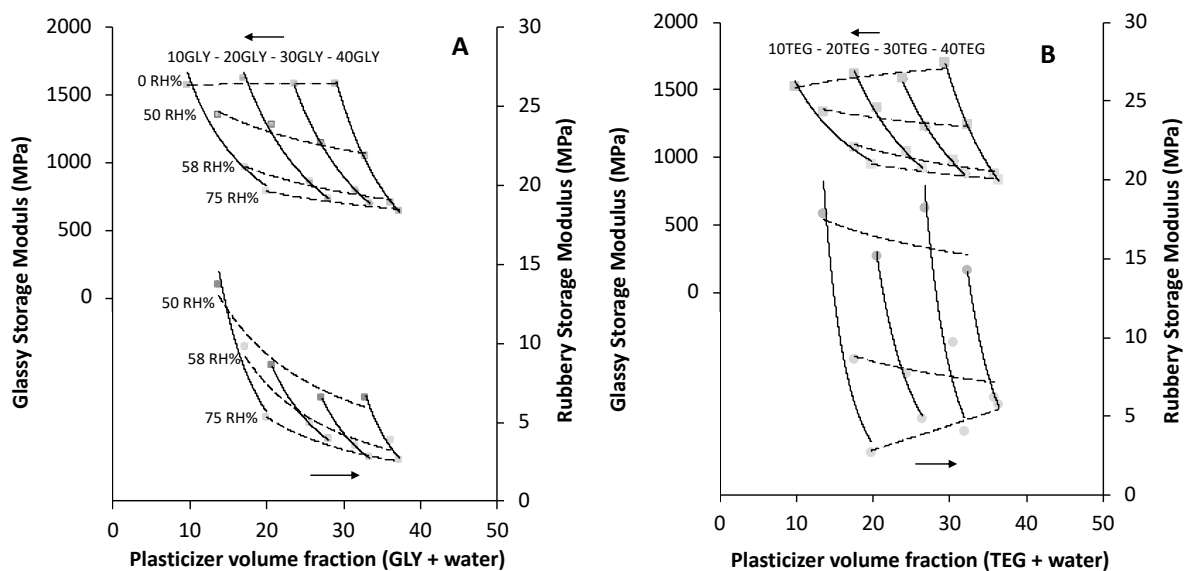


Figure 2. Glassy (upper) and rubbery (lower) storage modulus of different formulations after conditioning as a function of total plasticizer content (polyol plus water). Solid lines represent constant plasticizer content, while dashed lines represent lines of constant relative humidity (0 RH% not shown for the glassy modulus).

The combined effect of water and polyol of the glassy and rubbery modulus is further exposed considering the point of equivalence (**Figure 3**). Relative to the POE ($\Delta N_{H-b} = 0$), the glassy modulus divided into two clusters above and below the POE. Below the POE the glassy modulus was higher and dropped rapidly to the lower values above the POE. For the rubbery modulus, there was a drastic change at the POE, as previously observed using other measurements.³ The polyol type did not have an effect

either of these properties. However, plasticization in protein systems should be considered as a combined effect of water and plasticizer, driven by phase separation and changes in protein secondary structure and strongly depends on the POE. DMA revealed the formation of more than one phase, and above the POE water played an important role in making the plasticizer-rich regions smaller and well dispersed in the polymer network.⁴

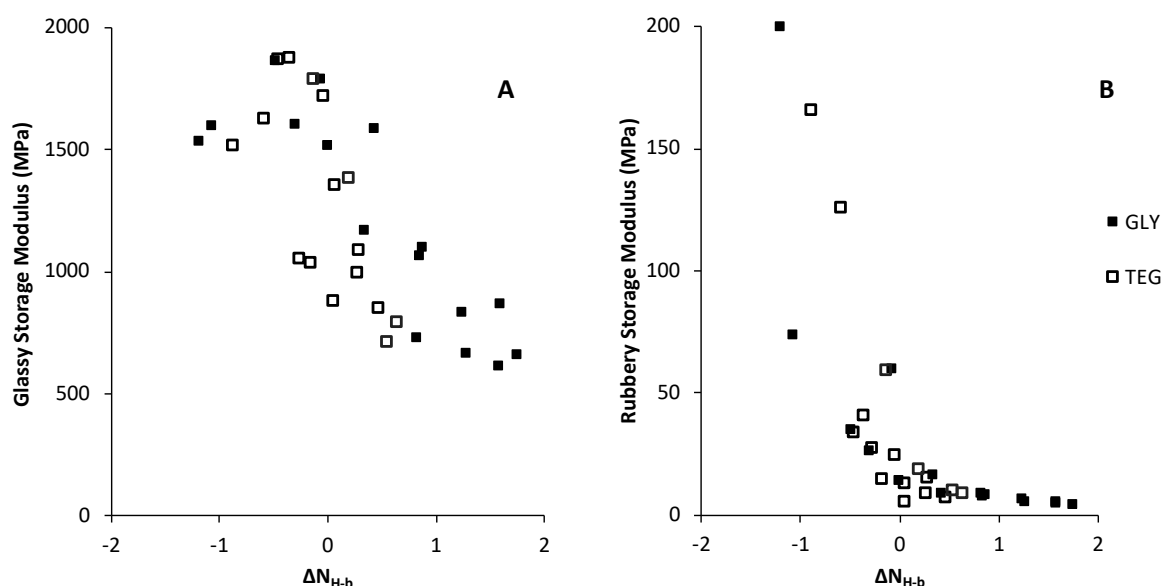


Figure 3. Changes in (A) glassy and (B) rubbery modulus as a function of theoretical hydrogen bonding sites relative to the POE ($\Delta N_{H-b} = 0$).

3.2 Loss Modulus

The loss modulus presents material's energy dissipation by molecular rearrangements and could be useful in assessing phase separation in ternary systems. In general, classical plasticization was observed, with higher energy dissipation at low temperature and a significant drop after the glass transition temperature (**Figure 4**). For both plasticizers, more than one thermal transition was observed depending on the total amount of plasticizer. The behaviour of TEG and GLY were similar, only differing in the magnitude and temperature where the transitions occurred.

The relative effect of water and polyol on the position and magnitude of these transitions were evaluated considering the conditioned loss modulus with and without polyol (Figure 4). An increase in water content expectedly shifted the transitions towards lower temperatures (Figure 4A). At low water content (or low RH%) it sharpened the transition region, eventually becoming almost indistinguishable at high

moisture content. In other words, as the protein becomes solvated, chain motion is activated leading to increased energy dissipation. As plasticization is increased (with water only) molecular friction would be almost negligible, leading to a low loss modulus.

For polyol plasticised samples in the absence of water (Figure 4B and C), the high transition temperature decreased with increasing plasticizer and so did the magnitude and agrees with what would be expected for any plasticised protein. However, a low temperature transition was also observed for highly plasticized samples, in agreement with the formation of a second phase. Molecular motion is restricted at low temperature and the observed energy dissipation is most likely that of a plasticiser rich fraction of the material.

When the total amount of plasticizer was increased a third transition was observed at low temperature (Figure 4D) and the combined effect of water and polyol has to be considered in terms of the POE. Below the POE little phase separation occurred and is also reflected in the relatively high magnitude of the loss modulus at high temperature. The intensity of the higher peak rapidly dropped with increasing total plasticizer content (water plus polyol) above the POE, suggesting that the polymer network was saturated with plasticizer. On the other hand, the intensity of the lower temperature peak increased dramatically. Increasing plasticizer content shifted the peak to lower temperatures to the extent that the temperature was below what was tested here and most likely signifies energy dissipation of shorter-range motions or that of smaller molecules, such as the plasticisers.

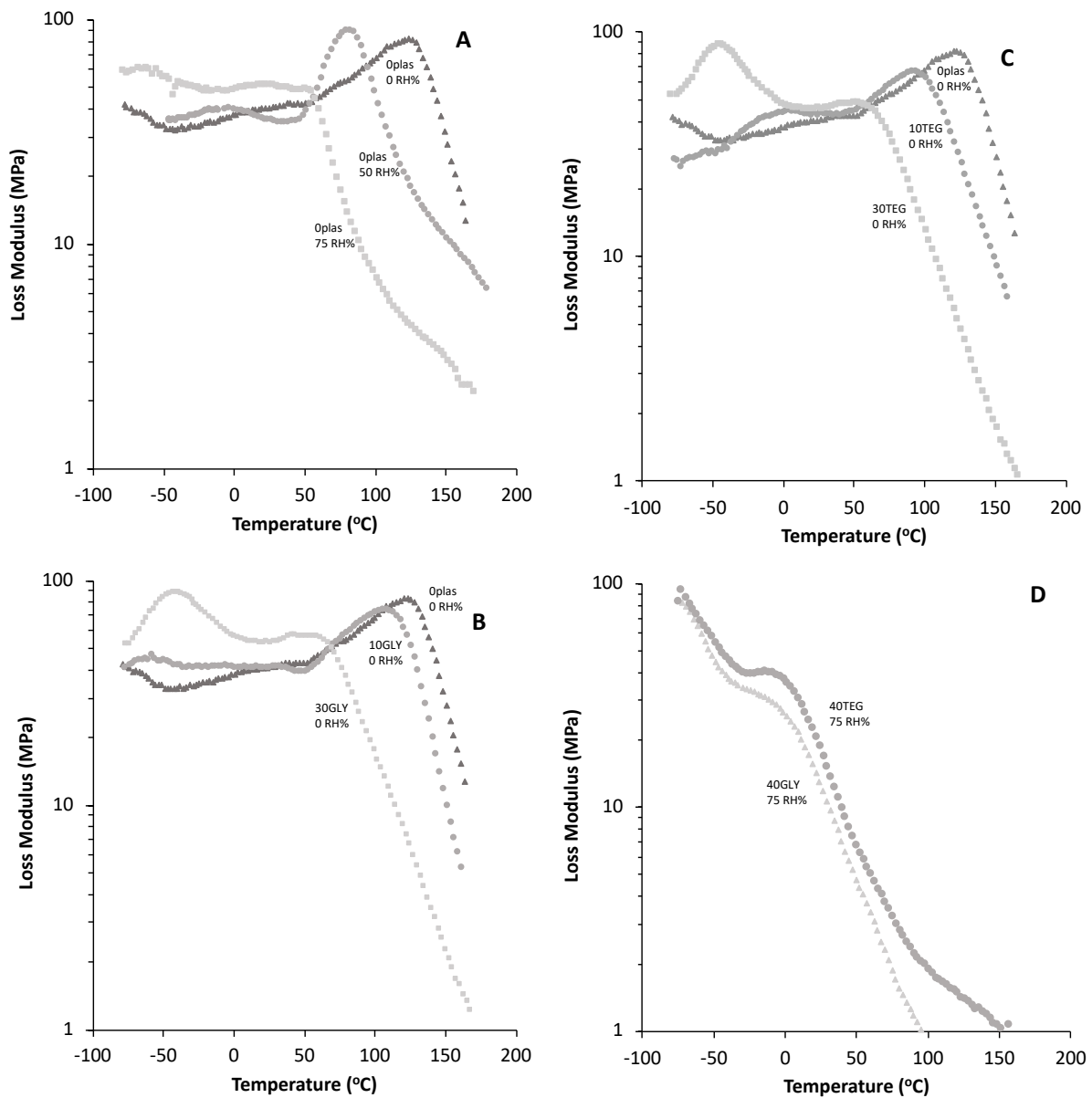


Figure 4. The loss modulus of Novatein plasticized with (A) no polyol, at 0, 50 and 75 RH% (B) 0, 10 and 30 pph_{BM} GLY, conditioned at 0 RH% (C) 0, 10 and 30 pph_{BM} TEG, conditioned at 0 RH% (D) 40 pph_{BM} GLY and TEG conditioned at 75 RH%.

3.3 The glass transition of multiple phases

Figure 5 presents $\tan \delta$ vs. temperature for the different samples measured at 1 Hz. For samples conditioned at 0 RH% or without plasticizer, a single transition was observed at high temperature. As expected, increasing the plasticizer content shifted the glass transition to lower temperatures and also led to the formation of two additional transitions. A clear difference between TEG and GLY was

observed, especially at the highest level of plasticization. In the case of GLY, two clear peaks and wider transition region was observed, whereas for TEG, the lower temperature was much more pronounced, with just a minor shoulder on the right-hand side, suggesting that TEG led to less pronounced phase separation.

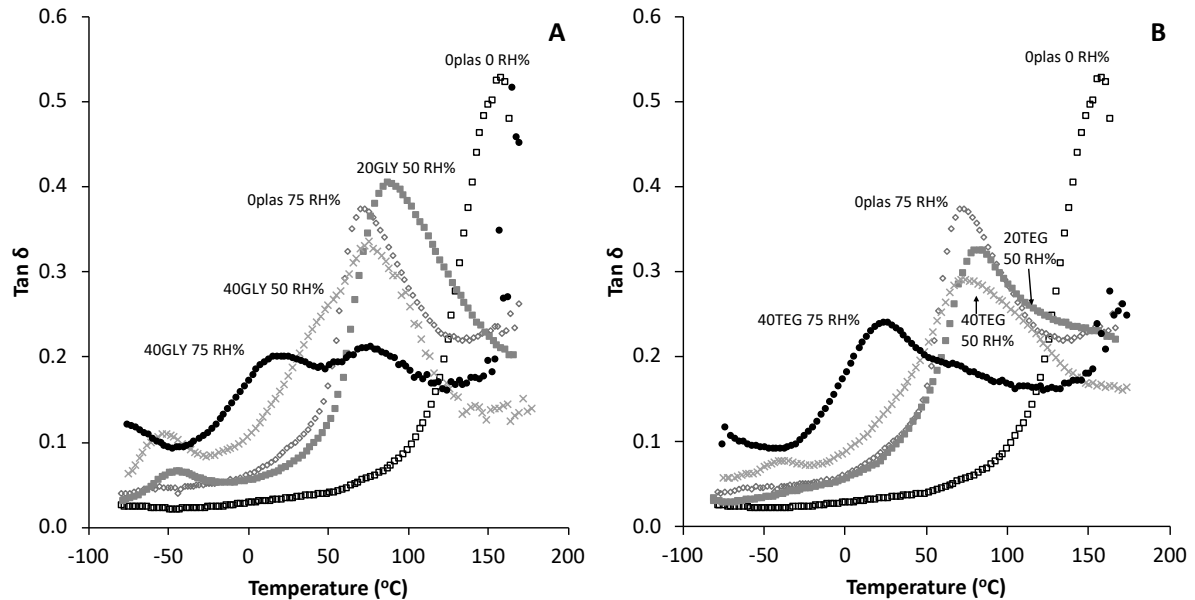


Figure 5. Tan δ for samples at low (no polyol at 0 and 75 RH%), medium (20, 40 pph_{BM} polyol at 50 RH%) and high levels (40 pph_{BM} polyol at 75 RH%) of total plasticizer (A) GLY and (B) TEG.

Similar to Duval et al's. work with wheat gluten, the formation of three different phases (polymer-rich, intermediate and plasticizer-rich phases) was observed due to changes in the level of total plasticization.⁸ The thermal transitions corresponding to the tan δ peaks were taken as the T_g of each phase. The coexistence of a protein-rich and protein-poor phase can be clearly observed from the 40GLY 75RH% composition. The lowest thermal transition was assumed to be polyol rich as the transition temperature corresponded closely to the T_g of the polyol itself. The formation of this phase was not observed for samples in the absence of a polyol. All the peaks exhibited a strong frequency dependence that deviated from Arrhenius behaviour, indicative of an α- rather than a β-transition (Appendix I and II).²

The glass transition temperatures for each phase (**Table 2**) was plotted against the volumetric hydrogen bonding density, $\left(R = \frac{x_w N_{OH}^w / M_w + x_p N_{OH}^p / M_p}{x_{BP} / M_{BP}}\right)$, as described by Djabourov's and van der Sman (**Figure 6**).^{3,7,9} R was calculated using the bulk composition, where x_i is mass fraction (water, w and plasticizer,

p) and x_{BP}/M_{BP} the moles of biopolymer (BP). Also shown in Figure 6 is the predicted T_g using bulk compositions and the Couchman-Karaszi equation (Equation 3), based on sorption isotherms presented in previous work.³

$$T_{CK} = \sum \frac{x_i \Delta C_{p,i} T_i}{x_i \Delta C_i} = \frac{x_{BP} \Delta C_{p,BP} T_{BP} + x_P \Delta C_P T_P + x_W \Delta C_W T_W}{x_{BP} \Delta C_{BP} + x_P \Delta C_P + x_W \Delta C_W} \quad (3)$$

ΔC_p for water and glycerol is $1.91 \text{ J g}^{-1} \text{ K}^{-1}$ and $0.88 \text{ J g}^{-1} \text{ K}^{-1}$ respectively, while a ΔC_p of $0.4 \text{ J g}^{-1} \text{ K}^{-1}$ was estimated for Novatein based on the value that have been used for various biopolymers.^{8,9} T_g for TEG and GLY was -95 and -93 °C respectively,¹² while the T_g for Novatein (191 °C) was based on the binary system calculations done with no polyol composition.

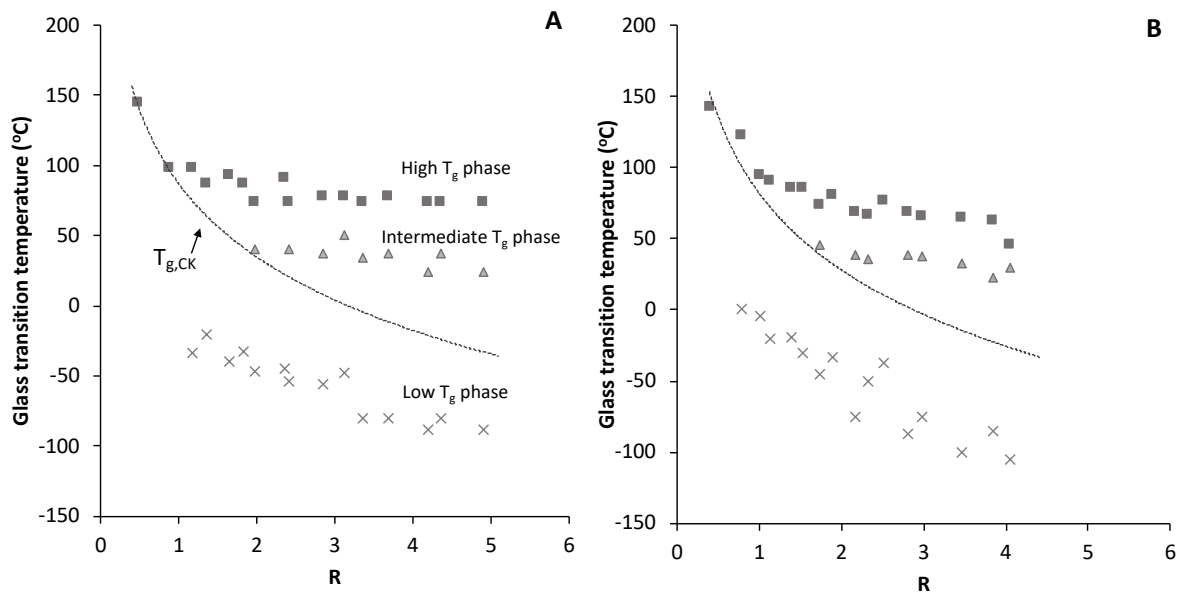


Figure 6. Glass transition temperature for each phase vs. volumetric hydrogen bonding density for (A) GLY and (B) TEG. Dashed lines represent the CK-model, assuming a single phase.

The results highlighted the problem applying the free volume theory to biopolymer plasticization. Plasticization is obvious, but equally obvious is the inability to plasticize the polymer network homogeneously, as plasticization led to simultaneous formation of up to three phases. The magnitude of $\tan \delta$ is conventionally related to plasticization efficiency (or the number of chains participating in the transition), but with biopolymers the magnitude often decreases.¹³ This is most probably a result of dilution due to phase separation.

Variation of the T_g for TEG and GLY were almost identical, however, GLY had a slightly higher total plasticization volume. Comparing Novatein to the behaviour of gelatin from Djabourov's data, the low T_g phase formed at much lower R-values.⁷ Also, maximum plasticization for gelatin was at $R = 18$, compared to 5 for Novatein, in line with the observation of a much lower saturation point for Novatein due to its very aggregated polymer network.⁴

Assuming that each separate phase is ideally mixed, the CK model can be used to estimate the composition for each phase where the EMC% can be estimated from sorption isotherms (Table 2).⁸ Both high- and intermediate- T_g phases were Novatein-rich, generally with a plasticizer content less than 25%. The low T_g phase, was polymer-poor, with relatively high water content. TEG's plasticization ability can be seen from the higher proportion of the intermediate phase, as well as a higher TEG content in the high T_g phase. The difference can be explained by TEG's compatibility with the protein network and also the difference in secondary structure changes for TEG and GLY. In previous work, TEG increased random structures, whereas GLY increased ordered structures.³ An increase in the random structures would reveal more potential hydrogen bonding sites from the aggregated protein fraction, leading to better plasticization in the amorphous protein network. The predicted T_g , based on the CK model were almost identical for TEG and GLY, however, it can never match with the data, as it does not account for phase separation.

Figure 7 presents the glass transition temperatures of all the phases, where the composition of each phase was used to calculate the change in theoretical hydrogen bonding sites (ΔN_{H-b}) relative to the POE.¹¹ Only the hydrogen bonding sites of the plasticizer (not water) was considered, with the assumption that they interact mostly with the protein during primary plasticization (below POE) or with water during secondary plasticization (above POE). The high and intermediate T_g phases clearly formed around and below the POE, whereas the polyol-rich fraction was above the POE. The constraint theory, which relates to hydrogen bonding of plasticizers, would be more applicable below the POE where a rapid decrease in T_g was observed with an increase in N_{H-b} . In contrast, the behaviour above the POE is explained well by the free volume theory, coinciding with phase separation. The effect of added hydrogen bonds gets diluted after the phase separation which can be seen from the different slope above the POE.

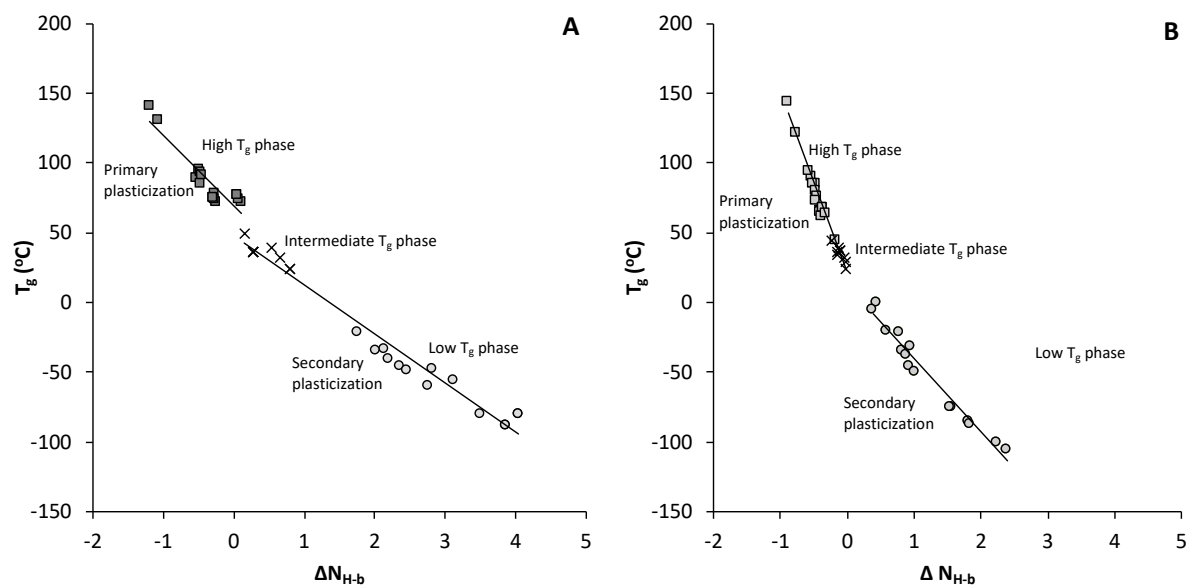


Figure 7. The glass transition temperature as a function of theoretical hydrogen bonding sites relative to the POE ($\Delta N_{H-b} = 0$) using the actual composition for each phase for specimens plasticized with (A) GLY and (B) TEG.

In Figure 7, the T_g for each phase for TEG and GLY samples formed a linear function of ΔN_{H-b} in which the slope is dependent on whether the plasticizer was acting as a primary or secondary plasticizer. With GLY, the intermediate phase formed above POE, with a similar slope to the plasticizer-rich phase. With TEG instead, the intermediate phase was below the POE, interacting strongly with the protein fraction. The result was also in agreement with the FTIR results presented in the previous study of authors.³ Less TEG was required to lower the T_g , suggesting TEG was more efficient as a plasticizer for Novatein. GLY plasticized samples had a much wider transition region in comparison to TEG (Figure 7), which has a negative implication for the material properties of Novatein; in addition to moisture sensitivity, the material will also be more temperature sensitive.

For the material as a whole, a single T_g cannot be found from experimental data, however, it is probably the most accurate way of describing the state of heterogeneous polymer system. In practice, this meant that at the highest plasticization levels, the polyol-rich phase dominated the material properties, also clearly visible from the loss modulus graphs. Using an average T_g for the material would therefore be

an illustrative tool to understand the effect of the combination of different phases, and can be determined using the CK model (Figure 6).

It can be concluded that the CK approach can be used for biopolymer systems if all the different phases are considered. In the same way, considering the T_g of the protein-rich fraction alone is not appropriate to understand the material properties. Highly aggregated polymers, such as Novatein can consist of up to 50% β -sheets which is strongly hydrogen bonded and does not melt nor can it be plasticized.² TEG and GLY have been found to have the opposite effect on secondary structure, which may explain why for GLY, the intermediate T_g phase was generally smaller compared to TEG. Especially in the case of Novatein, the role of a phase separated fraction may play a significant role in enabling thermo-mechanical processing, such as extrusion, or brittle to ductile transformation in terms of mechanical properties.⁴ On the other hand, the highly plasticized fraction has the potential to dominate the material properties. This also explains the requirement that, in the absence of a plasticizer, at least 70 pph_{BM} water is required to extrude Novatein, despite water alone can reduce the T_g of the protein-rich fraction below the processing temperature.

4 Conclusions

Plasticization in aggregated protein systems should be considered as a combined effect of water and plasticizer, complicated by phase separation and changes in protein secondary structure. Plasticization occurs as primary and secondary plasticization, depending on whether the plasticizer is interacting mostly with the protein or if its effect is diluted by the presence of excess water. The point of equivalence (POE) is based on the amount of added hydrogen bonding sites from the plasticizer and successfully differentiated between these plasticization mechanisms, explained by either the constraint or free volume theory.

For Novatein, three phases formed after plasticization; polymer-rich, plasticizer-rich and an intermediate phase. The Couchman-Karasz (CK) model was used to estimate the proportion and composition of each phase and it was concluded that using TEG generally resulted in a greater proportion of the intermediate phase, compared to GLY. If each phase is assumed to be ideally mixed, the CK model can be used to determine the T_g of each phase and a linear correlation was observed relative to the change in hydrogen bonding sites with different slopes above and below the POE.

For highly aggregated polymer systems, such as Novatein, the role of the intermediate phase can be considered crucial in terms of the total plasticization effect. Since a single T_g for the system does not exist, the CK model can be used to predict an average T_g which is a good indication of the overall material behaviour for Novatein.

5 Acknowledgements

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Declarations of interest: none

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7 Tables

Table 1. Formulations used prior to conditioning at four levels of relative humidity. Plasticizer and water content are based on 100 parts blood meal and all other additives were kept constant at 3 pph_{BM} sodium dodecyl sulphate (SDS) and 3 pph_{BM} sodium sulphite (SS).

Formulation	Plasticizer (pph _{BM})		Water (pph _{BM})
	TEG	GLY	
1	0	0	60
2	10		50
3	20		40
4	30		40
5	40		40
6		10	50
7		20	40
8		30	40
9		40	40

Table 2. Mass fraction protein, plasticizer and water in in the three different phases.

	RH (%)	Plasticizer (pph _{BMI})	High T _g phase (polymer-rich)				Intermediate T _g phase					Low T _g phase (plasticizer-rich)					
			Fraction	T _g (°C)	X _{BP}	X _{gly}	X _w	Fraction	T _g (°C)	X _{BP}	X _{gly}	X _w	Fraction	T _g (°C)	X _{BP}	X _{gly}	X _w
GLY	0	10	100%	144	95%	2%	2%	0%	-	-	-	-	0%	-	-	-	-
		20	100%	93	87%	9%	4%	0%	-	-	-	-	0%	-	-	-	-
		30	97%	87	86%	10%	5%	0%	-	-	-	-	3%	-21	56%	33%	12%
	50	40	85%	87	86%	10%	5%	0%	-	-	-	-	15%	-33	51%	36%	13%
		10	93%	98	90%	5%	5%	0%	-	-	-	-	7%	-34	53%	31%	16%
		20	82%	93	89%	6%	6%	0%	-	-	-	-	18%	-40	50%	33%	17%
	58	30	68%	91	88%	6%	6%	0%	-	-	-	-	32%	-45	48%	35%	17%
		40	44%	78	86%	8%	6%	18%	50	80%	12%	8%	39%	-48	47%	36%	18%
		10	58%	74	86%	6%	8%	29%	40	81%	10%	9%	13%	-47	49%	31%	20%
		20	38%	78	87%	6%	7%	34%	37	78%	12%	10%	28%	-56	45%	34%	21%
	75	30	34%	78	87%	6%	7%	34%	37	78%	12%	10%	31%	-80	31%	44%	26%
		40	41%	74	86%	6%	8%	18%	37	78%	12%	10%	41%	-80	31%	44%	26%
10		50%	74	88%	3%	9%	32%	33	79%	9%	12%	17%	-54	48%	29%	23%	
TEG	0	20	38%	74	88%	3%	9%	32%	40	81%	8%	11%	31%	-80	33%	38%	28%
		30	39%	74	88%	3%	9%	26%	24	77%	11%	13%	35%	-88	28%	42%	30%
		40	37%	74	88%	3%	9%	22%	24	77%	11%	13%	41%	-88	28%	42%	30%
	50	10	100%	144	95%	4%	2%	0%	-	-	-	-	0%	-	-	-	-
		20	87%	122	91%	6%	3%	0%	-	-	-	-	13%	0	65%	24%	11%
		30	88%	90.5	86%	9%	4%	0%	-	-	-	-	12%	-21	58%	29%	13%
	58	40	76%	85	85%	10%	5%	0%	-	-	-	-	24%	-31	54%	31%	15%
		10	89%	94	88%	8%	5%	0%	-	-	-	-	11%	-5	65%	22%	13%
20		79%	85.5	86%	9%	5%	0%	-	-	-	-	21%	-20	60%	25%	15%	
75	30	68%	80	85%	9%	6%	0%	-	-	-	-	32%	-34	55%	29%	17%	
	40	34%	76	85%	10%	6%	25%	40	77%	14%	9%	42%	-37.5	53%	30%	17%	
	10	54%	73	85%	8%	7%	30%	45	79%	12%	9%	16%	-45	51%	29%	20%	
	20	32%	66	84%	9%	7%	44%	35	77%	13%	10%	24%	-50	49%	30%	21%	
75	30	30%	65	83%	9%	7%	41%	37	78%	13%	10%	30%	-75	36%	39%	26%	
	40	22%	62	83%	10%	8%	43%	24	74%	15%	11%	35%	-85	29%	43%	28%	
	10	48%	68	85%	7%	8%	36%	38	79%	10%	11%	16%	-75	37%	35%	28%	
	20	32%	68	85%	7%	8%	45%	38	79%	10%	11%	23%	-87	30%	40%	31%	
	30	21%	64	85%	7%	8%	54%	32	78%	11%	11%	25%	-100	20%	45%	35%	
40	20%	45	81%	9%	10%	51%	29	77%	11%	12%	28%	-105	16%	48%	37%		

8 Figure legends

Figure 1. The storage modulus of Novatein plasticized with (A) no polyol, at 0, 50 and 75 RH% (B) 0, 10 and 30 pph_{BM} GLY, conditioned at 0 RH% (C) 0, 10 and 30 pph_{BM} TEG, conditioned at 0 RH% (D) 40 pph_{BM} GLY and TEG conditioned at 75 RH%.

Figure 2. Glassy (upper) and rubbery (lower) storage modulus of different formulations after conditioning as a function of total plasticizer content (polyol plus water). Solid lines represent constant plasticizer content, while dashed lines represent lines of constant relative humidity (0 RH% not shown for the glassy modulus).

Figure 3. Changes in (A) glassy and (B) rubbery modulus as a function of theoretical hydrogen bonding sites relative to the POE ($\Delta N_{H-b} = 0$).

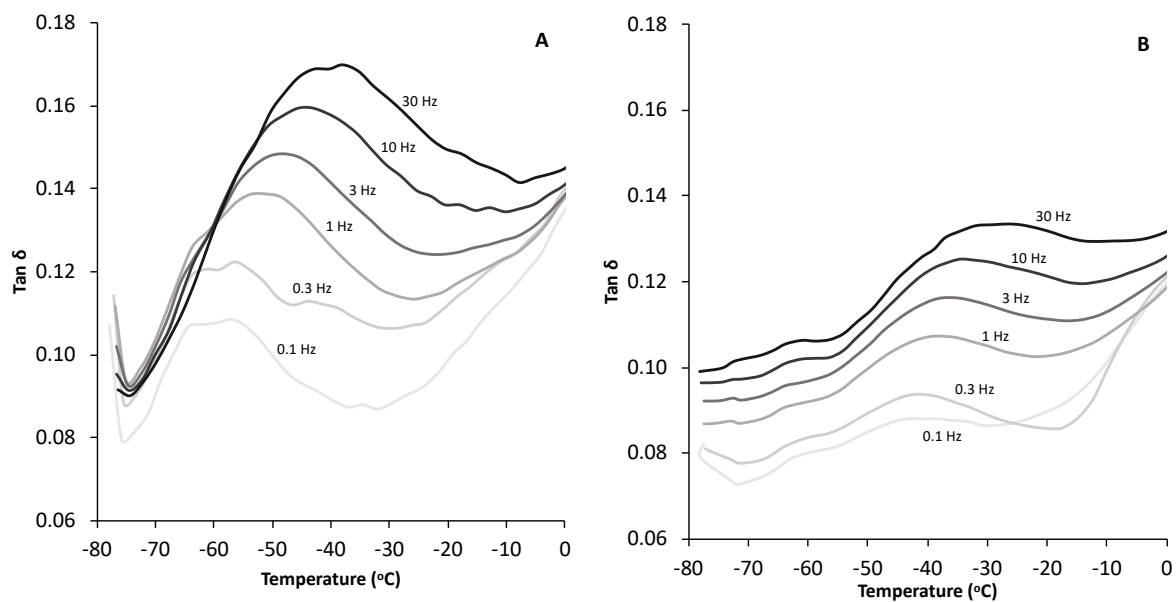
Figure 4. The loss modulus of Novatein plasticized with (A) no polyol, at 0, 50 and 75 RH% (B) 0, 10 and 30 pph_{BM} GLY, conditioned at 0 RH% (C) 0, 10 and 30 pph_{BM} TEG, conditioned at 0 RH% (D) 40 pph_{BM} GLY and TEG conditioned at 75 RH%.

Figure 5. Tan δ for samples at low (no polyol at 0 and 75 RH%), medium (20, 40 pph_{BM} polyol at 50 RH%) and high levels (40 pph_{BM} polyol at 75 RH%) of total plasticizer (A) GLY and (B) TEG.

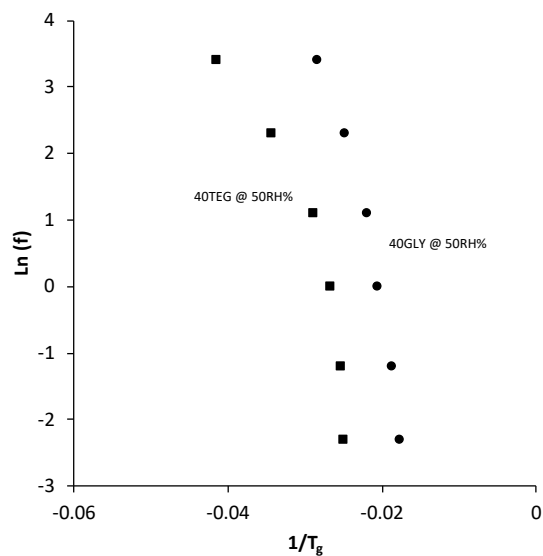
Figure 6. Glass transition temperature for each phase vs. volumetric hydrogen bonding density for (A) GLY and (B) TEG. Dashed lines represent the CK-model, assuming a single phase.

Figure 7. The glass transition temperature as a function of theoretical hydrogen bonding sites relative to the POE ($\Delta N_{H-b} = 0$) using the actual composition for each phase for specimens plasticized with (A) GLY and (B) TEG.

Supplementary information



Appendix I. Frequency dependency of the low T_g phase for 40GLY (A) and 40TEG (B) at 50RH% both with 0.1 offset.



Appendix II. Relaxation frequency-temperature dependency of the low T_g peak of 40TEG and 40GLY at 50RH% both indicating the alpha relaxation behavior.

6

The shear and extensional viscosity of a thermally aggregated protein-based thermoplastic polymer

A paper submitted in

Journal of Polymers for Advanced Technologies

By

Jussi M. Uitto, C.J.R Verbeek and Carlos Bengoechea

Shear and extensional viscosity of a thermally aggregated protein-based thermoplastic polymer

Chapter 6 investigates the rheological behaviour of Novatein with a focus on understanding shear and extensional viscosity. The aim is to apply the theories of plasticization from the previous chapters to manipulate rheology and understand why Novatein does not perform as well in comparison to conventional plastics.

With regards to main thesis objectives, the focus was to understand Novatein's rheology and relate it with conventional plastics, and modify it to improve sheet extrusion.

As first author of this paper, the PhD candidate conducted most experimental work under the guidance of his supervisor, and prepared the initial draft manuscript, which was refined and edited with consultation with the supervisor, who is credited as co-author. Experimental work was done with a support of visiting Professor C. Bengoechea who have been credited as co-author.

Full Paper

Shear and extensional viscosity of a thermally aggregated protein-based thermoplastic polymer

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ABSTRACT

Novatein is a thermoplastic produced from blood meal and is used in different agricultural applications. Novatein has some unique processing challenges and its rheology was studied using screw-driven capillary rheometry, with a particular focus on sheet extrusion. Ethylene glycol, glycerol, propylene glycol or triethylene glycol were used as plasticizers and the rheology was compared to polypropylene. The apparent shear viscosity of highly plasticized samples was similar to polypropylene, however, the processing behaviour was very different. This was mainly attributed to Novatein's secondary structure consisting of highly ordered regions that do not melt into a fully amorphous state during processing. Entrance pressure contributed up to 80% of the total pressure drop, but this was significantly reduced by plasticization or increased temperature. It was concluded that Novatein generally does not form fully developed flow based on an observed upward curvature in the Bagley plot. Polyol addition led to higher true shear viscosities in comparison to no polyol plasticization, most likely due to improved chain mobility resulting in orientation effects. Elongational flow was dominated by primary plasticization of the protein-rich phase and changes in secondary structure whereas secondary plasticization (phase separation into a polyol-rich phase) played a significant role in the reduction of the shear viscosity. Of the selected plasticizers, propylene glycol showed the most efficient plasticization in both shear and elongational flow. When combined with the beneficial secondary structural changes brought about by triethylene glycol, the sheet forming ability of Novatein was drastically improved.

1 Introduction

Products from protein-based bioplastics can be manufactured via thermo-mechanical processing techniques such as extrusion, injection molding and even blow molding [1-5]. Novatein is one such material and is manufactured using blood meal, a by-product of the meat processing exceptionally high in protein [6]. Blood meal is converted to a thermoplastic polymer via compounding with a variety of additives and plasticizers at relatively low temperature [6].

Like many conventional polymers, protein-based thermoplastics have a semi-crystalline structure, however, their behaviour during processing differs significantly. The complex structure of proteins is sensitive to structural changes such as denaturation, however, it does not necessarily imply complete unfolding into a fully amorphous material as true melting would suggest [7]. For example, ordered structures, such β -sheets, has been shown to remain intact at standard processing temperatures [8]. Uncontrolled processing may even lead to a more aggregated polymer network and impede processability [3,7]. The processability of aggregated proteins could, in fact, be closer to filled polymers, consisting of nano-crystallite aggregates, rather than a semi-crystalline polymer that has reached a molten state.

Plasticization of the amorphous phase is almost always required as the T_g of proteins are generally above their degradation temperature [9,7]. Polyols are often used as plasticisers in biopolymers, but, in addition to their plasticization effect, modifies the phase composition and water sorption behaviour of biopolymers [10,11]. The phase separated fraction, in particular the polyol-rich fraction, plays a significant role in providing processability for highly aggregated polymer networks [12]. It has been shown that a polyol and water are required for micro-scale phase separation to be effective in mimicking ideally mixed conditions [13]. However, water evaporates at high temperatures, changing the rheological properties of the biopolymer, and some studies have concluded that water is not a suitable plasticizer for this reason [14].

There are some rheological studies on protein-based polymers, including the shear rate dependence of viscosity [15,14,16-22]. However, most studies focus on understanding apparent shear viscosity and

fail to convey the core difference between protein-based thermoplastics and conventional plastic materials. To the authors' knowledge, only three studies have considered elongational viscosity [16,3,14], which may be the dominating factor in some of the more challenging thermo-mechanical processes [23]. One of the key findings is a significantly higher Trouton's ratio which is dependant on plasticization, temperature and protein type [16].

Thermoplastic proteins' behavior may differ significantly based on their natural structure and processing history [3]. For example, Oliviero et al. tested zein with different processing histories and found a strong correlation between a high α -helix to β -sheets ratio and the success in blow molding when plasticized with PEG400 [3]. With Novatein, the polymer network is highly aggregated due to excessive heat treatment during blood meal production, leading to a low α -helix to β -sheets ratio [13]. Novatein contains almost 50 % β -sheets that do not melt nor can it be plasticized. Optimising the process parameters, plasticizers and additives play a significant role in its thermoplastic nature [24,25]. For example, TEG was able to interact with the polymer network improving flexibility and reducing ordered secondary structures, whereas glycerol aggregated the polymer network more [26,27].

In this study, an extruder-based, screw-driven capillary rheometer was used for characterising the rheology of Novatein, in particular investigating the unique behaviour of thermoplastic proteins, compared to conventional polymers. Due to the combination of high water content and processing temperatures above water's boiling point, a continuous characterisation method is required to provide a steady-state process for rheology characterisation. Shear and extensional viscosity were characterised at different temperatures, water and plasticizer content with a particular focus to understand processability and factors affecting it.

2 Method

2.1 Materials

Blood meal was obtained in powder form from Wallace Corporation, Waitoa, New Zealand and sieved to 700 μm . Technical grade sodium dodecyl sulphate (SDS) was obtained from Biolab NZ and analytical grade sodium sulphite from BDH Lab supplies. Plasticisers were obtained from Merck Millipore New Zealand.

2.2 Sample Preparation

Novatein has been developed and patented by Aduro Biopolymers, New Zealand. Thermoplasticity is achieved by blending 100 parts by mass blood meal (pph_{BM}) with 3 pph_{BM} sodium dodecyl sulphate (SDS) and sodium sulphite (SS), water and five different levels of plasticisers, including one without plasticizer. The compositions were selected based on the results of the previously published work [27]. Four different plasticizers were selected; ethylene glycol (EG), glycerol (GLY), propylene glycol (PG) and triethylene glycol (TEG). In this paper, the term ‘plasticizer’ refers to external additives used as a plasticizer, but not water which can be defined as a plasticizer as well.

Twelve different compositions were prepared by dissolving SS, SDS and the plasticizer in the appropriate amount of water. The Novatein granules were prepared using reactive extrusion by changing the ratio between blood meal and the chemical cocktail fed by a gravimetric feeder and a peristaltic pump. A water content of either 60 or 70 pph_{BM} was selected, with a plasticizer content changing between 0 – 40 pph_{BM} . For EG, GLY and PG only 10 and 30 pph_{BM} were used, representing the levels at which primary and secondary plasticization occurs, respectively. The compositions used are summarised in **Table 1**.

Table 1. Compositions used, based on 100 parts blood meal (pph_{BM}).

Name	Plasticizer type	Polyol content	Water content
			pph _{BM}
60W	-	-	60
70W	-	-	70
10TEG	Tri(ethylene glycol)	10	70
20TEG	Tri(ethylene glycol)	20	70
30TEG	Tri(ethylene glycol)	30	70
40TEG	Tri(ethylene glycol)	40	70
10GLY	Glycerol	10	70
30GLY	Glycerol	30	70
10EG	Ethylene glycol	10	70
30EG	Ethylene glycol	30	70
10PG	Propylene glycol	10	70
30PG	Propylene glycol	30	70

Samples were prepared using a Labtech co-rotating twin-screw extruder at a screw speed of 200 rpm. The temperature profile increased over 11 barrel sections from 100 °C at the feed throat to 120 °C at the die. The L/D ratio of the extruder was 44 with a single 10 mm circular die. The mass flow rate of blood meal was maintained at 100 g min⁻¹ with plasticizer content changing based on composition. Die pressure and torque varied depending on the level of plasticization in a way that higher plasticization led to lower torque and pressure, as expected. The thermo-mechanical effect for all the compositions was not equal, and for example, structural changes caused by the difference in specific mechanical energy (SME) was considered as a characteristic property of certain plasticiser and plasticiser level. The experiment was designed in a way that all the compositions could be processed and the residence time in the extruder would be similar. Extrudates were granulated in a way that they could be fed with the gravimetric feeder in actual rheology measurements.

2.3 Rheology measurements

The rheological tests were done with the same extruder used in the previous section, fitted with a custom made rheological insert installed at the die-end of the extruder. Three different length capillaries (50 mm, 37.5mm and 25 mm) and an orifice, all with a 2.88 mm diameter were used. Granules were gravimetrically fed and the pressure transducer was fitted close to capillary entry. Temperature was controlled using the heating elements of extruder and a separately controlled external heating element fitted around the capillaries.

For each test, the mass flow was varied between 20 g/min to 100 g/min, corresponding to an apparent shear rate between 125 to 628 s⁻¹, or when a pressure limit of 100 bar was reached. For samples without plasticizer and those containing TEG, data was collected using three capillaries and an orifice. For EG, GLY and PG, only the shortest capillary and the orifice were used. During the measurements pressure, mass flow rate, torque and temperature were collected for the data analysis.

Analysis

The collected data was processed to assess the true rheology of the materials. The most important parameters are capillary length (L), capillary radius (R), pressure drop (P) and volumetric flow rate (Q).

An apparent viscosity of a material can be defined by calculating the ratio between apparent shear stress

($\tau_a = \frac{\Delta P}{2(L/R)}$) and apparent shear rate ($\gamma_a = \frac{4Q_v}{\pi R^3}$)

$$\boldsymbol{\eta}_a = \frac{\boldsymbol{\tau}_a}{\boldsymbol{\gamma}_a} \quad (1)$$

However, for calculating the true shear stress on the wall the Bagley correction is needed [28]. In this method the approximated entrance effect (ΔP_e), using either orifice value of extrapolation of three different length capillaries, will be deducted from the total pressure drop from which the true shear stress (τ_w) can be calculated [29].

$$\boldsymbol{\tau}_w = \frac{\Delta P - \Delta P_e}{2L/R} \quad (2)$$

The Rabinowitsch correction is required to calculate the true shear rate, considering the shear thinning character of the polymer (Equation 4). The pseudo-plasticity index (n) of the power law equation is defined by the slope of the straight line of a plot of $\log(\tau_w)$ vs. $\log(\dot{\gamma}_w)$ in Equation 3. True viscosity values can be calculated when true shear stress and true shear rate are known.

$$\tau_w = K\dot{\gamma}^n \quad (3)$$

$$\dot{\gamma}_w = \frac{3n+1}{4n} \dot{\gamma}_a \quad (4)$$

Extensional viscosity is calculated using the Cogswell equation [30]. The extensional viscosity (Equation 7) can be calculated when extensional stress and extensional strain rate are calculated using Equation 5 and Equation 6 respectively.

$$\sigma_e = \frac{3(n+1)}{8} \Delta P_0 \quad (5)$$

$$\dot{\epsilon} = \frac{4}{3} \frac{\eta_a \dot{\gamma}_a^2}{(n+1)P_0} \quad (6)$$

$$\eta_e = \frac{\sigma_e}{\dot{\epsilon}} \quad (7)$$

2.4 Synchrotron FTIR

Spatially resolved FTIR experiments were undertaken on the infrared microspectroscopy beamline at the Australian Synchrotron, Victoria, Australia. Spectra were collected using a Bruker Hyperion 3000 with an MCT collector and XY stage using Opus 6.5 software (Bruker Optik GmbH 2009). For each sample, two visually representative spots of the microtomed sample were selected for measurement of 13 x 13 sized maps with a 5 x 5 μm spot size revealing the structural characteristics from two different 650 x 650 μm areas of the sample. For each point, 32 spectras were collected in transmission mode with a resolution of 4 cm^{-1} between 3900 and 700 cm^{-1} and averaged using Opus 6.5 software (Bruker

Optik GmbH 2009). The secondary structure characterisation was done and histograms built in accordance to the method presented earlier [13,27].

3 Results

3.1 Apparent viscosity

Processing Novatein presents unique challenges over conventional thermoplastics and as background, the apparent viscosity of three compositions (W60, W70 and 40TEG), extruded at 120°C, have been compared to PP (Fig. 1). As with PP, typical non-newtonian, shear thinning behavior was observed in addition to plasticization (water or TEG) that lowered the apparent shear viscosity. However, W60 was very difficult to process and required very high pressures in the extruder, evident from a significantly higher viscosity compared to PP. Increasing water only, was not effective to reduce the viscosity significantly and required the combined effect of water and TEG.

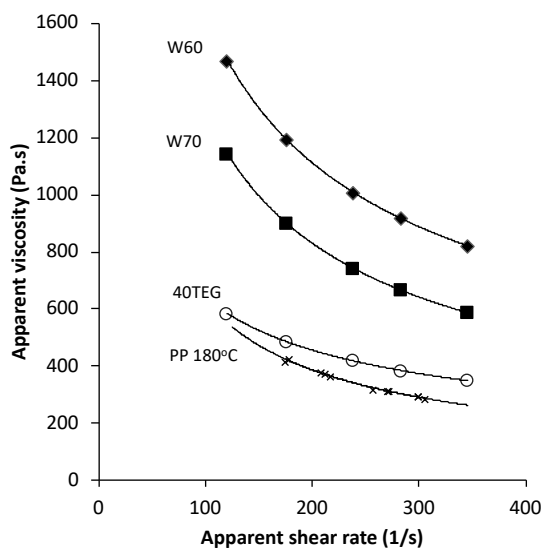


Figure 1. Apparent viscosity of Navatein at 120°C compared to PP at 180°C.

Even though the viscosity of the 40TEG sample was relatively close to PP, the behaviour during extrusion differs significantly. For example, attempts to produce sheets proved problematic (Fig. 2). For Novatein, pressure requirements were very high and the material flowed unevenly through the die, compared to PP (Fig. 2c). The reason for this behaviour is two-fold; extended residence time may lead

to excessive aggregation and crosslinking of the protein network [9] or the rheological nature of Novatein itself, similar to other proteins [31,14,32].

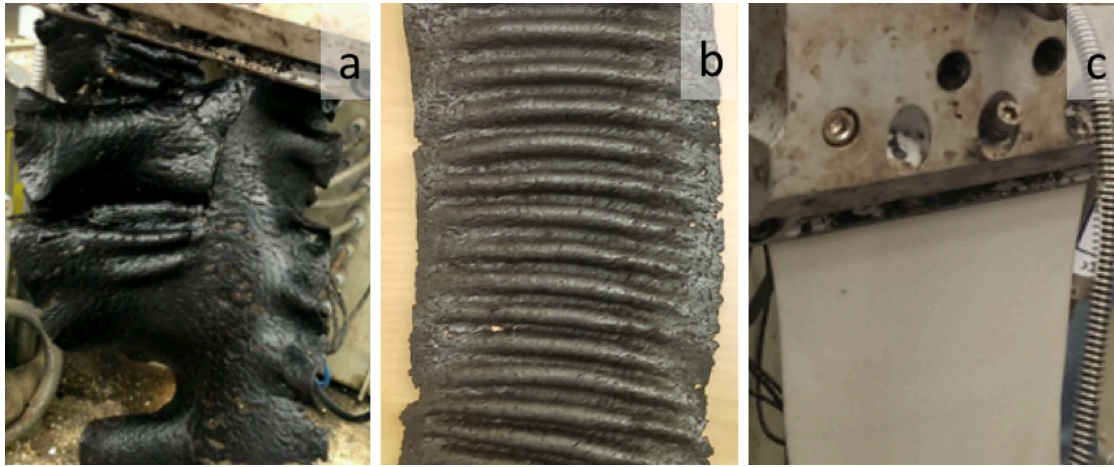


Figure 2. Sheet extrusion attempts using 40TEG at 120 °C (a and b) and PP at 180 °C (c).

One of the reasons for the unique rheology of Novatein lies in the secondary structure of proteins [3].

Fig. 3 a and f represents the secondary structure distribution of the no-polyol, and polyol plasticized samples. α -helices and β -sheets do not melt during processing, meaning that protein chains do not unfold into a fully amorphous state which furthermore limits extensional properties, as required during sheet extrusion. The average ratio of α -helix to β -sheet content was between 0.4:1 (no-polyol) to 0.6:1 (30TEG) whereas other studies on zein protein blow-moulding concluded that a 4:1 ratio was required [3]. No polyol and 10pph_{BM} samples of all the plasticizers showed a similar distribution, whereas 30pph_{BM} made a drastic difference, according to results presented previously [13]. The histograms also represent the spatial distribution of these structures, with TEG addition leading to a slightly narrower distribution (the spatial variation of α -helices and random structures are shown as inserts). Random structures (or amorphous regions), not reported in Oliviero et al, plays a significant role in plasticization of thermoplastic proteins [9,12].

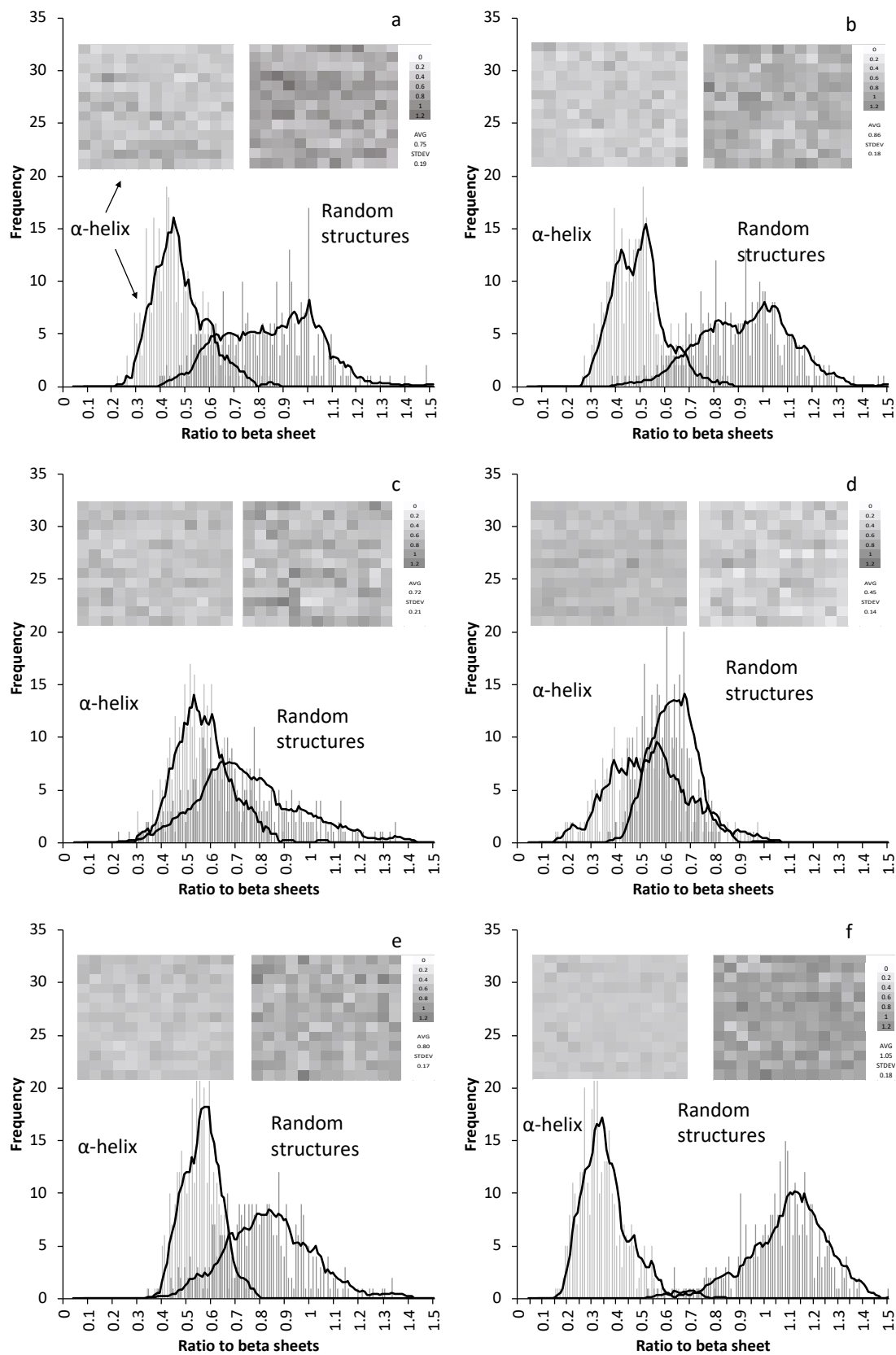


Figure 3. Histograms of secondary structure ratios of no-polyol (a), 10TEG (b), 30EG (c), 30GLY (d), 30PG (e) and 30TEG (f) with spatial maps as inserts.

Because ordered structures do not melt, the mechanism of consolidation is different to conventional thermoplastic materials. For example, a 20 – 30 bar pressure peak occurred before consolidation led to flow. Consolidation could be described as a sintering-like phenomenon in which material has to be in a state at which random structures could fuse together. This process is composition-dependant and significant pressure is required for the polymer chains to diffuse and entangle in a way that the material can flow.

3.2 Entrance pressure and total pressure drop

It is well known that the apparent shear stress needs to be corrected for entrance effects, as a portion of the pressure required for flow is from the pressure it takes for the material to enter the capillary (P_e). P_e is normally estimated by either the Bagley correction or using a zero-length capillary (orifice) [28,29]. The results showed the expected trend; longer capillaries and higher shear rates required higher pressures and was the same for all the compositions (Fig. 4).

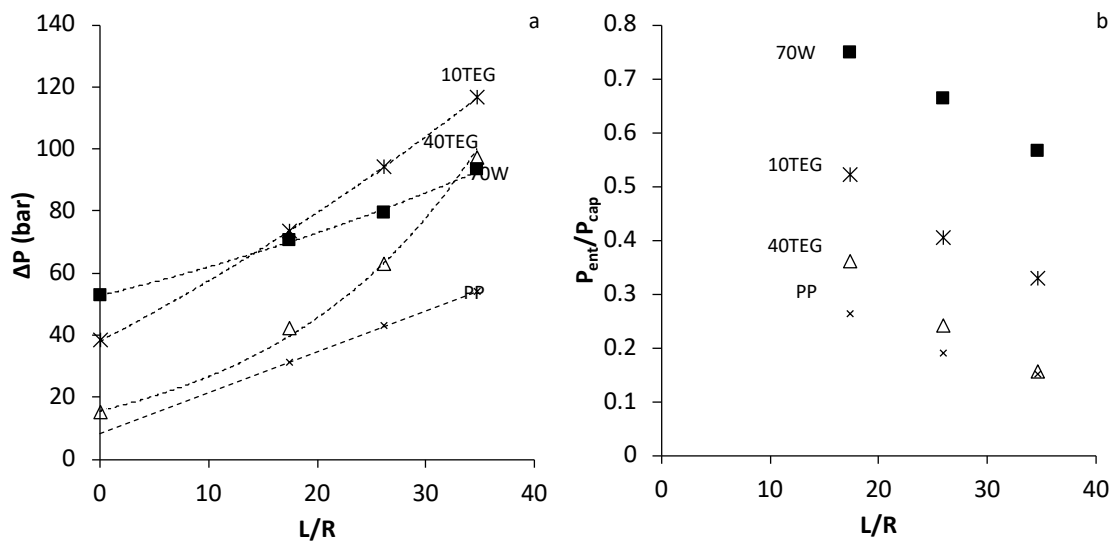


Figure 4. Bagley plots at a shear rate of 345 s^{-1} for three different Novatein composition and PP (a) and the ratio between of entrance to capillary pressure for the same compositions (b).

Linear extrapolation of pressure drop vs. L/R corresponded reasonably well with the orifice data ($R^2 > 0.96$) for only the 70W sample. For all the other formulations, linear extrapolation would have resulted in a negative entrance pressure. However, the data is well modelled using an exponential trendline (Fig. 4A). The effect of plasticizer is two-fold; increasing TEG led to a reduction in apparent viscosity as

well as an increase in the degree non-linearity (upward curvature). Nonlinear behaviour is often linked to pressure dependent viscosity, orientation effects (upwards curvature) or wall slip (downwards curvature) [33,34]. An upward curvature meant that polyol plasticization led to higher pressure drops for the longest capillary, but at the same time the $P_{ent}:P_{cap}$ -ratio was reduced (**Fig. 4B**). Chain orientation is expected due to the elongational flow at the capillary entrance with orientation diminishing along the length of the capillary, leading to an unexpectedly long flow-development region [35]. The elongational viscosity of Novatein will dominate in this case, and is explored further in later sections.

For Novatein, consolidation is known to be pressure dependent and would suggest that the material is better consolidated at higher pressure leading to a increase in viscosity, affected further by increasing polyol content. Similar nonlinear behavior has been observed in biopolymers such as wheat and soy as well as synthetic liquid crystalline polymers (LCPs) and some polystyrenes [35-39].

Alternatively, the observed nonlinearities can be explained by the two-fluid theory in which a fluid consists of a low and high viscosity phase where only the low viscosity phase tend to orient during flow [40]. In previous work, Novatein was shown to consist of at least two phases; one protein-rich and the other plasticizer rich [12,13]. Chain alignment is most likely occurring in the plasticizer-rich phase, which means the orientation effect will become more prominent with increasing plasticizer. The orientation effect is likely to become even stronger with increasing random structures which can be affected by the choice of plasticizer.

The effect of elongational viscosity can be understood by considering the entrance pressure (**Fig. 4**). The decreased entrance effect due to TEG was most probably due to an increase in the proportion of the low viscosity, polyol-rich phase (plasticization effect) and a more efficiently plasticised protein-rich phase [12]. The entrance pressure (P_e) was very significant in terms of the total pressure drop, especially with the 70W composition. The entrance effect decreased dramatically with increasing TEG, but also as a function of shear rate, whereas it increased the total pressure requirement for the longest capillary for the reasons mentioned earlier. This was somewhat unexpected, and highlights the peculiar processing characteristics of this thermoplastic.

3.3 True viscosity

The nonlinearity in the Bagley plot resulted in three different viscosity curves for the same material (Fig. 5a). The shear stress is first corrected for the entrance effect using the entrance pressure of the orifice (Bagley correction, Fig. 5b) followed by the Rabinowitsch correction for shear rate. To account for the non-linearity in the Bagley plot the degree of underdeveloped flow is assessed by further corrections to the shear rate:

$$Q_{total} = Q_{laminar\ flow} + Q_{plug\ flow} \quad (8)$$

$$\gamma_{corrected} = \gamma_{measured} - \gamma_{underdeveloped\ flow} \quad (9)$$

With underdeveloped flow it is assumed that 1) the lower viscosity measured using the shortest capillary has not reached fully developed flow, 2) the true viscosity of the same material with different capillary lengths is the same, 3) the orifice value is pressure-independent, and 4) flow is fully developed in the longest capillary. The difference in shear rate due to underdeveloped flow is iteratively determined using Excel to produce a single viscosity vs. shear rate curve for all capillaries (Fig. 5c).

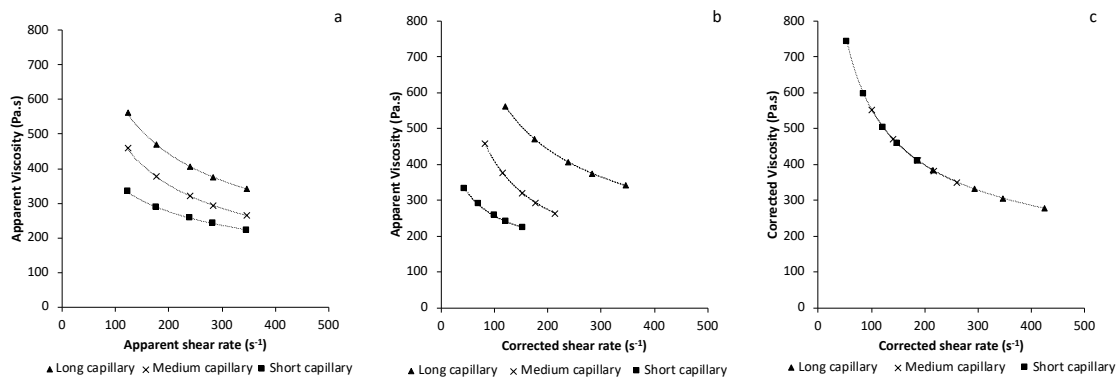


Figure 5. 40TEG's apparent viscosity vs apparent shear rate (a), apparent viscosity vs corrected shear rate (b) and corrected viscosity vs. corrected shear rate (c).

The true shear and extensional viscosities for 0-40pph_{BM} TEG plasticized Novatein is presented in the Fig. 6. The extensional viscosity (Fig. 6a) followed the expected trend based on the entrance pressure presented in the previous section. 40TEG had almost a one order of magnitude lower extensional viscosity in comparison to 70W at the corresponding elongation rate and would explain the significant difference in its ability to be sheet extruded.

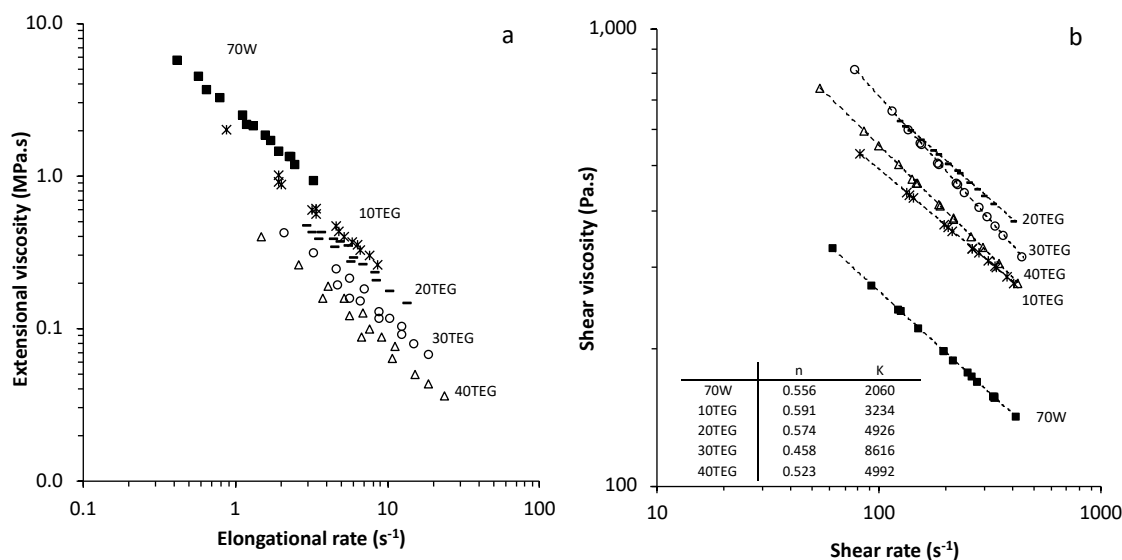


Figure 6. Fully developed elongational (a) and shear (b) viscosities for no polyol and TEG compositions.

The shear viscosity (**Fig. 6b**) followed quite a counterintuitive trend in terms of what is expected from plasticization. 10TEG had the lowest shear viscosity of the polyol plasticized compositions, being closest to 70W. The other compositions (20-40pph_{BM} TEG) followed the expected trend of decreasing shear viscosity with increasing plasticizer content. However, this was in agreement with the two-fluid deorientation mechanism that plasticization, together with secondary structure of the protein, made it easier for the material to reach fully developed flow. With 10TEG, the plasticizer interacted mostly with the protein network (primary plasticization) leading to a lack of phase separation and plasticization [12], thereby making the material more similar to 70W. In other words, even though the apparent shear rate has been corrected, the measured viscosity of formulations with low plasticizer content appeared lower because of their inability to form fully developed flow. However, the corrected viscosity values are not much different even comparing no-polyol to the TEG plasticized samples, and the true difference between these formulations lie in their elongational viscosity.

If the longest capillary were to be used to calculate the apparent viscosity of 40TEG, it was comparable to 70W (in contrast to **Fig. 1**). In the other words, in the case of fully developed flow the beneficial effect of plasticization in lowering the extensional viscosity can be also lost, e.g. the drastic difference in the sheet extrusion ability between the no-polyol and 40TEG compositions (**Fig. 2**). The existence

of a plug flow component could be seen as a beneficial effect in some circumstances and similar to wall slip, it can have a positive effect in terms of material processing even though it is often associated with the flow instabilities [41]. This is very similar to highly filled wood composite materials which has some similar characteristics to Novatein [42].

3.4 Effect of plasticizer and temperature

The effect of temperature on the shear and extensional viscosity of Novatein and PP can be seen from **Fig. 7** after Bagley and Rabinowitsch corrections (power law constants can be found in **Table 2**). Even though the longest capillary provided results closest to fully developed flow, in practice it is a challenging testing method with a narrow mass flow rate range and requiring very high pressure. For comparative purposes, only the shortest capillary and the orifice were used here. Due to underdeveloped flow, the shear viscosity could be lower in comparison to true shear viscosity. The selected plasticizer levels were based on previous findings that 10 and 30pph_{BM} polyol content was below and above the saturation point, where significant changes in physical properties were observed [13,12].

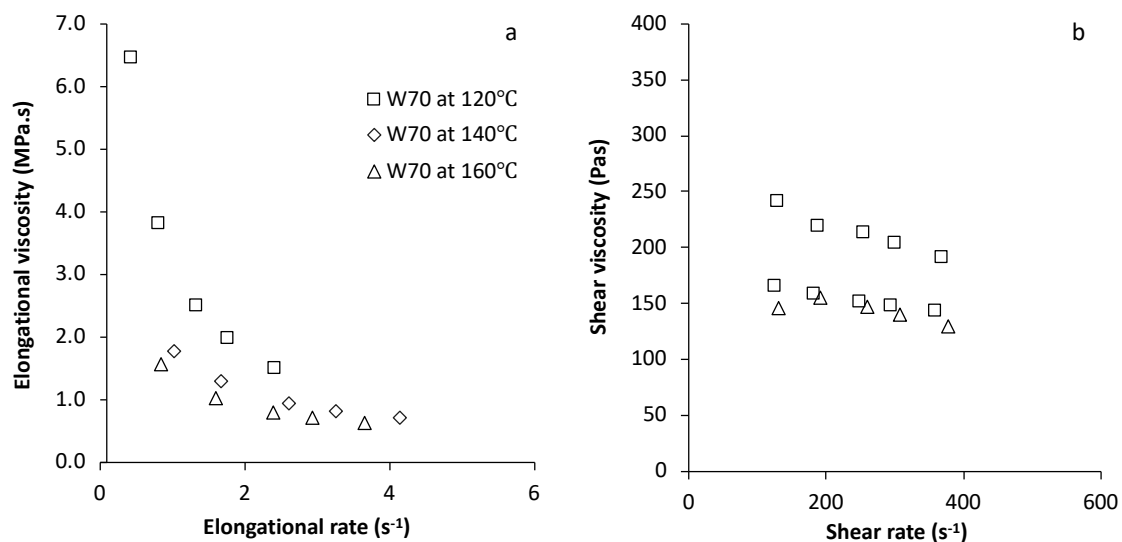


Figure 7. Elongational (a) and shear (b) viscosities of different temperatures.

Increasing temperature decreased the extensional viscosity for formulations without polyol. Judging from the power law constants, at higher temperature the elongational viscosity was slightly less dependent on deformation rate. For shear viscosity, the effect of temperature was the opposite; shear

thinning behaviour (n) increased with increasing temperature and K was the lowest at 120°C. This is most likely due to the competitive effect of increased chain motion and loss of plasticizer (water evaporation). It is notable that at 160°C some indication of degradation was evident in the extrudate. High water content and higher temperatures made flow easier, however, processability is less controlled, also observed by others [14].

The extensional viscosity for differently plasticized samples clearly separated in three regions, mostly based on the amount of plasticizer (**Fig. 8**). Adding 10pph_{BM} polyol lowered the elongational viscosity, independent from the plasticizer type. At this level, primary plasticization dominated, where the polyol partially replaced some hydrogen bonding between the protein and water. At 30pph_{BM}, secondary plasticization dominated, accompanied by well dispersed phase separation (dependent on type of plasticizer), leading to some differentiation between the effect of plasticizer type [12]. PG and TEG were most effective in lowering the elongational viscosity at 30pph_{BM}, followed by EG and GLY. The total effect is a combined mechanism of primary and secondary plasticization as well as secondary structure changes in the protein. The efficiency to lower elongational viscosity followed the same order as was observed for the same polyols' ability to plasticize the protein network where TEG's efficiency can be attributed to its ability to increase random structures (secondary structure) [27].

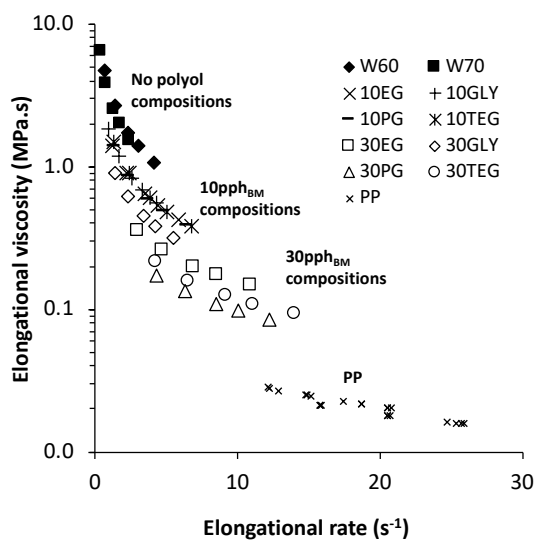


Figure 8. Elongational viscosity comparison of different compositions.

In comparison, the elongational viscosity of PP was drastically different compared to Novatein. The ratio between the elongational and shear viscosities for PP was 1:10 whereas for 70W this was closer to 1:400. Neither increasing temperature nor increasing water lowered this ratio significantly. The shear viscosity became slightly more shear thinning with increasing temperature, similar to observations for gluten (and soya) [16]. This would imply that processability can generally not be improved significantly by only increasing temperature or the amount of water.

Increasing water from 60 to 70p_{ph_{BM}} lowered the shear viscosity significantly (**Fig. 9a**) as at these levels, the network is saturated with water and secondary plasticization would dominate [12]. Flow is most likely underdeveloped, as discussed earlier, and also explains the high elongational viscosity.

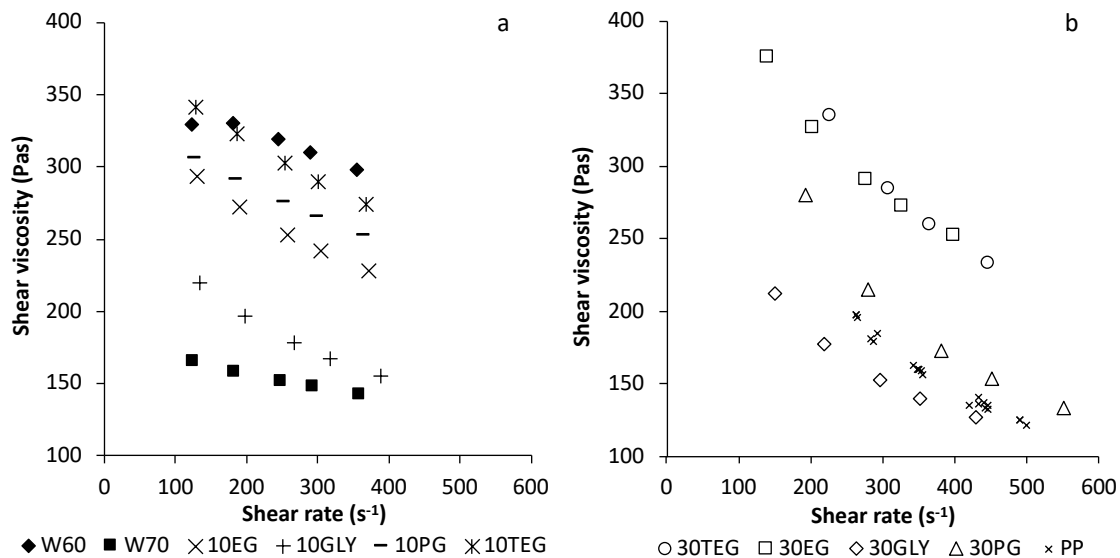


Figure 9. Shear viscosities of 0 and 10p_{ph_{BM}} polyol content (a) and 30p_{ph_{BM}} polyol content and PP (b).

Upon adding 10p_{ph_{BM}} plasticizer, the shear viscosity was higher compared to 70W, probably due to underdeveloped flow in the absence of polyol, if seen in conjunction with the high elongational viscosity. GLY lowered the shear viscosity the most, and could be explained by its inability to plasticize the protein network efficiently, leading to an apparent low shear viscosity as a result of underdeveloped flow [27]. The other polyols were more efficient at plasticizing Novatein, leading to higher shear viscosity, for the same reason as mentioned before.

Table 2. Power law indices of the different Novatein compositions collectively.

Composition and temperature*	Power law parameters ($\eta=Ky^{(1-n)}$)					
	Shear viscosity curves			Extensional viscosity curves		
	n	K Pa.s ⁿ	R ²	n	K MPa.s ⁿ	R ²
PP	0.29	10491	0.99	0.28	0.16	0.952
60W	0.904	534.97	0.8183	0.15	3.55	0.9999
70W	0.86	327.38	0.995	0.15	3.21	0.9999
70W 140°C	0.796	645.77	0.9637	0.38	1.8	0.9999
70W 160°C	0.73	634.65	0.8994	0.37	1.4	0.995
10TEG	0.79	949.75	0.9831	0.156	1.89	0.9999
20TEG	0.39	10188	0.999	0.39	0.84	0.9999
30TEG	0.46	6120.7	0.999	0.29	0.6	0.9999
40TEG	0.61	1839	0.999	0.29	0.35	0.9999
10EG	0.76	952	0.9937	0.21	1.7	0.9999
30EG	0.623	2420	0.999	0.31	0.76	0.9999
10GLY	0.67	1121	0.997	0.19	1.81	0.9999
30GLY	0.51	2488	0.999	0.2	1.21	0.9999
10PG	0.82	742	0.982	0.2	1.7	0.9999
30PG	0.297	11331	0.999	0.32	0.46	0.9999

*Temperature 120°C if not mentioned

Increasing the polyol content to 30pph_{BM} did not lead to a drastic reduction in shear viscosity, although the shear rate dependence was more significant. When considered with elongational viscosity, polyol plasticization did improve processability, as the elongational viscosity was significantly lowered. Interestingly, the shear viscosity of 30TEG is relatively high despite its beneficial effect on secondary structure [12].

It was concluded that elongational viscosity is driven by the changes in the plasticization of the protein-rich network whereas changes in shear viscosity is driven by the formation of phase a separated polyol-rich region. A significant improvement in sheet extrusion was achieved combining the effect of TEG (changes in secondary structure) with that of PG (reduction in elongation viscosity) as well as manipulating temperature and residence time (**Fig. 10**).



Figure 10. Extruded Novatein sheet, using a combination of 60W, 20TEG and 20PG, processed at 160 °C.

4 Conclusion

Even though the apparent shear viscosity of Novatein and PP may look similar, their processing during sheet extrusion was significantly different. The secondary structure of Novatein consisted largely of ordered structures that do not melt or unfold into a fully amorphous state during processing. This makes its processing closer to a highly filled polymer rather than a fully molten polymer. In addition, random structures, indicative of a protein's plasticization potential, was found to be lower with GLY in comparison to TEG.

Novatein's extensional viscosity was significantly higher in comparison to PP and its entrance pressure accounted for up to 80% of the total pressure drop (P_{ent}/P_{tot}). With increasing polyol content the ratio decreased significantly but led to non-linear behaviour in pressure drop vs. capillary length. This was attributed to a combination of pressure dependency and structural effects affected by the elongational flow. At low polyol content, Novatein's flow profile with the shortest capillary was closer to plug flow leading to low apparent shear viscosities. Chain orientation is expected due to the elongational flow at the capillary entrance with orientation diminishing along the length of the capillary, leading to an unexpectedly long flow-development region. At higher polyol content the shear viscosity was highly dependant on the capillary length. In practice, this might lead to higher than expected viscosities, as the total pressure drop can become higher in comparison to non-plasticized compositions.

Increasing polyol content and temperature were found to decrease elongational viscosity and resulted in an increase in shear viscosity. The effects were attributed to primary plasticization in the protein-rich phase and secondary plasticization in the polyol-rich phase. The elongational flow was dominated by primary plasticization and changes in secondary structure. However, the same mechanism also increased shear viscosity as a result of close to fully-developed flow in the shorter capillary. Phase separation (secondary plasticization) led to the reduction of shear viscosity for all the plasticizers, providing the plasticizer content was above 10pph_{BM} to ensure secondary plasticization. PG showed the most significant change in shear and elongational viscosity and combined with secondary structure effects of using TEG, the sheet extrudability of Novatein was improved drastically.

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Keywords: phase separation; extrusion; extensional viscosity; shear viscosity; proteins, rheology

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7

Concluding discussion

Concluding discussion

The latest wave of banning single-use plastics in large geographical areas such as India and EU represents a significant change in the materials sector. The driving force behind these actions is based on a large amount of uncontrolled waste and its accumulation in the environment. This has created a consumer and industry-driven demand for more sustainable material alternatives that actually degrades in the undesired event of uncontrolled disposal. As most semi-synthetic bioplastics require elevated temperatures (industrial composting) to trigger biodegradation, biomass-based polymer products such as Novatein, is one of the only alternatives that can fulfill these degradation requirements. Various by-product and waste streams are considered preferred resources for biomass-based plastics, as these are often not competing with human food supply.

One of the challenges of the biomass-based plastics is that the same chemical structures that make degradation possible, also make processing more challenging. In extrusion processing of Novatein, thermal degradation occurs below the melting temperature of ordered structures. This makes the material processing rather similar to rubbery-like materials or a filled polymer, consisting of nanocrystallite aggregates, rather than a semi-crystalline polymer that has reached a molten state. In addition, biopolymers are very hygroscopic and may influence degradation properties and make material properties highly dependent on environmental humidity. Thus, in addition to the temperature-dependence of properties that are normally considered with conventional plastics, biomass-based products are often limited to a narrow range of environmental conditions in which it can be used. For example, a ductile-to-brittle transformation of the mechanical properties is mostly undesirable and is dependent on relative humidity. These factors form the landscape for products and process design of Novatein and other biomass-based plastics and should also be when interpreting results presented in the following sections.

Blood meal has undergone excessive heat treatment as a part of its production making the structure different to many other proteins. During the heat treatment, the native state of all the proteins in blood meal is irreversibly denatured and the high heat further leads to aggregation and crosslinking. Turning

blood meal into Novatein is a process of counteracting crosslinking and increasing chain mobility. The state of aggregation and the protein's secondary structure were identified using Synchrotron FT-IR and XRD revealing that almost 50% of Novatein was β -sheets and typically hindered processing. However, crosslinking and aggregation led to mechanical properties that were comparable to conventional plastic products. This also meant that plasticization played a significant role in processability as the role of chain mobility and the presence of α -helices and random coils become more important.

Due to the hygroscopic nature of Novatein, water acts as a natural plasticizer. The equilibrium water content (EMC) was determined by relative humidity and depends on the polymer structure which influences the availability of hydrogen bonding sites. Due to its very small size water is able to diffuse readily in the polymer network and efficiently interact with the polymer's hydrogen bonding sites. Water reduces the T_g by about 10 °C for every 1% added water, at low plasticization levels. Water is therefore also a crucial processing aid for Novatein. However, the presence of water in Novatein also poses a problem; water evaporation causes shrinking and often also leads to products becoming brittle. For this reason, less-volatile polyol plasticizers were introduced in combination with water.

Plasticization using polyols were explained as primary and secondary plasticization. In primary plasticization, the plasticizer interacted directly with the protein network by replacing the protein's hydrogen bonding sites with water. This decreased the equilibrium moisture content (EMC) of the material in comparison to that without a plasticizer and even increased the tensile strength. In secondary plasticization, the polymer network became saturated and the polyol's hydrogen bonding sites became available for hydrogen bonding with water, leading to an increased EMC. The effect is substrate and plasticizer dependant; for example, secondary plasticization is very prominent for Novatein as the saturation point is reached much faster compared to other biomass-based polymers due to the lack of available hydrogen bonding sites (due to aggregation). This underlined the need for qualitative optimization for plasticization.

Phase separation was confirmed by FT-IR, assessing the relative absorbance of the primary alcohol groups to the amide III region. Of the selected plasticizers, GLY clearly showed the highest tendency

to phase separate followed by TEG, PG, and EG. The plasticizer content at which the EMC of a specific plasticizer becomes equal to the no-plasticizer composition was called as the point of equivalence (POE). This was also the point at which secondary plasticization occurred, evident by phase separation and was linked to the brittle-to-ductile transformation of the mechanical properties. Of the tested plasticizers the POE values varied from 19.9 pph_{BM} to 29.1 pph_{BM} in the order of GLY, TEG, PG, and EG. However, TEG had the lowest POE on a molar basis and nearly three times the molar amount of EG was required to reach the POE in comparison to TEG, whereas for GLY it was only 1.3 times. This indicated that molecular size, chemical structure and the amount of potential hydrogen bonding sites of the polyol affected the interaction with the protein network.

The plasticizers ability to change the EMC was related to the theoretical amount of hydrogen bonding and plasticizer's tendency to phase separate, quantified by using the POE. TEG was the most effective plasticizer due to its ability to plasticize the amorphous fraction, but also because of its ability to increase the amount of random coils. On the other hand, GLY showed the largest change in secondary structure, increasing the α -helix content at the cost of random coils. Thus, the mechanisms of plasticization between TEG and GLY was considered to be very different despite relatively similar mechanical properties. TEG's plasticization is based on its ability to interact efficiently with the protein network and modify the secondary structure sufficiently. GLY, instead, showed a strong tendency to phase separate leading to the highest hydrogen bonding potential above the POE, and therefore also raised the EMC above that of TEG. Despite the increase in the ordered secondary structures, GLY led to the highest strain at break, which was attributed to phase separation.

TEG and GLY were selected for further investigation to understand the role of water in the ternary system. Changes in the EMC was a result of structural properties of Novatein, polyol type and amount, and relative humidity. EMC was the dominant factor determining mechanical properties with a brittle-to-ductile transformation observed at 8% EMC, independent of polyol content and type. However, it is important to understand that the polyol content and type played a significant role in determining whether the material was below or above the POE. The constraint theory, relating to hydrogen bonding plasticizers was more applicable below the POE, whereas the free volume theory and the formation of

micro-scale phase separation, described material behaviour above POE when ideal mixing conditions applied in each phase. The role of water was found to be significant in forming ideally mixed phases (not between phases), explaining the applicability of the free volume theory. For dried samples, TEG and GLY formed clusters in the polymer network in the absence of water, whereas for hydrated samples the plasticizer was well distributed through the polymer network, albeit in micro-separated regions.

Water's role in improving ideal mixing conditions was also related to the brittle-to-ductile transition, although this cannot be seen in without also considering the polyol and phase separation. In other words, neither polyol or water could solely plasticize the polymer network in a way that the material would provide ductile properties, even if the water content was above 8%. The mechanism behind this is related to the protein is both hydrophilic and hydrophobic. Despite water being an efficient plasticizer, it is able to plasticize only the hydrophilic regions. Polyols, being amphiphilic, provides interaction with the hydrophobic regions as well, however, requires water to provide ideal mixing in the phase separated fractions. Water's effect on the secondary structure was found negligible.

The effect of phase separation was further studied using DMA and provided further insight into the POE approach and the applicability of the constraint and the free volume theories. Both TEG and GLY showed three different phases, protein-rich, intermediate and polyol rich. TEG was able to plasticize the protein-rich phase more efficiently in accordance to results from secondary structure analysis and the observed phase separation. The free volume-based Couchman-Karasz model was effective in describing plasticization and was used to calculate the fractional composition and amount of each phase. The role of the intermediate phase was crucial in terms of total plasticization, and led to better total plasticization performance with TEG. The drastic difference between TEG and GLY was attributed to changes in secondary structure.

In light of the understanding around plasticization, the rheology of Novatein was characterised using screw-driven capillary rheometry. Even though the apparent viscosity of highly plasticized Novatein was comparable to polypropylene, in practice it was not possible to extrude Novatein into sheets. This was attributed to Novatein's particularly high extensional viscosity. It was thought that this is most

likely due to the protein's ordered structures that cannot melt into a fully amorphous state during processing (in contrast to PP that fully melts). Novatein's high extensional viscosity was by very high entrance pressure, which accounted for up to 80% of the total pressure drop. Increased polyol content decreased the ratio significantly and led to nonlinear behaviour in pressure drop vs capillary length. The result is that with increasing plasticiser the total pressure drop and consequently the shear viscosity could become even higher. The behaviour was found to be a result of better flow development, suggesting that Novatein without polyol was behaved closer to plug flow. For Novatein, fully developed flow might not be the desired effect as it alters the material behaviour to pressure levels really unpractical in terms of processability.

Increasing polyol content and temperature decreased the extensional viscosity and increased the shear viscosity. However, due to secondary plasticization, the shear viscosity increased due to better flow development. Elongational flow was dominated by primary plasticization of the protein-rich phase and changes in secondary structure whereas secondary plasticization (phase separation into a polyol-rich phase) played a significant role in the reduction of the shear viscosity. PG showed the most efficient plasticization in both shear and elongational viscosity, which was attributed to the combination of small molecular size and its ability to enhance both primary and secondary plasticization. With other plasticizers, the results were in a very good agreement with the fundamental understanding of the plasticization. GLY, acting mostly as a secondary plasticizer, had the worst elongational properties and the best shear viscosity properties of the selected compositions. TEG, as an efficient primary plasticizer and an ability to modify the secondary structure, performed exceptionally well in terms of extensional viscosity considering its high molecular weight. The shear viscosity was comparable to EG that was shown to diffuse very efficiently in the polymer network. In general, the performance of TEG and PG was better in comparison to EG and GLY, which could be attributed with the lower fraction of hydrophilic groups in the polyol structure, and furthermore, its ability to interact better with the hydrophobic regions of Novatein.

As a whole, understanding rheology and plasticization in combination, presented a significant process improvement when using a combination of PG and TEG for sheet extrusion. A significant improvement

in deformation properties was observed, evident from the formation of a well consolidated, full-width sheet (Chapter 6). However, there was still room for improvement as the high process temperatures (close to 160 °C) led to significant water evaporation and also some small surface irregularities. Also, even as the material is very flexible directly after extrusion, it gets a bit stiffer over the time when the moisture content equilibrates. These can be seen as subjects for future research and development. The aim of achieving a fundamental understanding of the interaction between protein structure, plasticization, and rheology, was considered to be fulfilled.

Recommendations for future work

With regards to for future work, the focus should be put on the whole value chain of different waste and by-product streams. Achieving the most out of these streams, the structural effects during processing would be good to be considered. For example, steam coagulation of blood meal is indisputably a necessary project, however, it does not pay any attention to the changes to the protein structure. Less aggregated blood meal, or any other biopolymer network, could lead to increased flexibility in future processes and could lead to properties closer to for example zein which has great extensional properties.

The potential of using the water content as a characterisation tool to understand the state of the biopolymer should be studied further. For example, with Novatein water content was a good tool to predict the mechanical properties. Also, the EMC was found to be a result of protein state, plasticization state, and RH%, and when normalised into POE it provided information about the state of plasticization as well. The applicability of POE and the effects around it should be tested with other biopolymers as well. Sorption isotherms could be used as a tool to predict structural behaviour but could also help in understanding the level of plasticization. For example, plasticizer migration of the polyol plasticizers is a well-known phenomenon. In terms of product quality control, as a simple moisture content test could provide further information on for example aging. Similarly, plasticizer volatility during the extrusion is one of the random variables that could be understood better when sorption isotherms are well understood.

The POE approach would be good to use as a tool for understanding plasticization mechanisms and the interaction between polyol and biopolymer. Plasticization, as found for GLY and TEG, may vary quite a bit and the POE approach could be used to provide tailored plasticization. One of the reasons GLY is the most often used plasticizers among the biopolymer network might be its higher tendency for secondary plasticization.

Furthermore, a combination of different plasticizers could be used based on whether the biopolymer requires primary or secondary plasticization. Some of the long chain plasticizers, such as PEG400, would theoretically provide the most suitable plasticization. However, in the scoping section of this thesis, it was left excluded because of the very poor extrudate quality. With this problem as well, the combination of plasticizers could be beneficial. With a well-tailored combination of primary and secondary plasticization, processing Novatein at higher temperatures, even without water could be possible.

Another focus area should be in process and experimental design. Despite biomass-based plastics following the same fundamentals and can be processed in the same equipment than conventional plastics, processing methods and experimental design optimised for the biomass-based materials should be considered. Optimal process design could be consisted of short residence time, as little deformation requirement as possible but providing still good back pressure for a proper consolidation. With optimised sheet extrusion equipment, the optimised Novatein composition is assumed to provide a solid-state sheet extrusion process with great film quality. However, due to the high β -sheet content, it might be highly unlikely that any plasticizer combination would be able to provide deformation properties required for extreme deformation during processes like blow molding.

Appendix 1.

Conference publications

Appendix: Conference publications

J. M. Uitto, C. J. R. Verbeek, M. C. Lay. Rheology of Protein-Based Thermoplastics. Poster presented at The 16th Asian Pacific Confederation of Chemical Congress. 2015, Melbourne, Australia

J. M. Uitto, C. J. R. Verbeek, M. C. Lay. Plasticization of bloodmeal-based bioplastic. Poster presented at The 8th Bioprocessing Network conference. 2015, Wellington, New Zealand

J. M. Uitto, C. J. R. Verbeek. Rheological modification of a bloodmeal based bioplastic accepted for oral presentation at The 32nd International Conference of the Polymer Processing Society, July 2016. Lyon, France.

Appendix 2.

Coauthorship forms



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Co-Authorship Form

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Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 2 - Bioplastics. An invited book chapter published in *Encyclopedia of Polymer Science and Technology*.

Nature of contribution
by PhD candidate

Although I was not first author of this work, it has been included in Thesis as my contribution considered 85 per cent of the total chapter preparation. As a co-author, I prepared the first draft of the chapter, which was together with my supervisor revised and edited the entire manuscript into the form submitted for publication

Extent of contribution
by PhD candidate (%)

85

CO-AUTHORS

Name	Nature of Contribution
Johan Verbeek	Guidance with experimental work and editing of manuscript

Certification by Co-Authors

The undersigned hereby certify that:

- ❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
Johan Verbeek		26/07/2018



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Chapter 3 – Phase Separation of Plasticizers in Thermally Aggregated Protein-based Thermoplastic. A paper published in *Advanced Polymer Technology*.

Nature of contribution by PhD candidate

As first author of this paper, the PhD candidate conducted most experimental work under the guidance of supervisor, and prepared initial draft manuscript, which was redefined and edited with consultation of supervisor, who is credited as co-author.

Extent of contribution by PhD candidate (%)

85

CO-AUTHORS

Name	Nature of Contribution
Johan Verbeek	Guidance with experimental work and editing of manuscript

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Chapter 4 – The Role of Water in Plasticizing Thermally Aggregated Protein-based Thermoplastic. A paper accepted in *Journal of Applied Polymer Science*.

Nature of contribution by PhD candidate

As first author of this paper, the PhD candidate conducted most experimental work under the guidance of supervisor, and prepared initial draft manuscript, which was redefined and edited with consultation of supervisor, who is credited as co-author.

Extent of contribution by PhD candidate (%)

85

CO-AUTHORS

Name	Nature of Contribution
Johan Verbeek	Guidance with experimental work and editing of manuscript

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Chapter 5 – The Role of Phase Separation in Determining the Glass Transition Behavior of Thermally Aggregated Protein-based Thermoplastic. A paper submitted in *Journal of Applied Polymer Science*.

Nature of contribution by PhD candidate

As first author of this paper, the PhD candidate conducted most experimental work under the guidance of supervisor, and prepared initial draft manuscript, which was redefined and edited with consultation of supervisor, who is credited as co-author.

Extent of contribution by PhD candidate (%)

85

CO-AUTHORS

Name	Nature of Contribution
Johan Verbeek	Guidance with experimental work and editing of manuscript

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Chapter 6 - Shear and extensional viscosity of a thermally aggregated protein-based thermoplastic material. Submitted in *Journal of Material Science*.

Nature of contribution by PhD candidate

As first author of this paper, the PhD candidate conducted most experimental work under the guidance of supervisors, and prepared initial draft manuscript, which was redefined and edited with consultation of supervisor, who have is credited as co-author. Experimental work was done with a support of visiting Professor C. Bengoeachea who have been credited as co-author.

Extent of contribution by PhD candidate (%)

85

CO-AUTHORS

Name	Nature of Contribution
Johan Verbeek	Guidance with experimental work and editing of manuscript
Carlos Bengoechea	Help with experimental work

Certification by Co-Authors

The undersigned hereby certify that:

- ❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

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