The Effect of Moisture Content and Extrusion Temperature on the Processing, Thermal and Mechanical Properties of Novatein®

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Izuchukwu Sandra C. P

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Abstract

The recent effect of petroleum based synthetic plastic on economy and environment has led to ever increasing study of renewable natural polymers such as proteins. Novatein Thermoplastic (NTP) is a protein based thermoplastic processed from bloodmeal. Bloodmeal is a by-product of the animal slaughter house and it is widely sold and used as low cost fertilizer and animal feed. It contains 90% dry weight of protein and it can biodegrade under suitable conditions making it a better alternative to petroleum based synthetic plastics. The objective of this thesis was to investigate the effect of processing water and extrusion temperature on the processing, thermal and mechanical properties of urea free NTP to optimize processing temperature and formulation for this material.

Literature revealed that extrusion processing of protein applies considerable amount of heat and shear into protein material to form polymer melt requiring sufficient chain mobility which could be achieved through the lowering of the glass transition temperature (Tg) of protein with the addition of water and other additives. Also literature revealed that optimization of material properties of protein thermoplastic is highly dependent on extrusion processing parameters such as extrusion temperature, initial moisture content (processing water), torque, pressure, screw-speed and specific mechanical energy (SME).

Four formulations of Novatein were processed using bloodmeal powder with Sodium Sulfite (SS), Sodium Dodecyl Sulfate (SDS), Tri ethylene glycol (TEG) and varying Water content at 30, 35, 40 and 45$pph_{BM.}$ The resultant thermoplastic was extruded using varying die temperatures of 120 °C, 130 °C, 140 °C and 150 °C. Test pieces were produced using injection moulding and conditioned for 7days

The effect of processing water and extrusion temperature was characterized by

- Extrusion Processing, processing water between 30 to 35pph_{BM} produced consolidated material because of protein chain unravelling and above this water content, material produced was mostly not consolidated due to

irregularities in measured torque and pressure as a result of inconsistent feed-rate which is caused by excessive plasticization of the material.

- Moisture Content, after conditioning assumed water content measured at oven drying ranged between 4% to 15% with 9 out of the 16 experiments falling within the accepted narrower range of 8% to 11%. The remaining 7, all experiment in formulation 4 had higher moisture content than the acceptable range and all experiment extruded at die temperature of 150 °C had lower moisture content than the acceptable range.
- Mechanical properties, water was shown to be critical for processing as it acted as plasticizer lowering the glass transition temperature (Tg) and denaturing temperature of the protein material. During conditioning moisture was lost resulting to a brittle material because of the new interaction between the protein chains after conditioning. There was no clear trend when considering the effect of extrusion die temperatures used on the mechanical properties of Novatein.
- DMA, increasing processing water decreased Tg while increasing temperature, the 9 experiment with the acceptable range, at $30pph_{BM}$ showed increase in Tg and at 35 and $40pph_{BM}$ no increase was observed, the 7 experiments outside the acceptable narrower range showed no significant difference in Tg within their range. There was an insignificant difference in Tg when considering the effect of added water and processing temperature therefore the difference is as a result of water remaining in the sample when they were tested.
- TGA, showed varying residual moisture content as after freeze drying and milling the mass loss observed varied between 3% to 17% for all experiments which could be as a result of samples not freeze dried to the same extent or protein absorbing water from the atmosphere during milling because of its hygroscopic nature. There is an insignificant difference in moisture content after conditioning and subsequent freeze drying. There is no effect on the decomposition behaviour and thermal stability of Novatein within the range of processing water and extrusion temperature used as there was an insignificant difference observed in the TGA thermograms after freeze drying and milling.

- WAXS, crystallinity of the tested formulation ranged from 15% to 27% where most were within the range of 19% and 22% which fell within the reported crystallinity range of Novatein from previous study demonstrating that the range obtained was the appropriate range for NTP therefore no change in crystallinity was observed.

The optimal processing temperature and formulation was achieved using 35ppph$_{BM}$ water and extrusion die temperature of 150 °C (experiment 8). It showed ease of processing, having good mechanical properties. Before conditioning it had tensile strength of 5.35MPa, Young's modulus of 253MPa, strain at break of 0.34mm/mm, toughness of 1.61Mpa and after conditioning it had tensile strength of 17.49MPa, Young's modulus of 1101MPa, strain at break of 0.02mm/mm and toughness of 0.18MPa. Thermal property showed no significant difference therefore there is no change in thermal property of Novatein with this optimal processing temperature and formulation.

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Table of Contents

Abstract ii

Acknowledgements v

Table of Contents vii

List of Figures x

List of Tables xi

1 Introduction 1

2 Literature Review 6

2.1 Introduction 6

2.2 Biopolymers 7

2.2.1 Micro-organism 9

2.2.2 Bio-monomers 9

2.2.3 Types of biomass 10

2.2.3.1 Cellulose 10

2.2.3.2 Starch 11

2.2.3.3 Chitin 11

2.3 Proteins as materials 12

2.3.1 Protein structure 12

2.4 Types of proteinous polymers, uses and limitations 15

2.4.1 Gelatin 15

2.4.1.1 Casein 16

2.4.1.2 Keratin 16

2.4.1.3 Corn proteins 16

2.4.1.4 Wheat gluten 17

2.4.1.5 Soy protein 17

2.4.1.6 Bloodmeal 18

2.5 Protein polymer processing 19

2.5.1 Wet vs Dry processing 19
2.5.2 Extrusion 20
2.5.2.1 Effect of extrusion temperature on proteins 21
2.5.2.2 Material composition 23
2.5.2.3 Common additives 24
2.5.2.3.1 Water 24
2.5.2.3.2 Other plasticisers 25
2.5.2.3.3 Other additives 26
2.5.3 Injection Moulding 27
2.6 Processing of Novatein 28
2.7 Conclusions 31
3 Equipment and Methods 32
3.1 Experimental Design 32
3.2 Material 33
3.3 Choice and preparation of formulations 34
3.4 Processing 35
3.4.1 Extrusion 35
3.4.2 Injection moulding 35
3.4.3 Conditioning 36
3.5 Characterisation 36
3.5.1 Mechanical properties 36
3.5.2 Thermal properties 36
3.5.2.1 Dynamic Mechanical Analysis (DMA) 36
3.5.2.2 Thermogravimetric Analysis (TGA) 37
3.5.3 Physico-chemical characterization 37
3.5.3.1 Moisture content 37
3.5.3.2 Wide Angle X-ray Scattering (WAXS) 38

4 Results and discussion 39
4.1 Changes in moisture content 39
4.2 Extrusion parameters 42
4.3 Injection moulding 45
4.4 Mechanical properties 48
4.4.1 Unconditioned injection moulded material 49
4.4.2 Conditioned parts 51
4.4.3 Recommendations based on mechanical properties. 54
4.5 DMA 55
4.6 TGA 58
4.7 WAXS 61
5 Conclusion and Recommendations 66
6 References 70

List of Figures

Figure 2-1: Categorization of Biopolymers and their sources 9

Figure 2-2: Protein structures [89] 15

Figure 2-3: Various stages of NTP processing 18

Figure 2-4: Methods of protein processing. 19

Figure 2-5: Extruder profile [110] 21

Figure 2-6: Injection moulder outline [110] 28

Figure 3-1: lay-out of experimental design 33

Figure 3-2: Temperature profile and extruder screw configuration 35

Figure 4-1: Mechanical properties of unconditioned test pieces with varying water content and extrusion temperature. Error bars denotes standard deviation of the mean. 49

Figure 4-2: Mechanical properties of conditioned test pieces with varying water content and extrusion temperature. Error bars denotes standard deviation of the mean. 51

Figure 4-3; Plots of tanδ against temperature for processed NTP before and after conditioning. Although three scans were performed for each added water content and extrusion temperature, only one is shown for each experiment for clarity. 56

Figure 4-4; TGA thermograms of freeze dried PNTP, ENTP, INTP and CNTP. Although three scans were performed for each experiment, only one is shown for each experiment for clarity. 60

Figure 4-5; base-line corrected plot of WAXS Although three scans were performed for each experiment at each step, only one is shown for each experiment for clarity. 64

Figure 4-6; process for de-convoluting WAXS data and solving for crystallinity 65

List of Tables

Table 2-1: Structures of the 20 amino acids found in protein [54] 12

Table 2-2: Comparison of hydrogen bonding site by mole and mass for water, Urea and TEG [19]. 30

Table 3-1: Table of experiments done showing factorial design 32

Table 3-2: Table of pre-extruded formulations used..... 34

Table 4-1: Added water, plasticiser and fractional mass loss in percentage during oven drying for each experiment at each stage of processing. Average and standard deviation from three measurements are shown..... 40

Table 4-2: Measured average of extrusion parameters obtained from reading taking every 3 minutes during extrusion. 43

Table 4-3: Injection parameters 46

Table 4-4; Glass transition temperatures of conditioned and unconditioned samples. Average and standard deviation from three separate scans is shown for each sample type. 55

Table 4-5; Residual moisture content in freeze dried samples determined from cumulative mass loss in TGA to 120 °C. Average and standard deviation from three replicates is shown for each step. 59

Table 4-6; Crystallinity of different processing stages with added water, added water and TEG, and processing die temperature 65

1 Introduction

There is ever increasing concern regarding environmental pollution in our society today. This has resulted in an increased interest in natural polymers which have the ability to biodegrade under specific conditions. In contrast, petroleum based synthetic polymers are widely used commercially because of their excellent properties but their inability to degrade contributes to environmental pollution when disposed.

Proteins are natural polymers that have received renewed attention for thermoplastic applications in the last two decades [1]. Protein-based feedstocks can be renewably derived from agricultural or horticultural activities. However, if dedicated growth of crops is required for polymeric materials, this may result in competition for land use with the purpose of food production and growing demand for biofuels. In the quest for an alternative feedstock, sourced from either waste streams or low value by-products of existing activities, bloodmeal was identified as a potential raw material [2]. Bloodmeal is dried blood produced as a by-product of meat industry and has high protein content of between 90 to 95% dry weight [3; 4], it is readily available at low cost and can biodegrade under suitable conditions [5; 6].

Research at the University of Waikato over the past eight years has shown that bloodmeal can be successfully processed into thermoplastic material that can be extruded and injection moulded on conventional polymer processing equipment. This requires the addition of water and another small polar molecule such as urea or tri-ethylene glycol to act as plasticizers and disrupt hydrogen bonding between protein chains, sodium sulphite to reduce cross linking by cleaving disulphide bridges and sodium dodecyl sulphate to disrupt hydrophobic interactions [1]. The process of producing this bloodmeal-based polymer was patented by the University of Waikato through its commercial arm Waikatolink Ltd [7]. The resulting material is now known as Novatein® thermoplastic protein (NTP) and is being commercialised by Aduro Biopolymers LP in Waikato Hamilton New Zealand.

NTP can be seen as a sustainable material as there is an on-going demand for meat for human consumption. New Zealand alone produces 350,000 tonnes of exported lamb, 341,000 tonnes of exported beef product and 165,000 tonnes of meat for the domestic market annually with about $4 to 5 billion dollars in sheep and beef export earnings per year [8; 9]. To achieve this, the meat industry in New Zealand slaughters and processes a combined total of 25 million beef and sheep carcasses a year [10]. Producing this much meat and meat carcasses requires a large agricultural and meat processing sector and means by-products are readily available, thereby making the on-going supply of bloodmeal sustainable.

As well as being produced from the red meat sector, NTP has been identified as an attractive material for several short time use products used in red meat processing industry. Examples of such products are weasand clips and abattoir rectal plugs. Weasand clips are used to seal one end of the digestive track to prevent contamination of exposed meat the undigested food in the animal gut while abattoir plugs are used to seal the other end to prevent faecal contamination of exposed meat as the carcass is processed. Polypropylene plugs have been used for this purpose but this introduces plastic contamination into useful rendered end products such as meat and bone meal. A new design of abattoir plug, known as the Port Jackson™, has been designed by Bestaxx Innovation, Australia in partnership with Aduro Biopolymers LP, New Zealand for production out of Novatein and has successfully been injection moulded and trialled during meat processing.

Port Jacksons produced from Novatein should easily break down during rendering because of its hydrophilic nature. It will not be a source of plastic contamination in the rendered product as Novatein itself is produced from a rendered material and predominantly consists of animal protein. Furthermore, the Port Jackson design introduces other design innovations aimed at improving performance during meat processing. The plug has an easier to use design, with a rotating rod paradigm instead of the normal pneumatic gun, along with a helical screw design for easy insertion and a tight seal [11]. These properties makes Port Jackson plugs produced from Novatein a better substitute for existing polypropylene products. However, for the Port Jackson to be produced in commercial quantities Novatein

must be successfully produced using extrusion and injection moulding processes easily on a large scale.

Unlike conventional polymers which are typically dried prior to extrusion, protein extrusion is complicated as it is extruded in the presence of water which acts as a plasticizer. Water content is the most important factor of the extrusion parameters and product properties of protein-based materials [12; 13]. Water is of paramount importance as it lowers the glass transition temperature (T_g) and denaturation temperature of the material ensuring processability at temperatures which avoid excessive protein degradation during processing. Moisture remaining in the polymer after processing also greatly influences mechanical properties due to effective plasticization thereby increasing elongation and reducing material strength [1; 14-16].

After processing, protein-based polymers such as Novatein are very sensitive to atmospheric water activity [17]. When processed Novatein is stored in a highly humid environment, moulded parts will absorb water resulting to a highly plasticized, rubbery material having more flexibility, higher elongation at break and lower tensile strength and modulus [17]. If stored in a low humidity environment, parts will lose water, resulting in increased tensile strength and Young's modulus but lower elongation at break, becoming increasingly brittle and rigid [17]. This severe effect on mechanical properties, may limit the use of protein based polymers in some applications where synthetic polymers are used. For the Port Jackson, this is not a current barrier as a formulation that was tailored to the required properties for its use is utilized but it is important in designing an optimal processing condition for future product ranges using protein polymers to know how to tailor the moisture content according to the desired properties.

The amount of moisture in the final product (moulded Novatein parts) may vary from the initial amount of water added to prepare Novatein due to some evaporation of moisture during extrusion and injection moulding processes [13] or further losses during subsequent storage. An additional limitation for production of thermoplastic products in commercial scale injection moulding is that excessive plasticisation with water can lead to an overly flexible material causing difficulty

with mould release. Other researchers have, in the past, reported on difficulties in processing NTP due to its hydrophilic nature [18].

Since NTP's initial development, further research has emerged to address these limitations through different formulations, blending with other materials or modifying processing parameters. Initially, it was found that to get a material that remains ductile after conditioning, a considerable amount of urea was necessary. Unfortunately, urea also leaches out of the moulded part over a period of time like water because it has a weaker interaction to protein than protein/protein interaction [13]. Several less volatile plasticizers were trialled as partial replacement for water in addition to urea and TEG was chosen [19]. During the development of the plug it was found that the crude standard Novatein formulation containing both urea and TEG was too flexible immediately after moulding for effective part release. To solve this problem, the formulation was reassessed and a new formulation without urea was developed by Aduro Biopolymers LP for the production of Port Jackson™ plugs. Other approaches to overcoming the limitations of thermoplastic protein have included reinforcing NTP with bentonite, showing that reinforcement of NTP with nano-fillers to improve its properties is possible [20] and blends with other polymers such as polybutylene succinate and polyethylene using compatibilizers [18; 21]. Water absorption of the hydrophilic NTP was decreased when blended with hydrophobic linear low density polyethylene (LLDPE) [21] but use of such a blend could contradict the main aim of removing synthetic polymers from waste streams. Nevertheless, there is still some merit to this approach as linear low density polyethylene (LLDPE) is believed to be able to decompose and disappear if blended with biodegradable thermoplastics of sufficiently fine particle size [22; 23] and research is on-going. There continues to be additional, on-going research [24-29], not directly related to this thesis but contributing to the commercial potential of NTP including the modification of bloodmeal's colour to obtain a translucent honey appearance [30] and the production of foamed objects using NTP [31].

This thesis is proposing that the effect of processing conditions, specifically moisture content and extrusion temperature, needs to be properly understood before any modification or reinforcement is applied to Aduro's new formulation.

This is because different processing conditions can have a large effect on control and optimization of mechanical properties of protein based materials [15; 17; 19; 32-38]. This is especially important for optimization of the extrusion process used to prepare NTP on a larger scale for the production of commercial products. The specific objectives of this thesis, therefore, were:

- To investigate the effect of processing water and extrusion temperature on the processing, thermal and mechanical properties of urea free NTP.
- To determine the optimal processing temperature and formulation as a function of moisture content for the production of urea free NTP.

These effects were assessed using dynamic mechanical analysis and thermogravimetry for thermal properties at different stages of processing, mechanical testing for material properties and wide angle X-ray scattering for structural information.

A limitation of this study is that it only varied processing conditions as a function of moisture content and extrusion temperature. There are other factors that also need to be considered for process scale up, including processing conditions such as specific mechanical energy, degree of screw fill and other additives. Variation of these was outside the scope of this thesis

2 Literature Review

2.1 Introduction

The economic and environmental issues facing the use of petroleum based synthetic polymers since the 1980s have drawn attention to natural polymers such as protein, starch and cellulose because of their ability to degrade and their sustainable origins. Proteins can be produced as by-products or renewable waste streams product of agricultural or horticultural activities. Proteins from plants (soy, sunflower, corn and wheat) and animals (whey, casein, bloodmeal, keratin and gelatin) have been successfully processed into thermoplastics [1; 2; 39; 40]. A limitation of much research in the areas is that it utilizes casting and compression moulding, rather than the common processing techniques of extrusion and injection moulding used to rapidly manufacture parts from thermoplastics. This limitation hinders the wide acceptance and commercialization of protein-based polymers [14].

The extrusion process applies considerable amounts of shear and heat to a polymer material which results in structural re-arrangement and the formation of new interactions which may affect the material's final properties. The large number of different functional groups in protein results in many possible chain interactions during extrusion processing and for successful processing this need to be controlled. Such control can be achieved through the modification of extrusion parameters and appropriate additives to interact with functional groups in a predictable manner.

The aim of this chapter is to review relevant literature on the extrusion processing of protein polymers and identify the anticipated effect of extrusion parameters such as extrusion temperature and initial moisture content on the final properties of a produced part and also provide a contextual background on the development of protein based polymer alongside other biopolymers.

2.2 Biopolymers

Polymers are macromolecules consisting of repeating structural units known as monomers. They play an essential role in our everyday life because of their useful properties such as resistance to weather, low density, durability, ease of processing, thermal and electrical insulation [25; 26; 41]. Polymers are used widely in the automobile, aviation and household goods industries amongst others. Individual polymers have different material properties and this is because of the several factors that affect the interaction between macromolecules. Some of these factors are the presence of additives, number of monomers in an individual macromolecule and the way the monomers are linked [25; 26].

Polymers are classified into two categories based on their origin; Synthetic polymers, which are derived from petroleum by-products and natural polymers, which occur naturally and can be extracted from plants, animals or microbes. Polymer origin and its chemical structure play an important role in degradation. [42-45]. Since natural polymers are extracted mostly from biological resources and considered to be biodegradable they are oftentimes referred to as biopolymers or biodegradable polymers. However, not all polymers from biological resources are necessarily biodegradable and therefore in this thesis they will be referred to as biopolymers, although with the anticipation that those discussed in this thesis will biodegrade. Biodegradable polymers are those that undergo significant deterioration in their properties under the influence of microorganisms in specific conditions aided by chemical reactions like photo-degradation, oxidation and hydrolysis [46; 47]. Biodegradation is a natural complex phenomenon that occurs through the action of micro-organisms and this is achieved through three stages; bio-deterioration, bio-fragmentation and assimilation. Bio-degradation does not depend on the actions of micro-organisms alone, conditions associated with weather and burying also initiate the process [48].

Biopolymers have been present on earth for billions of years making them even older than synthetic polymers for which widespread use on an industrial scale only started in the 20th century. Examples of the early presence of biopolymers is DNA which is the building block of life made from long chain of repeating units of sugar, phosphate and nitrogen base; starch, which is the energy storage molecule in many plants and proteins serving many biological functions including

catalysis and structural roles. In nature, bio-polymers often have a well- marked structure. The composition and sequence of bio-polymer monomer units determine their primary structure and the unique way they fold makes up their secondary and tertiary structure. Bio-polymers have long been used in everyday products. For example, keratin which is present in hair and wool, is used as tough fibres to make clothes, also Cellulose-based bio-polymers are used in pure state as cotton in making clothes, wood used in making papers and as cellophane in packaging materials, However, the rapid growth of the oil industry resulted in the replacement of biopolymers for many applications [49].

There is an increasing research interest in thermoplastic biopolymers for short-life range applications such as packaging, agricultural use and bio-medical applications because they are good alternatives for commonly used synthetic polymers such as polyethylene (PE), polyethylene terephthalate (PET) and polypropylene (PP) due to their degradability, carbon neutral nature and sustainability. The use of biopolymers would help address the environmental issues caused by synthetic polymers such as waste streams and waterways pollution [50; 51], landfill use when buried at the end of life and atmospheric pollution through the release of fossil carbon when burnt at the end of life [52].

The most common and widely used thermoplastic biopolymers are polylactic acid (PLA), starch based plastic such as thermoplastic starch (TPS), poly-itaconic acid (PIA), sugar based PE and bio-composites that use plant fibre in place of synthetic glass or carbon fibre [53]. Thermoplastic starch is used for creating conventional products by extrusion and injection moulding, Sugar-based bio-polymers (polyactides) are used for medical purposes (surgical implants) as polyactides degenerate naturally in human body without producing harmful side effects [45; 54; 55] and to replace synthetic plastic used in conventional products such as lids for disposable cups, disposable table ware, compost bags and loose fill packaging.

Thermoplastic bio-polymers themselves can be further categorized into three groups based on their origin and production. Figure 2-1 shows the three group of bio-polymers and their sources [46; 56; 57]; polymers extracted from biomass, polymers produced by micro-organisms and polymers produced by chemical synthesis from biologically derived monomers [45]. In this thesis, the primary

concern is on polymers extracted directly from biomass, but the other two categories are also commercially relevant and will be discussed briefly first.

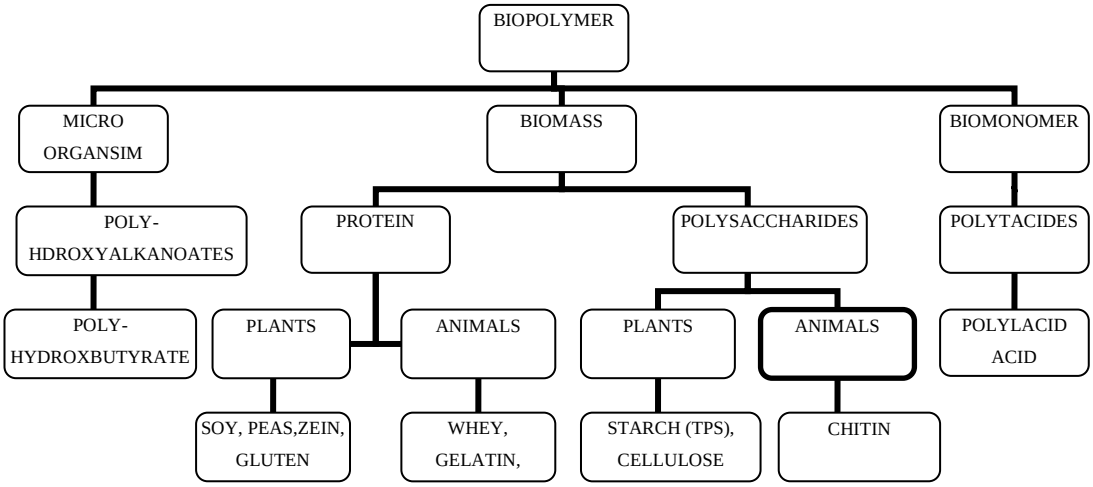


Figure 2-1: Categorization of Biopolymers and their sources

2.2.1 Micro-organism

These biopolymers are manufactured by culturing micro-organisms under different nutrient and environmental conditions, or sometimes directly in the cells of transgenic plants [58; 59]. The main example of bio-polymer developed through this process is the polyhydroxyalkanoate (PHA) family of bio-derived polyesters of various chain lengths [45; 60]. Feedstocks obtained from crops such as corn, soy beans, sugar cane or waste streams such as industrial waste water are converted into intracellular PHA through microbial fermentation [61; 62]. The most commonly used PHA is polyhydroxybutyrate PHB which has a methyl substituent group and similar properties to many industrial polyolefins [45; 60; 63]. The properties of PHA differ depending on the size of the alkyl substituent and the organisms and growth medium used. Brittle or flexible plastics can be obtained [64; 65].

2.2.2 Bio-monomers

These biopolymers are developed using biological processes, such as fermentation, to produce monomers that can be polymerized. An example of a biopolymer produced using this process is poly (lactic) acid (PLA). PLA is produced by chemical synthesis using renewable bio-based monomers [66]. Feedstock such as corn sugar is fermented to produce lactic acid, the monomer, and then this lactic acid is polymerized to produce PLA [26; 67]. In comparison to

other bio-polymers, PLA is considered to be favourable and versatile because of its properties such as transparency, bio-compatibility, good sealability and high mechanical strength [68]. PLA is used in biomedical applications such as orthopaedics, drug delivery and scaffold, also in packaging, fibres and paper coating [43; 68-71]. PLA has a low degradation rate compared to other bio-polymers and also is a typical brittle material; this limits its range of use [72; 73]. This limitation can be addressed and other properties of PLA modified by copolymerization of lactide and another monomer.

2.2.3 Types of biomass

Different kinds of thermoplastic material have been developed directly by extracting polymers from biomass with the intention of addressing the depletion of fossil resource [74]. Biomass polymers are produced by modification of natural polymers through extrusion, casting, compression and injection moulding. Biomass-based polymers are classified into two types; polysaccharides and proteins. Polysaccharides are made up of only one or a few different monomers and are carbohydrates consisting of long chains of mono-saccharides units bonded by glucosidic linkage while proteins are made up of several different amino acids. This section will first deal with polysaccharides. There are three basic polysaccharide biomasses and they are starch, cellulose and chitin.

2.2.3.1 Cellulose

This is the most abundant polysaccharide in nature consisting of β (1→ 4) linked glucose units and is the main structural component in cell walls of plants [69]. Glucose is a triol containing one primary and two secondary alcoholic hydroxyl groups [54]. Cellulose is a cheap raw material but its hydrophilic nature, crystalline structure and insolubility are limiting factors making it difficult to use as a thermoplastic, although a lot of cellulose derivatives are produced commercially [75]. Cellulose is poorly soluble in common solvents and is not melt-processible in its native state and is therefore converted to its organic ester before processing [69; 76]. The most commonly used cellulose derivatives are cellulose acetate, cellulose acetate propionate, carboxymethylcellulose and cellulose acetate butyrate [76]. These cellulose derivatives have wide usage in several areas such as food, bio-medicals and cosmetic applications [77].

2.2.3.2 Starch

This is another glucose based polysaccharide, but consists of α (1→ 4) linked glucose unit. The structure and composition of starch depends on its origin and it is composed of a mixture of linear amylose and highly branched amylopectin. Starch is one of the principal polysaccharides of interest for polymer material production [78]. Starch is a widely available raw material as it is the main storage supply molecules in a wide variety of plant species. Starch granules can easily be isolated from a number of different crops such as corn, wheat, potato and rice. Native starch is chemically and physically modified with the exception of filler application to produce reinforced plastics [79]. Starch is processed into thermoplastic using injection moulding and extrusion with an addition of plasticizers which disrupt hydrogen bonds between starch molecules, changing semi-crystalline starch granules into a homogeneous material that can be processed below its decomposition temperature [69]. Depending on the plasticizer level and starch source, a wide range of properties may be obtained [80].

2.2.3.3 Chitin

Chitin is a structural biopolymer found mostly in the exoskeleton of arthropods such as crustaceans (crabs, lobsters and shrimps) and insects. Chitin is the second most abundant polysaccharide in nature and its structure and function is comparable to that of cellulose in plants. Just as cellulose is produced in plant's cell wall, chitin is produced in insect and crustacean shells; making them related polysaccharides which provide strong unbroken structures in both plants and animals respectively [81]. Chitin consists of 2- acetamido-2-deoxy-B-O-glucose linked through a β (1 —> 4) and resembles cellulose with hydroxyl at position C-2 replaced by an acetamido group [26; 82]. Chitosan is produced through deacetylisation processing using chitin [25; 26; 82], it is used in purification of water to absorb greases, toxic substances, metals and oil. It is also used in medicine to treat obesity, high cholesterol and Crohn's disease. Chitin is used as a chelating agent in photography due to its resistance to abrasion and in cosmetics as artificial skin, but its applications are limited due to its reactivity and processability [82-84].

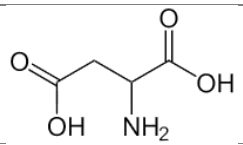
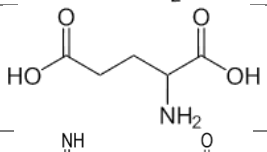
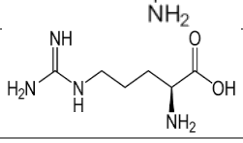
2.3 Proteins as materials

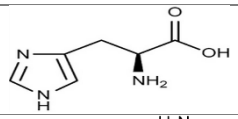
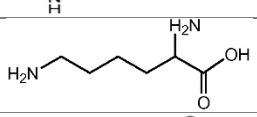
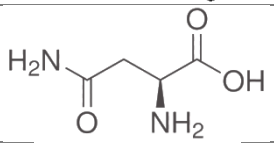
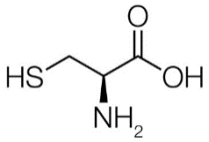
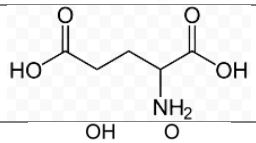
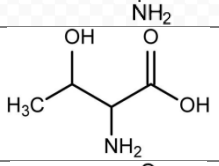
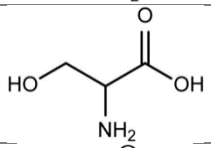
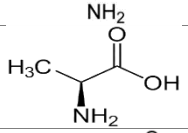
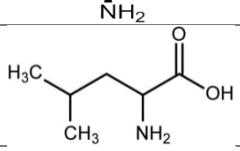
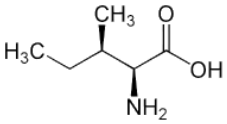
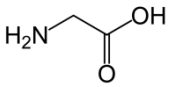
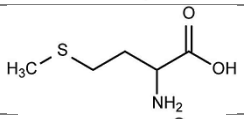
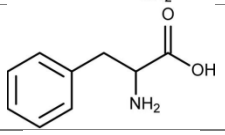
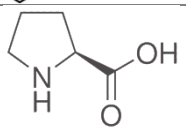
Proteins are natural polymers of α-amino acids from which many biological materials are derived. Protein based materials can be said to be three dimensional, macromolecular networks stabilized and strengthened by hydrogen bonds, hydrophobic interaction and covalent crosslinks [14]. Proteins have diverse and complex structure and function. They are made from 20 different amino acids which vary in the structure of their side chains. Different proteins have different combinations of these in different orders. Proteins are important renewable resources commonly obtained from both plants and animals. Examples are wheat, corn gluten meal, cotton, soy, pea, potato for plant proteins and casein collagen, keratin for animal protein [54; 85]. Proteins are used in the food industry, in medicine, for fibres and many biological processes including catalysis which is essential to life [2; 86]. Proteins have found used in early years as polymeric product such as blood-based wood glue and caseinate buttons but these uses were basically for thermosetting resins. In recent years attention has shifted to thermoplastic-based protein which can be extruded and injection moulded.

2.3.1 Protein structure

As mentioned earlier, proteins are complex polymers having 20 amino acid monomers with different R-group or side chains which can either be polar, non-polar, acidic or basic [54; 86] Table 2-1 shows the structure, name , side group and molecular weight of 20 amino acids found in protein

Table 2-1: Structures of the 20 amino acids found in protein [54]

R- CHAIN	NAME	STRUCTURE	Mw (g/mol)
Acidic amino acids (ionized polar)	Aspartic acid		133
	Glutamic		147
	Arginine		174

Basic amino acids (non-ionized)	Histidine		155
	Lysine		146
Polar Amino acids	Asparagine		132
	Cysteine		121
	Glutamine		146
	Threonine		119
	Serine		105
Non polar Amino acids	Alanine		89
	Leucine		131
	Isoleucine		131
	Glycine		75
	Methionine		149
	Phenylalanine		165
	Proline		115

In Protein, the amino acids' amine and carboxylic acid groups are joined together by a peptide bond (amide) creating polypeptide chains. Some proteins are

comprised of a single polypeptide although the majority consist of two or more aggregated polypeptides. The polypeptide can be folded into three structural levels such as secondary, tertiary and quaternary structure. Proteins can be classified according to the shape and solubility as fibrous, globular or membrane [87].The way in which peptide chains are folded in space affects the properties as well as their amino acid components and their protein chain bonding sequence.

The protein primary structure refers to the different amino acids that make up a protein and the order of sequence in which they are joined by peptide bonds. The Secondary structure refers to localised coiling and bending of the polypeptide chain due to hydrogen bonding between a carbonyl group of one amino acid and an amine of another group. The most common stable secondary structures of protein are the α-helix and β-sheet [45; 88]. Most proteins do not acquire a completely uniform conformation of either of these structures and the way their chains fold and bend into more complex three dimensional shapes is referred to as tertiary structure of a protein. Quaternary structure is the way multiple folded polypeptide chains aggregate into a larger structure.

The final folded conformation of a protein molecule is influenced by a number of intermolecular interactions. These include hydrogen bonding of amide carbonyl groups to NH groups, ionic interactions occurring between positively charged side chain and negatively charged side chains, repulsion of hydrophobic regions to minimize interaction of the non-polar amino acids with water and covalent disulphide linkages between cysteine residues. A protein is said to be denatured when disruption occurs to the secondary, tertiary or quaternary structure and degraded when the primary structure is broken.

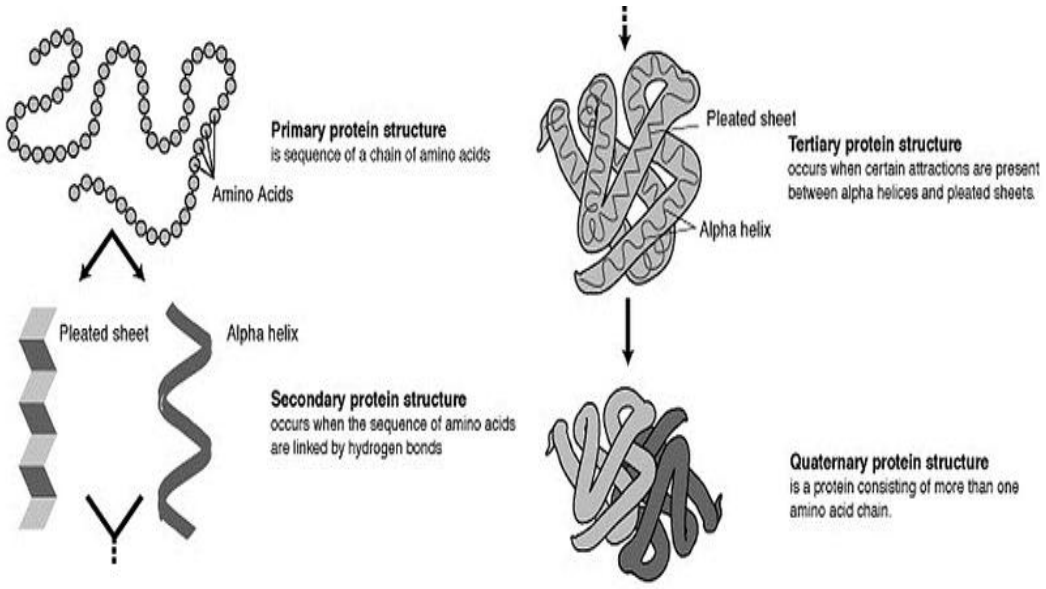


Figure 2-2: Protein structures [89]

2.4 Types of proteinous polymers, uses and limitations

There are different sources and types of protein polymers but proteins used for manufacturing of materials are those found in greater quantities [90]. Some common sources of protein polymers are animal proteins such as collagen, keratin, casein and bloodmeal, plants such as corn gluten meal (zein), wheat gluten meal, sunflower and soybean, and bacterial sources such as dehydrogenase, chymotrypsin and fumarase.

2.4.1 Gelatin

This is a high molecular weight, translucent, colourless and brittle material which is derived from the chemical denaturation of collagen. It is made up of 19 amino acids and it is soluble in water. It is an animal based protein which is naturally abundant in the cornea, cartilage, bone, blood vessels, gut, intervertebral disc, tendon, ligament and skin [91]. Gelatin is a mixture of α-chains (a single polymer chain), β-chains (two α-chains that are covalently cross-linked) and γ-chains (three α-chains that are covalently cross-linked) [92]. There are two different types of gelatin which can be produced based on the method used to pre-treat the collagen. Type A gelatin is produced from acidic pre-treatment and this processing method does not affect the amide group a great deal while type B gelatin is produced from alkaline pre-treatment which targets the amide group of asparagine and glutamine hydrolysing them into carboxyl group, as a result

converting many of these residues to aspartate and glutamate [91; 93]. Gelatin is commonly used in biomedical, adhesive and pharmaceutical applications because of its degradability and biocompatibility.

2.4.1.1 Casein

Casein is an animal protein which is abundant in mammalian milk, it makes up about 80% of protein in cow milk and about 65% of protein in human milk [94]. Casein has an open random coil structure. When acid precipitated casein is treated with alkali solution, casinate is produced. Casein is found useful across a wide range of applications such as food additive, adhesives, a binder for safety matches, biomedical areas and controlled releases, it is also a major component of cheese [91].

2.4.1.2 Keratin

Keratin has two main agricultural sources which are wool and feathers. Keratin has a large amount of cysteine amino acid compare to other protein polymer sources. Cysteine is a sulphur containing amino acid which can form sulphur-sulphur (disulphide) bonds with other cysteine molecules in the same or different polypeptide chain. Intermolecular cysteine bonds, crystallinity and hydrogen bonding give keratin high strength and stiffness but cysteine bonds create a hindrance in the melt processing of keratin. This can be modified using a reducing agent to break the cysteine bonds [95].

2.4.1.3 Corn proteins

Proteins form about 9% of dry corn weight and these are mainly zein, glutelin, albumins and globulins. Zein is one of the main components in the co-products from both wet milling and dry milling processing of corn to produce ethanol [96]. Zein is a highly hydrophobic protein but it is soluble in alcohol. Zein is one of the few plant proteins that are been extracted in a relatively pure form. The molecular weight of zein is between 9600g.mol^{-1} and 44000g.mol^{-1}. There are four different types of zein based on its differential solubility, they are α, β, γ and δ [87]. Zein has been used mainly in fibre industries since the early 19th century, but such fibres are made using wet spinning technology. However, extrusion techniques are also used to process zein at a moderate level [97-100].

2.4.1.4 Wheat gluten

Wheat gluten is a low cost, high quality protein by-product from the starch fabrication and bio-ethanol industries. It contains two main groups of protein which are gliadin and glutenin. Gliadin is made of single chain polypeptide which is held together by intra-molecular disulphide bond [78]. Glutenin contains gliadin like sub units which are held together by intermolecular disulphide bonds in a large aggregate. Both groups are typically present in equal amounts and have comparable amino acid composition with high concentration in glutamine and proline. Wheat gluten is widely used in packaging, films, coatings on paperboard and material for the insulation of houses because of its excellent viscoelastic, thermoplastic, film foaming and gas barrier properties [101-103]. The properties of gluten are greatly influenced by the size distribution, amount and macromolecular structure of glutenins and gliadins.

2.4.1.5 Soy protein

Soy protein is a by-product from soybeans produced through an extraction process and it is made of dehulled, defatted soybean meal. Soy protein consists of a mixture of albumins and globulins. On a dry basis, soybeans contain 38% to 42 % crude protein, 16% to 20% triglycerides and about 33% carbohydrate [78]. Dehulled and defatted soybeans are processed commercially into three types of product such as soy flour, concentrate and isolate. Soy protein is a low cost material and is abundant in nature. Soy protein was the first developed agricultural biopolymer that was used in the production of moulded parts. Ford manufactured some of its car parts in 1930s using a phenol formaldehyde and soybean flour blend [104], but because of cost and availability of a cheaper synthetic plastic, the use of this formulation was withdrawn.

2.4.1.6 Bloodmeal

Bloodmeal is a low value by-product of meat processing industry which is produced by drying of blood at temperatures above 100 ºC to remove water and destroy any pathogenic organisms resulting to a denatured protein. To produce bloodmeal, blood is collected from slaughter houses and the collected blood is filtered to remove fragments, coagulated using stream injection at 90 ºC [105] and centrifuged to separate the coagulated blood which is then dried between 100 ºC to 175 ºC using a rotating drum. The resultant bloodmeal is made into powder using a hammer mill [41]. Bloodmeal contains about 90%wt protein [13] but this protein is not collected in hygienic way fit for human consumption.

Novatein Thermoplastic protein (NTP) is a protein-based thermoplastic material produced from bloodmeal. NTP is produced using sodium sulfite, sodium dodecyl sulfate and urea or tri-ethylene glycol in combination with water. These additives are used to provide sufficient disruption to inter and intra molecular interaction between polymer chains to ensure successful production of thermoplastic protein. After production of NTP, it is then extruded and granulated for further processing. The pictures below are the various stages of NTP production Figure 2-3.

I II III IV

Figure 2-3: Various stages of NTP processing

I. Pre-extruded NTP

II. Extruded NTP

III. Granulated NTP

IV. Injection moulded NTP pieces

2.5 Protein polymer processing

2.5.1 Wet vs Dry processing

There are two main methods of processing materials from proteins; these are the wet and dry processes [106]. There are two types of wet processing; they are solution casting and reaction medium. Wet processing is based on first dispersing and solubilizing the protein in a solvent medium and then removing the solvent by drying or solvent reaction [1; 45]. Zein films have been processed through casting from acetone and water mixture. More solvents that can be used to process proteins are methanol, acetone and isopropyl alcohol. The dry process is based on mixing the proteins and appropriate additives under relatively lower moisture conditions (about 30% or less) followed by thermo-mechanical shaping using hot pressing, compression moulding or injection moulding. The use of dry processing for protein polymers is very low compared to synthetic polymers. This poses a big challenge in commerlization of protein polymers.

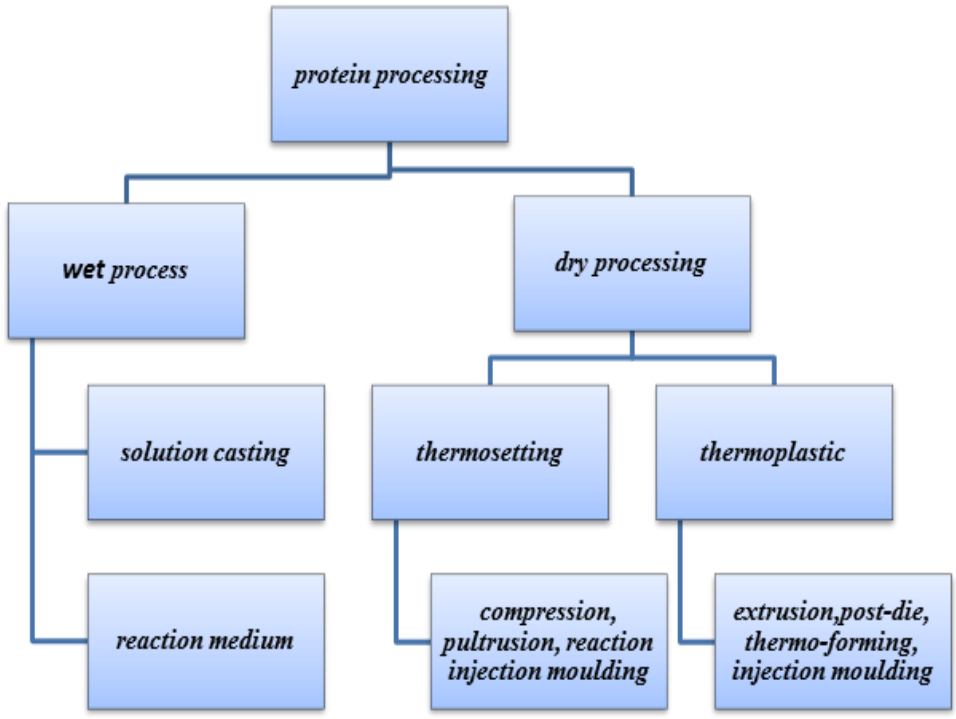


Figure 2-4: Methods of protein processing.

The dry process can be further broken down into two classes of processing (Figure 2-4), Thermosetting processing involves two process stages. In the first

stage, the protein polymer is prepared and second stage is using a desired mould shape to cross-link the prepared polymer. Material produced using this process is irreversibly cross-linked. Curing occurs under pressure when thermosetting a material either during heating and/or addition of a catalyst to form a cross-link [107]. Thermoplastic Processing involves melting a polymer using heat from radiation, conduction or mechanical work to overcome inter-chain reactions; allowing it to be shaped under pressure before cooling the material to re-introduce chain interactions to maintain the newly formed shape. Processing proteins thermo-plastically requires non-covalent interactions to be overcome without damage to the covalent linkages in the amino acid chain. These non-covalent interactions, for example, hydrogen bonding in protein secondary structures, contribute fixation of the formed shape [108].

In the past, all these methods have been employed to process protein-based biopolymers into potentially useful materials, although the majority of research has been focused on casting and compression. In recent years, attention has shifted to extrusion processing. For integration into common plastic processing routes, wider acceptance and commercialization of protein polymers, it is paramount that preferable plastic-processing techniques such as extrusion and injection moulding is utilize [33; 109]. Therefore, this thesis is concerned with dry processing of proteins into thermoplastic by extrusion and injection moulding.

2.5.2 Extrusion

In extrusion, screws convey thermoplastic material through a heated barrel inducing shear forces and increasing pressure to form a continuous viscous fluid along the barrel before it is pushed through a die where cooling and solidification occurs. Such an extrusion process implies elevated temperatures are used [110]. In most processes, the heated barrel contains several independently controlled heating zones with allowing different heating profiles for different materials. During extrusion, considerable amounts of heat and shear are induced into a polymer material which may result in degradation having a negative effect on the properties of the finished product [111; 112] therefore it is very important that a suitable temperature profile is used. Generally, temperature is gradually increased from feed zone to the die [113].

There are two common types of extruders that have been used to produce protein-based bio-polymers and they are single and twin screw extruders. Single screw uses the frictional forces between the screw and the barrel to force the material towards the die. The twin screw utilizes intermeshing screws to act as a positive displacement providing better mixing conditions more suitable for bio-plastic compounding [14]. Different sizes of extruder with different L over D and screw configuration can be used for the processing of protein.

Extrusion technology is effective and efficient and is used widely for the processing and compounding of polymers and biopolymers for various applications [114-119]. However, extrusion processing can alter the properties of proteins [14; 120]. It is therefore important that parameters which can be adjusted to control the extrusion process be understood. These include the temperature profile along the barrel, the material composition such as the initial water content and other additives, the screw-speed and the feed-rate. These will all affect the torque, pressure, specific mechanical energy (SME) and actual melt temperature that the polymer is subject to.

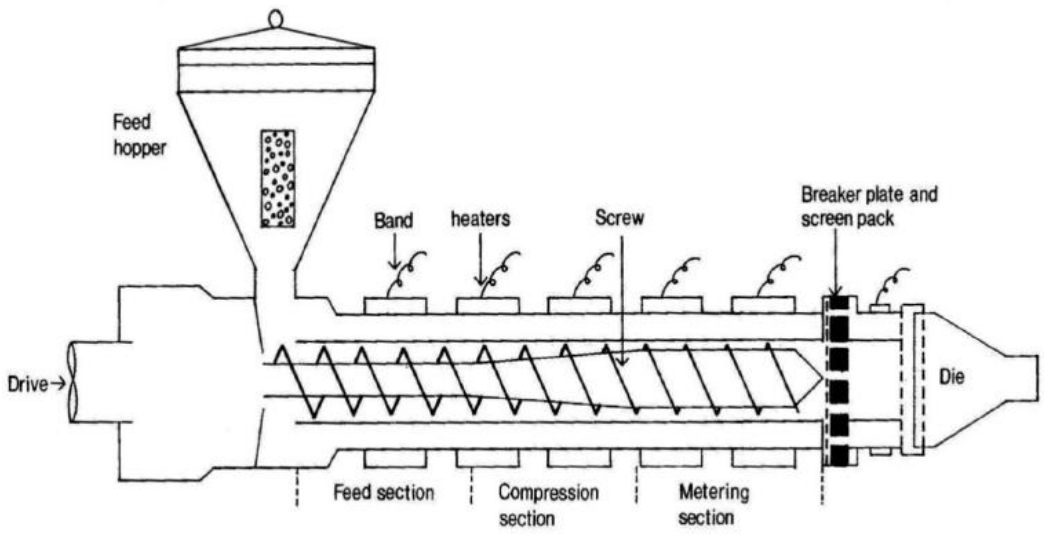


Figure 2-5: Extruder profile [110]

2.5.2.1 Effect of extrusion temperature on proteins

The transition of a semi-crystalline polymer's viscoelastic behaviour through different states from glassy, to leathery, rubbery, rubbery flow and then the

viscous state is a function of temperature and additives [1]. Polymer extrusion depends on this transition, and proteins should typically be processed at a temperature similar to the rubbery flow region. This is the material's softening point and is normally 40ºC above the glass transition temperature for protein [14; 121].

For proteins still in the native state, denaturation is also a very important phenomenon which oftentimes leads to structural changes thereby causing changes in material's processability. Denaturing is the process of protein transformation into a non-native conformation (transforming the secondary, tertiary or quaternary structures). Formation of a continuous consolidated material requires denaturation, which can be induced by thermal or chemical means [108]. The amino acid sequence of a protein, processing method and type of additives used determine the denaturation temperature of proteins. Denaturation temperature also strongly depends on moisture content [122].

Cross-linking and plasticization are also very important in processing of proteins. An appropriate amount of heat supplied during extrusion to achieve the above transitions is of great importance for proteins, but excessive temperatures can induce aggregation which will result in physical cross-linking thereby reducing the melt flow-rate and affecting mechanical properties. A balance must be maintained between protein de-aggregation as a result of mechanical stress and aggregation due to heating [16; 117; 123]. Several researchers have explored the relationship of temperature to extrusion of protein polymers. In feather keratin blended with glycerol, water and sodium sulfite, low extrusion temperatures resulted in the polymer softening just before die leading to high viscosity, whereas high extrusion temperature resulted in a lower viscosity and the material softening earlier inside the barrel [117]. In extrusion of Zein, structural changes slowly occurred over the range from 100 ºC to 220 ºC and tensile properties began to deteriorate above 140 ºC. Extrusion above 220 ºC yielded a material that could not be moulded [32]. When a starch and D-limonene mix encapsulated in milk protein was extruded, the temperature, along with the screw-speed and capsule level, had significant effects on processing and resulting properties of the product [124].

Thermoplastic extrusion of protein requires that most of the extrusion reactions would occur immediately before and after exiting the die [2; 125]. Therefore, a temperature profile that induces formation of melt early in the barrel is not desirable as it could cause excessive aggregation of protein in the barrel.

2.5.2.2 Material composition

Proteins have a large number of different functional groups resulting in an array of possible chain interactions that can cause issues during extrusion if temperature alone is used to promote processing [14]. As mentioned earlier, extrusion processing results in protein structural re-arrangement and formation of new interactions. The formation of a thermoplastic material can be disrupted by the formation of covalent cross-linkages during extrusion. Therefore, to produce a thermoplastic from protein these cross-linking and other non-covalent interactions have to be controlled and this can be controlled with the use of additives.

As discussed above, proteins need to be processed at above their glass transition temperature (T_g). For successful extrusion processing, the T_g needs to be lowered to reduce the onset of rubbery flow and this can be achieved by the use of plasticizers [14]. Plasticizers and protein materials are mixed together prior to thermo-processing to form a highly viscous melt material. The amount and type of plasticizer influences the required melt temperature, the equilibrium moisture content and viscosity. The diffusion of a plasticizer into a protein depends mainly on the type of plasticizer used and the amino acids contained in the protein itself [126; 127]. Increasing the amount of plasticizer will lower the melt temperature and viscosity of the blend [128]. A good plasticizer will reduce intermolecular interaction between polymer chains, increase the flexibility of a moulded product and impart greatly on extensibility of the material [129; 130]. Protein's hydrophilic hydroxyl groups are believed to be their active sites for plasticizers creating hydrogen bonding between polymer/plasticizer, interfering with polymer/polymer interaction and increasing chain mobility [131; 132].

Plasticizers induce movement between polymer chains by reducing interactions between adjacent chains and increasing its free volume [133]. Many theories have accounted for the mechanism of how plasticizer works [134] but there are two basic theories on mechanism of how plasticizers work; Lubricity theory, where it

is assumed plasticizers act as a lubricant facilitating movement between macromolecules and gel theory which considers the disruption of protein-protein interactions. Gel theory is more typically used to describe the mechanism of how plasticizers work [129]. Gel theory states that the study of plasticization is the study of ways to increase free volume and this was useful in clarifying the lowering of glass transition by plasticizers [126]. Free volume is temperature dependent because of the increased molecular motion and it is categorized as the space between polymer molecules. Increasing free volume enables increased movement of the protein chain thereby decreasing the glass transition temperature [128].

Not only processing, but also the resulting mechanical and thermal properties of protein polymer are influenced by the addition of plasticizers through the alteration of hydrogen bonding, hydrophobic interaction, ionic bonding and van der Waal's forces. If these short range interactions are strong enough then the strength of the material may still be high [106; 131].

2.5.2.3 Common additives

The most common additives used to produce thermoplastic proteins which can be injection moulded and extruded are plasticisers, including water and other less volatile molecules. Other additives including urea, SDS, SS are sometimes also used. These additives are chosen because of their abilities to interact with protein considering that protein has a large amount of possible amino acid composition.

2.5.2.3.1 Water

This is a good and natural plasticizer for protein; and also acts as a dispersion medium and solvent promoting the functions of other plasticizers [135]. Water influences mechanical properties of protein polymers by increasing elongation and reducing strength and toughness [1; 14; 16; 131; 136]. The diffusion activity of water increases in the rubbery state as water and other plasticizers reduces the amount of polymer-polymer interactions increasing free volume between chains [131; 137; 138]. This accounts for the distinct drawback of water activity on protein polymer over time. Loss of water from protein polymers results to loss of processability and reduction in mechanical properties. Moisture loss during and

after processing, could result in a brittle material and also the loss of material functionality [13; 117; 139].

Moisture content can have a very large effect on the glass transition temperature of proteins. In soy protein, consisting of the globulins conglycinin and the T_g of the Conglycinin (7s) fraction ranged from 114 °C to 67 °C with moisture content between 0% and 35% while the glass transition of the glycinin (11s) fraction ranged from 160 °C to -17 °C with moisture content between 0 and 40% [140]. When soy protein is converted to a plastic sheet, with an increase in processing moisture content both tensile strength and modulus decreased rapidly and there was a greater increase in elongation [15]. Paetau and Ink investigated the effect of preparation and processing on mechanical properties and water absorption of soy isolate and soy concentration, they reported that moulding temperature and initial moisture content significantly affected the tensile strength of soy protein [141].

Garcia and Onwulata investigated the effect of water plasticization on extrudate made from meat, bone meal and Sodium caseinate; they reported that the influence of moisture content on the glass transition temperature reflected the plasticization of this material by water [142].

Water has low boiling temperature and protein processing requires high temperatures resulting in water evaporation before reaching the required viscosity level for successful thermoplastic processing of protein. Therefore, hydrophilic additives which are less volatile such as tri-ethylene glycol and glycerol which can interact with protein polar group are used in combination with water.

2.5.2.3.2 Other plasticisers

Polyols such as glycerine, ethylene glycol, polypropylene glycol, propylene glycol, glycerol and diethylene glycol are commonly used as plasticisers for proteins [143-148]. Jane and Wang et al investigated the effect of glycerol and water on soy protein-based thermoplastic at a fixed moulding temperature, they reported that both glycerol and water significantly increased flexibility but greatly decreased the tensile strength of the soy-based thermoplastic [47]. A study by Di Gioia and Guilbert on corn protein-based thermoplastic with wide range of plasticizers suggested that polar plasticizer interacts easily with accessible polar

amino acid while amphiphilic plasticizers can interact with more difficult to access non-polar zones buried with the protein [126].

TEG is a commonly used plasticizer in the processing of biopolymers. It is an amphiphilic plasticizer containing hydrophobic groups and this makes it slower to percolate than water. The use of TEG as a plasticizer is to reduce plasticizer evaporation and migration during and after processing and improve storage stability of bio-plastic because of its hygroscopic nature. As TEG has both polar and non-polar regions it easily interacts with more difficult to access polar zone in the polymer. TEG has a high boiling point (290 ºC). Plasticizers, therefore, help to reduce protein degradation due to high processing temperature.

Another commonly used plasticizer for the production of protein polymers is urea [149-151]. It is used to denature protein molecule [14; 145; 149]. It prevents protein-protein interaction through the formation of hydrogen bonds with amino acids. Research has shown that urea leaves residue overtime on produced piece [152] showing that protein-protein interactions are stronger than urea/protein interactions resulting to the leaching of urea out of the material. Urea was found to reduce the evaporation of plasticizer during processing of soybean and wheat gluten protein polymers but its evaporation overtime results to a brittle material therefore requiring less volatile plasticizer [16; 152; 153].

2.5.2.3.3 Other additives

Reducing agents such as sodium sulphite are to disrupt chemical crosslinks through the reduction of disulphide, improving processability. Disulfide bonds hinder processing by locking the protein chains preventing viscous flow formation. Disulfide bonds are heat resistant but sodium sulfite is used to reduce formation of sodium disulfonate derivative [2; 117]. Conversely, sometimes deliberate crosslinking agents may be used. Galietta and Giovani et al investigated on the effect of both plasticizers and cross linking agents on mechanical and thermo-mechanical properties of film-based whey protein. Increased plasticizer increased the percentage material solubility in water but decreased the mechanical resistance, Young's Modulus and glass transition temperature of whey protein [154]. However, cross-linking agents improved mechanical properties, insolubility behaviour and increased glass transition temperature.

Surfactants may also be used in processing thermoplastic protein to disrupt hydrophobic interactions in order to facilitate the formation of new interactions during extrusion. One such surfactant is SDS, and anionic surfactant commonly used to denature protein. SDS also acts as a plasticizer, disrupting hydrogen bonding and increasing repulsion between adjacent chains but this is dependent on amount of SDS and type of protein used [155]. SDS interacted electrostatically with the positively charged groups on the modified soy protein isolates surface and then bonded hydrophobically to non-polar groups through the dodecyl chain [156].

2.5.3 Injection Moulding

Injection moulding forces a molten polymer into a closed mould of desired shape under high pressure. It is the most widely used technique to shape thermoplastic material [107]. Granulated material is fed through a hopper to the machine and the material enters the barrel by gravity through the feed zone. On entering into the barrel the extrudate is heated to the desired temperature and is injected into the mould by a reciprocating screw. The material is ejected after cooling in the mould [41; 157; 158]. The injection moulder has two mould plates, a fixed plate and a moving plate. The machine has four zones; feed zone, heating zone, injection zone and moulding.

The core of an injection moulder is essentially a single screw extruder. Extrusion and injection moulding processes therefore relate to each other, however, they differ as extrusion is a continuous process. If extrusion of a material fails then the injection processing of the material will likely not be possible therefore it is right to say that injection moulding depends on the successful extrusion of a material. Previous research has reported on the injection moulding performance and mechanical properties of Novatein® [109]. Figure 2-6 shows the outlines of a typical injection moulder.

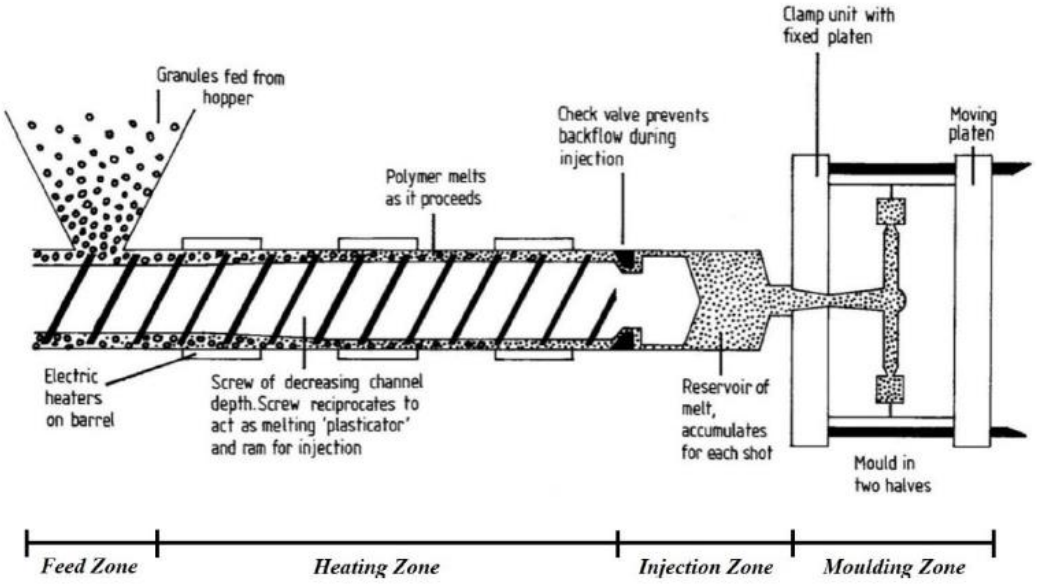


Figure 2-6: Injection moulder outline [110]

2.6 Processing of Novatein

Bloodmeal has a glass transition temperature (Tg) of 220 ºC, which is very high for extrusion processing of the material without excessive degradation which is why additives such as Water, SS, SDS, and TEG are used to reduce the Tg to about -80 ºC for successful thermoplastic processing [27]. Bloodmeal and NTP both retain some protein secondary structure which affects properties and processability in a manner resembling crystalline regions in synthetic semi-crystalline polymers [159]. According to spatially resolved FTIR analysis, bloodmeal particles consist of an amorphous core and β-sheet rich edges with α-helices evenly distributed throughout [28].

The early formulation used to process bloodmeal into thermoplastic utilized Sodium Sulfite (SS) to reduce covalent cross-links through cleaving disulfide bridges, Sodium Dodecyl Sulfate (SDS) and Urea to reduce inter and intra molecular forces, allowing plasticization and the extrusion temperature used was 100 °C for the barrel regions and 120°C for the die region [2]. The current formulation used in this thesis is different from previously published NTP formulations as there is need to produce a material that can be stiffer immediately after injection moulding to help improve mould release and also have reduced

plasticizer evaporation after conditioning, which was a problem encountered with early formulations. Lisa showed that there were statistically significant factors affecting loss of water during processing and conditioning of a bloodmeal material containing SS and water but material having SS, water and Urea has less water loss. This showed that Urea helped to reduce plasticizer evaporation by creating strong hydrogen bonding with the amino acids disrupting protein/protein interactions but protein/Urea interactions are weaker than protein/protein interactions thereby forcing Urea to leach out of moulded parts over a period of time leaving a white residue on material's surface [16; 152] resulting to a brittle material. Polymers produced with Urea show less flexibility and less extensibility compared to polymers produced using alternative plasticizers. Bier J, showed that TEG and water can be used to plasticise bloodmeal without Urea, TEG was disrupting protein secondary structure, helped to reduce plasticizer evaporation as samples with higher ration of TEG to water retained higher level of plasticisation after conditioning and higher plasticiser content increased chain mobility allowing chain rearrangement to the applied force [19], this was same reasons for using Urea [13]. Amphiphilic plasticisers having both polar and non-polar regions have shown to be more effective compared on a molar or H-bonding capability basis (see Table 2-2) to polar molecules such as Urea or water alone and TEG is one of such plasticisers Therefore a new formulation that favours TEG was used.

Water, urea and TEG were used but it resulted in an overly flexible part immediately after molding causing difficulties in part release. It was later found that TEG and water can be used in the absence of urea as TEG functions as plasticizer disrupting protein-protein bond just as urea but also helped to reduce plasticizer evaporation after processing favoring the use of TEG and Water over Urea.

NTP has been successfully extruded and injection moulded into abattoir rectal plug known as Port Jackson™ and it easily breaks down during rendering because of its hydrophilic nature. Although NTP has been successfully extruded and injection moulded using water in combination with other additives, during extrusion and storage, water evaporates from NTP resulting in a very brittle material with low mechanical property. This limits its potential application. Research has been carried out to understand the properties of NTP, resolve its

limiting factors such as difficulty of release during injection moulding, water sensitivity and storage instability [18; 21; 27; 133; 159; 160]. In order to select appropriate reinforcement materials and optimal processing windows that would be used to improve mechanical property, there is need to understand the effect of processing conditions such as extrusion temperature, moisture content, screw speed and type of plasticizer used on the property of NTP.

Many research works have been carried out on Novatein utilizing extrusion as processing step [2; 14; 19; 133; 153; 161] but only few have considered the effect of moisture content during extrusion on processing and properties [13; 17; 19; 161].

The selection of effect of water over the other added additives is because water contributes greatly to the amount of moisture present in a protein material compared to the other additives therefore, water will be varied to investigate the effect of moisture content on NTP. Also, studies have shown that extrusion can alter the properties of NTP [1; 14; 133; 159; 161]. However, these studies did not show how processing conditions such as extrusion temperature changed or affected the mechanical properties of NTP during extrusion. Improved understanding of how the mechanical properties are affected during extrusion process at various moisture contents and extrusion temperatures will be of utmost importance in defining the NTP processing limits.

Property	Water	Urea	TEG
molar mass [g·mol^{-1}]	18	60.6	150.17
melting point [°C]	0	133	−7
boiling point [°C]	100	decomposition	285
H-bonding hydrogen [mol·mol^{-1}]	2	4	2
lone electron pairs [mol·mol^{-1}]	2	4	8
H-bonding hydrogen [mol·g^{-1}]	0.11	0.07	0.01
lone electron pairs [mol·g^{-1}]	0.11	0.07	0.05
hydrophilic groups [%]	100	100	44

Table 2-2: Comparison of hydrogen bonding site by mole and mass for water, Urea and TEG [19].

2.7 Conclusions

Relevant literature on the extrusion of protein showed that successful thermoplastic processing of protein is only possible within a small processing window. Extrusion processing applies considerable amount of heat and shear into a protein material to form polymer melt which requires sufficient chain mobility. To ensure sufficient chain mobility during extrusion processing, the glass transition temperature of protein needs to be lowered to reduce the onset of rubbery flow and this can be achieved by the use of compatible plasticizer with low molecular mass and low volatility..

Optimization of mechanical property of protein thermoplastic is highly dependent on the extrusion processing parameters such as extrusion temperature, initial moisture content, SME, torque, pressure and screw-speed.

High extrusion temperature results in low viscosity and material softening earlier in the barrel which is not desirable, while low extrusion temperature results in polymer softening just before die leading to high viscosity. Also high extrusion temperature leads to deterioration of material's tensile properties.

To prevent excessive aggregation of protein in the barrel, temperature profile that induces formation of melt early in the barrel should be avoided as extrusion reaction is desirable immediately before and after exiting the die.

Preventing excessive formation of cross-linking after extrusion could stabilize protein structure resulting to improved mechanical properties [2; 162].

Water acts as plasticizer reducing Tg of protein material through disruption of protein/protein interaction presenting a processible material. Water influences mechanical properties of protein polymer by increasing elongation and reducing strength and toughness. Loss of moisture during and after processing results in a brittle material and loss of material functionality.

Understanding the effect of extrusion temperature and initial water content on the properties of NTP would help in optimizing the best processing window for successful production of protein thermoplastic.

3 Equipment and Methods

3.1 Experimental Design

This section deals with the factorial design used in the processing and testing of NTP. Four formulations were used varying the amount of water content at 30, 35, 40, 45 pph_{BM} and also varying die temperature at 120 °C, 130 °C, 140 °C and 150 °C, with corresponding increase in preceding zones, resulting in 16 experiments (Table 3-1).

Table 3-1: Table of experiments done showing factorial design

	DIE TEMPERATURE			
FORMULATION	120 °C	130 °C	140 °C	150 °C
1	EXP. 1	EXP. 2	EXP. 3	EXP.4
2	EXP.5	EXP. 6	EXP. 7	EXP. 8
3	EXP. 9	EXP. 10	EXP. 11	EXP. 12
4	EXP. 13	EXP. 14	EXP. 15	EXP. 16

There were several stages of sample preparation, processing and analysis done starting from the feedstock (bloodmeal) to conditioned thermoplastic protein as shown in figure 3.1 The samples prepared for mechanical testing were produced by injection moulding into ASTM [163] standard tensile specimens bars and samples for thermal and physcio-chemical testing were obtained at each processing step; pre-extrusion, extrusion, injection moulding and conditioning. After injection moulding, both samples conditioned in standard conditions and unconditioned samples were analysed to investigate both how the material will behave in real life use and how it compares under standardised conditions. Changes in moisture content were monitored between each processing steps; pre-extrusion, extrusion, injection moulding and conditioning.

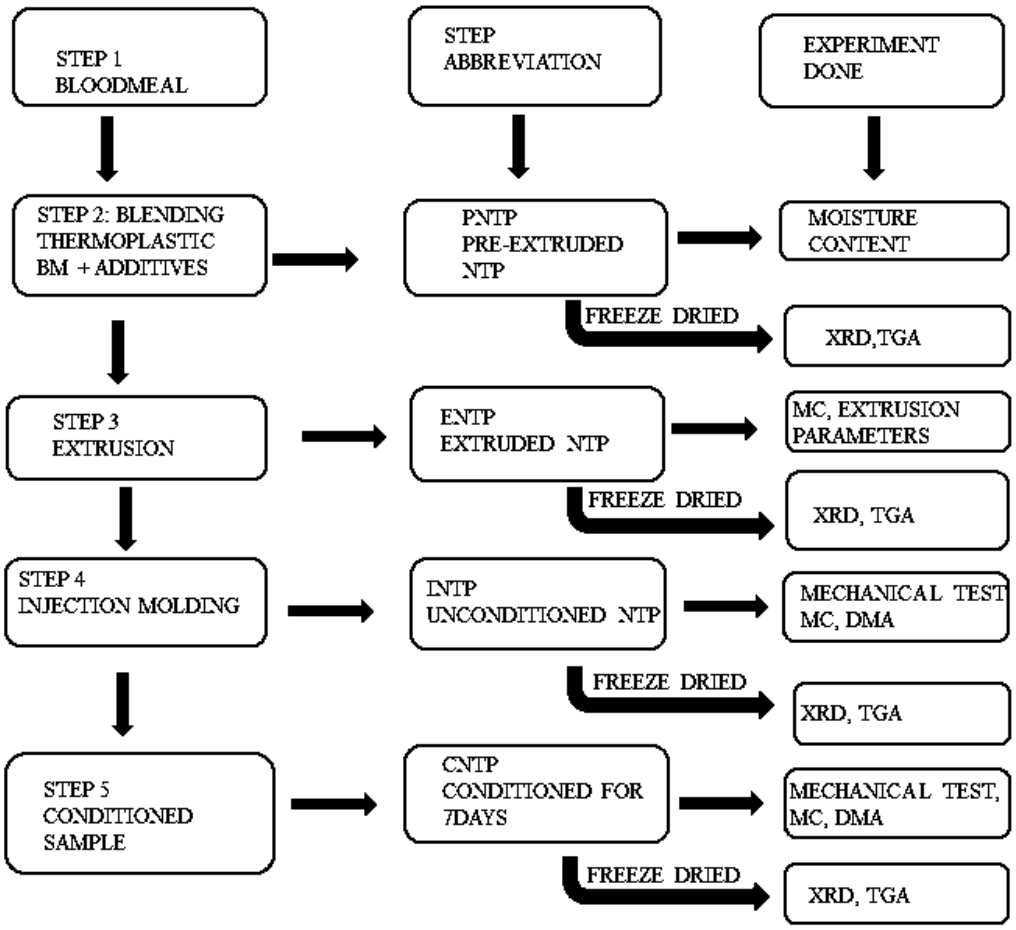


Figure 3-1: lay-out of experimental design

3.2 Material

Bloodmeal with p = 1300kg/^{m3} was obtained from Wallace Corporation Limited, New Zealand. It was sieved through a 700µm micro sieve. Technical grade Sodium Dodecyl Sulphate (SDS) and Tri Ethylene Glycol (TEG) were obtained from Bio-lab New Zealand and Sigma Aldrich respectively. Analytical grade Sodium Sulphite (SS) was obtained from BDH Lab supplies. Distilled water was produced onsite at the University of Waikato. Polymer processing additive JV46F was obtained from the Struktol Corporation of America.

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3.3 Choice and preparation of formulations

The formulations in this work were adapted from Aduro Biopolymer's urea free IR3020 injection moulding grade Novatein. The reasons for using a urea-free grade were outlined in Chapters 1 and 2. Thermoplastic protein was prepared by blending 100parts bloodmeal with 1 part per hundred bloodmeal ($pph_{BM)}$ SS, 1 pph_{BM} SDS, 20 pph_{BM} TEG and varying water contents; 30parts, 35parts, 40parts and 45parts respectively. Samples were prepared by heating the required amount of water to 60 °C on a hotplate stirrer to dissolve SS and SDS. Bloodmeal and the resultant were mixed in a high speed mixer for 5 minutes allowing denaturing to occur then TEG was added and mixed for another 5 minutes to ensure a homogeneous mixture was obtained. The resultant mixtures were stored overnight below 4 °C in a double sealed plastic bag to avoid loss or gain of moisture prior to extrusion. Formulations used are shown in the Table 3-2.

Table 3-2: Table of pre-extruded formulations used

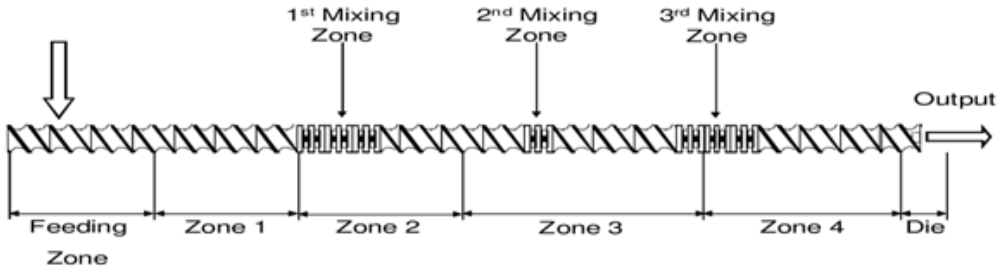
Material	Formulation 1 (pph_{BM})	Formulation 2 (pph_{BM})	Formulation 3 (pph_{BM})	Formulation 4 (pph_{BM})
Sodium Sulfite (SS)	1	1	1	1
Sodium Dodecyl Sulfate (SDS)	1	1	1	1
Tri Ethylene Glycol (TEG)	20	20	20	20
Water	30	35	40	45

Before injection molding of the extrudate, process additive Struktol JV 46fF (a mold release agent) was added at 2parts per hundred extruded ENTP.

3.4 Processing

3.4.1 Extrusion

Formulated samples were extruded using a ThermoPrism TSE-16-TC twin screw extruder. The screw speed on the extruder was 150±2rpm for all formulation. The screw diameter is 16mm and the length to diameter ratio (L/D) is 25 and the die used was a single 10mm circular die. Typical torque values ranged from 33% to 65% of the maximum allowed in the extruder (12nm per screw maximum). The extruder has five heating zones and extruder's die temperature varied from 120 °C to 150 °C depending on the formulation. The actual melt temperatures were within 2 -5 °C of the set temperature. Strands of the extrudate were pelletized using a Tri-blade granulator from Castin Manufacturing Limited. The temperature profile and screw configuration used is shown in the Figure 3-2 below.



Temperature 1	70	110	110	110	120
Temperature 2	70	110	110	120	130
Temperature 3	70	110	110	130	140
Temperature 4	70	110	110	140	150

Figure 3-2: Temperature profile and extruder screw configuration

3.4.2 Injection moulding

Extruded Novatein (NTP) was simultaneously injection moulded into type 1 ASTM Standard tensile test specimens [163] and ISO 179-1:2010 impact test specimens [164] using a BOY 35A injection moulding machine. Specimens were

injected through a cold runner into a 40 ^{o}C water heated mould. The injection molder has five temperature zones and the temperature profile used was 150 °C (die zone temperature), 150 °C, 150 °C, 125 °C, 100 °C (feed zone temperature), screw-speed of 150rpm,back pressure of 10bar, mould holding time of 2sec, injection rate of 2secs and holding pressure of 80bar was used. Produced specimen bars were used for the evaluation of mechanical and thermal properties and also physico-chemical characterization.

3.4.3 Conditioning

Samples were conditioned for 7days at 23 ºC temperature and 50% relative humidity. Moisture content was determined on both conditioned and unconditioned specimens

3.5 Characterisation

3.5.1 Mechanical properties

Tensile strength (TS), Young's modulus, toughness (energy to break) and elongation at break were determined according to ASTM standard D638-03[163] using an Instron model 33R4204 with extension rate of 5mm/min and extensometer gauge length of 50mm. The injection moulded samples were the standard dog bone sample shape with a cross section of 12mm width and 3mm thickness in the region. Six replicates each of conditioned and unconditioned samples were tested.

3.5.2 Thermal properties

3.5.2.1 Dynamic Mechanical Analysis (DMA)

The effect of extrusion moisture content and temperature on the resulting glass transition of NTP was established using a Perkin Elmer DMA8000 fitted with a high temperature furnace and cooled with liquid nitrogen. Rectangular samples of approximately 30mm long, 4mm thickness and 9mm width were cut from moulded specimens and tested in a single cantilever bending mode using 0.03mm displacement, multi frequency temperature scan range of -60 °C to 180 °C, programmed heating rate of 2 °C/min and 12.5mm free length. The frequencies

tested were 0.10, 0.30, 1, 3, 10 and 30Hz. The peak in tan δ at 1Hz was taken as the glass transition temperature.

3.5.2.2 Thermogravimetric Analysis (TGA)

The decomposition behaviour (Thermal stability) of NTP was measured using a Thermogravimetric analyser (SDT 2960 TA instruments, New Castle DE). Approximately 10mg of sample was heated from 25 °C to 800 °C at 10 °C/min in the presence of air with a flow rate of 150ml/min. each was scanned in triplicate. Samples were freeze-dried and milled using a commercial blender (magic bullet) before being scanned.

3.5.3 Physico-chemical characterization

3.5.3.1 Moisture content

Moisture contents were assessed after each processing step; pre-extrusion, extrusion, injection moulding and after conditioning by overnight oven drying. Three specimens of each sample type were weighted (M_I) in aluminium dishes of known weight and dried for 24hours in an air circulating oven (Conthen digital series oven) at 100-104 °C. Samples were reweighed (M_F) after drying to determine fractional mass loss using **Equation 1** as the average of the three specimens.

$$\mathbf{ML = \frac{MI - MF}{MI}} \qquad \textbf{……Equation 1}$$

Where; ML = fractional mass loss
MI = initial sample weight
MF = final sample weight

3.5.3.2 Wide Angle X-ray Scattering (WAXS)

Wide angle x-ray scattering data was collected after each processing step; pre-extrusion, extrusion, injection moulding and after conditioning using a Panalytical Empyrean X-ray diffractometer operating at 40KV and 40MA using CuKα. For each sample type, scans were performed with automatic divergence slit (ADS) with an irradiated distance of 10mm with no Incident anti scatter slit. The scattering intensity was collected in the range from 2Θ values from 2.9^{o} to 36^{o} at step size of 0.1050(ADS) where Θ is the X-ray beam's angle of incidence. A soller slit of 0.04rad, fixed incidence beam mask of 10mm were used. A Pixcel 3D area detector was used to detect the X-rays. Samples were freeze dried and milled using a commercial blender (magic bullet) before being scanned in a powder sample holder spinner stage. Crystallinity was determined from the ratio of the crystalline region to amorphous halo areas. Braggs law ($n\lambda = 2d \sin\Theta$) was used to relate the scattering of peaks to inter-chain distance and Gaussian peak was fitted under the curve to represent scattering by amorphous region and to estimate the degree of crystallinity.

4 Results and discussion

In the previous chapters, it was mentioned that non-covalent and some covalent bonds between protein chains in bloodmeal must be disrupted to improve processing conditions such as melt flow and chain rearrangement during extrusion and this can be achieved by the use of SS, Water, SDS and TEG. It was also mentioned that the added moisture content is an important factor in both extrusion processing and final product properties of protein polymers. Moisture content greatly influences mechanical properties of protein polymers by acting as an effective plasticizer but evaporation during or after processing will result in changes to mechanical properties. The purpose of this chapter is to report and discuss the effect that moisture content during extrusion and extrusion temperature had on the properties of NTP. It is important to note that the "added water" used to prepare a blend prior to extrusion will not be the same as its moisture content after subsequent processing.

4.1 Changes in moisture content

The mass loss measured during direct oven drying of samples from each experiment at each processing stage for 24 hours is shown in Table 4-1 long with, the amount of added water and combined added plasticizer (water plus TEG) for each experiment. Plasticization is a very crucial part of thermoplastic processing of protein and water is one of the effectively and mostly commonly used plasticizers [15; 106; 135; 139]. Water's low molecular mass enables proper plasticization but due to its low boiling temperature and high protein processing temperature, water evaporates during processing losing its effectiveness [139]. It is for this reason that less volatile additives such as TEG is used in combination with water.

Table 4-1: Added water, plasticiser and fractional mass loss in percentage during oven drying for each experiment at each stage of processing. Average and standard deviation from three measurements are shown

Expt No.	Form No.	Water (pph_{BM})	Extr. Die Temp	Mass fraction					
				Added water	Water + TEG	Lost during overnight oven drying			
						PNTP	ENTP	INTP	CNTP
1	1	30	120	0.20	0.33	0.18±0.00	0.14±0.01	0.18±0.00	0.10±0.00
2			130				0.12±0.00	0.16±0.00	0.08±0.00
3			140				0.11±0.01	0.16±0.00	0.08±0.02
4			150				0.16±0.00	0.14±0.00	0.04±0.00
5	2	35	120	0.22	0.35	0.31±0.00	0.22±0.00	0.18±0.00	0.10±0.01
6			130				0.21±0.00	0.17±0.01	0.09±0.00
7			140				0.19±0.00	0.16±0.00	0.11±0.02
8			150				0.20±0.00	0.16±0.00	0.07±0.01
9	3	40	120	0.25	0.37	0.27±0.01	0.21±0.00	0.18±0.00	0.09±0.00
10			130				0.22±0.00	0.18±0.00	0.10±0.00
11			140				0.21±0.00	0.17±0.00	0.09±0.00
12			150				0.15±0.00	0.14±0.00	0.06±0.00
13	4	45	120	0.27	0.39	0.36±0.01	0.30±0.01	0.23±0.00	0.15±0.00
14			130				0.27±0.00	0.22±0.00	0.14±0.00
15			140				0.27±0.00	0.21±0.00	0.13±0.00
16			150				0.26±0.00	0.20±0.00	0.13±0.00

Observing Table 4-1, it can be seen that mass loss greater than the amount of added water was seen during oven drying of all equilibrated PNTP samples except for formulation 1. This additional mass loss suggests the evaporation of something else in addition to water. Such a result (the loss of additional volatiles other than water during drying period) is a common error of the direct method of measuring moisture content in material [165]. Other known sources of error that may be found in oven drying method include the decomposition of the product and the incomplete removal of water [165]. The additional mass loss is believed to most likely be the loss of TEG. The loss of TEG has been previously reported during thermally resolved synchrotron FT-IR experiments on other PNTP formulations [153]. Although beyond the scope of this thesis, more insight could be obtained by monitoring NTP samples containing different amount of TEG at different drying times. The loss of TEG is less apparent after extrusion although

some moisture has also been lost during the extrusion process therefore it is impossible to account for the exact loss of TEG after extrusion processing. The loss of other volatiles would be more severe with PNTP powder than with moulded samples because moulded samples have consolidated. This introduces more closely bonded/packed molecules than in powdered samples therefore making the evaporation of plasticisers more difficult for the moulded part compared to the loose powder. Surface area of powder is greater compared to consolidated sample and volume ratio is smaller than consolidated sample thereby the loss of moisture is facilitated in powder than in a consolidated sample.

The results from oven drying of extruded, moulded and conditioned samples demonstrate that some moisture or plasticizer has been lost during each of these processing stages, however, not to same extent for each experiment. ENTP at formulations 2, 3 and 4 have the highest mass loss compared to all processing stages (excluding PNTP) and formulation 4, with the most added water had the highest overnight mass loss compared to the other formulations after extrusion. This is expected to affect material properties such as tensile strength, Young's modulus and the glass transition temperature. There also seems to be some irregularity on the measured content of ENTP in comparison to INTP at formulation 1 as it showed an increase in mass loss after injection moulding instead of the expected decrease, nevertheless mechanical properties were measured after injection moulding and conditioning which showed consistency and similar trends with extrusion temperature across the different formulations.

For injection moulded (INTP) and conditioned (CNTP) samples, Formulation 4 consistently lost more mass during overnight drying compared to the other formulations. As Table 4-1 shows, within the groups of samples in the same formulation, mass loss during overnight drying decreased as extrusion temperature was increased. For example, across all the experiments, samples extruded with 120 ^{o}C die temperature showed more mass loss during overnight drying than those extruded with 150 ^{o}C.This shows that more water or plasticiser was lost from each formulation during processing at higher temperature.

Comparing INTP and CNTP samples, it can also be seen from Table 4-1 that samples containing the most water before conditioning also had higher moisture content than samples from other experiments after conditioning. The difference in measured moisture content after conditioning could be attributed to the fact that at 7 days of conditioning the material has not fully equilibrated or alternatively the that equilibrium moisture content is affected by processing with a different amount of initial added water.

After conditioning, assumed water content measured by oven drying ranged from 4% to 15% for all samples. 9 out of the 16 experiments were in a narrower range of 8% to 11%, this is consistent with other reported values for conditioned NTP [13; 153]. Therefore these 9 experiments can be expected to have similar mechanical properties, unless the different moisture content and temperature during extrusion has induced structural changes in the protein. Of the remaining 7 experiments, all extrusions of formulation 4 led to a higher conditioned moisture content than the typical range, and all extrusions of the other formulations with a die temperature 150 ºC is lower than the narrower range. At 45ppph$_{BM,}$ 150 ºC (experiment 16) the extra moisture during extrusion has a bigger effect on the mass loss than the other extrusion temperature at 150 ºC. The experiments outside the narrower range are excepted to have different mechanical property because the highest added water and highest temperature suggests an induced structural changes. Based on the data from table 4.1, experiment 13 (45ppph$_{BM}$ at 120 ºC) would be preferred as optimal processing window as it still shows the material to be more flexible than the others having more mass loss meanings that it is more plasticised after conditioning.

4.2 Extrusion parameters

Visible differences in the appearance of the extrudate could be observed as the extrusion temperature was increased from 120 ^{o}C to 150 ^{o}C and initial water content increases from 30 pph$_{BM}$ to 45 pph$_{BM}$. The extrudate for formulation 3 and 4 between 140 ^{o}C to 150 ^{o}C had darker colour compared to other samples and it

appeared water was leaving the material faster within the barrel and upon exiting due to the combination of higher temperature and increased initial water content.

In order to obtain a good understanding of how moisture content and extrusion temperature affects the extrusion processing of NTP, it is also important to consider other extrusion parameters such as screw speed, feed-rate, torque, pressure, mass and specific mechanical energy input (SME). SME is a measurement of severity of extrusion conditions and it is calculated using the equation 2 below. Unfortunately, along with different moisture content came difficulties in obtaining a consistent feeding rate, leading to some variability in these parameters. Table 4-2 is a recorded average data of extrusion parameter during processing.

$$\mathbf{SME} = \frac{\mathbf{torque} * \mathbf{screw\ speed}}{\mathbf{mass\ flow\ rate}} \quad \textbf{.....Equation 2}$$

Table 4-2: Measured average of extrusion parameters obtained from reading taking every 3 minutes during extrusion.

Expt No.	added water (pphBM)	die temp (°C)	screw speed (RPM)	feed setting (Hz)	torque (%)	pressure (Mpa)	Mass (g/min)	SME (KJ/Kg)
1	30	120	150	47	43	18	27	138
2		130	150	47	39	17	25	126
3		140	150	47	42	15	28	133
4		150	150	46	37	16	27	122
5	35	120	153	60	51	20	27	130
6		130	153	75	44	27	20	89
7		140	153	81	62	21	26	118
8		150	153	77	45	20	18	90
9	40	120	152	46	42	18	13	136
10		130	151	52	35	18	15	102
11		140	152	63	37	22	16	89
12		150	151	81	41	25	17	77
13	45	120	138	71	44	14	18	85
14		130	140	71	65	24	20	127
15		140	142	71	37	14	19	73
16		150	142	72	33	12	17	66

Formulation 1 fed easily compared to the other formulations, which contributed to higher mass flow. Cracking and breaking of the extrudate was observed at Exp. 1 and 2, when formulation 1 was processed at the lower two temperatures. Exp. 3

ran smoothly and continuously for a time before breaking and cracking and Exp. 4 had a continuous strand throughout. Exp.1 had the highest disruption compared to Exp. 2, 3 and 4. The breaking and cracking reduced as temperature increased and this suggested that higher temperature promoted protein chain unravelling which was desirable.

Formulation 2 fed next most easily but not as well as to formulation 1, requiring a higher feed rate setting to try and match the mass flow of formulation 1. It produced mostly (>70%) continuous strand before cracking and breaking. Exp. 5 had the highest disruption compared to Exp. 6, 7 and 8. This is consistent with the observations at formulation 1.

Formulations 3 and 4 were very difficult to feed into the machine due to high moisture content causing clumping and clogging in the feeder and feed throat. Experiments 9, 10, 13 and 14 produced shorter disrupted strands but for much of the extrusion, material popped out in small chunks or emerged from the die the still resembling the initial powder and Exp. 11, 12, 15 and 16 produced some (30% - 70%) continuous strand before cracking and breaking. Exp. 9 and 14 had the highest level of disruption among their formulations; The popping out in small chunks or material remaining powdery was thought to be as a result of material not consolidating or the protein chain not unravelling which could be because of the irregularities in measure torque and pressure as a result of inconsistent feed-rate. Nevertheless enough consolidated material was able to be obtained from each experiment for further processing of sample pieces. It was observed that higher temperature had the least disruption which suggests that at increase added water content (>30pph_{BM}), high temperature was promoting protein chain unravelling. Other researchers have reported that disruption of extrudate results from excessive cross-linking brought about by high SME and product temperature, low SME input is required for effective extrusion because it mainly prevent excessive cross-linking [14; 166] but in this report disruption of extrudate was seen to be as a result of protein chain not unravelling sufficiently due to low temperature and overly plasticized material. The fluctuating torque and pressure values was accompanied by the unstable flow of the extrudate and blocking of die caused by either increasing moisture content or extrusion temperature.

Considering the results from Table 4-2, it was found that higher temperature and increasing water content from 30 to 35 pph_{BM} promoted consolidation because of the unravelling of protein chains as well as lowering torque resulting to low SME which is also desirable for successful extrusion processing of proteins. It was also observed that irregularities in torque and pressure made it difficulties to produce a consolidated material. Experiment 1, 3 and 5 produced the highest mass, Of these, exp. 3 had the lowest extrudate disruption and was easier to extrude compared to all three experiments and exp. 5 has the highest torque, pressure and feed-rate therefore the preferred optimal processing window is chosen to be experiment 3 because of its ease of processing.

4.3 Injection moulding

During injection moulding, parameters related to the ease of production were monitored for each sample (Table 4-3). These parameters were plasticizing time, injection time and injection pressure. The injection moulder used can be used in one of three mould cycle operating modes. These are automatic, semi-automatic and manual operational mode. In the automatic operational mode, the screw pushes the material in to fill the mould. The time it takes the mould to fill and pressure that the screw applied to push the material is recorded as injection time and injection pressure. The screw rotates to push more material to the front of the barrel which is recorded as plasticisation time, the barrel retracts after the mould fill, followed by the retraction of the mould's moving plate. As the mould opens the moving plate is pulls out having the part and the sprue with it, then the ejector pins push out and the part falls out of the mould. Then the mould closes and the cycle restarts on its own repeating the same process. In semi-automatic mode the whole process is completed by the injection moulder but restarting a new cycle is done manually after the part is ejected while in the manual mode, each step has to be manually controlled. In this thesis samples were processed utilizing the automatic and semi-automatic mode but not to same extent for each experiment as not all part self ejected thereby requiring interruption of the cycle to eject them.

For some experiments most (>70%) did, in other experiments some (30% - 70%) did and still others only a few (<30% but not <10%) or none did.

Table 4-3: Injection parameters

Expt No.	added water (pphBM)	die temp (°C)	injection time (sec)	plasticizing time (sec)	injection pressure (Bar)
1	30	120	1.2	9.1	121
2		130	1.3	8.3	132
3		140	1.3	10.9	138
4		150	1.3	11.1	140
5	35	120	0.4	10.2	114
6		130	0.4	12.9	121
7		140	0.5	15.9	148
8		150	64.2	20.6	145
9	40	120	1.3	8.5	124
10		130	1.3	8.1	117
11		140	1.3	14.7	125
12		150	1.4	13.0	162
13	45	120	0.5	6.4	125
14		130	0.5	8.7	129
15		140	0.5	8.3	135
16		150	0.5	8.3	130

Observing table 4.3, the highest maximum plasticizing times and lowest injection time were observed with formulation 2 showing it was difficult to fill the barrel and mix the Struktol processing additive with ENTP but the mould fill was easier compared to other formulations. Formulations 4 had lower plasticising time showing that barrel fill and Struktol mixing with ENTP was easier compared to the other formulations. Formulations 1, 2 and 3 showed the highest plasticization time at the highest extrusion temperature and this can be attributed to the effect of moisture loss during extrusion resulting to less plasticised material. Also at the highest temperature, the highest injection pressure was observed suggesting that due to loss of moisture, the pressure required to push the material through the die was higher.

In formulation 1, Exp. 1 and 2 some to most of the parts self ejected out of the mould (completed the full automatic cycle) while a few parts remained on the stationary plate and a few sprues pulled out correctly but parts stuck to the moving

plate. For Exp. 3, a few remained stuck on the stationary plate after mould opening, but most were split evenly between self-ejecting and getting stuck on moving plate. For Exp. 4, only a few parts self-ejected, with some sticking to each plate..

With Formulation 2, for Exp. 5 only a few parts were caught with the sprue on the stationary plate, with most part pulling out the sprue with the moving plate but only some parts ejecting out of the mould. In Exp. 6 most parts pulled with sprue on the moving plate but none ejected out of the mould. In Exp. 7 and 8, again only a few were caught with the sprue in the stationary plate. Most pulled out with sprue but remained on the moving plate but none ejected out of the mould.

Formulation 3, Exp. 9 all pulled the sprue but remained on the moving plate, without self-ejecting. With Exp.10 most parts pulled the sprue but remained on moving plate and a few parts self-ejected from the mould. Exp. 11, a few were caught with the sprue in the stationary plate, although most pulled but remained on the moving plate, fewer part filled and remained on the stationary plate and fewer part pulled with sprue but remained on the moving plate. Exp. 12, fewer parts were caught on the sprue, fewer part ejected out of the mould and most part pulled with sprue but remained on the moving plate.

Formulation 4, none self-ejected out of the mould but all pulled out with sprue but remained on the moving mould except for Exp. 16 that had few caught on the sprue.

Considering table 4.3, the best processing formulation in injection moulding is formulation 4 as they have the lowest plasticizing time and injection time showing they were easier to mix ENTP with Struktol and fill the mould but the optimal processing window would be for experiment 13 as it has the lowest plasticizing time and injection time though all pulled out with the sprue but remained on the moving plate, this can be addressed with the use of external mould release spray.

4.4 Mechanical properties

In the previous section, it was shown that despite issues feeding at higher moisture content and difficulties obtaining a continuous extrudate for some experiments, all 16 experiments could be injection moulded successfully into test pieces suitable for mechanical testing. Mechanical properties of protein polymers are heavily influenced both by processing conditions and the actual moisture content in the material when it is tested. To account for this, specimens were tested both as they were moulded (INTP) and after a conditioning step (CNTP) intended to bring each to similar moisture content before testing. As previously observed, (table 4.1), conditioned specimens for 9 of the 16 experiments had consistent moisture contents of in the narrow range of 8% to 11%, however 7 of the experiments were outside this range after conditioning. It is important to note that when "added water" is mentioned it refers to the water added before extrusion, which is different from the moisture content of the parts after processing and conditioning.

The mechanical properties of unconditioned samples had low tensile strength (ranging from 3Mpa to 5Mpa) and low modulus (typically 100MPa - 300MPa), though samples were ductile – having high strain at break of between 13% and 31%, contributing to moderate energy to break (toughness) After conditioning, tensile strength increased greatly to between 12Mpa to 18Mpa with high moduli ranging from 981Mpa to 1899Mpa but elongation at break dropped dramatically to between 1% and 2%. This embrittlement is due to loss of moisture loss during conditioning.

4.4.1 Unconditioned injection moulded material

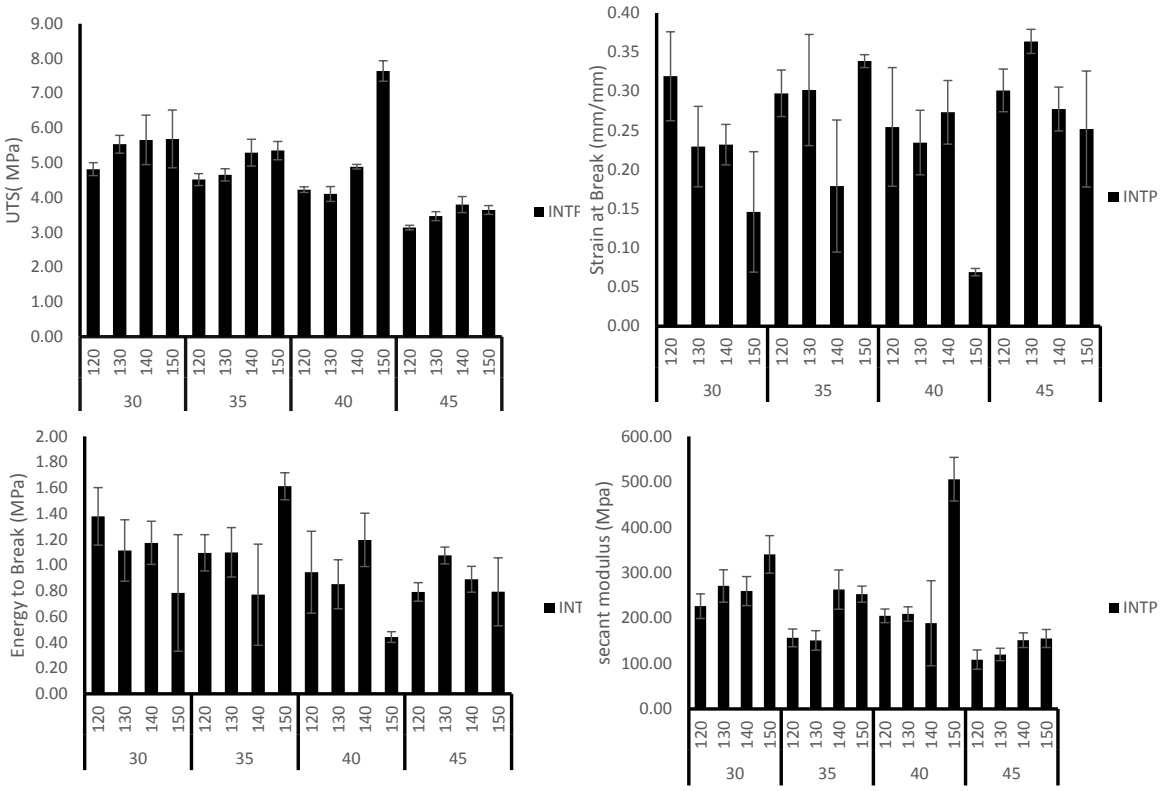


Figure 4-1: Mechanical properties of unconditioned test pieces with varying water content and extrusion temperature. Error bars denotes standard deviation of the mean.

For unconditioned material (Figure 4-1), added water strongly influenced the mechanical properties of NTP. Increasing added water content typically decreased tensile strength and modulus showing that added water remaining in the samples after processing acted as a plasticizer for example all samples in formulation 4 showed decreased modulus and tensile strength compared to other formulation and also samples at die temperature 140 °C as water increases from formulation 1 to formulation 4. A higher plasticizer content increases chain mobility and reduces strength allowing some chain rearrangement in response to the applied force resulting in a tougher material with higher strain to break.

Looking within formulation groups in Figure 4-1, it seems that typically the tensile strength and Young's modulus of the unconditioned samples increased with extrusion temperature. Strain at break was more variable, but typically decreased as temperature increased except for formulation 2 where strain at break increased from 0.30mm/mm when extruded at 120 °C to 0.34mm/mm when extruded at 150 °C. However, given the variation seen for strain at break, this increase is not likely to be significant. An increase in strength and young's modulus as temperature increases means the material is stiffer and more rigid, likely due to the loss of additional moisture as temperature was increased. Experiment 12 is a typical example, looking at Table 4-1 it can be observed that samples from this experiment had the smallest amount of water remaining in them after extrusion and injection moulding showing that it is less plasticised which is why it has a higher strength and lower elongation than other samples. To control for this effect samples were also tests after conditioning which was intended to make the moisture contents similar.

The best processing conditions based on unconditioned mechanical properties appears to be experiment 8 (150 °C with formulation 2 – 35pph$_{BM}$ added water) with the highest toughness (energy to break) due to having both one of the higher tensile strengths and higher strain at breaks from the 16 experiments. For formulation 1, the best processing conditions appeared to be experiment 1 as strength only increased small amounts at higher temperatures, while strain at break dropped more severely. For formulation 2, as mentioned, experiment 8 was preferable. For formulation 3, experiment 11 is chosen as desirable, with strain at break dropping sharply when the temperature increased from 140 °C to 150 °C. Experiment 13 had the best unconditioned properties for formulation 4, but it should be noted that between formulations 1 and 4 the modulus (stiffness) dropped from 151MPa to 341MPa.

4.4.2 Conditioned parts

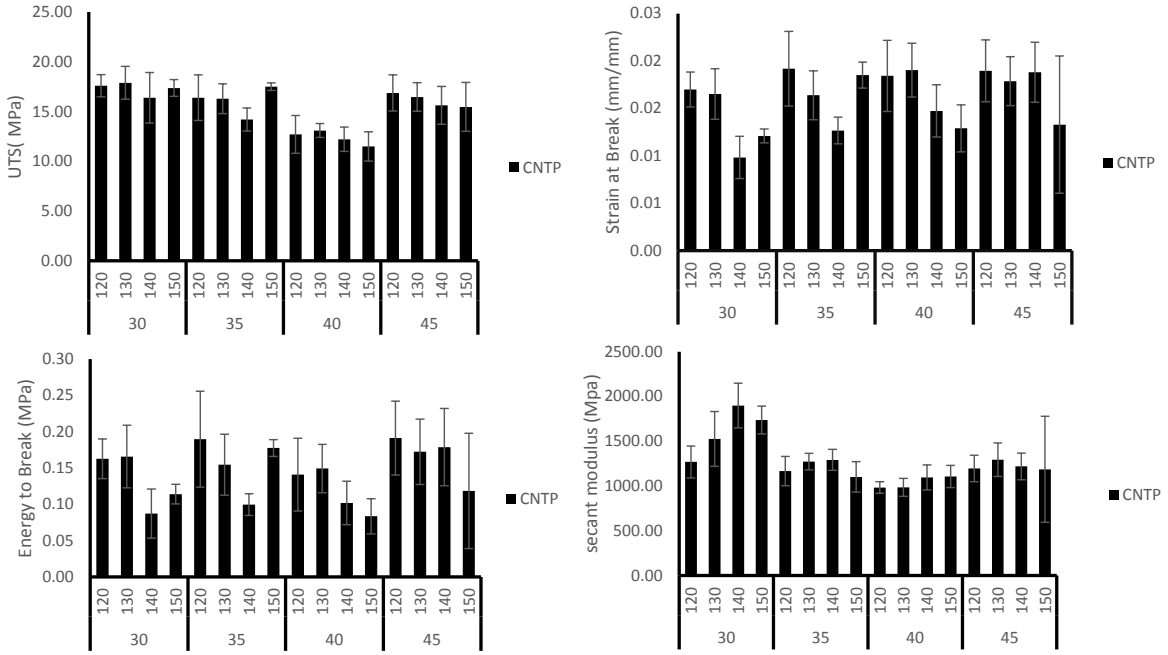


Figure 4-2: Mechanical properties of conditioned test pieces with varying water content and extrusion temperature. Error bars denotes standard deviation of the mean.

The mechanical properties of CNTP from each of the experiments with different added water and extrusion temperatures are show in Figure 4-2 all samples tested became brittle after conditioning this is evident by the very large decrease in strain at break and very low toughness when compared to the unconditioned value in Figure 4-1. However, the modulus increased dramatically (experiment 3 increased from 260MPa to 1899MPa and experiment 4 from 341MPa to 1737MPa) after conditioning, demonstrating that the material became much more rigid. During conditioning water evaporates from the material allowing new secondary interactions to occur between chains. Increasing added water content, both tensile strength and young's modulus decrease. This is consistent with results previously reported by other researchers [13].

Considering the effect of added water during extrusion on the mechanical properties of a subsequently conditioned sample, a gradual decrease in tensile strength was observed with increasing water up to formulation 3 before a noticeable increase occurred at formulation 4. This indicates that even though water is lost during conditioning, the resulting tensile strength is very sensitive to

what the water content was during extrusion. Pervious research on Novatein reported an increase in tensile strength was observed at low water activity when using 25pph$_{BM}$ and 30pph$_{BM}$ TEG before a rapid decrease occurred suggesting tensile strength is not sensitive to moisture below about 10wt% [17]. From the fractional mass loss in Table 4-1, it can be observed that after conditioning formulation 4 had moisture content above 10wt%. when Novatein with urea and TEG was extruder at 2 different moisture content (30pph$_{BM}$ and 40pph$_{BM}$), it was reported that there was no differences in the mechanical property after conditioning [19] while early Novatein formulation with urea and without TEG having different amount of water (45pph$_{BM}$ and 60pph$_{BM}$) and same amount of urea showed a difference in mechanical properties after conditioning having higher tensile strength at lower added water content [13]. In this thesis, TEG was kept constant and water was varied at 30pph$_{BM,}$ 35pph$_{BM,}$ 40pph$_{BM}$ and 45pph$_{BM}$, it was observed that there was a clear difference in mechanical properties after conditioning. The conditioned samples showed an increase in tensile strength and modulus and it is also observed that lower added water content showed the highest increase in tensile strength.

As discussed earlier, the measured moisture content of conditioned samples varied between 6% and 15%, but 9 of the experiments fell within a narrower range of 8% to 11% (Table 4-1). From these 9 experiments, it was still observed that as added water increases, tensile and Young's modulus decreased while strain at break and energy to break remains the same. These 9 experiments have same moisture content therefore the difference in strength is due to a difference in protein-protein interactions after processing at different moisture content. Tensile strength ranges from 16Mpa to 18Mpa for added water of 30pph$_{BM}$, 14Mpa to 16Mpa for added water of 35pph$_{BM}$ while for added water of 40pph$_{BM}$ only ranged from 12 to 13Mpa, Similarly, Young's modulus ranges from 1268Mpa to 1899Mpa for added water of 30pph$_{BM}$, 1165Mpa to 1291Mpa for added water of 35pph$_{BM}$ and 982Mpa to 1095Mpa for added water of 40pph$_{BM}$. Strain at break was about the same for all added water of 30pph$_{BM,}$ 35pph$_{BM}$ and 40pph$_{BM}$ ranging between 0.01mm/mm to 0.02mm/mm. Likewise, the energy to break range is same for all added water of 30pph$_{BM,}$ 35pph$_{BM}$ and 40pph$_{BM}$ ranging between 0.2Mpa to

0.1Mpa. There is little or no clear effect of die extrusion temperature on the 9 experiments within the narrower ranges

The 3 experiments which conditioned to a lower moisture content of 4% to 7% mass loss in overnight over drying all had the same die temperature of 150 ºC but different added water contents. In these it was also observed that as added water increases, tensile and Young's modulus decreases while strain at break and energy to break remains the same following same trend as at lower extrusion temperatures. Tensile strength ranged from 11Mpa with 40ppph$_{BM}$ added water to 17Mpa with only 30ppph$_{BM}$, while Young's modulus ranges from 1106Mpa to 1737Mpa. Considering the changes in value ranging, it showed a significant figure in the tensile strength and young modulus though the changes in strain at break and energy to break is insignificant therefore it is right to say that the 3 experiments below the narrower range showed changes in their mechanical properties.

Considering the 4 experiments with the highest overnight mass loss after conditioning, they fell in the same added water content of 45ppph$_{BM}$ so the effect of added water cannot be compared within this group. It was observed that as temperature increased tensile strength and young's modulus changed a little while strain at break and energy to break again remained similar. Tensile strength ranged from 15Mpa to 17Mpa and Young's modulus ranges from 1186Mpa to 1292Mpa. Strain at break ranged from 0.01mm/mm to 0.02mm/mm. Energy to break ranged from 0.02Mpa to 0.01Mpa. Considering the small changes in value range, these four experiments showed the same mechanical properties.

There is no clear trend when considering the effect of extrusion die temperature, therefore the best processing temperature cannot be chosen based on mechanical properties.

Exp. 8 was chosen as good compromise between toughness and strength with the best strain at break of all 16 experiments as the difference between its toughness and best toughness has no significant difference (example experiment 8 has toughness of 0.18 while experiment 5 and 13 had toughness of 0.19MPa with lower strength of 16.38MPa and 16.89MPa respectively compared to experiment

8 with 17.49MPa. but it is out of the narrower range. Exp. 2 was chosen as the best among formulation 1 because it has higher strength, best strain at break and best toughness. Exp. 10 was chosen as the best among formulation 3 because it had higher strength, best strain at break and best toughness of all experiments in formulation 3. Exp. 13 was chosen as a good compromise between strength and toughness with the best strain at break of formulation 4.

4.4.3 Recommendations based on mechanical properties.

From the data presented in Figure 4-1and Figure 4-2, the most desirable experiment for the effect of added water and extrusion temperature on the mechanical properties of NTP was experiment 8 for both unconditioned and conditioned, which had 35ppph$_{BM}$ water and was extruded at 150 °C, because of its ease of production in comparison to all 16 experiments. Also it has better strain at break and tensile strength for both conditioned and unconditioned stages compared to all 16 experiments. Experiment 8 has approximately double the toughness compared to experiment 13 which is the next best experiment (1.61Mpa compared with 0.79Mpa) at unconditioned stage while the difference at the conditioned stage (experiment 13 with 0.19Mpa compared to experiment 8 with 0.18Mpa) is small enough to be insignificant. It is also recommended that the optimal processing window should fall within those that conditioned to a consistent narrower range, which also had the best processing properties therefore formulation 1, experiment 2 at 130 °C was chosen as optimal.

4.5 DMA

Dynamic mechanical analysis (DMA) was used to investigate the effect of added moisture content and extrusion temperature on the glass transition temperature (T_g) of INTP and CNTP. T_g is a reversible transition of the amorphous regions within a semi-crystalline material or a completely amorphous material. It is the transition from a brittle or glassy state into a rubbery or molten state. For both INTP and CNTP, only one peak was seen in the tan δ curves for each experiment (Figure 4-3) which would normally indicate a single phase with a single T_g is present however although there is a single peak, it is broad and consistent with pervious experiments in Novatein powder pocket [27]. From the tanδ curves, an increase was observed in the tanδ peak values when comparing unconditioned to conditioned samples (Figure 4-3). The glass transition temperature determined from this peak for each experiment is shown in. Table 4-4

Table 4-4; Glass transition temperatures of conditioned and unconditioned samples. Average and standard deviation from three separate scans is shown for each sample type.

Expt. No	Formu. No.	water pphBM	Added water (%)	Water + TEG (%)	Die temp °C	INTP (Tg °C)	CNTP (Tg °C)
1	1	30	0.20	0.33	120	64±1	82±2
2					130	67±2	83±1
3					140	67±1	86±3
4					150	66±3	79±1
5	2	35	0.22	0.35	120	60±1	80±1
6					130	60±0	81±1
7					140	59±3	80±1
8					150	61±2	80±0
9	3	40	0.25	0.37	120	61±1	78±1
10					130	60±3	77±1
11					140	62±2	79±1
12					150	62±2	77±1
13	4	45	0.27	0.39	120	59±1	80±1
14					130	60±3	79±1
15					140	54±3	78±2
16					150	60±2	80±1

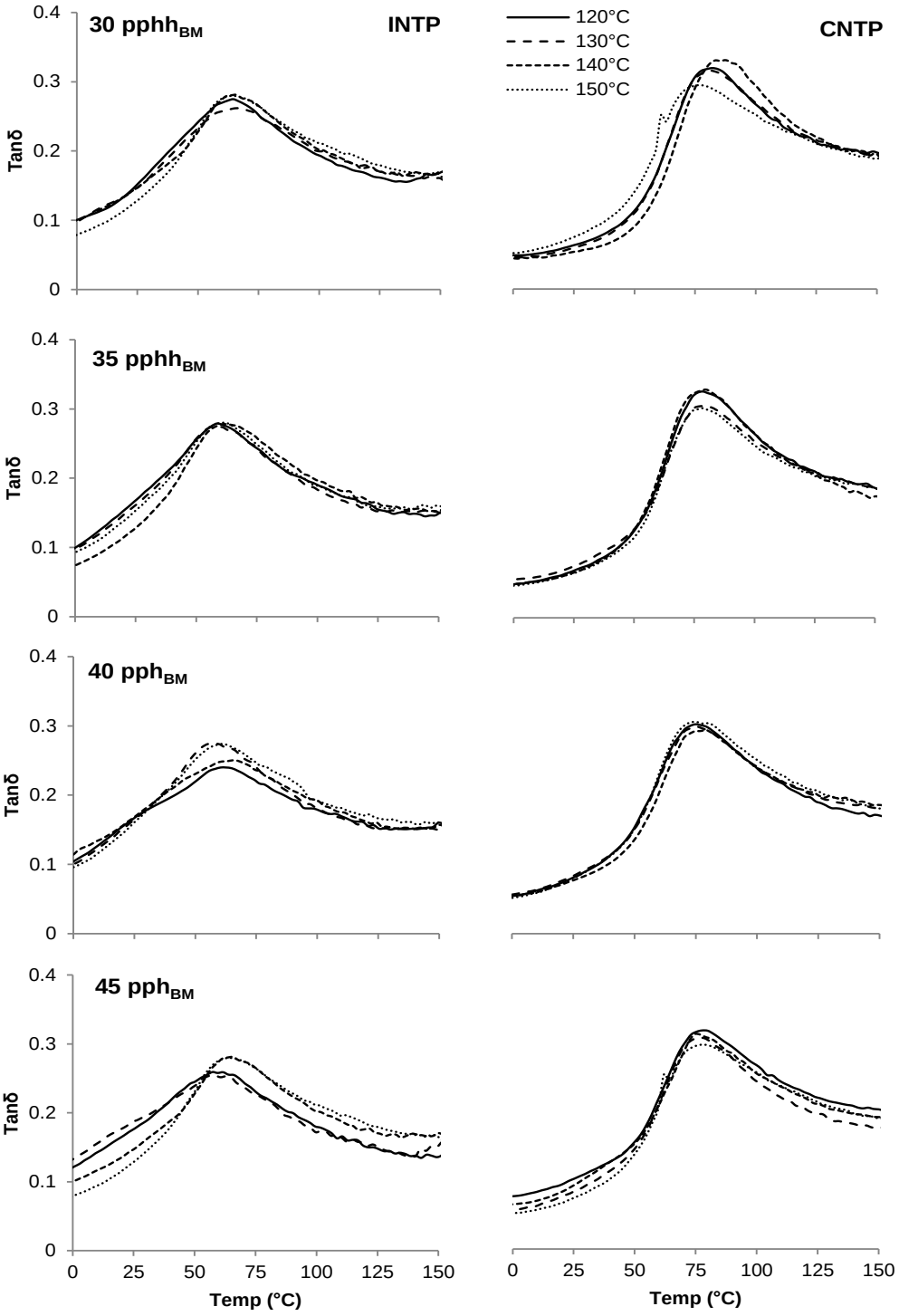


Figure 4-3; Plots of tanδ against temperature for processed NTP before and after conditioning. Although three scans were performed for each added water content and extrusion temperature, only one is shown for each experiment for clarity.

Previous research has identified the glass transition of bloodmeal to be about 220 °C which can be lowered by the addition of water to -10°C and the addition of other additives to -80 °C for PNTP due to reduction in chain interactions [27]. In this thesis without urea, T_g was seen to vary between 54 °C to 67 °C for INTP and 77 °C to 86 °C for CNTP (Table 4-4). This increase in T_g after conditioning demonstrates a decrease in chain mobility of the protein molecules and this is as a result of moisture loss during conditioning. This is consistent with changes observed in mechanical properties measured at room temperature after conditioning.

From the tanδ curves, an increase was observed in the tanδ peak values when comparing unconditioned to conditioned samples (Figure 4-3).

Increasing added water content showed a decrease in Tg for both INTP and CNTP. Similar result has been reported by previous researcher about soy protein sheet showing a decrease in Tg from 50 °C to -7 °C with moisture content ranging from 26% to 2.8% and 30part of glycerol.[15] . The 9 experiments with consistent conditioned moisture content, at $30pph_{BM}$ Tg increased with an increasing temperature while at $35pph_{BM}$ and $40pph_{BM}$ no increase was observed as temperature increase. For the four formulation above narrower range no changes was observed, they have about same Tg and the 3 experiment below narrower range which was extruded at 150 °C, they have similar Tg. INTP $30pph_{BM}$ showed a higher Tg than the other experiments at INTP stage. The other 15 experiments at INTP stage have about same Tg.

From Figure 4-3, it can be seen that CNTPs has sharper peaks than INTPs. At $45pph_{BM}$ the higher temperature has higher and sharper peaks than the lower temperatures; this is consistent with the lower mass loss during oven drying shown in Table 4-1. It was also observed that CNTP from experiment 4 had a lower and broader peak and lower T_g than the other experiments in formulation 1 but also had the lowest mass loss during oven drying. This is not expected as lower mass loss during drying means less moisture was in the sample during processing and it therefore should have a higher T_g. unless the action of other

added additives had an additional effect on protein-protein interactions to reduce the T_g.

There is an insignificant differences observed for the most part of this experiments which should be as a result of the different added amount of moisture content and extrusion temperature. The difference in the amount of water remaining in the sample when they were tested has a great effect on the material.

4.6 TGA

Thermogravimetic analysis (TGA) analysis was performed to examine the thermal decomposition behaviour of freeze dried then milled samples prepared for WAXS analysis. In TGA, there are four main stages of mass loss which represent the thermal decomposition of plastic material [2; 40; 167; 168]. The first stage for protein is observed between room temperature to 150 °C (but mostly observed at 120 °C) which relates to the loss of water indicating the existence of residual moisture in materials. The second stage is at about 150 °C to 230 °C which relates to the evaporation/decomposition of other additives/plasticizers such as TEG indicating an interaction between protein polymer and the additive (hydrogen bonds) and the beginning of decomposition of the protein chain. The third is associated with weak bond cleavage which can be seen at about 230 °C – 380 °C and the fourth is associated with strong bond cleavage which can appear at above 380 °C. This behaviour has been reported by previous researchers on protein based polymer and is consistent with what was observed for all formulations here. For this thesis, residual moisture content in the freeze dried samples was estimated as cumulative mass loss to 120 °C, where a plateaux region was seen (Figure 4-4) after the first stage. The second and third stage of mass loss overlapped, but were centred at 312 °C, relating to the evaporation of TEG and the onset of decomposition of protein chain. The third stage was completed by about 510 °C and the fourth was observed at 660 °C relating to strong bond cleaving.

Table 4-5; Residual moisture content in freeze dried samples determined from cumulative mass loss in TGA to 120 °C. Average and standard deviation from three replicates is shown for each step.

Water	die temp	Added Water (%)	Expt. No	PNTP Mass loss (%)	STDEV (%)	ENTP Mass loss (%)	STDEV (%)	INTP Mass loss (%)	STDEV (%)	CNTP Mass loss (%)	STDEV (%)
30	120	0.20	1	0.17	0.02	0.03	0	0.09	0	0.08	0
	130	0.20	2			0.03	0	0.09	0	0.07	0
	140	0.20	3			0.03	0	0.09	0	0.07	0
	150	0.20	4			0.04	0	0.08	0	0.06	0
35	120	0.22	5	0.10	0.01	0.08	0	0.08	0	0.06	0
	130	0.22	6			0.09	0	0.08	0	0.06	0
	140	0.22	7			0.08	0	0.06	0	0.06	0
	150	0.22	8			0.07	0	0.10	0	0.06	0
40	120	0.25	9	0.04	0.00	0.05	0	0.05	0	0.07	0
	130	0.25	10			0.06	0	0.09	0	0.07	0
	140	0.25	11			0.05	0	0.08	0	0.07	0
	150	0.25	12			0.04	0	0.08	0	0.06	0
45	120	0.27	13	0.02	0.02	0.03	0	0.07	0	0.07	0
	130	0.27	14			0.05	0	0.10	0	0.10	0
	140	0.27	15			0.04	0	0.10	0	0.10	0
	150	0.27	16			0.04	0	0.09	0	0.09	0

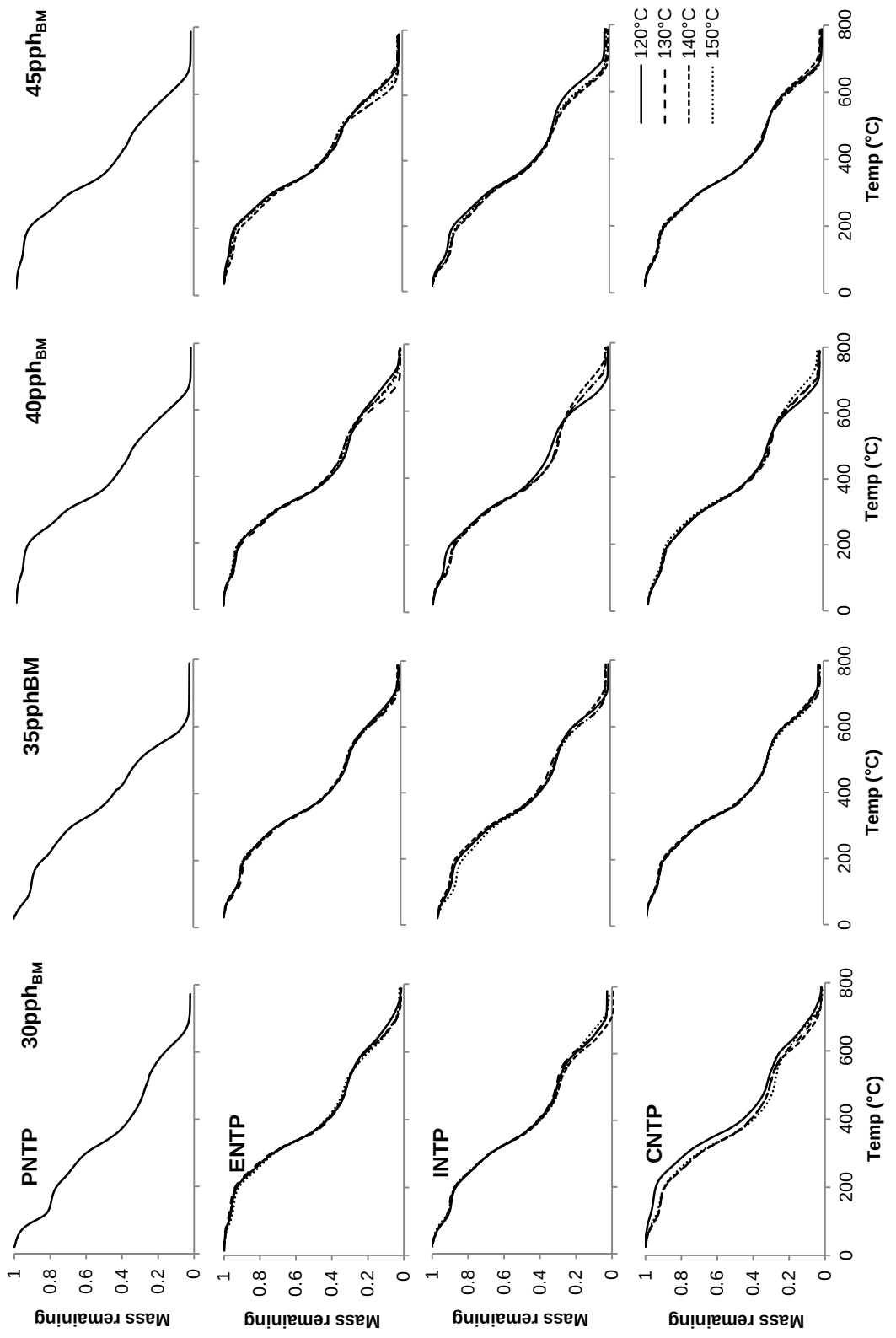


Figure 4-4; TGA thermograms of freeze dried PNTP, ENTP, INTP and CNTP. Although three scans were performed for each experiment, only one is shown for each experiment for clarity.

After freeze drying and milling the mass loss in TGA to a plateaux at 120 °C varied between 3% to 17% for all experiments, suggesting varying residual moisture contents (Table 4-5) and these differences could be due to either the samples did not freeze dried to same extent or due to the hygroscopic nature of proteins absorbing water from the atmosphere during milling process.. It can be observed that after conditioning and subsequent freeze drying there is little difference in the moisture content of the formulations, although formulation 4, with the highest added water, still had the highest mass loss to 120 °C. Between conditioned and unconditioned samples there is again little difference in the mass loss to 120 °C after freeze drying and milling the samples.

From Figure 4-4, it can be observed that freeze dried and milled ENTP have lower mass loss than similarly treated INTP and CNTP. PNTP were not milling as they were already granulated.

Based on the information obtained from Table 4-5 and Figure 4-4, it can be concluded that the ranges of added water and extrusion temperatures trialled had no effect on the decomposition behaviour and thermal stability of NTP as there is the only observed differences in the TGA thermograms after freeze drying and milling were small amounts of residual water.

4.7 WAXS

Wide angle X-ray Scattering (WAXS) can be used to estimate the degree of crystallinity of semi crystalline polymers. This was used to determine the degree of crystallinity in NTP processed with different moisture content and different extrusion temperature. The materials tested were freeze dried before testing to control for the effect of water still in the samples after processing and to make milling into a powder easier.

WAXS curves of ENTP, INTP and CNTP displayed two main peaks which indicate a crystalline structure and it can be seen from Figure 4-6. These peaks originated from the semi crystalline structure of NTP. When Braggs law ($n\lambda = 2d \sin\theta$) is used to relate the scattering angles of these peaks to inter-chain distance

these two peaks are found at $2\theta \approx 8° - 9°$ ($d \approx 10$Å) and $2\theta \approx 19°$ (d$\approx 4 - 5$Å) for the wavelength used in this thesis. Such peaks are typical in proteins and suggest the presence of inter-helix packing in helix rich proteins, inter-sheet separation in beta-sheet rich protein and either hydrogen bonding along the backbone in helix or bonding between strands in β- sheets [19; 133; 169; 170].

As temperature and added water increased, the scattering intensity and 2Θ angle of the two peaks remained the same across most of the experiments suggesting that an increase in temperature or water during extrusion caused little change to the packing of ordered secondary structure. However, some of the experiment had a considerable lower intensity but their angles were the same. Three scans were done for each experiment although one is shown, all three had same issue.

There is no clear trend as to which have lower intensity across the different formulations, processing steps and extrusion temperature. With PNTP at 30pph$_{BM}$ added water, this lower intensity was first thought to be as a result of higher residual moisture content after freeze drying but this was not borne out in the other experiments that showed lower intensity (Figure 4-5). For example, experiment 5 for ENTP, 4, 6 and 16 for INTP and 4 and 12 for CNTP have the same or similarly amount of residual moisture content with the other experiments in their formulation that showed higher intensity (Table 4-5). However when processed to obtain crystallinity values, the experiments with lower intensity had consistent crystallinity values compared to experiments within their formulation and processing steps for example experiment 6 and 7 have same crystallinity values despite the differences in their intensities.

For the estimation of the degree of crystallinity, a Gaussian peak representing scattering by amorphous regions was fitted under the curve (Figure 4-6). Five peaks were then seen (peaks A to E) and this was similar for all experiment. Peaks A and B were observed at 2Θ = 10.26Å and d spacing = 8.61Å, 2Θ = 19.00Å, and d spacing 4.67Å respectively, corresponding to the two peaks mentioned earlier. Peak C was at 2Θ = 24.01Å and d-spacing = 3.70Å, peak D was at 2Θ = 31.06Å and d-spacing of 2.88Å and peak E was at 2Θ = 31.06Å and d-spacing = 4.43Å. Peak D and E relates to the interatomic scattering not assigned to secondary

structure [133] and the study of this is outside the scope of this thesis though they were taken into account during crystallinity calculations. For the peak at $2\Theta = 19°$ (second peak), the d spacing of 4.7Å was observed in all samples. The persistence of this peak indicates that protein-protein H bonding interaction was maintained in the presence of the amounts of plasticiser and other additives used here.

Table 4-6 shows the degree of Crystallinity for each experiment at each stage of processing. As added water increased, crystallinity increased a small amount and decreased as more water was added. This increase was observed across formulation 2 in all stages of experiment suggesting that increasing water disrupted more protein-protein interaction.

Increasing temperature of INTP formulations 1 and 3 showed a decrease before an increase in Crystallinity and formulations 2 and 4 showed an increase before a decrease but for conditioned sample, formulation 1 and 2 had a decrease before an increase while formulations 3 and 4 showed an increase before a decrease. As no clear trend was observed with regard to extrusion temperature and crystallinity, one can infer that in these experiments, the thermal history during extrusion had little effect on the structure after injection moulding.

Previous research showed crystallinity in PNTP varied between 22% and 34% as TEG was varied [19], while in this thesis, the crystallinity ranged from 15% to 27% most are within the range of 19% to 22%. Although the previously studied formulation was different to the one used in this thesis (urea was used) this demonstrates the crystallinity ranges in this thesis is in the appropriate range for NTP therefore there is no change in crystallinity as previously observed [19]

From the data presented in Table 4-6 and Figure 4-5, it can be said that within the range explored in this thesis, the thermal history during extrusion of NTP has only a small effect on the structure of the material after injection moulding and conditioning.

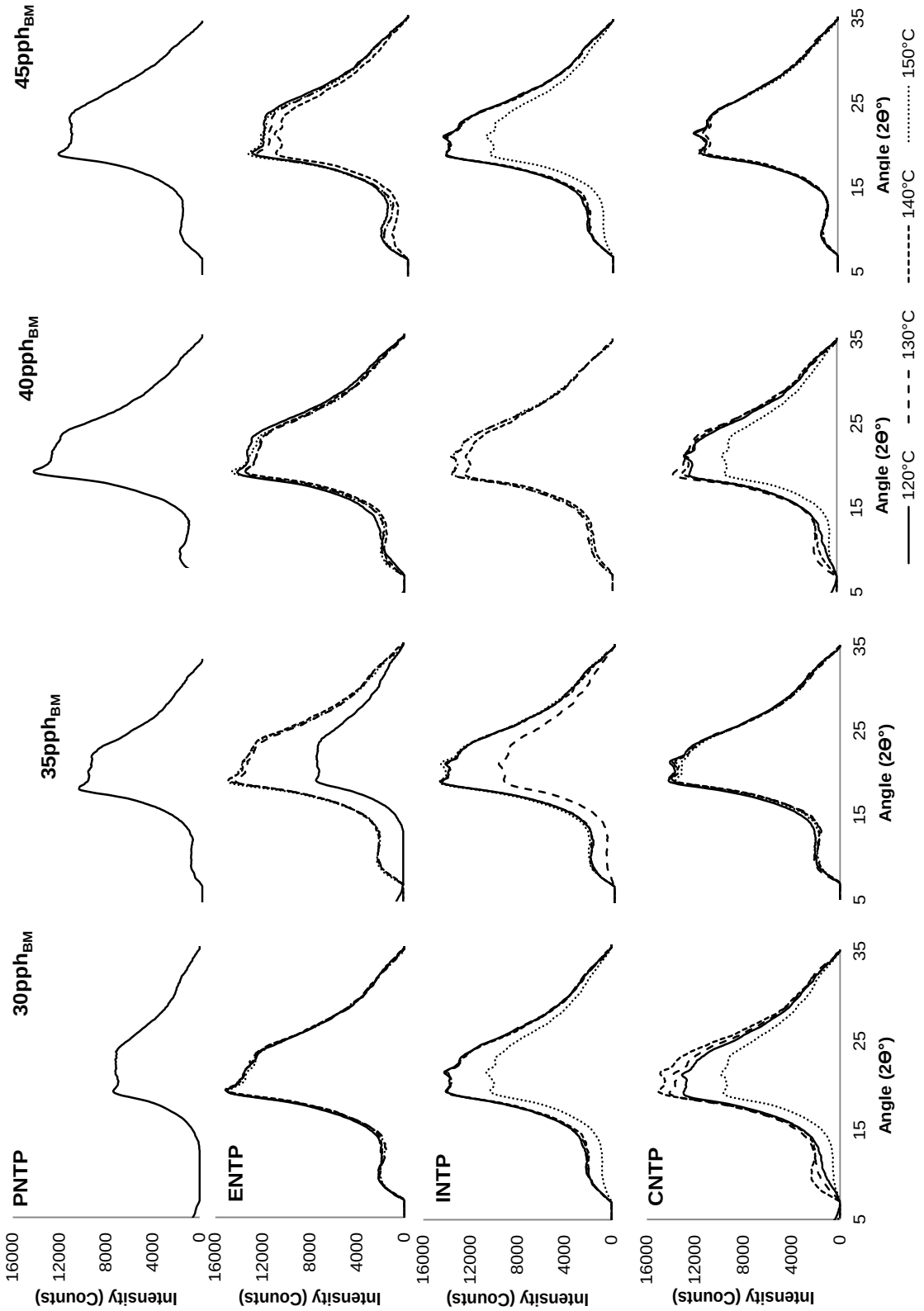


Figure 4-5; base-line corrected plot of WAXS Although three scans were performed for each experiment at each step, only one is shown for each experiment for clarity.

Table 4-6; Crystallinity of different processing stages with added water, added water and TEG, and processing die temperature

Expt. No	Formu. No.	water pphBM	Added water (%)	Water + TEG (%)	Die temp °C	PNTP crystallinity (%)	ENTP crystallinity (%)	INTP crystallinity (%)	CNTP crystallinity (%)
1	1	30	0.20	0.33	120	22	19	20	21
2					130	22	24	19	19
3					140	22	19	22	20
4					150	22	24	19	23
5	2	35	0.22	0.35	120	20	20	23	23
6					130	20	21	24	24
7					140	20	20	24	26
8					150	20	20	21	20
9	3	40	0.25	0.37	120	27	18	22	19
10					130	27	21	18	19
11					140	27	20	19	19
12					150	27	23	19	22
13	4	45	0.27	0.39	120	15	18	22	23
14					130	15	22	21	23
15					140	15	22	20	22
16					150	15	23	19	22

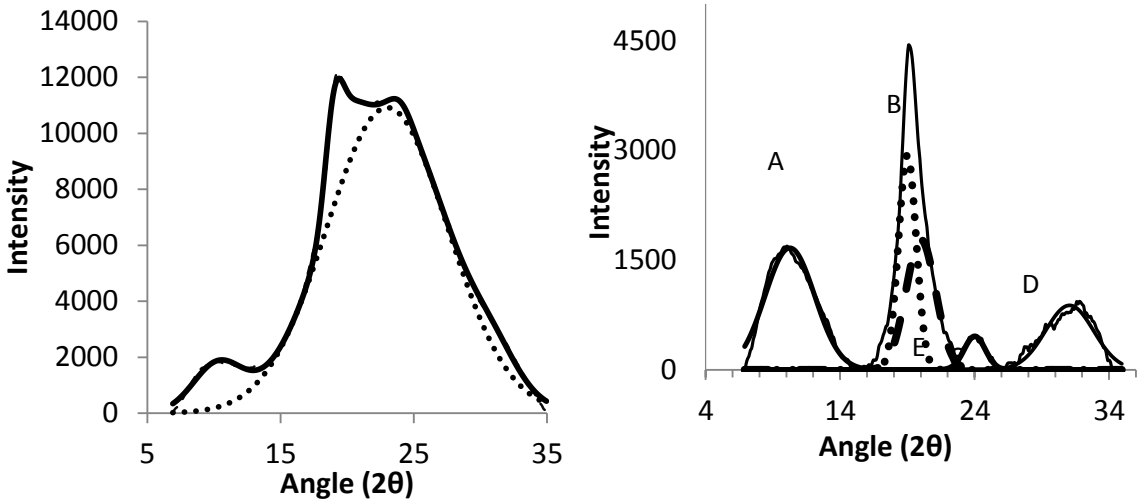


Figure 4-6; process for de-convoluting WAXS data and solving for crystallinity

5 Conclusion and Recommendations

The objectives of this thesis was to investigate the effect of processing water and extrusion temperature on the processing, thermal and mechanical properties of urea free NTP and determine the optimal processing temperature and formulation as a function of moisture content for the production of urea free NTP.

From literature it showed that successful thermoplastic processing of protein is only possible within a small processing window. Extrusion processing requires sufficient chain mobility for successful processing of proteins and to ensure sufficient chain mobility during extrusion processing. The glass transition temperature of protein needs to be lowered to reduce the onset of rubbery flow and this can be achieved by the use of compatible plasticizer with low molecular mass and low volatility. Water acts as plasticizer reducing Tg of protein material through disruption of protein/protein interaction presenting a processible material. Water influences mechanical properties of protein polymer by increasing elongation and reducing strength and toughness. Loss of moisture during and after processing results in a brittle material and loss of material functionality.

Optimization of mechanical properties of protein thermoplastic is highly dependent on the extrusion processing parameters such as extrusion temperature, initial moisture content, SME, torque, pressure and screw-speed.

To prevent excessive aggregation of protein in the barrel, temperature profile that induces formation of melt early in the barrel should be avoided as extrusion reaction is desirable immediately before and after exiting the die.

Four formulations of Novatein were processed through extrusion processing with varying the amount of water content at 30, 35, 40, 45ppph$_{BM}$ and extrusion Die temperature of 120 °C, 130 °C, 140 °C and 150 °C. Test pieces were produced using injection moulding.

Extrusion processing of Novatein showed that the processing parameter had different effects on the material with different formulations. it was found that higher temperature and increasing water content from 30 to 35 pph$_{BM}$ promoted

consolidation because of the unravelling of protein chains as well as lowering torque resulting to low SME. Irregularities in torque and pressure made it difficult to produce a consolidated material. Experiment 3 had the lowest extrudate disruption and was easier to extrude compared to all experiments therefore the preferred optimal processing window is chosen to be experiment 3 because of its ease of processing.

The best processing formulation in injection moulding is formulation 4 as they have the lowest plasticizing time and injection time showing they were easier to mix ENTP with Struktol and fill the mould but the optimal processing window would be for experiment 13 as it had the lowest plasticizing time and injection time though all pulled out with the sprue but remained on the moving plate, this can be addressed with the use of external mould release spray.

Furthermore, it was shown that optimization of processing temperature and formulation as a factor of processing water and extrusion temperature could be characterized by:

- Moisture content, it was found that samples containing the most water before conditioning also had higher moisture content after conditioning. There were observed differences in measured moisture content of all experiments after conditioning showing that at 7 days of conditioning the material had not fully equilibrated or alternatively equilibrium moisture content was affected by processing with a different amount of initial added water. After conditioning, assumed water content measured by oven drying ranged from 4% to 15% for all samples. 9 out of the 16 experiments were in a narrower range of 8% to 11%, for the remaining 7 experiments, all extrusions of formulation 4 led to a higher conditioned moisture content than the typical range, and all the die temperatures at 150 ºC were lower than the narrower range. Based on moisture content, experiment 13 (45ppph$_{BM}$ at 120 ºC) was preferred as optimal processing window as it still showed the material to be more flexible than the others having more mass loss meaning that it was more plasticised after conditioning.

- Mechanical properties, it was concluded that the most desirable experiment for the effect of added water and extrusion temperature on the mechanical properties of NTP was experiment 8 for both unconditioned and conditioned, which had 35pph$_{BM}$ water and was extruded at 150 °C, because of its ease of production in comparison to all 16 experiments. Also it had better strain at break and tensile strength for both conditioned and unconditioned stages compared to all 16 experiments. Experiment 8 had approximately double the toughness compared to experiment 13 which was the next best experiment (1.61Mpa compared with 0.79Mpa) at unconditioned stage while the difference at the conditioned stage (experiment 13 with 0.19Mpa compared to experiment 8 with 0.18Mpa) was small enough to be insignificant. It was also recommended that the optimal processing window should fall within those that conditioned to a consistent narrower range, which also had the best processing properties therefore formulation 1, experiment 2 at 130 °C was chosen as optimal formulation and processing window.
- Thermal properties, it was found in DMA that increasing added water content showed a decrease in Tg. The 9 experiments within consistent conditioned moisture content, at 30pph$_{BM}$ Tg increased with an increasing temperature while at 35pph$_{BM}$ and 40pph$_{BM}$ no increase was observed as temperature increased. The four formulations above narrower range showed no changes, they had about same Tg and the 3 experiments below narrower range which were extruded at 150 °C had similar Tg. There were insignificant differences observed for the most part of these experiments which should be as a result of the different added amount of moisture content and extrusion temperature. The difference in the amount of water remaining in the sample, when they were tested, had a great effect on the material
- TGA showed that after freeze drying and milling the mass loss in TGA to a plateaux at 120 °C varied between 3% to 17% for all experiments, suggesting varying residual moisture contents and these differences could be due to either the samples did not freeze dry to same extent or due to the hygroscopic nature of proteins absorbing water from the atmosphere

during milling process. It was also found that after conditioning and subsequent freeze drying there was little difference in the moisture content of the formulations. It was concluded that the ranges of added water and extrusion temperatures trialled had no effect on the decomposition behaviour and thermal stability of NTP as the only observed differences in the TGA thermograms after freeze drying and milling were small amounts of residual water.

- WAXS, it was found that the crystallinity of formulation tested ranged from 15% to 27% most were within the range of 19% to 22%. From previous study this demonstrated the crystallinity range was the appropriate range for NTP therefore there is no change in crystallinity as previously observed. It was then concluded that within the range explored in this thesis, the thermal history during extrusion of NTP had only a small effect on the structure of the material after injection moulding and conditioning.

An optimal material formulation and processing temperature based on the effect of moisture content and extrusion temperature on material properties contained 34pph$_{BM}$ water and was extruded at 150 °C die temperature. It showed good mechanical properties compared to other samples. Before conditioning it had tensile strength of 5.35MPa, Young's modulus of 253MPa, strain at break of 0.34mm/mm, toughness of 1.61Mpa and after conditioning it had tensile strength of 17.49MPa, Young's modulus of 1101MPa, strain at break of 0.02mm/mm and toughness of 0.18MPa. Depending on an intended application added water content and extrusion temperature can be altered to yield a material with desired quality. It was found that the loss of moisture content during processing led to embrittlement of Novatein.

It is recommended that further study be undertaken to investigate the effect of other extrusion parameters such as SME, torque, pressure and screw-speed on the structure of Novatein in-order to maintain or improve the mechanical properties and processing conditions.

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