Effects of Drying Conditions on Protein Properties of Blood meal

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Abstract

Blood meal is a by-product of the meat industry produced through drying of animal blood. It contains about 85 wt.% proteins. Drying has been used as a method to preserve biomaterials and involves lowering of the water activity of biomaterials. The purpose of this research was to study the drying kinetics of producing blood meal in an oven dryer, to evaluate suitable drying models for describing the drying process and to determine the effects of drying conditions on the physio-chemical properties of blood meal.

Moisture content and drying rates were determined by drying coagulated blood at different temperatures (60 °C, 100 °C and 140 °C) for a constant period of 24 hours. The initial moisture content of coagulated blood was about 60.7 wt% on a wet basis. A drying temperature of 140 °C was found to be the optimal for routine moisture determination for coagulated blood as equilibrium moisture was achieved within 24 hours period. A constant drying rate period was not observed in any of the conditions tested, and the initial increasing rate period as followed by a short transition phase prior to the falling-rate period. Thus, moisture removal from the coagulated blood was governed by a diffusion-controlled process.

The experimental drying data for coagulated blood was used to fit the Lewis, Page, Modified Page, Logarithmic and Henderson and Pabis models and the statistical validity of models tested were determined by non-linear regression analysis. The Page model had the highest R^2 (0.9999) and lowest χ^2 (0.0001) and RMSE values. This indicated that Page model adequately described the oven drying behaviour of coagulated blood.

Blood meal samples were produced by drying coagulated blood at different temperatures (60 °C, 100 °C, 140 °C) to varying moisture contents (5 %, 10 % and 15 %). A drop penetration test using water and/or sodium dodecyl sulphate dissolved in water were used to determine the wettability of the samples produce while thermal analysis techniques such as Thermo-Gravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC) and Dynamic Mechanical Analysis (DMA) were used to investigate the thermal properties of bloodmeal. X-ray scattering was used to investigate conformational changes in blood meal proteins during drying.

Drying conditions had substantial effects on both physicochemical and thermal properties of blood meal. It was established that drying temperature had a more significant effect on the wettability of blood meal than the final moisture content. However, the final moisture content had larger contribution to the thermal stability of blood meal than drying temperature. Blood meal produced at 60 °C to 15% moisture content was the most stable sample while blood meal produced at 140 °C to 10% moisture content was the least stable.

Protein denaturation was observed at 92 °C to 122 °C, depending on moisture content and drying temperature. DMA results revealed that different relaxations occurred when drying coagulated blood. A dry glass transition temperature for samples was observed between 219 °C - 226.8 °C. This suggested that bound water does not act as plasticiser in blood meal. Glass transitions observed in DSC were therefore, considered more accurate and reliable for blood meal samples containing moisture. Drying of coagulated blood was observed to have drastic effect on the structural arrangement of coagulated blood in XRD. Increase in moisture content was observed to have an effect on the β-sheets structure of samples dried at 60 °C and 100 °C. Since bloodmeal produced at 60 °C and 100 °C did not show complete denaturation of proteins, future thermoplastic processing should consider blood meal produced within this temperature range for improved properties.

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Nomenclature

- Moisture ratio- MR
- Equilibrium moisture content of sample (g water/g dry solids)-M*e*
- Experimental dimensionless moisture ratio- MR*exp, i*
- Predicted dimensionless moisture ratio- MR$pre_{, i}$
- The moisture content at any time (g water/g dry solids)- M*t*
- Moisture content, dry basis- M_{db}
- Moisture content, wet basis M_{wb}
- Mean bias error- MBE
- R^2- Coefficient of determination
- X^2 – Reduced Chi-square
- Root mean square error- RMSE
- X-ray diffraction-XRD
- Thermogravimetric analysis-TGA
- Dynamic mechanical analysis-DMA
- Dynamic Scanning Calorimetry-DSC
- Sodium dodecyl sulphate-SDS
- Analysis of variance-ANOVA
- Moisture content- MC
- Enthalpy-ΔH
- Denaturation temperature- Td
- Glass transition temperature-Tg

CHAPTER 1

Introduction

The red meat industry has been a principal driver of New Zealand's economy and identity, with exports worth NZD$6.8 billion a year [1]. The meat processing industry produces large amounts of blood as one of the by-products. Approximately 80 000 tonnes of blood is collected in New Zealand every year [2]. However, blood cannot be discharged into the environment due to its high biological oxygen demand and contributes highly to total pollution [3; 4]. The use of blood and its derivatives in various industries has been explored to some extent. Blood's high protein content means potential to be used in higher value applications. For example plasma proteins are used as emulsifiers and whole blood is used in some traditional products such as black pudding. It is estimated that the food industry utilises about 30% of blood produced from slaughterhouses [5; 6]. However, the religious constraints as well as negative consumer perception of blood for direct consumption have contributed to its limited use in food applications. Therefore, blood from abattoirs is normally collected, stored, steam dried and produced as blood meal. Blood meal contains at least 85 wt.% proteins and less than 10% moisture.

Because of its high protein content, blood meal is mostly used as a high-nitrogen fertiliser and a high protein animal feed (N=13.25%, P=1.0%, K=0.6%) [7]. Bloodmeal can also be mixed with urea, sodium dodecyl sulfate (SDS), sodium sulphite and triethylene glycol (TEG) to produce a bio-based thermoplastic [2; 8; 9]. Historically, blood meal production is achieved by drying blood at temperature above 100^{o}C using different drying methods [3]. The major objective in drying agricultural product is the reduction of excess water to a level, which allows safe storage over a period of time and to destroy pathogenic organisms. Drying is also done to minimise packaging, storage and transportation costs [10]. However, when proteins are heated they usually undergo thermal denaturation. Temperature is known a strong denaturing factor for proteins. During the drying period, proteins conformation changes and the degree of protein unfolding determines the type and proportion of hydrophobic interactions, ionic and hydrogen bonds interactions that can be formed between protein chains [11]. The presence of covalent cross-links is known to be unfavourable before and during thermoplastic processing [12].

It has been shown that bloodmeal produced through convectional method had poor digestibility and doubtful value for livestock feed [13-15]. Chemically available lysine value of dried poultry blood have also been reported to decrease as the time and temperature exposure increases [16]. However, Kramer [13] pointed out that the nutritional quality of blood meal may be related to protein damage during processing. The food industry provided evidence that different drying methods affect the product quality [17]. In polymer science, the effects of drying method and thermal history have been shown to influence the relaxation time, diffusivity, stability and glass transition temperature of the materials used to produce thermoplastics [17; 18]. Additionally, the mechanical properties of protein-based plastics are known to be highly associated with the distribution and concentration of inter-and intra-molecular forces. Evidence from studies on different biopolymers suggests that many properties of polymers, especially mechanical and rheological properties are strongly related to its phase transition temperature and processing conditions [19; 20]. Up to now, there has been no unique explanation for drying effects on protein properties of blood meal as a precursor of Novatein thermoplastic.

A full understanding and appreciation of the stresses encountered by blood meal proteins during drying and the influence of thermal history on the physio-chemical properties of bloodmeal is therefore of importance in choosing the appropriate drying process and to optimize the process for high quality Novatein precursor product. The specific objectives of this thesis, therefore were:

- To study the drying kinetics of producing blood meal in hot air dryer to evaluate a suitable drying model for describing the drying process.
- To determine the effects of drying conditions (drying temperature and moisture content) on the thermal and physio-chemical properties of bloodmeal.

This study was limited to a laboratory scale and the study varied the processing conditions as a function of drying temperature and moisture content.

CHAPTER 2

Literature review

2.1 Introduction

Drying has been used as a method to preserve biomaterials and involves lowering of water activity of a biomaterial. The removal of moisture prevents survival of micro-organisms and minimizes many moisture-mediated deteriorative reactions. It brings about substantial reduction in volume and weight, lowers storage and packing space, transportation cost and it enables storability of the product under ambient temperatures. However, during drying important physical transformations such as shrinkage, crystallization, chemical and microbial degradation may take place in the product. In a nutshell, drying affects the final quality of the product. Blood meal is dried blood produced with high protein content of at least 85% [3]. Currently several methods such as spray dryers, rotary gas-filled dryers and ring dryers are most widely used in drying coagulate blood at temperatures above 100 ˚C to produce blood meal [3; 13].

The conformation of a protein is related to its functionality and is dependent on the chemical bonds which connect peptides to form different structures. However, the chemical bonds are sensitive to environmental changes such as pressure, pH and temperature. Therefore, any change in the environment has the potential to alter the chemical bonds thus changing the conformation and as a result alter functionality properties. This is to conclude that in protein quality may be due to protein denaturation during drying. This has been a major problem in protein-based materials [11; 21]. The interaction between physicochemical nature of proteins and the drying conditions is very important. Hence, the aim of this chapter was to review relevant literature on proteins, production and uses of other protein based bio-materials. It also provides information on drying kinetics, drying models of bio materials and identify the anticipated effects of drying on different protein-based materials.

2.2 Proteins

Proteins exist in all living cells and are commonly obtained from plants and animals. They are major constituent in skin, tendon muscle, hair, nerve tissue, blood, enzyme and hormones [22]. They are also present in plants playing a role in structural support and biological activity [23]. Examples of plant proteins include wheat- and corn gluten meal, soy proteins, pea proteins and potato proteins. Animal proteins include bloodmeal, casein, whey, collagen and keratin [24].

Proteins have unique properties that can be exploited for several technical applications as shown in Table 1**.** Proteins have been used as biopolymers to obtain new biomaterials with a high added value [25]. Proteins are renewable, biodegradable with great potential to improve the quality and stability of a large range of products using several processing techniques. The high value added products from proteins help reduce waste and preserving petroleum by substituting synthetic polymers [24].

Table 1. Examples in which Proteins can be used on Technical applications [24]

Protein	**Technical application**
Wheat gluten	Detergent, cosmetics, adhesives
Gelatin	Photographic emulsions, adhesives, encapsulations
Soy proteins	Ply wood adhesive, plastic, paper coatings
Corn zein	Grease proof paper, floor coatings, printing inks
Keratin	Cosmetics. Textile
Casein	Adhesives, leather finishes, paper coatings
Bloodmeal	Fertiliser, thermoplastic

According to De Graaf and Kolster, both animal and plant proteins (Table 1) are relevant for technical applications because of the following specific properties of proteins [24];

- Surface active properties
- High resistance toward U-V radiation and oil/organic solvents
- Adhesion to various substrates

- Good film forming properties and good mechanical properties of the films
- High barrier properties for gases such as oxygen and carbon dioxide

Protein properties depend on both the amino acid structure and other modifications that are performed to improve other specific properties [24]. Nevertheless, proteins have limitations. For some applications, it will be necessary to improve for instance the mechanical properties and water resistance of the materials. The thermal stability of proteins and other biopolymers have been found to be limited [26].

2.2.1 Plant proteins

2.2.1.1 *Soy proteins*

Soy proteins are primarily composed of two protein structures; 7s (β-Conglycinin) and 11s (glycinin) globulins [27]. Different method of production produces different categories of soy proteins (soy protein isolate, soy protein concentrates and textured soy protein). The proteins have a wide range of molecular weight of 8kDa to about 600kDa [28]. Soy protein isolate is a protein obtained from soybean seeds. It contains at least 90% protein on dry weight basis [29]. Soy protein isolate is normally produced by extracting defatted soy flour with water at 1:10 to 1:20 solids to solvent ratio at 60 °C under alkaline conditions. The insoluble fiber will be removed by centrifuging and the extract is acidified to pH between 4 and 5. The resulting slurry will be centrifuged and the precipitate collected is washed and centrifuged again. The washed precipitate is then spray dried or neutralized with calcium hydroxide or sodium and spray dried [30].

Soy protein concentrate is basically soybean without the water-soluble carbohydrates and contains about 70% protein. Soy protein concentrate preparation involves counter current extraction. The three common processes for manufacturing soy protein concentrate include: acid wash, aqueous alcohol wash and hot water leaching [27]. In the acid-wash process, defatted soy flour are leached with water at pH 4.5 to remove soluble sugars. The insoluble material is normally adjusted to neutrality and spray dried. The resulting soy protein concentrate will contain about 67% protein, 5.2% moisture content, 3.4% crude fiber and 4.8% ash [27]. In the aqueous alcohol wash, soy flour is extracted with 60% ethanol. A ethanol soy protein concentrate will contain 66% protein, 6.7% moisture content, 3.5% crude fiber and 5.6% ash [27]. As for the hot water leaching process, defatted soy flour is subjected to moist heat to denature the protein, followed by water leaching and

drying. The resulting soy protein concentrate contains 70% protein, 3.1% moisture content, 4.4% crude fiber and 3.7% ash [27]. Soy protein ingredients are mainly used in compounded foods for their functional properties, as plastic polymer, synthetic fibres, fire foams and paper coatings [31].

2.2.1.2 *Pea proteins*

Yellow field pea (*Pisum sativum L.*) have been used as an alternative source of seed proteins [32]. Like other legume seeds it is characteristically rich in proteins (18-30%) [32-34]. Pea seed storage proteins are composed mainly of vicillin (7S), legumin (11S) and albumins (2S) and the majority of pea protein isolates contain globular 11S and 7S proteins [32; 34]. As for its amino acid profile, pea protein mainly contains glutamic acid (19%), aspartic acid (12%), lysine (8%) and arginine (8%) [35]. Pea proteins can be processed into pea protein isolates, pea protein concentrate and pea flour. Production of pea proteins involves dry and wet processing. In the dry processing, whole or dehulled field pea seeds are pin milled to yield flours with specific density and particle size. Flours are the further separated into protein (fine structures) and starch (coarse fractions) using a air classifier [36]. The optimum moisture levels for resulting product are between 7% and 9% [37]. In the wet processing, after an alkaline, acid or water solubilisation of the proteins, the insoluble material is removed by centrifugation. The protein will be precipitated isoelectrically and the protein product can be dried either with drum, spray or freeze drying method [36]. Peas are traditionally used as swine feed, due to their high nutritive value [35]. Technical applications of pea protein are limited to surfactants, films and microspheres in cosmetics [38].

2.2.1.3 *Wheat gluten*

Wheat gluten is a 'cohesive, visco-elastic proteinaceous material prepared as a by-product of the isolation of starch from high quality wheat flour' [39]. It contains approximately 75% proteins, 8% moisture and varying amounts of fibre, starch and lipids [40]. Wheat gluten contains two main groups of proteins, gliadin and glutenin. Gluten contains hundreds of protein components which are either present as monomers or molecules with disulphide bonds. They are unique in terms of their amino acid composition, which are characterised by low contents level of amino acids with charged side groups and low molecular weight [41]. Glutenin comprises aggregated proteins, with a three-dimensional structure. There are several well-known processes used to separate wheat gluten from wheat. However, most of these

technologies are operated as “trade secret” processes. Mostly, wheat gluten processing also involves, wet-milling and dry-milling [42]. The protein content of wheat varies from 11% to 19%, based on the type of wheat and variety [43]. Their molecular weight is at least ten times higher than that of gliadins [41]. Wheat gluten is mostly used in the food industry, producing natural adhesives, packaging, films and useful in cosmetics, lotions and hair preparations [39].

2.2.2 Animal proteins

2.2.2.1 *Collagen*

Collagen is the primary protein component found in the animal connective tissues including tendons, cartilage, skin and blood vessels. It makes up about 30% of the total animal protein [44]. It is composed of three peptides chains, each approximately ~1011 amino acids. About 75 % of the amino acids are hydrophobic while 16 % amino acids contain charged side groups that are distributed along its length. The preparation of collagen protein includes: selection of the materials, pre-process, extraction, separation and purification. The most common methods used for collagen extraction are salting out method, acid method, alkaline method and enzyme method [45]. The molecular weight of collagen is approximately 100-200kDa [46]. Collagen is enzymatically degradable and has unique biological properties. It has been mainly used in food industry, photographic filming, biodegradable materials and extensively investigated for biomedical applications [47].

2.2.2.2 *Casein*

Casein comprises the largest fraction of cow’s milk proteins (80% total protein). The caseins are divided into, α_{s1}-, α_{s2}-, β- and κ-casein, distributed in proportions of 40 %,10 %,40 %,10 %, respectively, of the total casein [48]. Casein has an open, flexible structure because it lacks secondary structure. The open structure exposes the hydrophobic parts of the amino acid chain which gives a higher surface hydrophobicity [49]. Casein proteins are separated from milk proteins by acid precipitation at pH 4.6 [50]. The major uses of casein were technical, non-food applications such as paper coating, adhesives for wood, leather finishing and in synthetic fibres [51].

2.2.2.3 *Keratin*

Keratin is a fibrous protein widely found in the integument in vertebrates; feather, hair, wool, horn, and hoof. It is abundantly available as a by-product from poultry, tanning, slaughterhouse and fur processing industry [52]. Keratin contains about 12% serine, a polar amino acid as well as 7% of hydrophobic amino acids, valine and leucine. The basic macromolecule that form keratin are polypeptide protein chains. These chains can either curl into α-helices or bond side-by side into β-pleated sheets [52; 53]. Extraction of feather keratin can be achieved by breaking the hydrogen and disulfide bonds. Reducing and oxidizing agents can be used to break the disulfide bonds [54]. Keratin has a molecular weight of 10.168 kDa [55]. Keratin can be used directly to produce variety of cosmetics, creams, hair conditioners, and pharmaceutical products. Keratins from feathers is also used for the purpose of textile, composite and agricultural industries [56]. Though keratin finds application in different industries, it is so difficult to degradation and its disposal leads to environmental problems [53].

2.2.2.4 *Bloodmeal*

2.2.2.4.1 Blood

Blood composition

A large amount of blood is produced during slaughter in abattoirs. Approximately 2-5% of the live weight of cattle is blood, comprised of 80.9 wt.% water, 17.3% protein, 0.23% lipid, 0.07% carbohydrate and 0.62% minerals [57]. Blood can be fractionated on a volume basis of plasma (65-70%) which contains suspended red blood cells and cellular mass (35-40%), [57]. The general chemical composition of whole blood and its fractions are shown in Table 2.

Bovine blood plasma contains 7.9% protein, mainly albumins (3.3%), immunoglobulins, globulins (4.2%) and fibrinogen (0.4%) [57]. Dehydrated plasma contains about 7% moisture, 80% protein, 7.9% minerals and ~1% fat [58; 59]. Because of the high protein content of blood, it is sometimes referred to as "liquid protein". The amino acid content of blood proteins varies with species. Bovine blood has been reported to have deficiency in isoleucine and methionine as compared to poultry blood with higher quantity of isoleucine [60].

Table 2. General chemical composition of whole blood and its fractions (wt.%) [57]

Component	Whole Blood	Plasma	Red Blood cells
Water	80	90.8	60.8
Salts	0.9	0.8	1.1
Fat	0.2	0.1	0.4
Protein	17	7.9	35.1
Albumin	2.8	4.2	-
Globulin	2.2	3.3	-
Fibrinogen	0.3	0.4	-
Haemoglobin	10	-	30
Other	1.1	0.4	2.6

Applications of blood

Animal blood has several potential application in other areas of the food industry. Blood has long been used as emulsifiers, to make blood sausages, blood cake, biscuits, blood curd, bread and blood pudding [61]. However, issues such as transimmission of spongiform encephalopathies on blood collected from slaughter houses have been raised [61]. Negative consumer perceptive as well as the religious constraints of blood for direct consumption have also contributed to its limited use in the food industry. This has resulted in animal blood being used in other industrial applications as shown in Table 3.

Table 3. Blood utilisation in different sectors [62]

Sector	Utilisation
Animal feed	Supplement, dary enricher, pH stabiliser
Food industry	Stabiliser, emulsifying, thickening
Fertilisers	pH stabiliser, mineral enricher, seed coating
Laboratory	Culture media, catalase, globulins, albumin
Medical	Plasminogen, fibrin, fibrinogen, immunoglobulins
Pharmaceutics	Comestics
Industry	Adhesive, insecticide coadjuant, cellular concrete,fire extinguisher, plastic additive, thermoplastic material

Blood collection and Processing

Blood from slaughterhouse animals can be collected via open drainage and closed drainage method [63]. In the open drainage method blood from animal is drained into buckets or trays. The method is more susceptible to contamination during the blood collection process [63]. Blood is more susceptible to bacterial growth because of its high nutritional value. For the closed draining system, blood from the slaughterhouse is not exposed to air and is drained directly from the animal. The method can be expensive and slows down production [64]. With the right collection procedure, blood components can be used as raw material for technical and human consumption products. Control of the temperature of the raw blood is necessary in order to ensure an end product that meets the exceptionally stringent requirements. Slaughterhouse blood can therefore, be utilised in novel ways as shown in Figure 1**.**

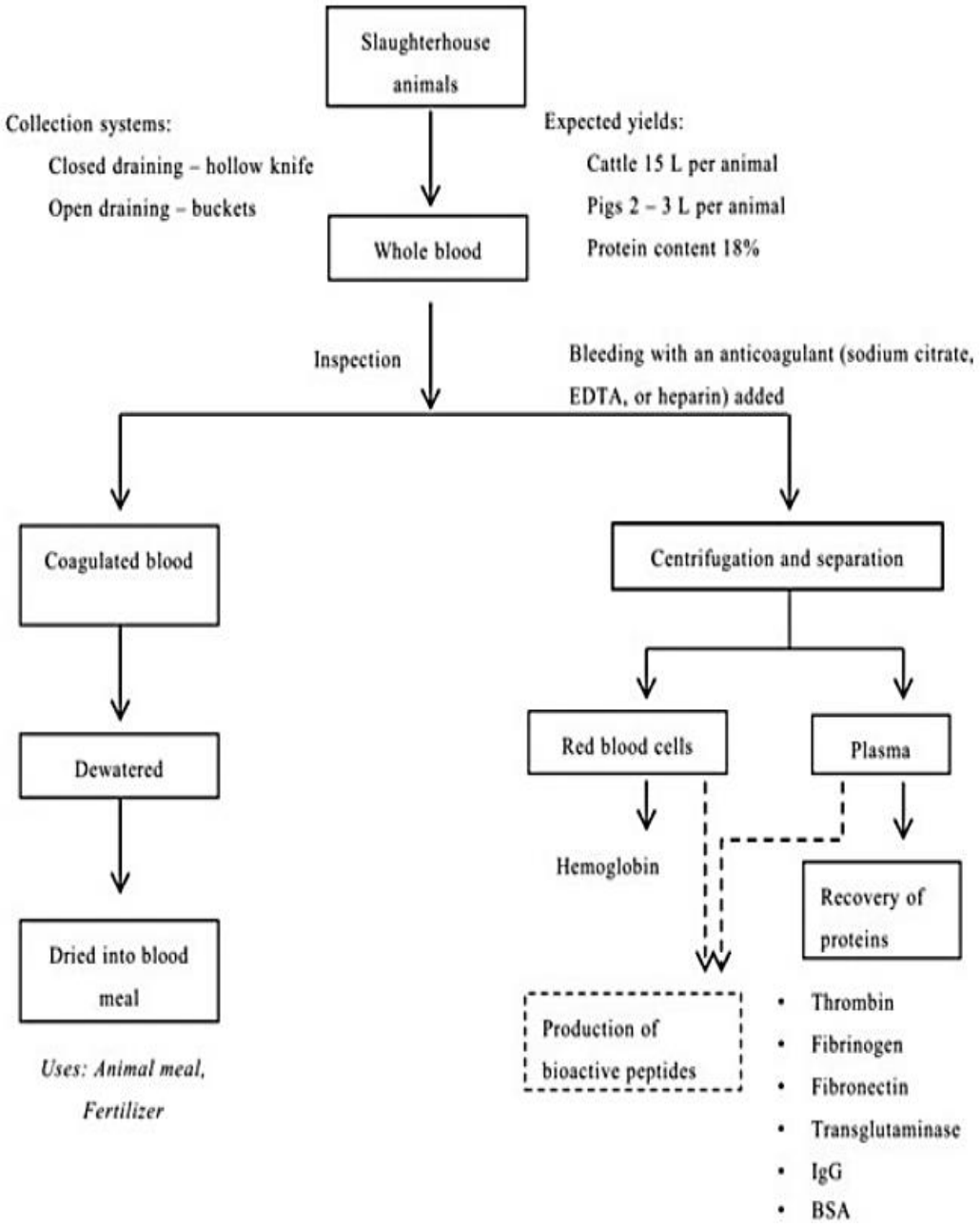

Figure 1. Animal blood collection and separation into usable fractions [65]

Blood meal is an insoluble powder with at least 80wt % protein and less than 10 % moisture, [3], produced by drying bovine blood. Bloodmeal is rich in the amino acid lysine and has a high cysteine content. However, about half of the amino acid of bloodmeal is polar with valine, leucine, isoleucine, phenylalanine and methionine as the most hydrophobic. Blood meal processing methods may be divided into coagulation and whole blood systems [13]. In the coagulate system, blood is normally filtered to remove fragments, coagulated using steam injection at

90 ˚C, [13] and centrifuged to separate the coagulated blood. The coagulated blood will be dried at temperatures above 100˚C using several methods such as spray dryers, rotary gas-filled dryers and ring dryers. The method requires less energy to produce a denatured protein [3; 13]. In the whole blood systems blood solids are not separated from the liquid portion before drying. It is usually used at smaller plants with old equipment. Indirect heat from a steam jacketed shell is applied to whole blood in a batch cooker to boil off water. Some processes use stirrers to increase heat transfer. Blood is dried until it reaches 2-10% moisture. Temperatures can reach up to 138 °C and drying times between 10-15 hours [3; 13; 66].

The solar and oven drying is well suited for small scale-operations. Blood is collected in large pans and slowly boiled while stirring constantly. When the moisture is sufficiently reduced to 10-12%, bloodmeal is spread on a clean cemented surface and sun-dried or oven dried. For the drum drying, the raw blood is finely comminuted to form a free-flowing slurry that is then deposited onto the descending side of the top of a heated dried drum and formed into a film by one or more spreader rolls. The drum is heated to a temperature between 120 ˚C and 175 ˚C [67]. The film is rapidly dried and scraped in the form of a dried sheet which can either be flaked or pulverized to provide a high-grade blood meal product. The drum is rotated at a speed sufficient to keep the blood solids at temperatures insufficient to destroy the protein and amino acid content of blood [67].

For the ring and flash drying method, animal blood is dispersed into the high velocity venture section of the system. The blood first encounters the hot drying airstream and the bulk of evaporation occurs. The product is then dried as it is conveyed up through the drying column. In spray drying animal blood is spray dried as whole blood, or after-separation into plasma and red albumin. Drying is done at low temperatures to prevent heat coagulation. Spray dried blood meal is of higher quality than the sun-dried blood meal since the duration of the heating period is lower than with cooking. Proteins and amino acids are better preserved and lysine content is higher [68]. Bloodmeal is mostly used as a low cost fertiliser and as animal feed. High protein account have made bloodmeal an attractive resource for Novatein thermoplastic [2].

2.2.3 Physicochemical properties

2.2.3.1 *General protein structure*

Proteins are a group of natural polymers with diverse biological, structural and functional characters. They are heteropolymers with monomeric units linked in specific sequence by a special type of amide bond called the peptide bond. Proteins are classified as condensation polymers because their synthesis involves elimination of water to form a peptide bond between amino acids as shown in Figure 2 [69; 70]. An amino acid is a compound that contains at least amine group ($-NH_2$) and at least one carboxyl group (-COOH).

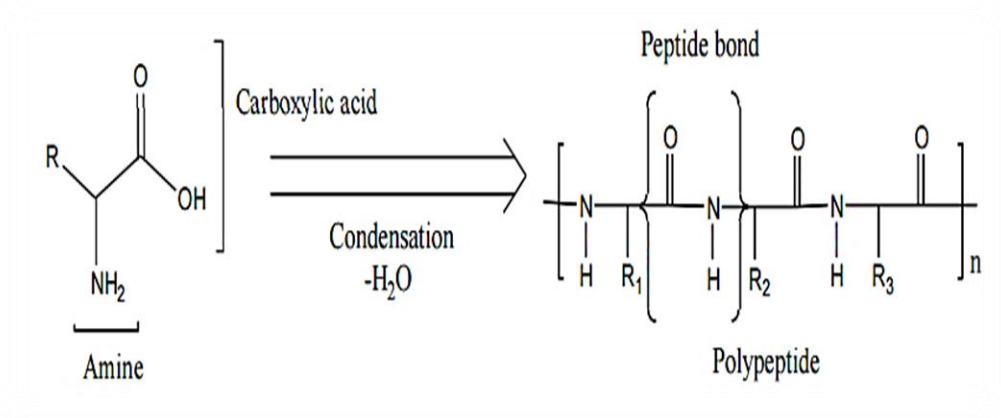

Figure 2. Condensation reaction of protein to form a peptide bond

The chemical and physical properties of proteins are determined by amino acids constituents. There are twenty common α-amino acids found in nearly all proteins. The amino acids differ from each other in the structure of the side chains bonded to their carbon atoms. The side chains can be electrically charged or uncharged, hydrophobic or polar as shown in Table 4. Different combinations of amino acids result in the formation of various three-dimensional structures. The protein structure is divided into four different levels; primary, secondary, tertiary and quaternary, each of which describes a different aspect of the 3D structure as shown in Figure 3.

The primary structure is the most basic level and only describes the amino acid sequence of the polypeptide chain [69]. The primary structure is held together by covalent/peptide bonds which are made during the process of protein translation. The Secondary structure describes how the molecule folds into highly regular 3D structures such as the alpha helices and beta sheets being formed. It involves hydrogen bonding between a carbonyl group of one amino acid and an amine of

another group [71; 72]. The tertiary structure explains the overall shape of the single protein molecule and demonstrates how the secondary structure relate to each and are driven by the hydrophobic interactions between the amino acids. The Quaternary structure involves how multiple folded polypeptide chains aggregate into a larger full protein structure [69]. The final folded conformation of a protein molecule is influenced by a number of intermolecular interactions [69].

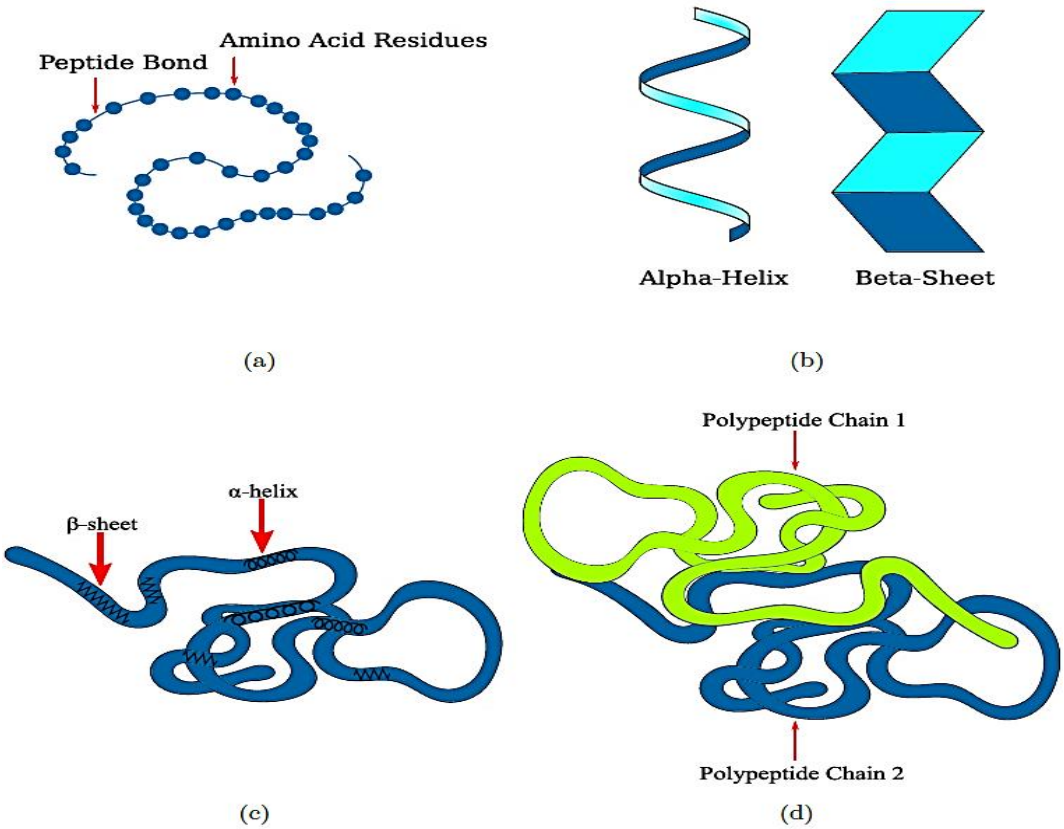

Figure 3. Different levels of a protein's structure: a) the primary structure, b) the secondary structure (alpha helices and beta sheets), c) tertiary structure, d) quaternary structure

Table 4. Standard Amino acids grouped according to their chemical properties of their side chains [73]

Group	Full name	Acronym	Side chain group	Additional side chain characteristics
Nonpolar	Glycine	Gly	–	Nonpolar, carry single hydrogen atom
	Alanine	Ala	Methane	The least hydrophobic
	Valine	Val	Propane	Intermediary hydrophobicity
	Leucine	Leu	2-Methylpropane	Highly hydrophobic
	Isoleucine	Ile	Butane	
	Proline	Pro	Pyrrolidine (Tetrahydropyrrole)	Side chain attach to the α-amino group, forming a pyrrolidine ring
	Methionine	Met	(Methylsulfanyl)ethane	Contain divalent sulfur atom, nonpolar, but capable to weakly interact with polar molecules
Polar neutral	Serine	Ser	Methanol	Contain hydroxyl group
	Threonine	Thr	Ethanol	
	Cysteine	Cys	Methanethiol	Contain thiol group (sulfhydryl)
	Asparagine	Asn	Acetamide	Contain carboxyamide, which can serve as hydrogen donor and acceptor
	Glutamine	Gln	Propanamide	
Electrically charged	Glutamate	Glu	Propanoate	Acidic, contain carboxylic group.
	Aspartate	Asp	Acetate	pKa = 4.3 (glutamate), pKa = 3.9 (aspartate)
	Lysine	Lys	Butan-1-amine	Basic, side chain includes nitrogen containing group
	Arginine	Arg	1-Propyl-guanidine	
	Histidine	His	4-Methyl-1*H*-imidazole	
Aromatic	Phenylalanine	Phe	Methylbenzene	Include benzene, overall nonpolar
	Tyrosine	Tyr	4-Methylphenol	Include phenol ring with hydroxyl group
	Tryptophan	Trp	3-Methyl-1*H*-indole	Include indole

The protein structure in its natural environment is stabilised through hydrophobic interactions, hydrogen bonding and electrostatic interactions between amino acid functional groups. Once folded, the structure may be stabilised further with strong covalent cross-links. However, the protein structure can be disrupted by external stress or compound such as change of pH, addition of chemicals, increasing temperature and when pressure is applied. The structure and protein functions depend on their amino acid sequence and their dynamic behaviour of protein conformations. The protein surface activity it is therefore governed by the protein size, shape, amino acid composition, net charge and charge distribution, amino acid sequence and their higher structure, surface hydrophobicity or hydrophilicity, molecular flexibility and rigidity [74].

2.2.3.2 *Protein denaturation*

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, [75]. The process is known as denaturing. It can be defined as the unfolding of the protein from a structured native state into an unstructured with no or little fixed residual structure, which is not far from a random coil. During denaturation, the hydrogen bonds, hydrophobic interactions, covalent disulphide and ionic interactions are affected, causing protein structure untangling into a random coil conformation. Protein denaturing can be reversible or irreversible. Reversible meaning the proteins can regain their native state when the denaturing influence is removed. Heat denaturation of proteins is usually irreversible at temperatures higher than 80 ^{0}C [76], because under these conditions proteins generally aggregate after heat treatment.

Denaturation can have several important consequences, such as increase in viscosity of protein solutions, decrease of solubility due to the exposure of hydrophobic groups, increase of reactivity of side groups, altered sensitivity to enzymatic proteolysis and altered surfactant properties [77]. Denaturation therefore can have both negative and positive effects; the consequences thereof are important for protein processing because a change in protein structure is usually associated with changes in physical-chemical and functional properties. Denaturation of proteins includes thermal denaturation, denaturation by using high concentration of urea, inorganic salts, organic solvents, detergents and changing pH.

In most literature denaturing is a thermodynamic phenomenon which depends on temperature and heat is known to be the most common factor that causes the denaturation of proteins. Heat treatment of proteins increases molecular motion, leading to the rupture of various intermolecular and intramolecular bonds of native protein structure [74; 78]. According to De Graaf denaturing occurs above medium water content (>5%) and above temperature approximately 75 ^{0}C [79]. Since heat is used to produce industrial proteins, bloodmeal has a risk of protein denaturation. However, the denaturing temperature of proteins differ per their source, additives and processing methods. Moreover, the denaturation temperature of the protein depends on the water content [79; 80]. The denaturing temperature decreases from 120 ^{0}C to 200 ^{0}C down to 80 ^{0}C between 0 and 20% w/w water [79]. In order for denaturation to take place in a material on a measurable time-scale, the molecular

mobility of the material should be sufficient that is above glass transition temperature [79].

2.2.3.3 *Hydration and dehydration*

Protein-water interactions play an important role in maintenance of the three-dimensional structure of proteins [74]. The thermodynamics of protein-water interaction directly affects wettability, dispersibility, solubility and swelling of proteins. Protein hydration is a process from its dry state to the solution state which occurs over a very wide water activity. Water molecules bind to both polar and non-polar groups in proteins via dipole-dipole, dipole-induced dipole and charge-dipole interactions. The hydration of a protein is therefore, related to its amino acid composition and is affected by conditions such as ionic strength, pH and temperature [78; 81].

Protein structure is largely determined by its interaction with water. However, dehydration results in the removal of "bound" water which is intimately hydrogen bonded at protein surface. Dehydration is normally a result of drying. Removal of 'bound water' is accompanied by a reduction in the total number of intermolecular hydrogen bonds between protein and water molecules [17]. Dehydration can therefore destabilize the protein native structure. Many charges of protein molecules will be exposed to each other when a protein is dehydrated during drying. However, the extent of protein dehydration may differ from one drying condition to the other [17]. Protein denaturation that occurs during drying is thought to greatly contribute to protein aggregation and loss of functionality. Aggregation is hydrophobically driven. Protein denaturation result in greater exposure of hydrophobic groups which in an aqueous environment leads to aggregation [82].

2.2.3.4 *Solubility*

The solubility of proteins is an important property that affects and predicts other functional properties. The solubility of proteins is a thermodynamic manifestation of the equilibrium between protein-solvent and protein-protein interactions. Solubility depends on proteins amino acid composition and sequence. The factors that influence the solubility of protein include; surface hydrophobicity, net charges and polarity [74]. Hydrophobic interactions promote protein intermolecular interactions, resulting in a decreased solubility. However polar and charged amino residues, promote protein-water interactions, resulting in increased solubility. As a rule, proteins containing more polar and charged groups, globular in shape,

relatively small in molecular weight have better solubility. Nevertheless, proteins with random structure or highly aggregated protein polymers and highly hydrophobic proteins, are generally insoluble in a solution. Many thermodynamic variables, such as pH, ionic strength and temperature affect protein solubility and compatibility. At constant pH and ionic strength, the solubility of most proteins increases with an increase in temperature [74; 78].

2.2.3.5 *Wettability*

Wettability plays an important role for the amount of conformational changes of adsorbed proteins [83; 84]. Wettability is known as the ability of a powder to be penetrated by a liquid due to the capillary forces [85]. According to Ji *et al* [86] wetting behaviour of proteins can be assessed through different procedures including, immersional wetting, capillary, spread wetting and condensational wetting as shown in **Figure 4**.

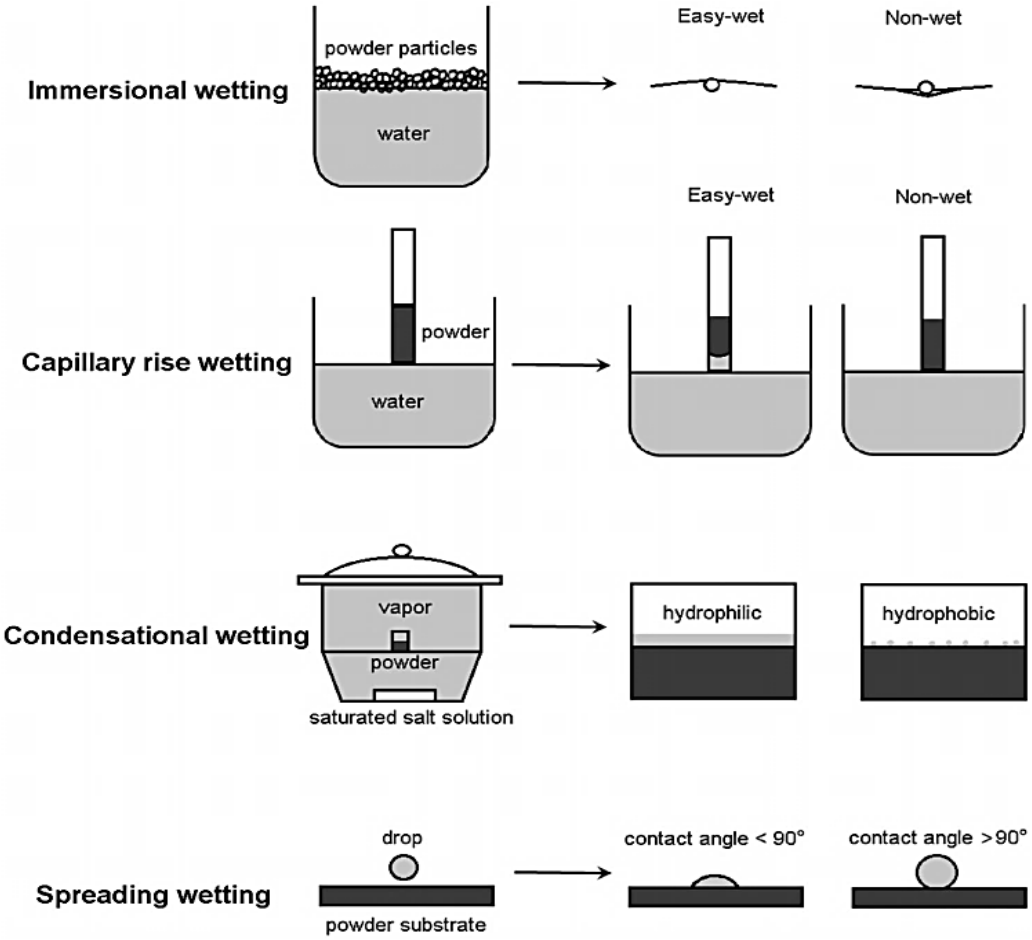

Figure 4. Different wetting procedures [86]

Protein adsorption/unfolding is considered to be hydrophobically driven [83]. The relationship between protein adsorption and hydrophobic interactions were indicated by researchers showing that the protein adsorption increased with decreasing of the protein wettability [87]. In a hydrophilic surface, water molecules exhibit a relative more-dense water structure in an extended 3D network of self associating molecules, while for the hydrophobic surface, water molecules are ordered in an ice-like structure at the surface [88]. The water structure therefore, makes a significant difference between hydrophilic and hydrophobic surfaces.

Proteins are adsorbed weakly with a conformation near to their native state. Therefore, protein adsorption on hydrophilic surfaces is reversible whereas the hydrophobic ones are not reversible as shown in Figure 5. Wettability affects the water structure near to the surface and therefore the protein adsorption and the cell behaviour [83; 89].

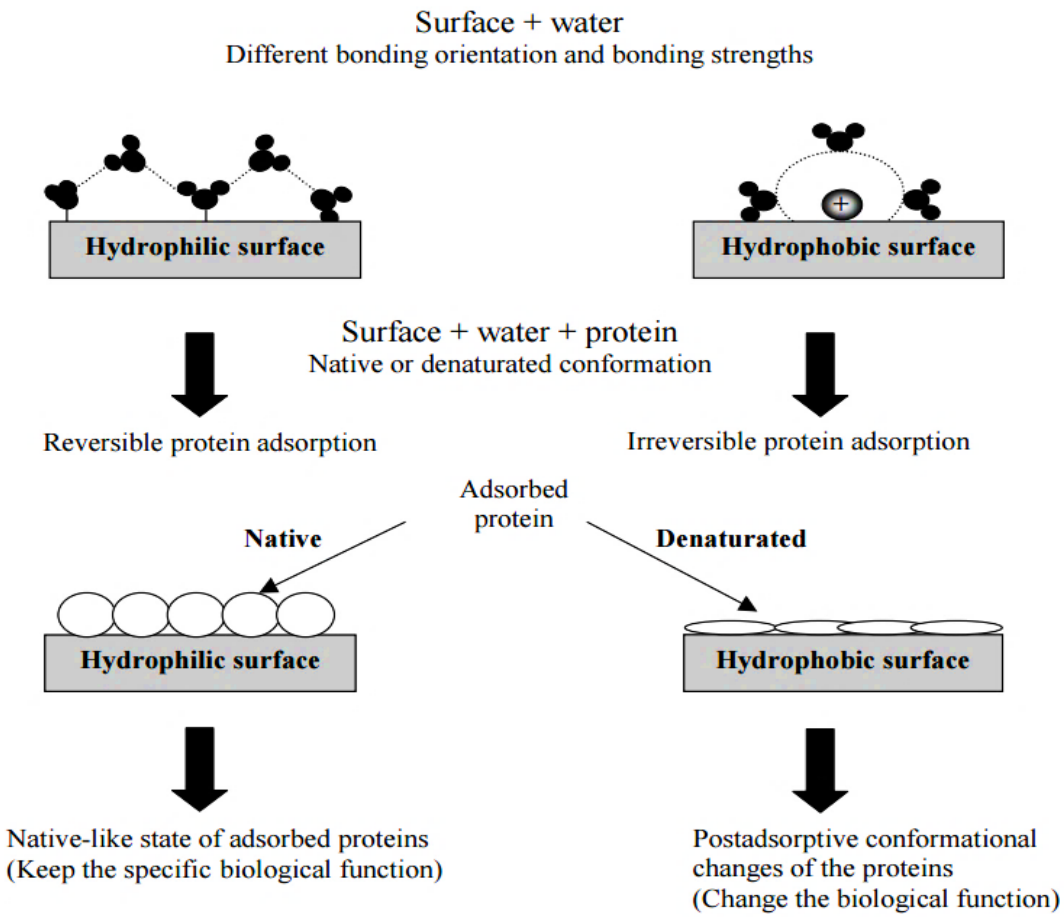

Figure 5. Schematic illustration of the events following after the blood (or plasma) contact with a hydrophilic or hydrophobic surface [89].

2.2.4 Thermal Properties

For a protein to behave like a synthetic polymer, the protein chain is required in an extended conformation enabling the formation of sufficient chain entanglement [75]. In order to achieve this multiple non-covalent and covalent interactions, chains need to be allowed to unfold [8]. Proteins containing small amounts of lysine and cysteine will less likely to form covalent cross links and will require temperature, pressure and a plasticiser to form a plastic [90; 91]. Cysteine is a sulphur-containing amino acid and can form sulphur-sulfur (S-S) cysteine bonds with other intra- or intermolecular cysteine molecules. The cysteine bonds impair the processing thermoplastic in the melt-state. Proteins such as keratins and blood meal are defined by a large amount of cysteine and lysine therefore requiring reducing agent to break the covalent crosslink as compared to other proteins [92; 93].

The transition of a protein from the glassy to rubbery and viscous flow states is also known to govern its processability. The transition is achieved with judicial application of heat, pressure and chemical additives. The protein primary structure and the protein natural state therefore influence each of these factors. Based on the structure of proteins, the thermoplastic processing requirement have been identified as follows [75];

- Breaking of protein stabilising intermolecular bonds by either chemical or physical means
- Orientating and arranging mobile chains in the desired shape
- Enabling formation of new intermolecular bonds and interactions to stabilize the three-dimensional structure.

2.2.4.1 *Glass transition temperature*

In order to predict the state and the behaviour of proteins during processing and storage, it is vital to understand the phenomenon of glass transition and its relationship with the physicochemical changes. Glass transition temperature (T_g) is defined to be the temperature at which the material softens due to the onset of long range coordinated molecular motion in the polymer main chain; from the immobile (glassy) to mobile (rubbery) state [94]. The transition from a glassy state to a rubbery state is not a change in the material but molecules are randomly disposed rather than being in definite crystalline structures [95]. A number of synthetic polymers exist in an amorphous state, which is a non-equilibrium state at

temperatures below the equilibrium melting temperature of the material [96]. Figure 6 shows various transitions an amorphous material undergoes with change in temperature.

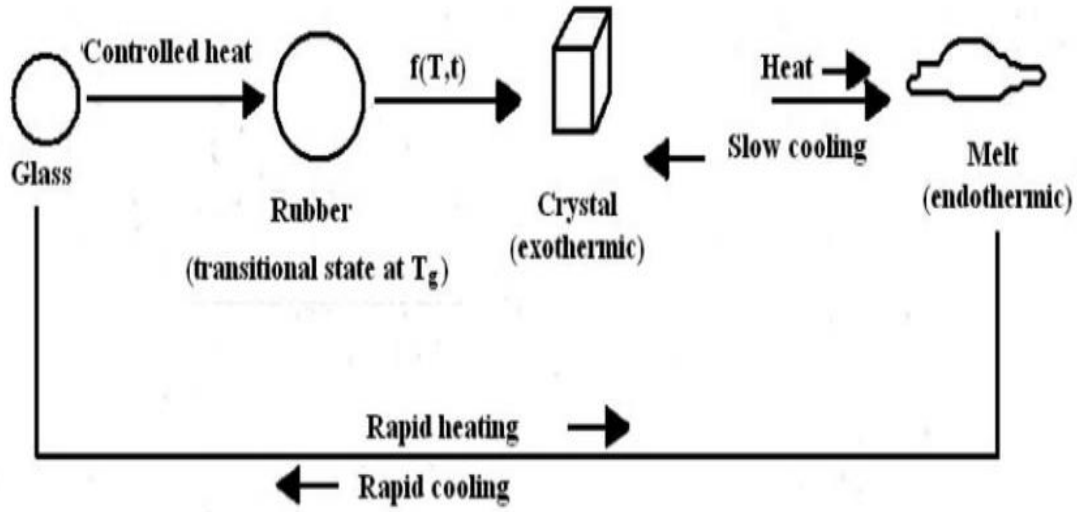

Figure 6. Changes of the Amorphous physical state through rubbery to crystalline state [97].

Above the glass transition temperature, polymeric materials softens and are in a soft, rubbery state[94; 98] and the large chain segments are able to move resulting in a mobile material [79]. At temperatures below the glass transition, amorphous solids are in a metastable glassy and stiff state [95]. According De Graaf, below T_g, only a maximum of four chain atoms is involved in motions. In the glass transition region 10-50 chain atoms attain sufficient thermal energy to move in a coordinated manner [99]. The physical properties associated with increases in molecular mobility are significantly affected and vary accordingly at the glass transition range [100; 101]. The glass transition is therefore influenced by function of temperature, time, pressure, composition, molecular weight and water activity [100; 102-105].

2.2.4.2 *Effects of water on Glass transition*

Glass transition temperature of hydrophobic polymers decrease significantly with an increase in moisture content. It has been observed that at higher moisture content the T_g decreases with increase in moisture content at a high rate than that observed at lower water content [106]. De Graaf and Sochava also observed that the dependence of T_g on the water content is stronger than that of denaturation temperature (T_D) on the water content [99]. T_g decreases more rapidly than T_D upon increasing water content [79; 80].This is thought to be due to a larger free volume

change created by the molecules of water adsorbed on the protein. Although this effect depends on the chemical nature of the protein [107]. As shown in Figure 7 freeze-dried carp surimi-trehalose mixtures with different moisture content had a clear significant decrease of glass transition temperature [108].

In soy proteins, the T_g of the Conglycinin (7s) fraction ranged from 114 ^{0}C to 67 ^{0}C with moisture content between 0% and 35% whilst the T_g of the glycinin (11s) fraction ranged from 160 ^{0}C to -17 ^{0}C with moisture content between 0 and 40% as shown in Figure 8 [109]. The influence of moisture content on the glass transition of an extrudate made from meat, bone meal and Sodium caseinate reflected plasticization of the material by water [110]. For blood meal, the T_g was observed well above 200 °C and reduced to about 130 °C when water and TEG were added as shown in Figure 9 [111]. Moisture or water content acts as a plasticizer due to the formation of hydrogen bonds with polymeric chains, hence increasing the distance between polymeric chains and decreasing Tg. Therefore, the effect of moisture in protein polymers is of utmost importance in determining processability and physical properties.

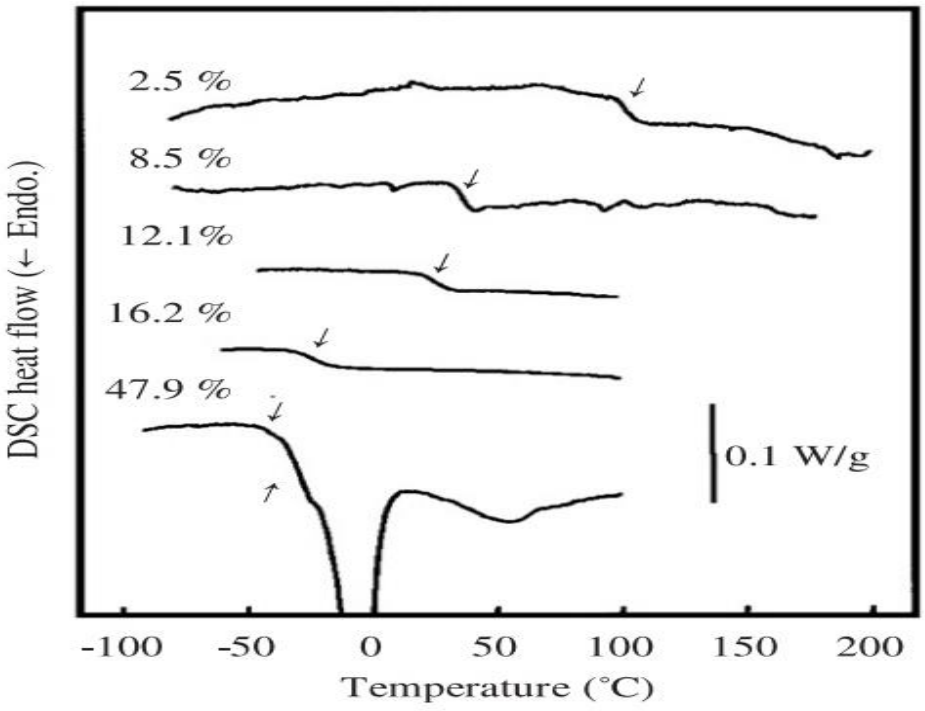

Figure 7. DSC thermograms for freeze-dried carp surimi–trehalose mixtures (80% trehalose in dry matter) of varying residual moisture. Down-arrows shown in the DSC thermograms for samples containing 2.5–16.2% (w/w) moisture indicate Tg. Down-arrow and up-arrow shown in the DSC thermogram for a sample containing 47.9% (w/w) moisture indicate T'g 1 and T'g 2, respectively [108]

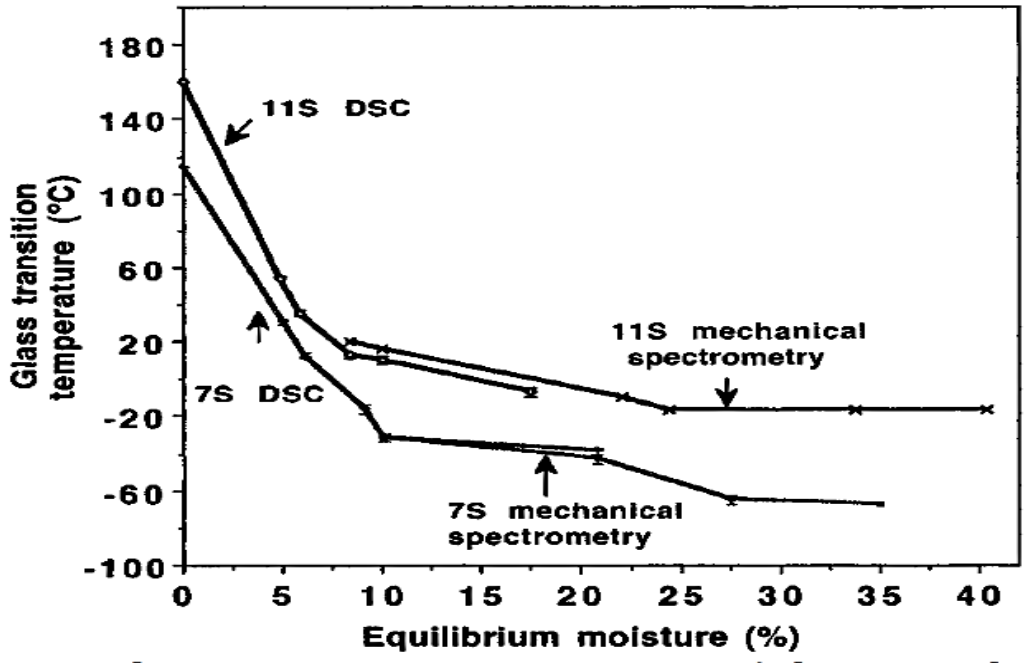

Figure 8. Glass transition temperature of the 7s and the 11s soy globulins as a function of moisture by differential scanning calorimetry and mechanical spectrometry [109]

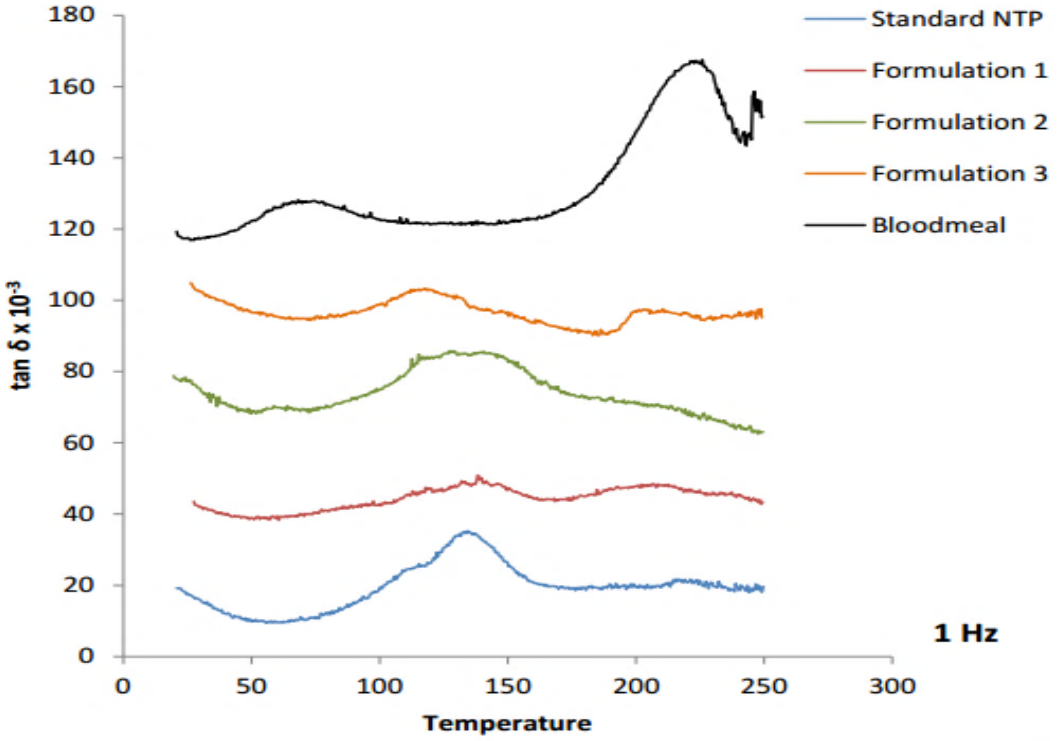

Figure 9. DMA results showing Tg of four different NTP formulations with raw blood [111].

2.2.4.3 *Effects of molecular weight and chain length in glass transition temperature*

The glass transition temperature values are strongly influenced by the molecular weight of a polymer material [105]. The low molecular weight polymers and monomers in their pure form have lower T_g than the longer chain molecules. Figure 10 shows the effect of molecular weight on the glass transition temperature of polystyrene [105]. Bizot *et al*, [112] considered the increase in glass transition temperature of linear polymers to be having a positive correlation with their molecular weight. Abiad *et al*, [101] also confirmed that increasing the molecular weight or cross-link density for a given polymer will decrease its specific volume, resulting an increase in glass transition temperature. Therefore, glass transtition temperature increase with an increase in chain length and molecular weight.

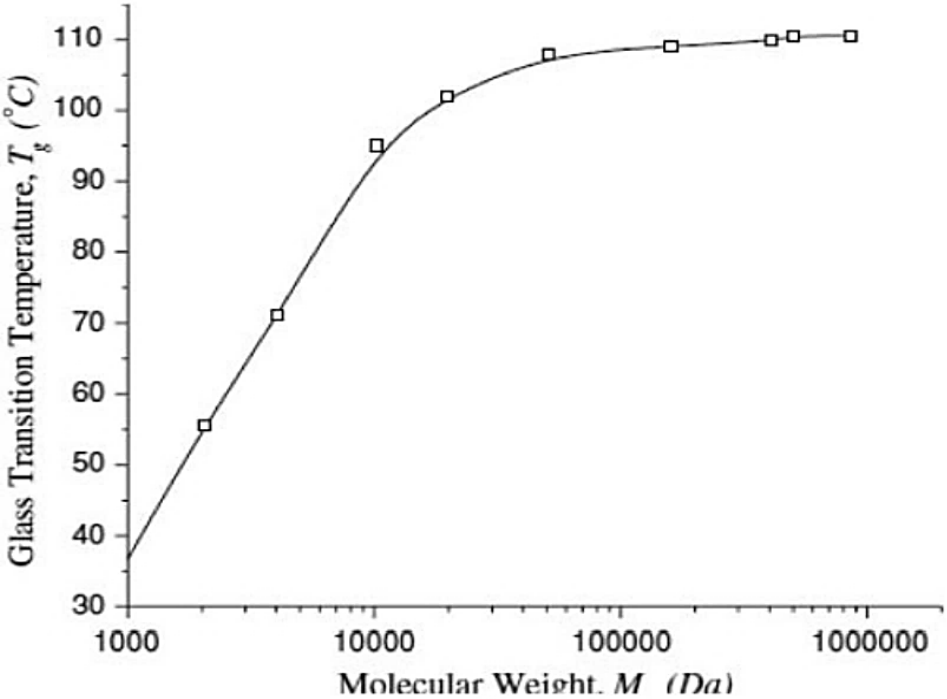

Figure 10. Effect of molecular weight on the Tg of polystyrene [105].

2.2.4.4 *Effects of Heating/Cooling Rate on Glass transition*

Glass transition temperature is dependent on the rate of heating and cooling [113]. Cooling rate is directly proportional to Tg. If the rate of cooling of molten solid is higher, it results in higher values of Tg and slower cooling rate results in lower Tg [114-116]. According Chaudhary [117] the glass transition temperature (Tg) can be

utilised to elaborate the relationship between the vitrification and metastability of systems in order to develop innovative product formulations and methods of processing. In choosing conditions for drying of proteins, glass transition temperature is a crucial factor that needs to be considered [118].

2.2.4.5 *Crystallization and Melting*

The release of water molecules from protein structure induce change in protein solubility which may either lead to crystallization or precipitation. Protein based thermoplastics, are not widespread crystals of entire structure but the presence of α-helix and β-sheet secondary structures resemble crystalline zones as other polymers [119]. The presence of crystalline phase in proteins introduce a potential melting event and give rise to glass transition temperature in amorphous regions of semi-crystalline polymers than in purely amorphous polymers [120]. This is because the semi-crystalline polymers differ in behaviour from completely amorphous system. The long range segmental motions associated with the glass transition become limited in the amorphous regions of partially crystalline polymers [121].

2.2.4.6 *Thermal Analysis Techniques*

Thermal analysis refers to a group of techniques with a common feature, in which a material's response to being heated, cooled or held isothermally is measured. The main aim is to establish the relationship between temperature and specific physical properties of material [122]. Thermal analysis is widely applied in fields, including polymer science, medicines, ceramics ands foods. It is mostly used in the quality control of production field, material acceptance inspection and process control. The most commonly used techniques are listed in Table 5. Of these techniques, DMA, DSC and TGA are widely used for characterizing thermal transitions and thermal stability. A selection of representative thermal analysis is presented in Figure 11**.**

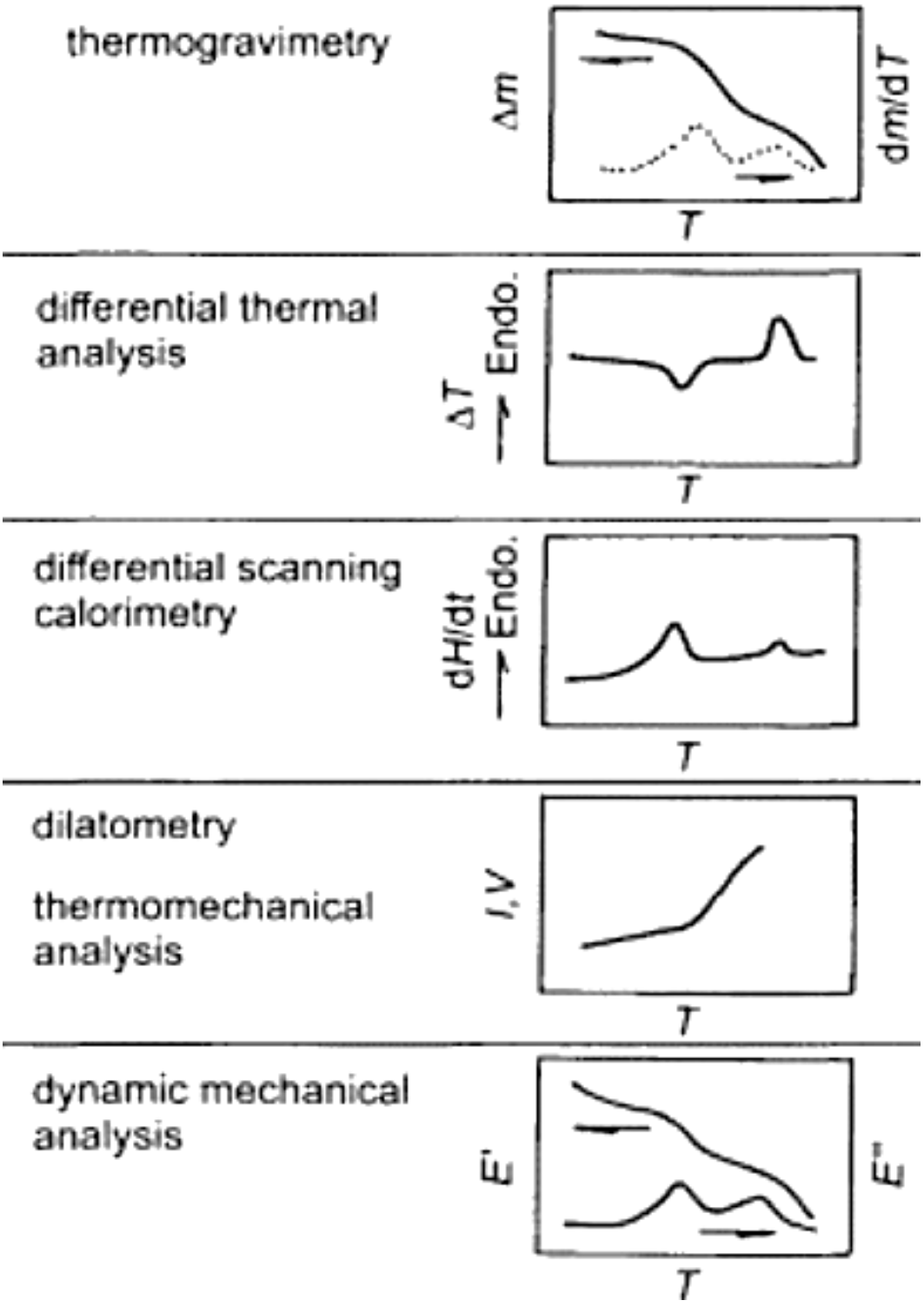

Figure 11. Représentatives TA curves [123]

Table 5. The Most Important General Methods and Techniques of Thermal analysis techniques [122; 124].

Technique	Acronym	Measures	Reveals
Dynamic mechanical analysis	DMA	Deformation under oscillating load	Glass transition Viscoelastic properties
Differential Scanning Calorimetry	DSC	Heat flow difference	Glass transition, heat capacity, phase changes
Thermal gravimetric analysis	TGA	Mass change	Decomposition temperature, oxidative temperature, volatilisation of moisture and plasticiser, moisture content
Differential Thermal analysis	DTA	Temperature difference	Exothermic and endothermic thermal events
Simultaneous DSC/TGA	SDT	Mass change and heat flow or temperature difference	Causes of mass loss events
Dilatometry	DT	Length or volume change	Creep, thermal expansion
Thermomechanical analysis	TMA	Deformation under constant load	Creep, thermal expansion, heat deflection temperature

2.2.4.6.1 Dynamic mechanical analysis

DMA is a frequency response analysis that uses a constant oscillating force, recording the materials response as a function of at selected temperatures and frequencies [123]. DMA is used to measure viscoelastic properties and glass transition (Tg) of polymeric amorphous materials. Under compression/tension deformations the measured viscous component of the material's response is referred to as the loss modulus (*E*''), while the energy stored by reversible deformation of the material or elastic component of the material's response is referred to as the

Storage modulus (*E*'). The ratio of the loss modulus to the elastic modulus is referred to as tan delta (δ) [125]

The glass transition region is identified as the region in which the temperature where a decrease *E*' or the maximum in tan δ. These contribute to a peak in the plot of tan δ versus temperature [99; 124; 126; 127]. Alternatively, the glass transition temperature could also be reported as the peak in the loss modulus or the onset of drop in storage modulus [90; 91]. However, materials do not always have a single glass transition, therefore Tg is referred to the range of temperatures in which the material undergoes drastic changes in its thermos-mechanical properties [125]. Frequency of testing is always reported since DMA results from are frequency dependent. DMA also reveal secondary transition below $T_{g.}$ In dry blood meal material pocket DMA detected T_g at about 200 ^{0}C along with smaller transitions at about 60 and -90 to 70 ^{0}C [128]. Guan *et al* also observed the same transition in native silks [129].

2.2.4.6.2 Thermal gravimetric analysis (TGA)

Thermal decomposition of protein bioplastics in inert gas can be used to characterise protein interactions. Thermal gravimetric analysis was used to analyse the thermal stabilities of native proteins and their derivatives [130; 131]. The TGA curve, (figure 9) generally consists of four parts: (1) water elimination; (2) plasticizer decomposition or elimination; (3) weak bond cleavage resulting in peptide bond cleavage; (4) stronger bond cleavage contributing to total degradation [93; 131]. The temperature at which the four events occur depends on the protein source, plasticizer content, processing techniques used and the chemical additives. The thermal decomposition temperature is normally shown by the derivative peak maxima in part (3) as shown in Figure 12 [93].

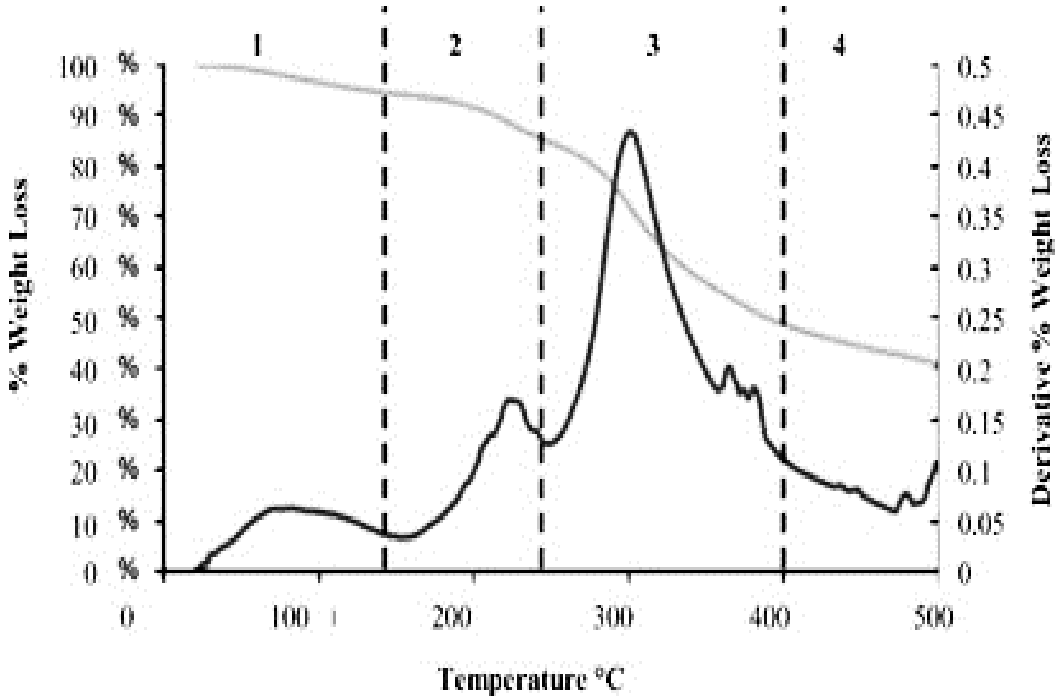

Figure 12. Typical TGA curve of a protein-based plastic [93]

The native plant proteins (gluten, zein, pea and soy proteins) degradation temperature occurs above around 330 °C and their acetylated derivatives were found to less stable than their counterparts [130]. However, the decomposition temperatures are higher than the required temperatures to soften the derivatives during processing [130]. The TGA scans of SPI resin indicates, the degradation of 30% glycerol-plasticized-SPI at 250 °C and the 25% stearic acid at 275 °C. Above 275 °C, the percentage weight loss dropped faster than below 275 °C [29]. Chen also indicated that glycerol plasticised soy proteins have three stages of weight loss during heating under nitrogen atmosphere. From room temperature to 120 °C mass loss was due to moisture evaporation; from 120 °C to 260 °C the loss was due to volatilisation of glycerol and beyond 250 °C was due to the decomposition of protein [132]. Samples of freeze dried-Pre-extruded NTP showed very little mass loss below 120 °C; the larger mass loss occurred between 150 °C and 250 °C which was corresponded to TEG evaporation [12]. A small mass loss determined from TGA of bloodmeal alone would be expected at approximately 5 wt%, corresponding to about 4 wt% of the blended NTP formulation [12]. Nevertheless, according to Verbeek and Bier, bloodmeal has a glass transition temperature of 220 °C, which corresponds to the onset of significant mass loss due to protein degradation when investigated using thermogravimetric analysis (TGA) [75].

2.2.4.6.3 Differential Scanning Calorimetry (DSC)

DSC is an effective analytical tool used to characterise the physical properties of a polymer. It measures the difference in heat flow between a specimen and a reference as a function of temperature. It enables determination of melting, crystallisation, characterisation of glass transition, the corresponding enthalpy and entropy changes and other effects that show either changes in heat capacity or a latent heat. The advantage of DSC compared with other calorimetric techniques lies in the broad dynamic range regarding heating and cooling rates, including isothermal and temperature-modulated operation [133].

DSC have been used to measure the thermal unfolding of proteins. It measures the heat absorbed by a protein as it slowly heated through its melting transition [134]. Protein denaturing is seen as an endothermic peak in DSC and a high uptake of energy occurs during this process [124; 134]. The thermoplastic proteins may or may not exhibit the endothermic peak in DSC, depending on the processing conditions and protein used. It was found that the soy protein plasticized with glycerol exhibited a denaturing peak around 152 °C corresponding with the denaturing temperature of soy protein [135]. The denaturing temperature of soy protein depends on the moisture content. With 11 % water as a plasticizer of soy protein, the denaturation of soy protein is around 180 °C [136]. A decrease in denaturation temperature of was a result of plasticization of soy protein with glycerol. An irreversible endothermic event was also observed in the glass transition of a thermoplastic produced from bloodmeal after mixing with sodium sulphite, sodium dodecyl sulphate, tri-ethylene glycol, water and urea [71].

2.3 Drying theory

Drying is traditionally defined as that unit operation which converts a liquid a liquid, solid or semi-solid feed material into a solid product of significantly lower moisture content, [137]. Drying is one of the oldest method used to improve storage life and reduce transportation costs for solids, liquids or slurries. In most cases, drying involves the application of thermal energy. It is a complex process involving simultaneous coupled, transient heat, mass and chemical and physical transformations which may in turn cause changes in product quality as well as mechanisms of heat and mass transfer [138]. Physical changes that may occur

include, shrinkage, glass transitions, crystallization and puffing [137]. In some cases, desirable/undesirable chemical changes may occur leading to change in odour, colour, texture or other properties of the solid product. For each and every product, there is a representative curve that describes the drying characteristics for the product at specific temperature, velocity and pressure conditions. The curve describes the change in drying rate as a function of time is often used to characterise evaporation of water during drying. The general drying rate curve consists of three main phases: the initial phase, constant phase and the falling phase [139; 140] as shown in Figure 13.

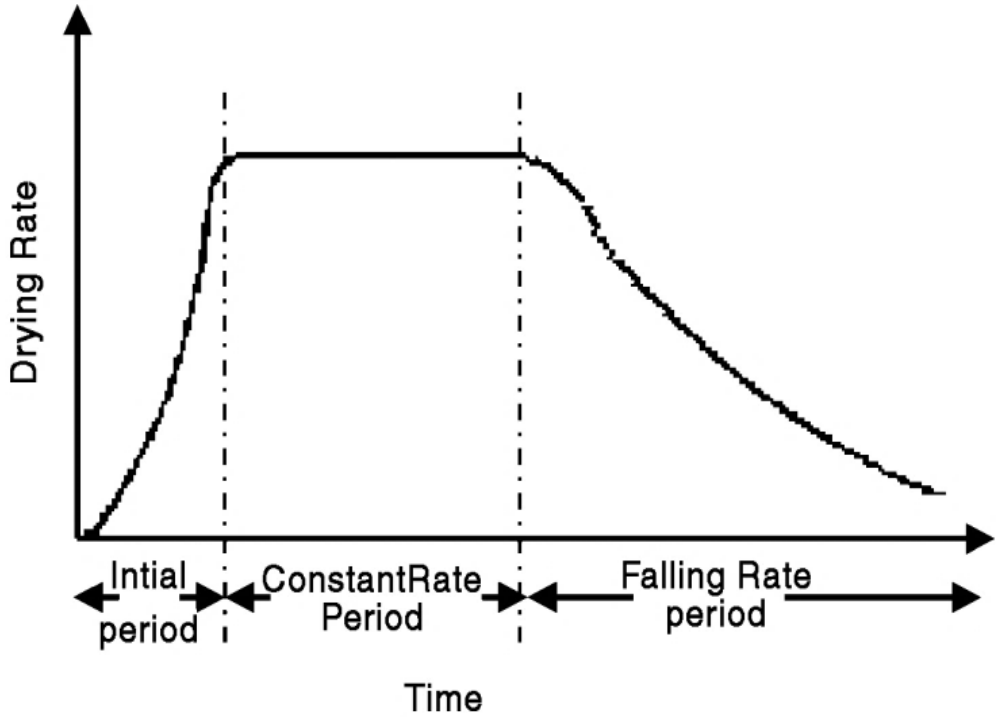

Figure 13. General drying rate curve [140]

The initial phase is where sensible heat is transferred to the feedstock and the contained moisture. The rate of evaporation increases dramatically, with mostly free moisture being removed in the initial phase. During the second phase, moisture removal from the droplet is at a near-constant rate representing the highest rate achieved in the dying rate curve. The falling rate phase involves migration of moisture from the inner interstices of each particle to the surface becomes a limiting factor that reduces the drying rate.

2.3.1 Drying methods

Drying occurs by effecting vaporization to the liquid by supplying heat to the wet material. Basic methods of heat transfer used in industrial dryers in various combinations include, convection, conduction and radiation. In the processing industries, most dryer's employ forced convection and continuous operation. With the exception of the indirectly heated rotary dryer and the film drum dryer, units in which heat is transferred by conduction are suitable only for batch use [138]. This limitation effectively restricts them to applications involving somewhat modest production runs. Radiant or so called "infra-red" heating is rarely used in drying materials such as fine chemicals, pigments, clays or synthetic rubbers. Its main application is in operations such as the drying of surface coatings on large plane surfaces. Because of the spectrum of duties required there is a great variety of dryers available. The correct choice depends on the properties of the feed material and the desired characteristics of the final product. However, dryers are either suitable for batch or continuous operation. The most common dryers are listed in Table 6.

Table 6. Dryer types as an aid to operation selection [141]

Dryer	**Operation**
Forced convection	Batch
Agitated pan	Batch
Double cone tumbler	Batch
Fluidized bed	Continuous
Conveyor band	Continuous
Film drum	Continuous
Pneumatic/flash	Continuous
Rotary	Continuous
Spin flash	Continuous
Spray	Continuous

Batch operated equipment is related to small production runs or operation requiring great flexibility. Thus, the batch type forced-convection unit certainly finds the widest possible application of any dryer used today. The majority of designs employ circulatory air systems incorporating large volume, low pressure fans which with the use of properly insulated enclosures, usually provide thermal efficiencies in the region of 50 to 60% [142]. However, the capital investment and installation cost are relatively low. The use of fan systems minimizes both power requirements and operating costs. In contrast, labour costs can be high.

In reference to the drying curves for the processing of materials in solid, filter cake, or wet powder form, the ultimate rate-governing factor is the rate of diffusion of moisture from the wet mass [143]. This becomes increasingly so during the falling rate period of drying. This situation, however, can be improved by preforming the product to increase the effective surface area presented to heat and mass transfer. The various batch dryers operate by means of forced convection. The transfer of thermal energy increases the vapour pressure of the absorbed moisture, while the circulated air scavenges the overlying vapour. Good conditions are maintained for continued effective drying. Alternatively, and where the material is thermo-sensitive implying low temperatures with consequently low evaporative rates and some improvement can be effected by the use of sub-atmospheric dryers [138].

They are different methods used for drying both thermo labile materials and thermo stable materials. Most of the methods can be used for the thermos stable materials, but due to decomposition problems of thermos labile materials like proteins few drying methods can be used [143]. Different drying methods include freeze-drying, spray drying and oven drying.

2.3.1.1 *Freeze Drying*

Freeze-drying or lyophilisation is the most common method used to dry proteins, to improve the stability and long term storage of liable products [144]. It can be defined as an efficient desiccation step where water (solvent), is removed from a frozen product by sublimation [145]. Technically freeze drying involves [146];

- Cooling of the liquid sample followed by the conversion of freezable solution water into ice, crystallization of crystallisable solutes and the

formation of an amorphous matrix comprising non-crystallizing solutes associated with unfrozen moisture.

- Sublimation of ice under vacuum
- Evaporation of water from the amorphous matrix
- Desorption of unfrozen water resident in the apparently dried cake

The fundamental principle in freeze drying is sublimation. Sublimation occurs when a molecule gains enough energy to break free from the molecules around it. There are two major factors that determine what phase (solid, liquid or gas) a substance will take: heat and atmospheric pressure, [147]. For a substance to take any phase, the temperature and pressure must be a certain range. Without these conditions, that phase of the substance can't exist [148].

Freeze drying can therefore be visualised in terms of three steps; that is, freezing, primary drying and secondary drying [144].

2.3.1.1.1 Freezing

Freezing is the first step of freeze drying and thus the stage where most of the water is removed. The system separates into multiple phases, and the interfaces between ice and protein phase form. Freezing often induces many destabilising stresses on proteins. These stresses include increase of protein concentration that enhances the protein-protein interaction leading to aggregation, reduced hydrophobic interactions due to the dehydration effect of ice formation that removes water from the protein phase, pH changes, limitless increase in ionic strength and formation of large ice aqueous interfaces[144]. On a larger-scale, freezing is usually done using a freeze-drying machine. The material is cooled below its eutectic point; the lowest temperature at which the solid and liquid phases of the material can coexist. Usually, the freezing temperatures are between -50 ˚C and -80 ˚C [148].

2.3.1.1.2 Primary drying

Primary drying or ice sublimation phase, is carried out at a low chamber pressure to improve the rate of ice sublimation. The chamber pressure impacts both mass and heat supplied to the material for the water to sublimate. The chamber pressure is normally below the vapour pressure of ice, and ice is transferred from the product to the condenser through sublimation and crystallisation onto cold coils (<-50 ˚C) in the condenser [144]. About 95% of the water in the material is sublimated in this phase [148]. The phase may be slow and is typically the longest stage of freeze

drying. However, if too much heat is added, the materials structure could be altered and freezing damage can occur with labile products such as liposomes and proteins. Removal of the hydration shell from proteins and liposomes during drying in the absence of the appropriate stabilizers can cause destabilisation of the protein structure and fusion of liposomes [148].

2.3.1.1.3 Secondary drying

Secondary drying aims to remove unfrozen water molecules. Some secondary drying occurs at the very beginning of primary drying as ice is removed from the region. The phase is governed by the materials adsorption isotherms. The temperature is raised higher than in the primary drying phase and can even be above 0˚C, [148] to break any physio-chemical interactions that have formed between the water molecules and the frozen materials. At the end of the operation, the final residual water content in the product is around 1% to 4% [148], which is extremely low. It normally takes only hours and the opportunity for time reduction by process optimization is limited [145].

Lyophilisation is therefore regarded as the best drying method to obtain products of the highest and excellent quality. Freeze dried products have a long shelf-life. The technique has been applied with success to diverse biological materials. Thus, freeze-dried powders offer advantages at the storage and shipping/distribution stages. The advantages of the freeze-drying process are superior product quality due to low temperature during sublimation and the maintenance of product structure that prevent deterioration reactions and microbial activities. Despites many advantages, freeze-drying has always been recognised as the most expensive process for manufacturing a dehydrated product.

2.3.1.2 *Spray drying*

In a spray drying process, the liquid material is atomized or sprayed into heated air within a drying chamber. The process is carried out in three fundamentals shown in Figure 14. The first stage involves atomization of a liquid feed into fine droplets. In the second stage, spray droplets evaporate from the droplets into the drying gas to solidify the droplets, thereby forming particles. The final stage involves separation of particles from the drying gas and collected in a chamber [140].

The spray dried products moisture contents are usually below 5% [149]. The spray drying process has found many applications in numerous industries because it is a

continuous process. If liquid feed can be supplied to the drying system, the spray-dried product will be produced continuously. The particle size, moisture content and flow properties of the final product can be controlled through the selection of equipment and manipulation of process variables. Spray drying is well suited for thermo-sensitive products because the process is almost instantaneous. Contact between the mechanical parts and materials is minimal, therefore, corrosive and abrasive materials can be accommodated. In addition, spray-drying process can be fully automated making it a labour-cost effective process [149-151].

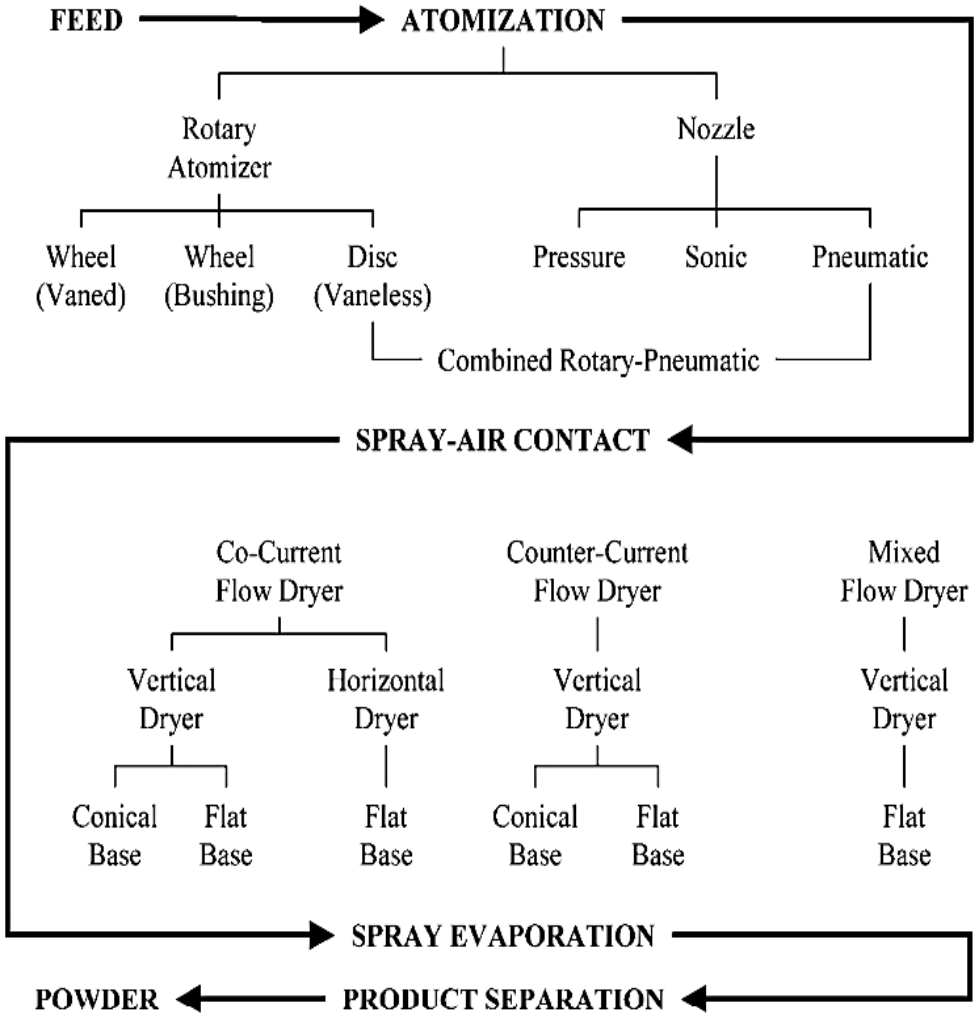

Figure 14. Schematic of spray-drying process shown in stages: stage I: atomization; stage II: spray-air contact and evaporation; stage III: product separation [151; 152]**.**

Like any other drying process, spray drying also has some limitations. the equipment can be very bulky and expensive with a low overall thermal efficiency. Spray drying an aqueous solution of a pure protein produces aggregation, unfolding and inactivation [153]. In case of the drying efficiency not being adequately scaled,

the solvent content of the product particles may increase undesirably. Increasing the drying rate also results in unsuitable particle morphology and/or size distribution for products with defined performance specifications [140]. Furthermore, changing the drying rate gas may substantially reduce the product yield.

2.3.1.3 *Vacuum Oven Drying*

The main working principle of a vacuum drying oven is to continually convey volume of hot air outwards through the exhaust fan replaced with fresh air, preventing the formation of potentially explosive compounds. The vacuum drying oven normally has a chamber volume of between 20 and 150 litres and a temperature range starting at between +5 °C and +15 °C above room temperature up to +250 °C; some appliances can also operate at up to 400 °C, [137]. The chamber load is heated or dried through direct contact with a heated surface. Vacuum drying has been applied widely to dry various heat-sensitive products in which the structure, colour and various vitamins are impaired at elevated temperatures [154]. Vacuum drying results in better product quality with respect to characteristics such as rehydration, flavour and fragrance [155]. Nevertheless, the cost of production is very high [156].

2.3.2 Effects of drying methods on proteins

Different studies have demonstrated that drying methods can exert a profound effect on the physicochemical and functional properties of proteins [13; 157-159]. Soy protein isolates were obtained by three different drying methods including: freeze drying, spray drying and vacuum drying [157]. The soy protein isolates were found to have different colours. The spray dried isolates had better emulsifying and foaming properties, low denaturation, free sulfhydryl's, surface hydrophobicity and had higher solubility than the other soy protein isolates obtained by freeze-drying and vacuum-drying [157]. Freeze-dried Soy Protein Isolates showed the highest surface hydrophobicity and contents of disulfide bonds, which might be caused by the formation of ice crystals during freezing. The formation of ice crystals led to high protein concentration in the unfrozen phases. When the proteins in aqueous solution adsorbed to the growing ice crystals, they could unfold and/or dissociate, exposing freer sulfhydryl groups and hydrophobic groups on the surface of protein molecules, which was favourable for Sulfhydryl groups/disulfide bonds interchange reactions. However, hydrophobic aggregation was unlikely a

predominant factor in the protein denaturation because of the use of low temperature [157].

Drying methods used to prepare chickpea protein concentrates were also found to have effect on the physio-chemical and functional properties [158]. Chickpea is one of the economic sources of plant proteins (25-30% of protein) [158]. The effects of freeze drying and convective drying at 40 ^{0}C and 50 ^{0}C on physio-chemical and functional properties on chickpea protein concentrates were investigated [158]. Freeze dried concentrate had the highest water and oil holding capacities and gave the lightest concentrate colour[158]. Heat treatment affects the thermal properties of Chickpea proteins concentrates. Moreover, freeze Dried Concentrates had the highest water-holding and oil absorption capacities. In general, concentrates prepared with convective drying had higher values of Emulsifying activity indices and Emulsifying stability indices compared to the freeze-drying extract [158].

As for freeze dried proteins, the freezing process can induce cold protein denaturation. According to prediction, cold denaturation of all known proteins in pure aqueous solutions should occur at temperatures below the freezing point of water [160]. The protein unfolding mechanism was described by Privalov [160] as a temperature-dependent interaction of protein nonpolar groups with water, which is inversely proportional to temperature. According to Wang [161], if too much heat is added to the freeze dried protein, the materials structure could be altered and freezing damage can occur with labile proteins . Liable proteins can be strongly affected while others tolerate the stresses to a certain degree [161]. Decreasing temperature weakens the hydrophobic interactions in a protein and decrease the rate of chemical reaction. Protein adsorption and unfolding are normally influenced by the ice-water interface generated during freezing. Removal of the hydration shell from proteins and liposomes during drying in the absence of the appropriate stabilizers can cause destabilisation of the protein structure and fusion of liposomes [148]. It can also lead to protein denaturation by dehydration. However, freeze drying proteins results in a decrease of alpha-helix and random structure and an increase in beta-sheet structure, which will be responsible for low activity of freeze-dried powders in nearly anhydrous media [147].

The effects of hot air drying, microwave vacuum drying and vacuum freeze drying methods on drying fish meat gel were also investigated [159]. The freeze dry method obtained the best sensory scores for acceptance, odour, highest water

absorption index and water solubility index and protein fraction [159]. Freeze drying is therefore recommended as the most suitable method that could effectively preserve fish proteins from degradation as compared to hot air drying and microwave vacuum drying. Different drying methods produce bloodmeal with different amino acids as shown in Table 7 [13]. Such variation in amino acid content not only influences the nutritive quality, but potentially the manufacture of thermoplastics [9]. It has been found that minor modification to amino acids may result in contribution of stabilising interactions but excessive changes may result in decreased strength, stiffness and elongation of thermoplastics [162]. However, Tilton *et al,* couldn't draw any conclusions about the general effects of temperature on protein structure. It is believed that protein molecules will have a range of thermal expansion behaviour depending on local atomic packing, secondary structure arrangement, solvent-protein coupling and molecular topology [163]. This concludes that the effect of a specific drying condition depends on various characteristics of the raw material during drying.

Table 7. Amino acids content from different types of dryers [13; 57; 164]

Amino acids	**Direct dried Bloodmeal**	**Coagulated bloodmeal**	**Spray dried bloodmeal**
Lysine	7.55	9.52	10.37
Histidine	3.76	4.23	6.38
Arginine	3.89	4.12	2.07
Aspartic acid	9.58	11.04	11.03
Threonine	3.36	5	5.11
Serine	3.36	4.78	5.47
Glutamic acid	8.7	10.11	8.09
Proline	4.02	4.16	3.24
Glycine	4.91	4.78	4.51
Alanine	7.17	8.74	9.47
Cysteine	0.57	9.76	-

Valine	7.78	1.02	8.5
Methionine	0.62	0.95	0.36
Isoleucine	1.01	0.92	-
Leucine	11.25	13.82	13.92
Tyrosine	2.22	3.1	2.39
Phenylalanine	6.06	7.9	8.19

2.3.3 Drying kinetics

Dying modelling is based on having a set of mathematical equations that are necessary for characterizing drying system, selection of suitable drying conditions and for prediction of mass and heat transfer during drying process [165]. The drying kinetics of products may be describe completely using their thermal diffusivity, moisture diffusivity, interface heat, mass transfer and thermal conductivity coefficients [166]. The drying conditions, characteristics of the particular product to be dried and the type of dryer all have an influence on drying kinetics [167]. Several methods have been proposed to simulate and analyse the drying process of different materials. In thin layer drying processes, three types of models can be used: empirical, theoretical and semi-theoretical.

The empirical models derives a direct relationship between drying time and average moisture content. The empirical models are usually obtained through simple mathematical analogies based on experimental data, dimensional and statistical analysis. The models do not posses a theoretical formulation but present a good fit for the observed data. The theoretical models consider the mechanism of both external and internal movement of moisture and its resistance. The models involve the geometry of the material its conductivity and mass diffusivity of the material [168]. The most widely used theoretical models are derived from Fick's second law of diffusion [169]. The semi theoretical models are derived from the Fick's second law of diffusion and some are derived by analogues with Newton's law of cooling. Factors that could determine the application of the models include the initial moisture content, relative humidity, drying air velocity, material thickness and drying temperature [167; 169; 170]. Under this conditions it can be noted that the

complexity of the models can be attributed to number of constants as shown in Table 8**.**

Table 8. Thin-layer drying models

Model no.	**Model equation**	**Name**	**References**
1	MR= exp(-kt)	Lewis	[171; 172]
2	MR= exp($-kt^n$)	Page	[173; 174]
3	MR= exp($-(kt)^n$)	Modified page	[175; 176]
4	MR= a exp(-kt)	Henderson and Pabis	[177; 178]
5	MR= a exp(-kt) +c	Logarithmic	[179]
6	MR= a exp($-k_0t$) +b(exp($-k_1t$)	Two-term	[180; 181]
7	MR= $1+at+bt^2$	Wang and Singh	[182]
8	t= a1n(MR)+ b$(1n(MR))^2$	Thomson	[183; 184]
9	MR=a exp(-kt) +(1-a) exp(-kbt)	Diffusion approach	[185]
10	MR=a exp(-kt) +(1-a) exp(-gt)	Verma et al	[186; 187]
11	MR=a exp(-kt) +(1-a) exp(-gt) +c exp(-ht)	Modified Henderson and Pabis	[187]
12	MR=a exp(-kt) +(1-a) exp(-kat)	Two term exponential	[188]
13	MR= a exp(c(t/L^2))	Simplified Fick's diffusion (SFFD) equation	[189]
14	MR=exp(-k$(t/L^2)^n$)	Modified page equation II	[189]

2.3.3.1 *Models derived from Newton's law of cooling*

2.3.3.1.1 Newton Model

The simplest model sometimes referred to as Lewis or the Exponential model. It has a single model constant as shown in Table 8. The model has been found suitable for describing drying behaviour of several bio materials [167; 190]:

$$MR=\exp(-kt) \quad (2\text{-}1)$$

where, MR is the moisture ratio and k is the drying constant (s^{-1}). The model provides simple and clear analytical solution to the moisture content and time relationship in thin layer of biomaterials. The model concept assumed that the material is thin enough, the air velocity is high and the drying air conditions are kept constant. However, according to Brooker *et al* [191], the challenge of the Lewis model is a high drying rate, but proper prediction of drying constants can solve this challenge.

2.3.3.1.2 Page model

Page model was the modification of Lewis model to get a more accurate model. This model was used successfully to model drying of soybean [192], shelled corn [193], short and medium rice [182] and barley [190]. The errors of using Newton model are minimised by the addition of a dimensionless empirical constant (n):

$$MR= \exp(-kt^n) \quad (2\text{-}2)$$

where, *n* is known as the model constant (dimensionless) and k is the drying constant (s^{-1}). The Page model has been adopted as Standard in thin-layer modelling of agricultural and biological products [167].

2.3.3.1.3 Modified Page Model

Overhults *et al* [175] modified the Page model to describe drying of soybeans. Three forms of Modified Page model (I, II, III) have been reported [169]. The Modified Page-I model:

$$MR= \exp (-kt)^n \quad (2\text{-}3)$$

The Modified Page-II model:

$$MR= \exp-(kt)^n \quad (2\text{-}4)$$

The Modified Page-III model was used to describe the drying of sweet potato slices [194] and onions [167]:

$$MR = \exp -k(t/d^2)^n \quad (2\text{-}5)$$

where, d is an empirical constant (dimensionless).

2.3.3.2 *Models derived from Fick's second law of diffusion*

2.3.3.2.1 Henderson and Pabis (Single Term) Model

Henderson and Pabis [195] used Fick's second law of diffusion to improve a model for drying. The new model of drying was used to describe the drying of corns and millet. The model has two constants:

$$MR = a \exp(-kt) \quad (2\text{-}6)$$

where, a represent the shape of the material used and generally named as model constant (dimensionless).

2.3.3.2.2 Logarithmic model

A modified form of Henderson and Pabis model with an empirical term addition [196], which was used to describe the drying of laurel leaves [179] and other vegetables [167]:

$$MR = a \exp(-kt) + c \quad (2\text{-}7)$$

where, c is a dimensionless empirical constant.

2.3.3.2.3 Two-Term Model

The second term general solution of the Fick's second law of diffusion proposed by Henderson [181] for correcting the errors of the Henderson and Pabis Model. The model assumes a constant product diffusivity and temperature throughout the drying process. The model contains 2 dimensionless empirical constants and 2 model constants which can be derived from experimental data:

$$MR = a \exp(-k_0 t) + b(\exp(-k_1 t) \quad (2\text{-}8)$$

where, a and b are defined dimensionless empirical constants, indicating the shape of the material and K_0 and K_1 are the drying constants. The first term of the model describes the last part of the drying process, while the second term describes the

beginning of the drying process [167]. The model was used to describe drying of grains [169]

2.3.3.3 *Empirical models*

2.3.3.3.1 Wang and Singh Model

This model was created by Wang and Singh [182] for the intermittent drying of rough rice. The model gives the best fit to the experimental data. However, Wang and Singh model has no physical or theoretical interpretation [167]

$$MR= 1+at+bt^2 \tag{2-9}$$

where, a (s^{-1}) and b (s^{-1}) are dimensionless model constants obtained from experimental data.

2.3.3.3.2 Thompson Model

Thompson et al [184] developed an empirical model with experimental data of drying of shelled corns in the temperature range 60-150 °C by correlating the drying time as a function of the logarithm of the moisture ratio:

$$t= a1n(MR)+ b(1n(MR))^2 \tag{2-10}$$

where, a and b are dimensionless empirical constants. The challenge of the Thompson model is lack of physical interpretation and has np theoretical basis. However, the model has been found suitable for describing the drying kinetics of green peas [167].

According to Erbay and Icier [169] mathematical modelling of drying often requires the statistical methods of regression and correlation analysis. Linear and nonlinear regression analyses are significant tools to find the relationship between different variables, especially for which no established empirical relationships exist. The validation of models for each material can be checked with different statistical methods. The most widely used method in literature is performing correlation analysis, reduced chi-square (χ^2) test and root mean square error (RMSE) analysis, respectively.

2.4 Conclusion

Relevant literature on proteins showed that proteins are commonly obtained from plants and animals. Plant proteins include wheat and corn gluten meal, soy proteins, pea proteins and potato proteins. Animal proteins include bloodmeal, casein, whey, collagen and keratin. Both plant and animal proteins have unique properties that can be exploited for several technical and industrial applications. However, protein properties depend on both the amino acid structure and other structural modifications induce by change of environment. Production of industrial proteins such as soy proteins, wheat and blood meal involves drying. Drying is a complex process involving simultaneous coupled, transient heat, mass and chemical and physical transformations which may in turn cause changes in protein quality. Drying can induce protein denaturation. Denaturation can have several important consequences, such as increase in viscosity of protein solutions, decrease of solubility due to the exposure of hydrophobic groups, increase of reactivity of side groups, altered sensitivity to enzymatic proteolysis and altered surfactant properties. Denaturation effects are of importance for protein processing.

Different drying methods can exert a profound effect on the physicochemical and functional properties of proteins. The spray dried isolates had better emulsifying and foaming properties, low denaturation, free sulfhydryl's, surface hydrophobicity and had higher solubility than the other soy protein isolates obtained by freeze-drying and vacuum-drying. The thermal properties of Chickpea proteins concentrates were affected by different heat treatments. Different drying methods produce bloodmeal with different amino acids. The denaturing temperature of soy protein depends on the moisture content. Glass transition temperature of hydrophobic polymers decrease significantly with an increase in moisture content. The dependence of T_g on the water content is stronger than that of denaturation temperature on the water content. The solubility of most proteins increases with an increase in temperature during drying. It has been found that minor modification to amino acids may result in contribution of stabilising interactions but excessive changes may result in decreased strength, stiffness and elongation of protein based polymers. It is also believed that protein molecules will have a range of thermal expansion behaviour depending on local atomic packing, secondary structure arrangement, solvent-protein coupling and molecular topology.

Several methods have been proposed to simulate and analyse the drying process of different materials. In thin layer drying processes, three types of models can be used: empirical, theoretical and semi-theoretical. The validation of models for each product can be checked with different statistical methods of regression and correlation analysis.

A full understanding and appreciation of the stresses encountered by blood meal proteins during drying is therefore of importance in choosing the appropriate drying process and to optimize the process for high quality Novatein precursor product.

CHAPTER 3

Experimental

3.1 Experimental Design

The aim of this research is to obtain drying kinetics of coagulated blood and to produce blood meal at different drying conditions. Coagulated blood was dried at different temperatures (60 °C, 100 °C and 140 °C) for 24 hours for moisture content determination and drying curves.

Moisture content obtained was therefore, used to estimate the weight of samples to be produced at different temperatures with various moisture content as shown in Table 9. The oven dryer was used to produce the sample specimen's. The blood meal samples obtained were characterised using thermogravimetric analysis, dynamic mechanical analysis, Dynamic Scanning calorimetry and X-ray diffraction.

Table 9. Experiments done showing factorial design

	Drying Temperature (°C)		
Moisture Content (%)	**60**	**100**	**140**
5	EXP 1	EXP 2	EXP 3
10	EXP 4	EXP 5	EXP 6
15	EXP 7	EXP 8	EXP 9

3.2 Materials

Coagulated blood was obtained in 10 litre buckets from Wallace Corporation Limited, New Zealand. Distilled water and technical sodium dodecyl sulphate (SDS) were obtained from University of Waikato and Bio-lab, New Zealand respectively.

3.3 Equipment

The following equipment were used in the experiments:

Table 10. List of equipment used for experiments

Equipment	Supplier
Oven	Contherm Thermotec 2000
Electronic balance	Sartorious CP 225D
Grinder	Magic bullet
Dynamic Mechanical analyser	Perkin Elmer DMA 8000
Thermogravimetric analyser	Sdt 2960 simultaneous DTA-TGA
Differential Scanning calorimetry	Perkin Elmer DSC 8500
X-ray diffraction	Panalytical Empyrean

3.4 Moisture content determination

Determination of initial moisture content is an important factor as it directly affects the dehydration process and also significant for modelling the drying process [197]. The determination of initial moisture content at different temperature for a certain time was an important part of this research.

3.4.1 Procedure for moisture content determination

Moisture content was determined by the oven drying method [198]. All drying experiments were carried out in a convection Contherm Thermotec 2000 oven and repeated twice. Coagulated blood moisture content was determined by three different temperature (60 °C, 100 °C and 140°C). Sample of coagulated blood of about 454.5 grams of weight was dried at different temperature of 60 °C, 100 °C and 140°C for a specific period of 24 hours. The initial weight and final weight for each sample was recorded and used to determine the moisture content. Sartorious CP 225D electronic balance was used to weigh samples. The moisture content (wet basis and dry basis) of coagulated blood samples were determined as per equation (3-1) and (3-2), respectively.

Moisture content, wet basis (M_{wb}) is the amount of water per unit mass of moist or wet sample.

Thus,

$$Mwb = \frac{(Wet\ mass - dry\ mass)}{Wet\ mass} \quad (3\text{-}1)$$

Moisture content, dry basis (M_{db}), is the amount of water per unit mass of dry solids present in the sample.

Thus,

$$Mdb = \frac{(Wet\ mass - dry\ mass)}{dry\ mass} \quad (3\text{-}2)$$

The two moisture contents are related by the following equation

$$Mdb = \frac{Mwb}{1 - Mwb} \quad (3\text{-}3)$$

Figure 15. Sartorious CP 225D electronic balance

3.5 Drying kinetics

3.5.1 Drying rates

Sample of coagulated blood of about 454.5 grams of weight was dried at a relatively slow process at 60 °C for 24 hours. The initial weight was recorded before the samples were subjected to drying using a Sartorius CP 225D electronic balance. Samples were removed and weighed on a digital balance every 30 minutes for the first 4 hours, hourly for a period of 2 hours and finally after 19 hours. The same procedure was repeated for samples dried at 100 °C and 140 °C. Drying was stopped when constant weight was reached with two consecutive readings.

The instatenous rate of drying was calculated using equation presented by Hu *et al* [199]. Which are expressed as g water/ g dry solids per min. Where Z_t and Z_{t-1} are the water contents of the dried sample at time t and the fresh samples on dry basis respectively.

$$Instatenous\ DR = \frac{M_t - M_{t-1}}{dt} \quad (3\text{-}4)$$

3.5.2 Mathematical modelling of coagulated blood drying curves

The moisture ratio (MR) of samples was calculated using the following equation:

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (3\text{-}5)$$

Where M_t is the moisture content at any time (g water/g dry solids), M_0 is the initial moisture content of sample (g water/g dry solids) and M_e is the equilibrium moisture content of sample (g water/g dry solids).

The values of M_e are relatively small compared to M_t or M_0, hence the error involved in the simplification is negligible [194]. The equilibrium moisture contents M_e were determined by drying samples until no further change in weight was observed for each drying temperature [197].

The drying curves data obtained were used to determine the drying rates. The drying rates were then fitted on different models which was proposed by other researchers to determine the best and most convenient model as shown in Table 11. Statistical analyses of the experimental data were performed by using software package (Statistica 13.2). The coefficient of determination (R^2) was one of the primary criterion for selecting the best equation to define the drying curves of coagulate blood. In addition to R^2, the various statistical parameters such as, reduced chi-square (χ^2), mean bias error (MBE) and root mean square error (RMSE) were used to determine the quality of the fit. The higher the value of R^2 and the lower the values of χ^2 and RMSE were chosen as the criteria for best fit [166; 200-202]. These parameters can be calculated as following:

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{N - n} \quad (3\text{-}6)$$

$$MBE = \frac{1}{N}\sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right) \quad (3\text{-}7)$$

$$RMSE = \left[\frac{1}{N}(MR_{pre,i} - MR_{exp,i})^2\right]^{1/2} \quad (3\text{-}8)$$

Where $MR_{exp,\ i}$ is the experimental moisture ratio found in any measurement and $MR_{pre,\ i,}$ is the predicted moisture ratio for this measurement. N and n are the number of observations and constants, respectively.

Table 11. Mathematical models used for coagulated blood drying curves

Model no.	**Model equation**	**Name**	**References**
1	MR= exp(-kt)	Lewis	[171; 172; 190]
2	MR= exp(-kt^n)	Page	[173; 174]
3	MR= exp(-$(kt)^n$)	Modified page	[175; 176]
4	MR= a exp(-kt)	Henderson and Pabis	[177; 178]
5	MR= a exp(-kt) +c	Logarithmic	[179]

3.6 Blood meal production

3.6.1 Drying to different moisture content

Moisture content obtained was used to calculate the weight of samples to be produced at different temperatures with various moisture content. Coagulated blood was sampled and dried at different temperatures (60 °C, 100 °C, 140 °C) to different moisture contents (5 %, 10 % and 15 %) to produce a total of 9 samples as shown in Table 9**.** The properties of the produced samples were analysed by means of drop penetration test, dynamic mechanical analysis, dynamic scanning calorimetry, thermo-gravimetric analysis and x-ray diffraction.

3.7 Physicochemical Analysis

3.7.1 Drop penetration test

Blood meal samples dried at different temperature to various moisture content were milled using a commercial blender. Samples were sieved to a grain size <32 mm. About 3.5g of the sieved samples was tapped into a 15ml falcon tube until it no longer compresses. The mass of the compressed powder into a falcon tube was measured using an electronic balance. 5 ml of water and/or 1% sodium dodecyl sulphate (SDS) was gently pipetted on top of the sample powder in the falcon tube as shown in Figure 16**.** The wettability of the samples was determined by measuring the amount of the solvent (water or sodium dodecyl sulphate) that penetrated the powder in 5 minutes. A video taken to obtain accurate times.

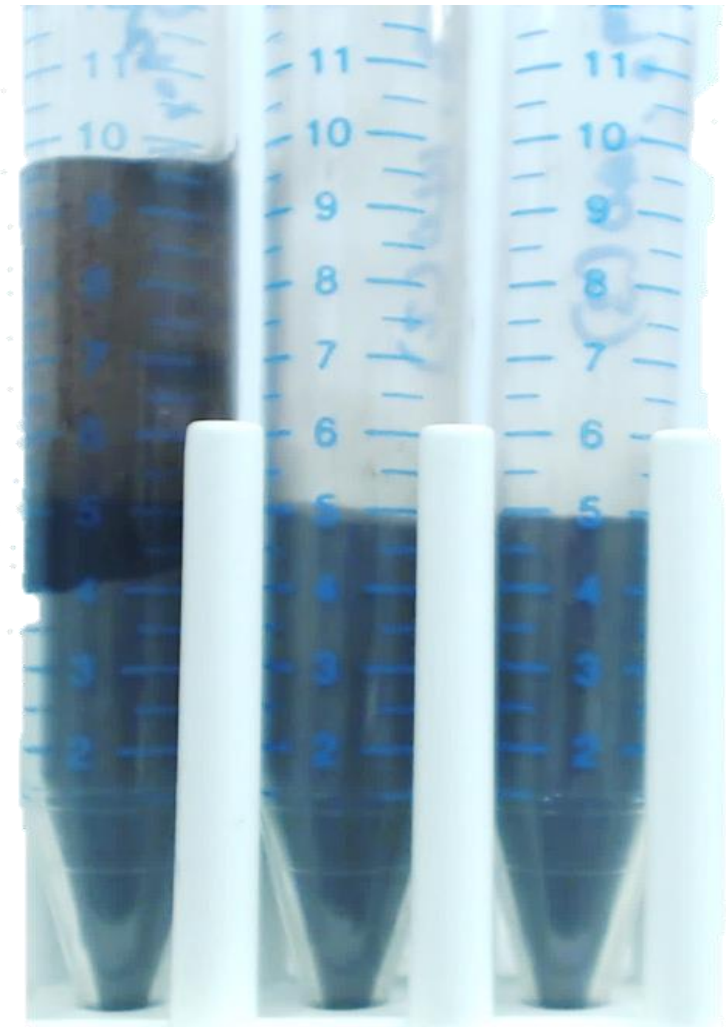

Figure 16. Water pipetted on top of blood meal sample compressed on a falcon tube.

3.8 Thermal Analysis

3.8.1 Thermo-gravimetric Analysis

The thermal stability of dried coagulate blood (bloodmeal) was determined using SDT 2960 simultaneous DTA-TGA by heating approximately 20 mg of each sample from 25 °C to 800 °C at a rate of 10 °C/min with an air flow of 150ml/min. Each sample was scanned in triplicates. The data obtained were used to determine the thermal behaviour of the sampled coagulate blood.

Figure 17. SDT 2960 Simultaneous DTA-TGA

3.8.2 Dynamic Scanning Calorimetry

Differential scanning calorimetry (DSC) was conducted using a DSC 8500 and analyzed using Pyris Software version 11.1.1.0492. Approximately 10 mg of sample were crimp sealed in 30 ml aluminum pans and run under constant nitrogen purge gas. Specimens were heated from -50 °C to 140 °C at 10 °C/min held for 1 min at 140 °C, cooled to -50 °C at 10 °C/min, held for 1 min at -50 °C after which the cycle was repeated using the same method. Samples were weighed to ensure no mass lost.

Figure 18. Perkin Elmer DSC 8500

3.8.3 Dynamic mechanical Analysis

The effect of drying conditions such as moisture content and temperature on the resulting glass transition temperature of bloodmeal was established using a Perkin Elmer DMA 8000 fitted with a high temperature furnace and cooled with liquid nitrogen. Blood meal samples were examined by mounting approximately 1.70 × 7.45 × 12.90 mm^3 powder pockets. The samples were scanned at -100 °C and 250 °C at a programmed heating rate of 2 °C/min in a single cantilever-bending mode with a dynamic displacement of 0.03mm. The samples were scanned at multi frequencies of 0.10, 0.30, 1, 3, 10 and 20 Hz. The peak in tan δ at 1Hz was identified as the glass transition temperature.

Figure 19. Perkin Elmer DMA 8000

3.8.4 X-ray diffraction

The structural characteristics of blood meal produced under different drying conditions was determined using PANalytical EMPRYEAN 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 4° to 60° at step size of 0.0263. 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 milled using a commercial blender before being scanned in a powder sample holder spinner stage. A linear baseline was fitted to the minima between 4° and 60°.

Figure 20. PANalytical EMPRYEAN X-ray diffractometer

CHAPTER 4

Results and Discussion

This research investigated mainly two aspects of blood meal. The first was to determine the drying kinetics of coagulated blood at different temperatures. Secondly, the blood meal produced at different temperatures to varying moisture content was characterised to determine the effects of drying conditions on the physio-chemical and thermal properties.

4.1 Drying kinetics

4.1.1 Determination of initial moisture content of coagulated blood

Coagulated blood was dried at different temperatures of 60 °C, 100 °C and 140°C for a specific period of 24 hours for initial moisture content determination. The moisture content after drying at various temperatures are given in Table 12. The initial moisture content of coagulate dried at different temperature varied between 60.7-58.8 % wet basis and/or 154.5-142.7 % dry basis. The coagulate blood dried at 140 °C showed a higher moisture content compared to the other drying temperatures. This shows that longer drying times were required for drying temperatures of 60 °C and 100 °C to ensure that the samples were fully dried. Therefore, the standard oven drying temperature of 140 °C for a period of 24 hours, should be used for routine moisture determination for coagulated blood.

Table 12. Mean moisture content and standard deviation for Coagulate dried at different Temperature

Drying Temperature (°C)	Initial Moisture Content (%wet basis)	Standard deviation (wet basis)	Initial Moisture Content (%dry basis)	Standard deviation (dry basis)
60	58.8	0.0003	142.7	0.002
100	59.2	0.002	145.1	0.010
140	60.7	0.001	154.5	0.009

Analysis of the experimental data shows that to reach a specific moisture content, drying time decreased with an increase in drying temperature. For example, it took 60 °C, 240 minutes, 100 °C, 90 minutes and 140 °C, 60 minutes to obtain a moisture ratio of 0.8 (Figure 21). This was caused by an increase of the air heat rate to the sample and the acceleration of water molecules mobility within the sample, resulting in a decrease of drying time. Similar results were obtained by other researchers when drying food and non-food products [197; 203; 204].

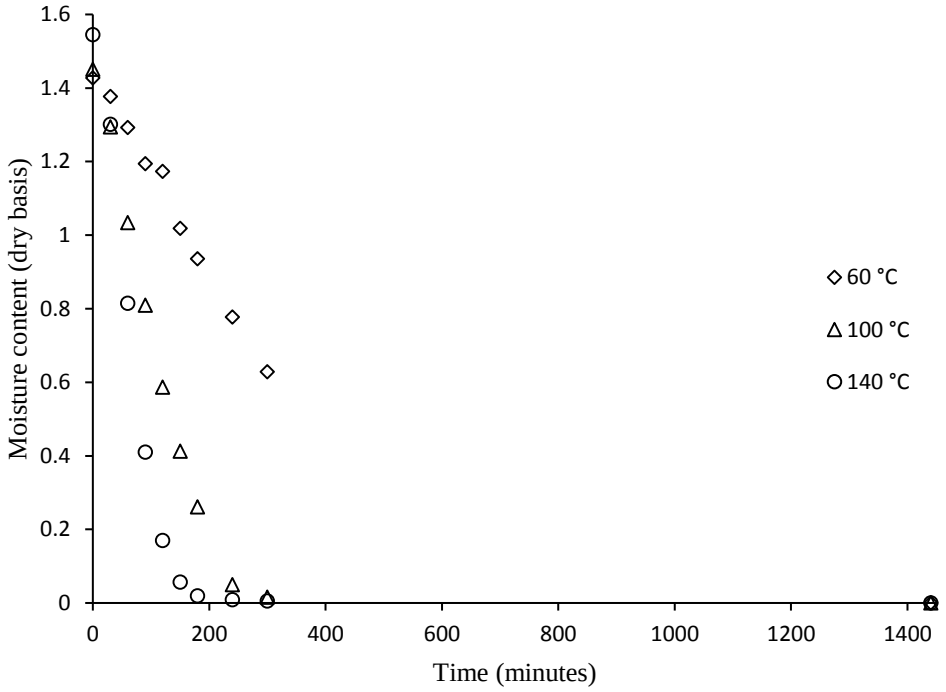

Figure 21. Moisture ratio versus drying duration of coagulated blood at different temperatures.

According to literature a typical drying rate curve consists of three main phases: the initial phase, constant phase and the falling phase [139; 140]. The drying rate curves for coagulated blood as a function of time at different air drying temperatures are presented in Figure 22**.** As expected, an increase in drying temperature showed an increase in drying rate. It was also observed that the drying rates decreased with an increase in drying period/time. Lahsashi [205] suggested that, this is due to an increase of air heat supply to the product and the acceleration of water migration inside the product, which leads to an increase in evaporation rate. In the range of experimental conditions tested, coagulated blood drying rate curves showed only

two drying phases: that of the initial rate period and the falling rate period as shown in Figure 22**.**

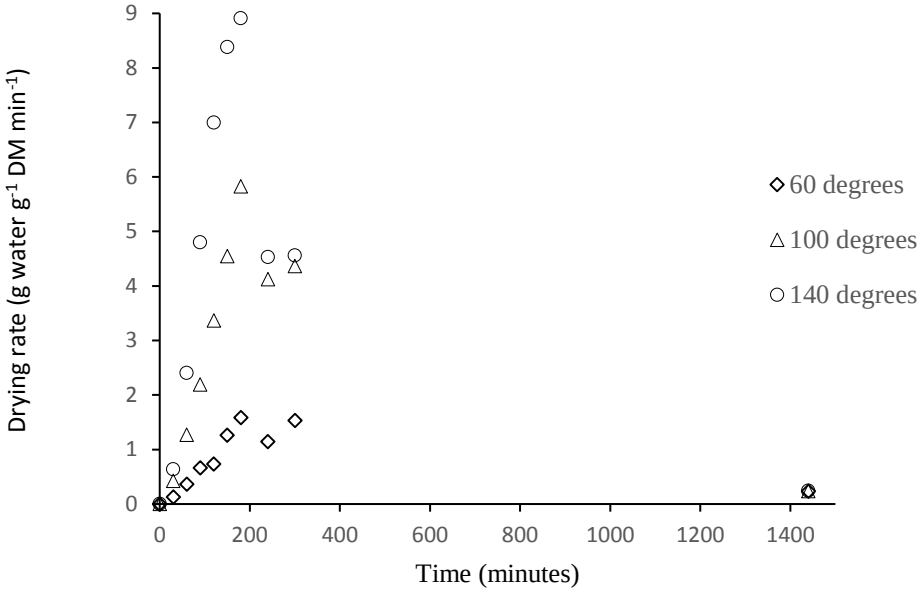

Figure 22. Drying rate of coagulate blood as a function of time at varying temperatures

The presence of two drying stages suggests that the coagulate blood had both free water and bond water; the water was lost during the initial increasing rate on the drying curve and the bond water was lost during the falling rate period. In the initial period, the drying rate for 140 °C and 100 °C dramatically increased compared to 60 °C. This is due to the increase of thermal energy to the product. According to de Lima [206] the increase in drying rate will continue to happen until it reaches a constant rate between mass and heat transfer occurs. This is referred to as constant rate period. However, no constant rate was observed for coagulated blood, indicating that drying process took place in a falling rate period. Therefore, it can be considered a diffusion-controlled process in which the rate of moisture removal is limited by diffusion of moisture from inside to the surface of the product. This phenomenon has been described to be true for most biological and agricultural products [191; 206-212].

The drying rate curves had a similar shape in the falling rate stage as shown in Figure 22. The drying rate drastically decreased for both three drying temperatures. The drying rate declined sharply within a short time. During this period, a reduction in moisture migration from the interior to the surface of the product occurred.

Similar results have also been reported by other researchers [206]. For both 60 °C, 100 °C and 140 °C drying rate curves in Figure 22, a transition was observed in the falling rates period. The same transition was also observed from the measurement of the drying rate of fish muscles [213]. The transition was ascribed as corresponding to the moisture content at which the monomolecular layer of water molecules bound to the underlying protein is uncovered [207; 213; 214]. During this stage, the product is fully in the hygroscopic stage and the drying rate decreases, reaching zero at equilibrium moisture content. The falling rate period corresponds to the equilibrium between the product and the surrounding drying conditions [207].

4.1.2 Mathematical modelling of coagulated blood

The moisture content data observed at the drying experiment were converted into the moisture ratio and fitted to the four models listed in Table 13. The models were evaluated based on coefficient of determination (R^2), the reduced chi-square (χ^2) and the Root mean square error (RMSE). The statistical results of the different models including the drying model coefficients and the evaluation criteria used are listed in Table 13. In all cases the four models expect the Logarithmic model obtained R^2 greater than 0.95 which is acceptable range for all drying temperatures [215]. The χ^2 and RMSE values were lower than 0.46 and 0.33, respectively (Table 13).

Among the selected models the Page and modified page models gave higher R^2 and lower χ^2 and RMSE values compared to other models, implying an excellent fit in all ranges of the drying temperatures. Therefore, based on R^2 and χ^2 values, Page and/or Modified Page model may be assumed to represent the drying behaviour of coagulated blood in an oven dryer within the examined range. Similar results were reported in the literature for various biological products [10; 216-218]. All the experimental values of the moisture ratio for the different drying air temperature and the fittings obtained for each case using the Page model are illustrated in Figure 23.

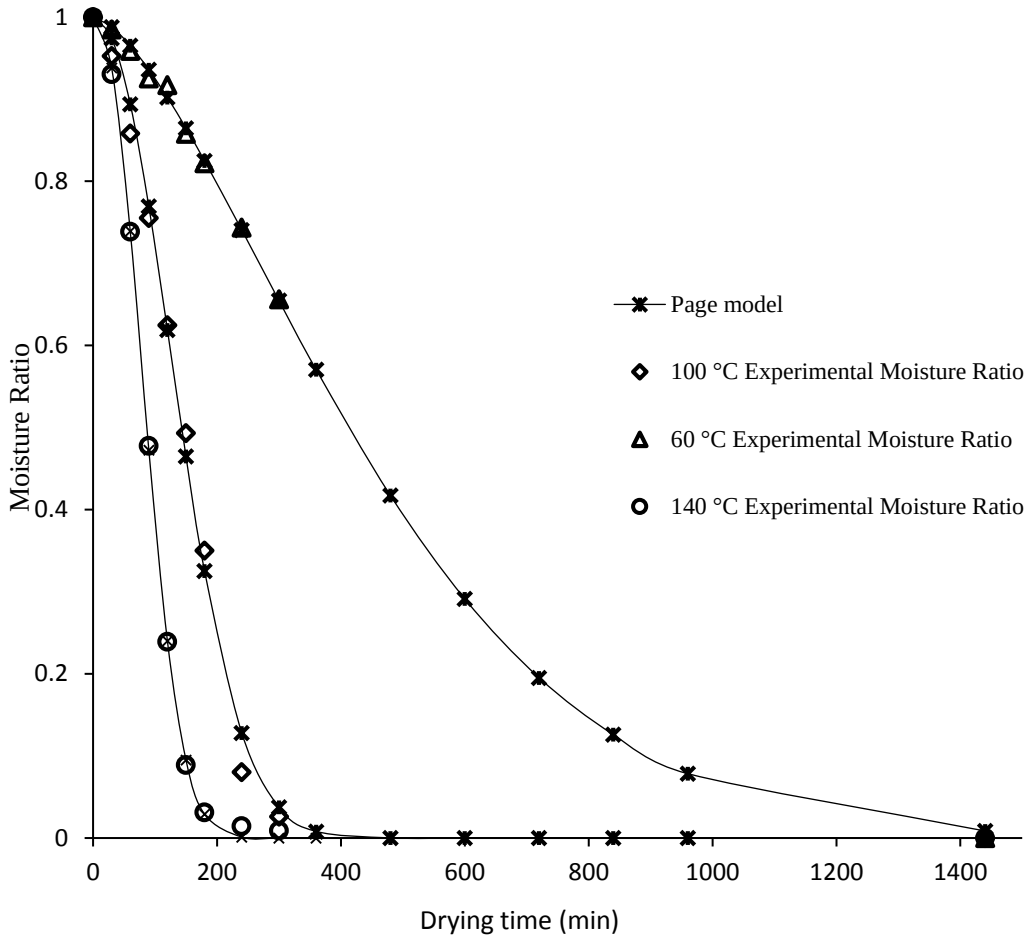

Figure 23. Experimental Vs predicted values using the Page model for different drying air temperatures for coagulated blood

Table 13. Mathematical models used for coagulated blood drying curves

T (°C)	Model name	Coefficient of determination (R^2)	Absolute Mean bias error (MBE)	Reduced Chi-square (χ^2)	Root mean square error (RMSE)
60	Lewis (k=0.0137)	0.9949	0.0141	0.0036	0.0567
	Henderson and Pabis (a= 1.061 k=0.0016)	0.9908	0.0066	0.0024	0.0437
	Logarithmic (a= 0.237 k=3.0 c=0.763)	0.2521	0.0000	0.1064	0.2729
	Page (k=0.00006 n= 1.542)	0.9997	0.0018	0.0001	0.0073
	Modified page (k=0.0019 n=1.542)	0.9997	0.0018	0.0001	0.0073
100	Lewis (k=0.0053)	0.9664	0.0119	0.0143	0.1134
	Henderson and Pabis (a= 1.127 k=0.00620)	0.9644	0.0121	0.0123	0.0992
	Logarithmic (a= 0.5397 k=1.00 c=0.460)	0.4432	0.0000	0.1533	0.3276
	Page (k= 0.00002 n=2.0988)	0.9980	0.0071	0.0007	0.0240
	Modified page (k=0.00587 n=2.09887)	0.9980	0.0071	0.0007	0.0240
140	Lewis (k= 0.00999)	0.9703	0.0001	0.4619	0.1116
	Henderson and Pabis (a= 1.131 k=0.01115)	0.9679	0.0184	0.0124	0.0997
	Logarithmic (a= 0.71881 k=1.0 c=0.2811)	0.5643	0.0000	0.1422	0.3155
	Page (k=0.00003 n=2.23966)	0.9999	0.0015	0.00005	0.0062
	Modified page (k=0.00977 n=2.23997)	0.9999	0.0015	0.00005	0.0062

4.2 Physicochemical Analysis

4.2.1 Drop penetration test

About 5ml of water and/or SDS was gently applied to the blood meal samples with different drying conditions. This procedure was done to determine the wettability of blood meal samples produced at different temperatures (60 °C, 100 °C and 140 °C) to different moisture contents (5 %, 10 % and 15 %). According to literature wetting is a straight forward technique used to show the hydrophobicity or hydrophilicity of protein powders [86]. Water and sodium dodecyl sulphate are considered to be one of the good plasticisers for proteins since they can increase the free volume of biomaterials and prevent hydrophobic interactions between protein chains [2; 219]. However, the migration rate of plasticisers in proteins depends on the physiochemical properties of the plasticiser and other factors such as environmental conditions and protein conformation [220]. Plasticiser migration may be reduced by a more aggregated protein structure [221]. The amount of water and/or SDS absorbed by different blood meal samples in a specific period of 5 minutes is shown in Figure 24**,** Figure 25 and Table 14**.**

Table 14. Total amount of water/SDS that penetrated blood meal powders produced at different drying conditions within 5 minutes.

Drying temperature (°C)	Moisture content (%)	Water volume (ml)	SDS volume (ml)
60	5	2.4	2
	10	2.2	2.4
	15	1.7	2.2
100	5	1.6	1.9
	10	1.5	1.9
	15	1.5	1.9
140	5	1.4	2
	10	1.4	1.7
	15	1.1	2.5

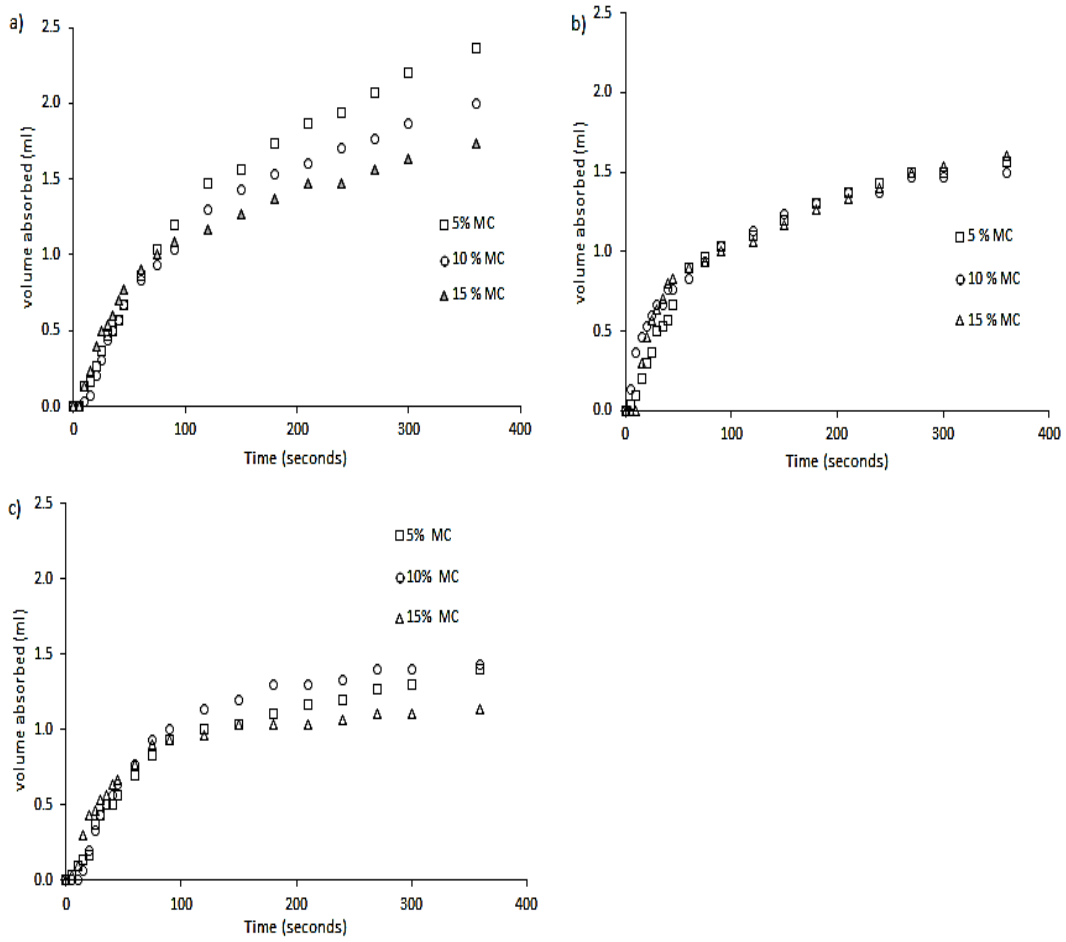

Figure 24. Relationship between water drop penetration and time for blood meal processed at different temperatures to various moisture content (5% MC, 10% MC, 15 % MC) a) bloodmeal dried at 60 °C, b) blood meal dried at 100 °C, c) blood meal dried at 140 °C.

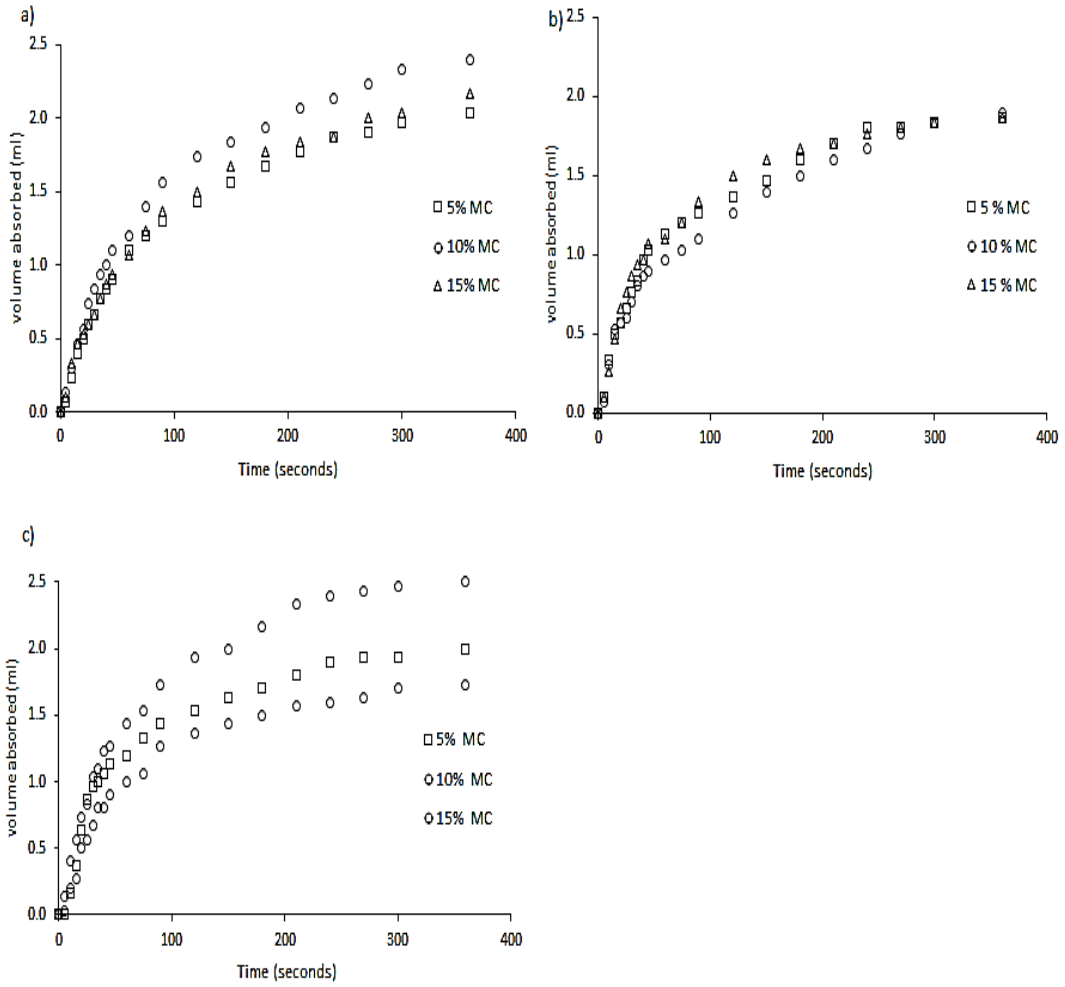

Figure 25. Relationship between SDS drop penetration and time for blood meal processed at different temperatures to various moisture content (5% MC, 10% MC, 15 % MC) a) bloodmeal dried at 60 °C, b) blood meal dried at 100 °C, c) blood meal dried at 140 °C.

The results show that blood meal produced under different drying conditions had varying degrees of wettability. Wetting of blood meal with water decreased with increasing drying temperature. Blood meal produced at 60 °C to 5% moisture content penetrated with 2.4 ml of water in 5 minutes while about 1.1 ml of water penetrated blood meal produced at 140 °C to 15% moisture content, suggesting that blood meal proteins are more difficult to wet when denatured at high temperature. It also indicates that water was faster to percolate blood meal surface dried at low temperature than at higher temperature. According to Wittaya [222] hydrophobic interactions increases when drying temperature increases. It is known that hydrophobicity reduces wettability of protein powders by making it difficult for water to penetrate into the powder [223]. However, at a constant temperature a decrease wettability was observed with an increase in moisture content. This suggest the saturation of the protein molecules with an increase in moisture content.

The amount of SDS that penetrated blood meal samples in 5 minutes was much higher than that of water, suggesting that SDS could enhance blood meal wettability. There was no definite trend observed on wetting of blood meal with SDS however, about 2.5 ml of SDS penetrated blood meal produced at 140 °C to 15 % moisture content, suggesting better wettability. Sodium dodecyl sulphate is an anionic surfactant. It is characterised as containing a hydrophilic head region and a hydrophobic tail region. SDS is known to reduce hydrophobic interactions between protein chains [224]. This suggest that SDS will tend to absorb strongly through hydrophobic surface as observed with blood meal samples dried temperature (Table 14**).** Analysis of variance (Table 15**)** revealed that the main effect of temperature on the amount of water that penetrated bloodmeal samples was significant (F (2,4) =11.3, p =0.023). This was expected since hydrophobic interactions are more temperature dependent than moisture content dependent.

Table 15. Main effects of drying temperature and moisture content on blood meal wetting with water.

Effect	SS	DF	MS	F	P
Drying Temperature	0.83	2	0.41	11.3	0.023
Moisture content	0.17	2	0.08	2.3	0.219
Error	0.15	4	0.04		
Total	1.14	8			

Table 16. Main effects of drying temperature and moisture content on blood meal wetting with SDS.

Effect	SS	DF	MS	F	P
Drying Temperature	0.14	2	0.07	0.87	0.485
Moisture content	0.10	2	0.05	0.61	0.585
Error	0.31	4	0.08		
Total	0.54	188			

4.3 Thermal Analysis

4.3.1 Thermogravimetric analysis (TGA)

Thermogravimetric analysis was performed to determine the thermal stability of bloodmeal dried at varying temperature and to a varying moisture content. Weight loss as a function of the temperature for blood meal processed at different temperature and to a varying moisture content is reported in Figure 26**.** Sample TGA and DTGA plots for each blood meal sample is represented in Figure 27. The degradation temperature for each sample is shown in Table 17. As shown in Figure 26 and Figure 27 the sampled blood meal degraded by a four weight loss steps. Similar behaviour has been reported by previous researchers on protein polymers [2; 21; 131; 225-228]. The weight loss in the first stage which is below 100 °C is assigned to the evaporation of residual water. The second step can be observed for all experiments at 310 °C, overlaps the third step, which is attributed to evaporation of bound water, weak bond cleaving and onset of protein chain decomposition. The third step was observed at about 495 °C and it is assigned to protein degradation and then the final step was observed at 540 °C, which is mainly due to strong bond cleaving.

It was found that blood meal dried at 60 °C to 15% moisture content had the degradation temperature at about 307.0 °C, which suggest that it was more thermally stable while the blood meal sample dried at 140 °C to 10 % moisture content had the lowest onset decomposition temperature at about 301.1 °C, which implies a reduction in thermal stability as shown in Table 17. The thermal stability of samples dried at 60 °C and 100 °C decrease with decreasing moisture content (Error! Reference source not found.a and b, respectively**).** However, the stability o f samples dried at 140 °C was very close, showing no difference of stability among samples. This could be due to hydrophobic interactions formed during drying at high temperature. According to literature the hydrophobic bond is one of the more important factors involved in stabilizing the folded configuration of proteins [229; 230]. According to Wolfenden [231], most protein side chains become more hydrophobic with rising temperature. Larger hydrophobic effects generally destabilizes proteins [232]. However, hydrogen bonds also make a large contribution to stability. The loss in conformational entropy when a protein folds is the major destabilizing force and the burial of charged groups also contribute to protein instability [229; 233; 234].

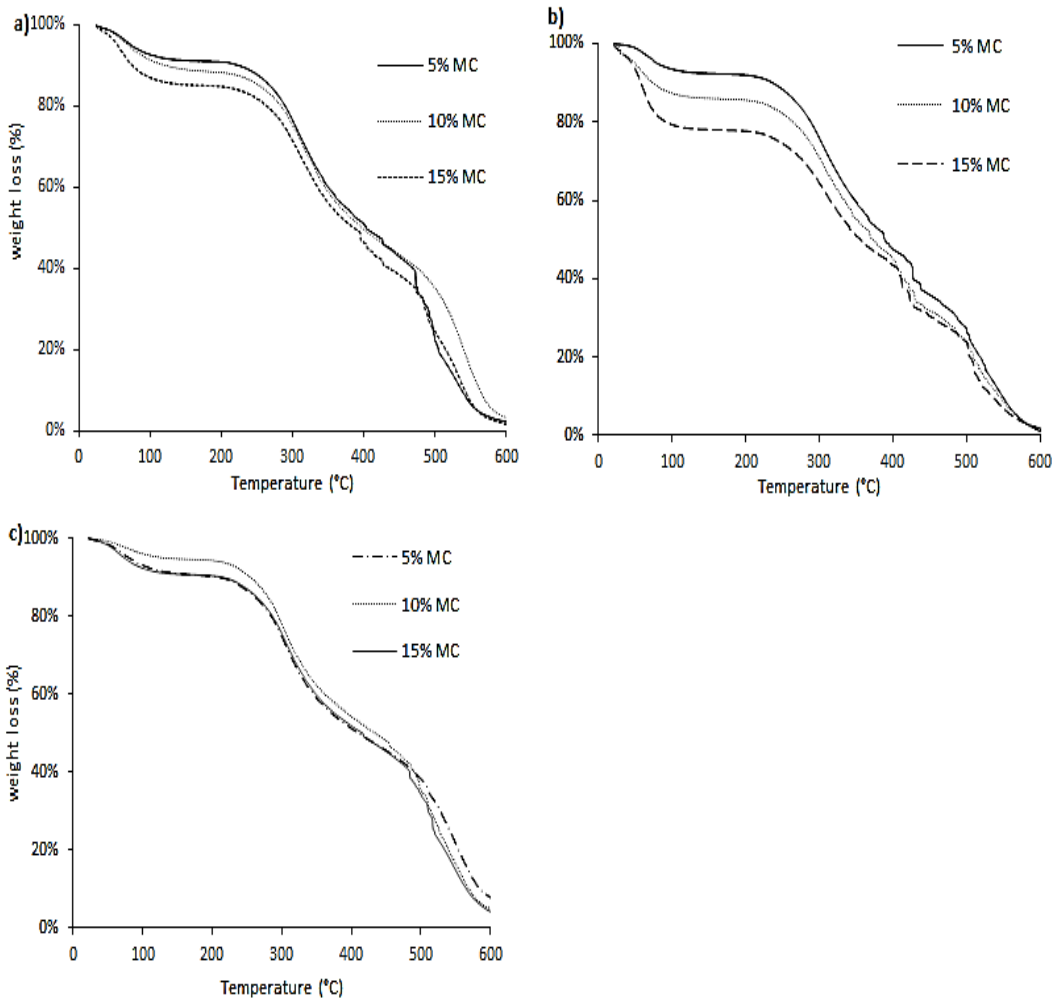

Figure 26. Thermal stability of blood meal dried at different temperatures: (a) 60 °C, (b) 100 °C, (c) 140°C to various moisture content (5%, 10% & 15%).

ANOVA and Response Tables for means (Table 18 and Table 19, respectively) were used to determine the effect of drying temperature and moisture content on the decomposition and thermal stability of blood meal. The main effect of drying temperature and moisture content was not significant ($F(2,4) = 0.971$, $p = 0.453$) and ($F(2,4) = 0.959$, $p = 0.457$), respectively. However, the response table for means (Table 19) made it clear that the moisture content had affected the thermal stability of blood meal more than drying temperature. Therefore, it can be concluded that blood meal dried at 60 °C to 15% moisture content was the most stable and blood meal dried at 140 °C, to 10% moisture content was the least stable within the parameters sampled.

Table 17. Degradation temperature for blood meal produced at different temperatures to various moisture contents

Moisture content (%)	Drying temperature (°C)	Decomposition temperature (°C)
5	60	305.0
	100	303.5
	140	303.8
10	60	305.2
	100	304.4
	140	301.1
15	60	307.0
	100	303.2
	140	305.9

Table 18. Main effects of drying temperature and moisture content on thermal degradation temperature

	SS	DF	MS	F	P
Temperature	16405.1	2	8202.5	0.971	0.453
Moisture content	16206.0	2	8103.0	0.959	0.457
Error	33804.2	4	8451.1		
Total	66415.3	8			

Table 19. Response Tables for means for the main effects influencing TGA thermal decomposition temperature

Level	**Temperature**	**Moisture content**
1	304.1	305.7
2	303.6	303.7
3	305.4	306.6
Error	1.8	2.1
Rank	**2**	**1**

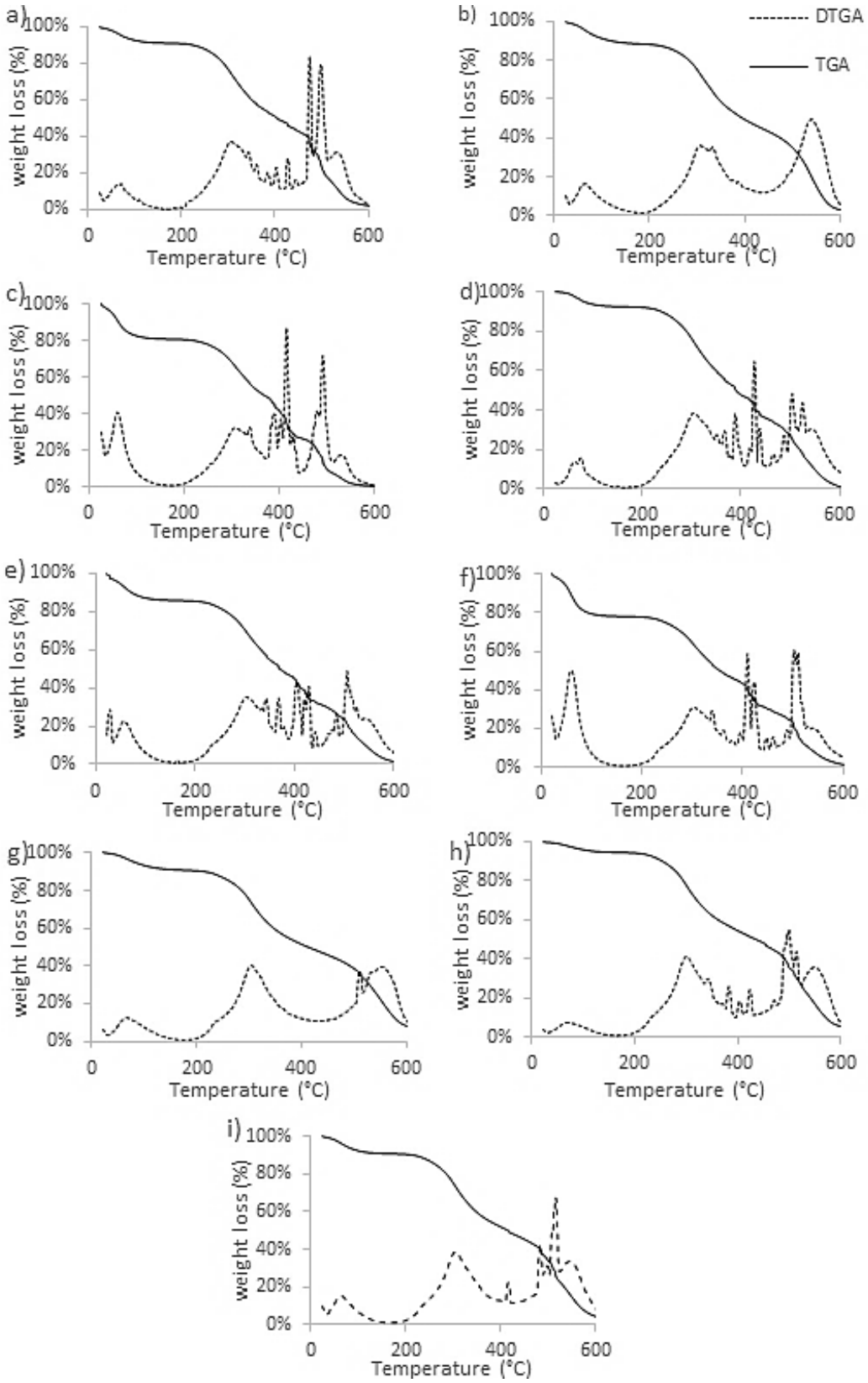

Figure 27.TGA and DTGA curves for each blood meal sample produced at different temperature (°C) to various moisture content (%): (a) 5% MC @ 60 °C, (b) 10% MC @ 60 °C, (c) 15% MC @ 60 °C, (d) 5% MC @ 100 °C, (e) 10% MC @ 100 °C, (f) 15% MC @ 100 °C, (g) 5% MC @ 140 °C, (h) 10% MC @ 140 °C and (i) 15% MC @ 140 °C.

4.3.2 Differential Scanning Calorimetry (DSC)

DSC is mainly used as an indicator for protein denaturation, which significantly affects the functionalities of the proteins. The onset denaturation temperature, the temperature at the maximum peak, peak height, peak area and enthalpy of denaturation (ΔH) were determined from thermograms and presented in Figure 28**.** According to Ma and Harwelkar [235], the maximum peak temperature indicates protein thermostability, while the enthalpy of denaturation (ΔH) is an indication of the extent of denaturation that the protein has undergone prior to analysis [236]. The protein denaturation and unfolding are accompanied by enthalpy changes, and denature proteins have a lower denaturation enthalpy (ΔH) than native proteins [157]. Two endotherm peaks were observed for each sample (Figure 28**).**

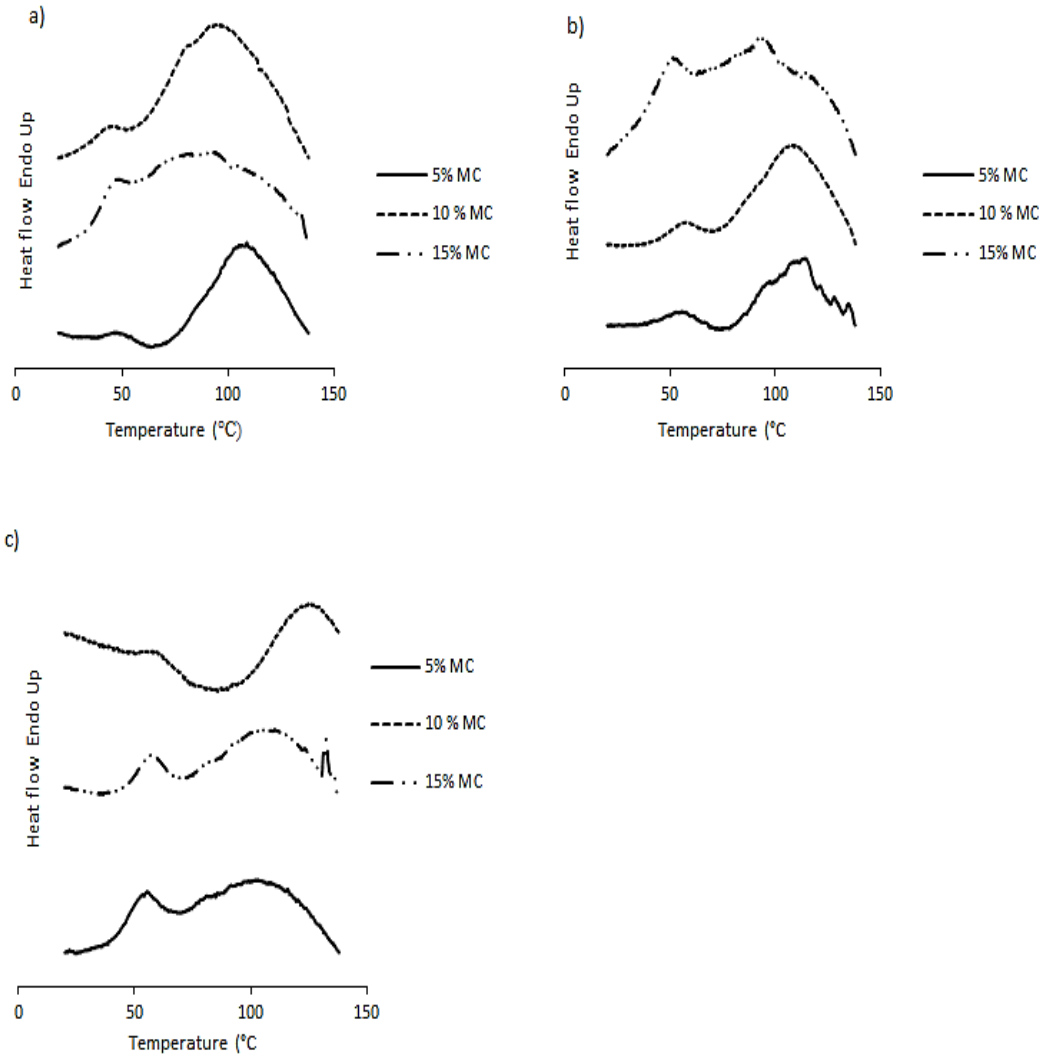

Figure 28. DSC thermograms for bloodmeal dried at different temperature to vary moisture content (5% MC, 10% MC, 15 % MC) based on the first heating scan: a) bloodmeal dried at 60 °C, b) blood meal dried at 100 °C, c) blood meal dried at 140 °C.

An endothermic peak was observed in all samples at lower temperature at around 43.7 °C -60 °C. Details for the first peak are shown in Table **20.** In dry or partially hydrated proteins the same endothermic peak has been ascribed to aging [127]. The same peak was also observed in soy proteins and Bovine Serum Albumin, both in the native and after thermal denaturation at water contents below 20% [237; 238]. However, previous studies detected the endothermic peak as the relaxation behavior where internal readjustments of the tightly packed chain take place through cooperative molecular motion [239]. At a constant moisture content, the peak temperature increased with increasing drying temperature, probably due to the adverse effect of high drying temperature of coagulated blood.

Table 20. 1st Peak temperature and ΔH of blood meal produced under different drying conditions.

Drying Temperature (°C)	MC (%)	Peak Temp (°C)	ΔH (J/g)
60	5	48.1	0.14
	10	43.7	0.15
	15	46	0.31
100	5	55.5	0.38
	10	56.4	0.25
	15	51.2	0.55
140	5	55.4	0.37
	10	59.1	0.26
	15	57.5	0.46

A second endothermic peak was observed in all samples at around 70-140 °C. The peak was thought to be evaporation of water, however Myers [240] suggested that high denaturation temperatures are expected for proteins with a high proportion of hydrophobic interactions. It was also mentioned that denaturation of dry proteins may be above 200 °C close to degradation [77]. It was observed that at a constant moisture content the peak area decreased with increasing drying temperature (Table

21), indicating that the transition was temperature dependent. The peak was therefore, ascribed to full denaturation window of protein membranes of blood meal. The same endothermic peak was observed in Bovine Serum Albumin [237]. Table 21 shows the details of the upper endothermic peak observed at around 70-140 °C, ascribed to denaturation.

Table 21. Denaturation peak properties of blood meal produced at different drying conditions

MC (%)	Drying Temperature (°C)	Td peak (°C)	Peak area (J/g)	ΔH (J/g)
5	60	108.8	4.35	4.35
	100	114.3	3.79	3.79
	140	121.9	1.2	1.24
10	60	96.3	7.75	7.75
	100	107.9	4.67	4.67
	140	108.8	2.62	2.62
15	60	92.1	4.36	4.36
	100	94.2	3.27	3.27
	140	105.9	2.07	2.07

The denaturation temperature of all samples was dependent on moisture content. At constant drying temperature, the denaturation shifted to higher temperature with decreasing moisture content. The sample with low denaturation temperature from DSC showed high thermal stability with TGA. Sample dried at 60 °C to 15% moisture content showed a lowest denaturation temperature of about 92.1 °C while sample dried at 140 °C to 5 % moisture content showed the highest denaturation temperature of about 121.9 °C. The denaturation for this samples, increased up 32.4%, indicating that a significant molecular organization took place. As expected, at constant moisture content the denaturation enthalpy (ΔH) for each sample

decreased with increasing temperature. Blood meal dried at 60 °C to 10% moisture content showed the highest denaturation enthalpy of about 7.75 J/g, which meant that a certain extent of denaturation took place in the sample by exposing the hydrophobic regions during drying, while blood meal dried at 140 °C to 5% moisture content showed the lowest denaturation enthalpy of 1.24 J/g, which could be attributed to higher protein denaturation, induced by hydrophobic interchains reactions among protein molecules during drying. This effect may also be due to a closely packed structure of the protein resulting in reduction of enthalpy [241; 242]. Analysis of variance (Table 22) revealed that the main effects of both drying temperature and moisture content on denaturation temperature were significant ($F(2,4)=26.54$, $p= 0.005$) and ($F(2,4)=14.56$, $p= 0.01$), respectively.

The denaturation peak temperature also increased with increasing drying temperature at a constant moisture content. Denaturation enthalpy and peak denaturation temperatures measured by DSC are usually affected by the heating rate and protein concentrations [157]. In the present study, Analysis of variance (Table 23), revealed that the main effect of drying temperature on the enthalpy of denaturation was significant ($F(2,4)=12.74$, $p=0.018$) The main effect of moisture content and drying temperature on the peak temperature (Table 24) was significant ($F(2,4)=26.54$, $p=0.005$) and ($F(2,4)=14.56$, $p=0.015$), respectively. This was expected because hydrophobic interactions are very temperature dependent and are weaker at lower temperature as compared to higher temperatures [160; 243]. Similar results were obtained for corn proteins [242].

Table 22. Main effects of moisture content and drying temperature on denaturation temperature

Source	**DF**	**SS**	**MS**	**F**	**p**
Drying temperature	2	471.6	235.8	26.54	0.005
Moisture content	2	258.8	129.4	14.56	0.01
Error	4	35.5	8.9		
Total	8	765.9			

Table 23. Main effects of moisture content and drying temperature on enthalpy of denaturation (ΔH)

Source	**SS**	**DF**	**MS**	**F**	**P**
Moisture	6.74	2	3.37	4.63	0.091
Drying Temperature	18.5	2	9.27	12.74	0.018
Residual Error	2.94	4	0.73		
Total	28.2	8			

Table 24. Main effects of moisture content and drying temperature on peak temperature

Source	**SS**	**DF**	**MS**	**F**	**P**
Moisture	471.61	2	235.804	26.54	0.005
Temperature	258.78	2	129.391	14.56	0.015
Residual Error	35.54	4	8.886		
Total	765.94	8			

4.3.3 Dynamic Mechanical Calorimetry (DMA)

Glass transition, Tg, is known as the temperature region at which an amorphous system changes from glassy to a soft, rubbery state. Dynamic mechanical analysis (DMA) was used to investigate the effect of drying temperature and moisture content on the glass transition temperature (Tg) of blood meal. For both samples three peaks were seen in the tan δ curves as shown in Figure 29**.** Coagulated blood showed no transitions at low temperatures. The first peak for samples dried at 60 °C and 100 °C was observed at low temperature between 35 °C and 70 °C was thought to be the gamma, (ɤ)-transition, which may be associated with water. The ɤ-transition observed in samples dried at 60 °C and 100 °C fell into a broad range depending on the moisture content. A similar observation for glycerol-plasticised soy protein was reported by Chen *et al* [244], where it was also assigned to the protein-water fraction.

As the temperature increased a shoulder-like peak appears between 85 °C and 100 °C, which would normally be attributed to beta, (β)-transition, indicating the material toughness. The β-transitions also varied substantially with moisture content for samples dried at 60 °C and 100 °C. According Zhang *et al* [245] the β-transitions varying with moisture content could be attributed to motions of the hydrated protein side groups which depend on moisture and/or the crankshaft motion of the main chains. Samples dried at 140 °C showed only ɤ-transition that happened between 40 °C and 60 °C, this could be attributed to hydrophobic interactions which increases with temperature which will result in chain movement restriction. The defined maximum temperature peak was thought to be dry glass transition (Tg), after evaporation of water during testing. The glass transition temperature for each sample are shown in Table 25.

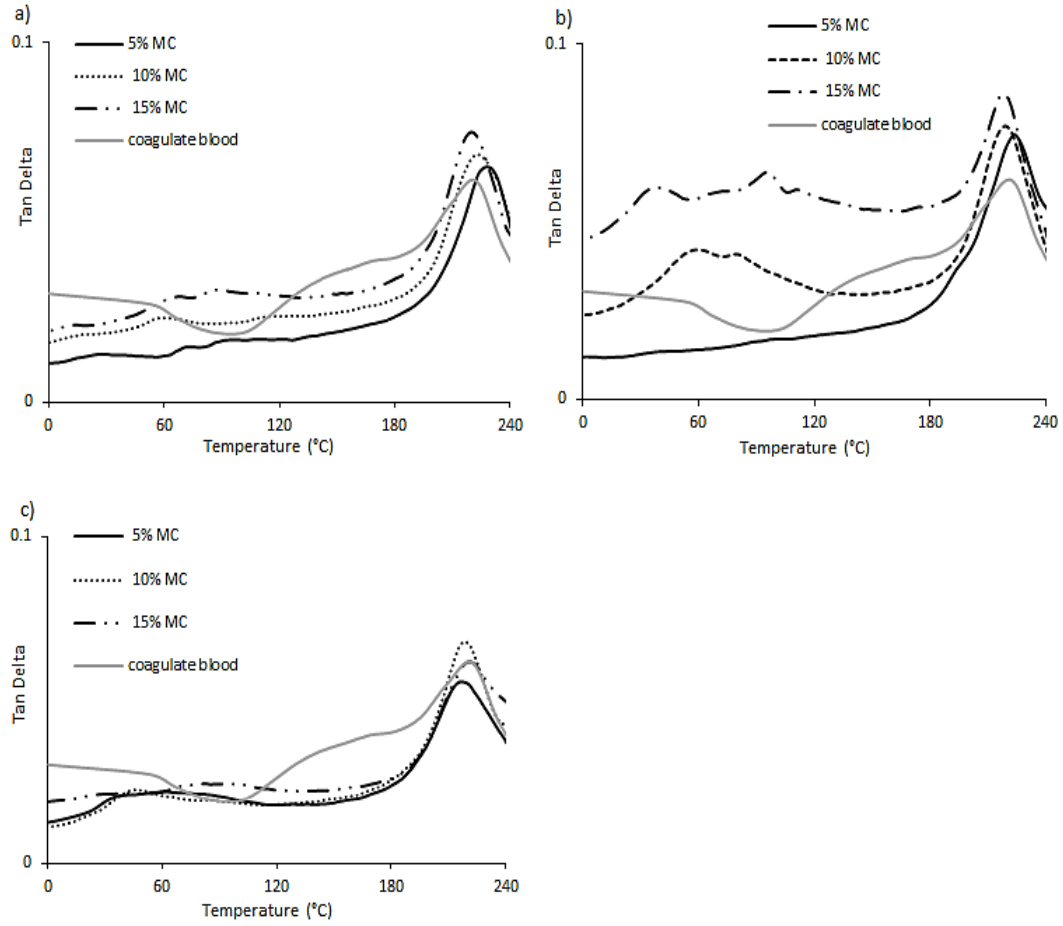

Figure 29. Tanδ for blood meal processed at different temperatures to various moisture content (5% MC, 10% MC, 15 % MC) at 1Hz: a) bloodmeal dried at 60 °C, b) blood meal dried at 100 °C, c) blood meal dried at 140 °C.

The glass transition temperature, Tg, vary between 222 to 224 °C for both coagulated blood and samples dried at 60°C, 219 to 226 °C for samples dried at 100 °C and 220 °C to 222 °C samples dried at 140 °C. This falls within the previously identified glass transition of blood meal to be about 220 °C [128]. Sample dried at 100 °C to 15 % moisture content show the lowest glass transition temperature of 219.3 °C, followed by sample dried at 100 °C to 10 % moisture with Tg of 220.7 °C. The lower the Tg of sample, the greater the degree of protein destabilization. The alpha relaxation of a thermoplastic protein material is heavily influenced by interactions with water. Water has a well-known plasticiser effect on proteins. Therefore, the glass transition temperature should decrease when the moisture content increases. Table 25 clearly showed that this was not the case with the blood meal samples. The lack of change with moisture content would be more consistent with local motions or sub Tg transitions than with long range mobility. It has been

pointed out that the highest temperature peak for blood meal would be the dry Tg after evaporation of water during processing [128].

Analysis of variance revealed that both drying temperature and moisture content were not statistically significant (P>0.05) on the influence of glass transition as shown in Table **26**. This suggest that within the experimental range explored bound water evaporated overtime hence showing no plasticization effect on blood meal.

Table 25. Glass transition temperature (average and standard deviation) of coagulate blood dried at different temperatures to various moisture content.

	Temperature (°C)		
Moisture Content (%)	**60**	**100**	**140**
5	224.6±3	226.8±2	220.4±2
10	224.7±5	220.7±2	222.1±2
15	222.6±2	219.3±3	221.7±3

Table 26. Main effects of moisture content and drying temperature on glass transition temperature

Source	**SS**	**DF**	**MS**	**F**	**P**
Moisture	2	11.22	5.608	0.90	0.476
Temperature	2	10.23	5.114	0.82	0.503
Residual Error	4	24.98	6.244		
Total	8	46.42			

4.3.4 XRD Analysis

X-ray scattering can be used to investigate conformational changes in proteins. The structural properties of blood meal dried at varying temperature to varying moisture content were studied by WAXS as shown in Figure 30**.** Three scans were done for each sample and similar diffraction patterns were observed for all samples, hence one scan shown**.** The peaks with the angle at 2θ around 10° and 20° in WAXS patterns of proteins belong to the α-helix and β-sheet structure, respectively [246]. Blood meal is semi-crystalline [75]. As shown in Figure 30**,** XRD analysis, α-helices and β-sheet are spaced about 10-12 angstroms apart from themselves, each other and protein strands which gives the peak at 10°. The loops in α-helices and β-strands are about 5 angstroms apart which gives the peak at 20°.

The intensity of a peak can reflect changes in the structure of a protein. The intensity of peaks for blood meal samples are shown Figure 30**.** Samples dried at 60 °C showed an increase in β-sheet structure with increasing moisture content. Moisture content had no effect on the α-helix structure. This suggest that increasing moisture content at a 60 °C disrupt more protein-protein interaction. According to literature bound water disrupts the intermolecular cohesive forces between protein chains; this result will promote chain movement in the non-crystalline regions and induce β-sheets [129; 247].

Increasing drying temperature to 100 °C showed a decrease in both α-helix and β-sheet structure as moisture content increased. This could be attributed to a reduction in stabilising interactions such as hydrogen bonding as drying temperature increases. No change was observed upon drying coagulated blood at 140 °C regarding increase of moisture content, perhaps due to protein structure denaturation that normally happens at higher temperatures. However, change in α-helix and β-sheet structure was observed between coagulated blood and samples dried, suggesting a change of structure as coagulated blood was dried.

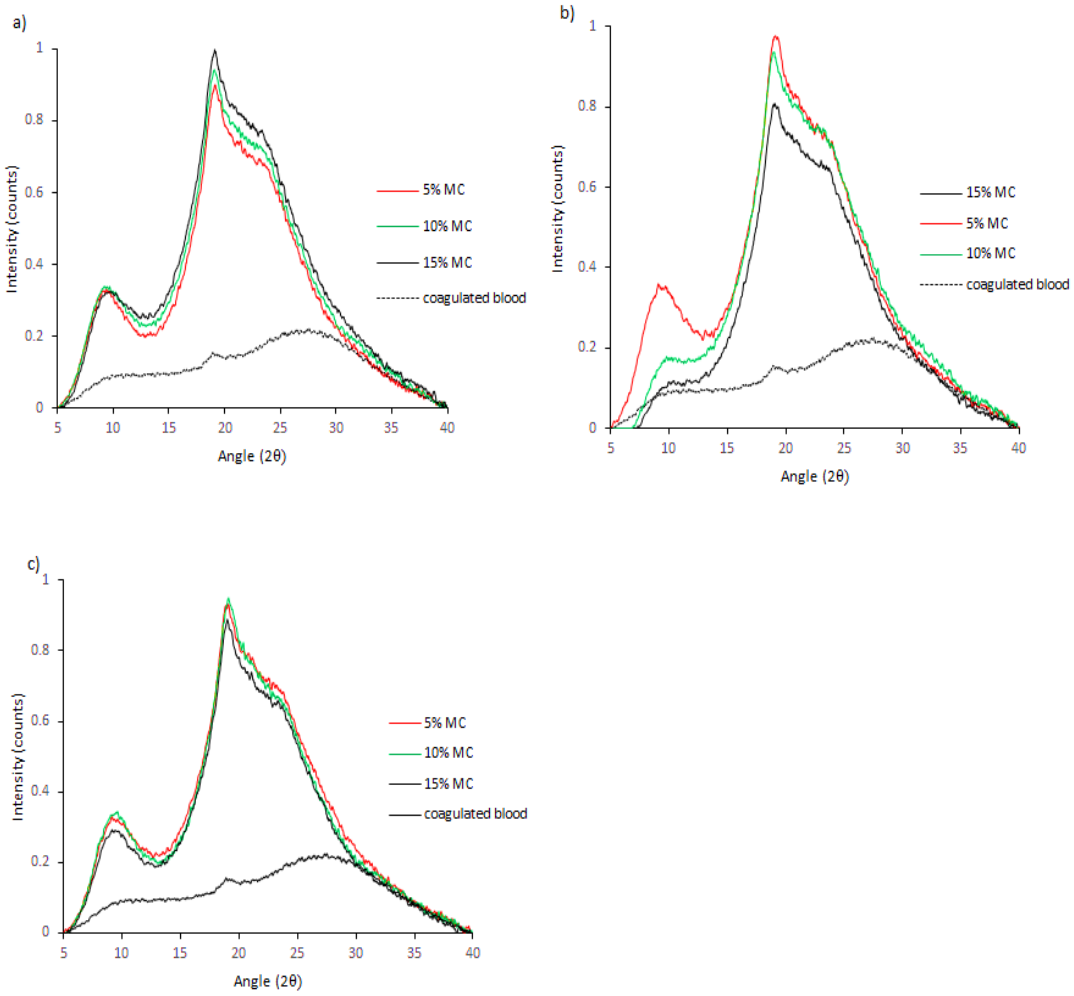

Figure 30. X-ray diffraction of blood meal dried at different temperatures to various moisture content (5% MC, 10% MC, 15 % MC) at 1Hz: a) bloodmeal dried at 60 °C, b) blood meal dried at 100 °C, c) blood meal dried at 140 °C.

CHAPTER 5

Conclusion

The following results may be drawn from the present work in which drying of coagulated blood by an oven dryer have been studied. Coagulated blood contains about 60.7 wt % moisture on a wet basis. The standard oven drying temperature of 140 °C was found to be the optimal for routine moisture determination for coagulated blood as equilibrium moisture was achieved within 24 hours period. Drying process of coagulated blood took place in a falling rate period. No constant drying rate was observed for present study, which implies that moisture removal from the material was governed by a diffusion-controlled process. The drying rate increased with increasing drying air temperature. Within the trialled mathematical models fitted, the page and/or the modified Page drying models were adequately described the oven drying behaviour of coagulated blood.

Wettability of blood meal with water was found to be slower, this was thought to be due to its high protein content which is usually hydrophobic. However, it was observed that SDS, could improve the wettability of blood meal. This could be because of sodium dodecyl sulphate having both polar and non-polar regions, making it easy to percolate in aggregated protein powder than water. Native blood meal dried at 60 °C to 10% moisture content had a higher wettability than denatured proteins dried at 100 °C and 140 °C. It was established that drying temperature has a more significant effect on the ability of water to penetrate through blood meal than moisture content.

Blood meal samples were observed to degrade at temperatures around 307 °C. Drying temperature has more contribution to the thermal stability of blood meal than moisture content. Drying temperature induced hydrophobic interactions, which destabilised the blood meal proteins. Samples dried at low temperature of 60 °C to 15% moisture content was the most stable sample while sample dried at high temperature of 140 °C to 10% moisture content was the least stable.

Two endothermic peaks were observed from DSC. An endothermic peak which was thought to be relaxation behaviour of tightly packed chain was observed at about

40-60 °C. The second endothermic peak was observed within 92 °C to 122 °C and it was identified as protein denaturation peak. The denaturation of blood meal proteins were moisture content and drying temperature dependent. Denaturation of samples shifted to a higher temperature with decreasing moisture content and it increased with increasing drying temperature.

DMA results revealed that different relaxations occurred when drying coagulated blood at different temperatures to varying moisture content. Blood meal samples dried at different drying conditions had a dry glass transition temperature between 219 °C - 226.8 °C. This suggested that bound water does not act as plasticiser in blood meal.

XRD revealed that blood meal is a semi crystalline structure consisting of α-helix and β-sheet sheets. Drying of coagulated blood was observed to have drastic effect on the structural arrangement of coagulated blood. Increase in moisture content was observed to influence the β-sheets structure of samples dried at 60 °C and 100 °C. Therefore, drying conditions had effects on both physicochemical properties and thermal properties of blood meal.

Recommendations for future work

Further investigation about the bed depth, air humidity, air velocity, effective diffusivity and activation energy on drying characteristics of coagulated blood is needed for obtaining optimized conditions to produce blood meal. It is also recommended that further study be undertaken to produce Novatein thermoplastic using blood meal produced under different drying conditions to determine the final properties of the product.

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