Starch modified with stearic acid and xanthan gum as a stabiliser in a fermented whey beverage

by

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DECLARATION OF ORIGINALITY

I, Jeandré Andrew Johnston declare that the dissertation, which I hereby submit for the degree MSc: Food Science at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: ……

DATE: ……

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ABSTRACT

Starch modified with stearic acid and xanthan gum as a stabiliser in a fermented whey beverage

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Degree: MSc Food Science

Whey is a by-product of cheese manufacturing and can be used to make a fermented whey beverage. However, the beverage rapidly separates by sedimentation. The addition of a stabiliser to the beverage can improve the texture of the beverage and prevent sedimentation due to the viscosity increase. Chemically modified starches are used as stabilisers in the food industry. Nevertheless, consumer concerns have demanded for less synthetic chemically modified ingredients in foods, and instead prefer clean-label ingredients. Starch modified with lipids and xanthan gum can be regarded as clean-label. The objective of this study is to determine the effect of modified starch treated with stearic acid and xanthan gum, on the quality of a fermented whey beverage.

Firstly, the properties of maize and high amylose maize starch (HAMS) modified with stearic acid and xanthan gum alone and in combination was determined. Normal maize starch was pasted with and without the modifying agents for two hours at 91 °C under atmospheric pressure. HAMS was pasted with and without modifying agents under pressure (500 kPa) for 10 minutes at 121 °C followed by 91 °C for 110 minutes. The gel strength and thermal properties of the pasted samples were analysed. The flow properties and characteristics of the beverages with the modified starches were evaluated at set intervals over a 15 day period. The IKA® LR 1000 reactor vessel used in upscale-processing could only operate under atmospheric pressure and could thus not fully gelatinise the HAMS. As a result, only maize starch (pasted alone and modified with stearic acid and xanthan gum, alone and in combination) could be used as a stabiliser in the fermented whey beverage.

Maize starch pasted with xanthan gum had an increased pasting viscosity relative to maize starch alone. The viscosity of HAMS pasted with xanthan gum was lower relative to the HAMS pasted

alone. The high temperature and pressure processing conditions that affect the pasting of HAMS could potentially have degraded the xanthan gum. This could be a reason why an increase in viscosity was not observed as with the maize starch. The presence of amylose-lipid complexes (with and without xanthan gum) in both the maize starch and HAMS that was pasted with stearic acid, resulted in an increased pasting viscosity. Furthermore, the amylose-lipid complexes prevented a gel formation in both starches, possibly by interfering with the retrogradation of amylose chains.

The differential scanning calorimetry showed that maize starch pasted with stearic acid had more crystalline amylose-lipid complexes than the maize control. Decreased amounts of Type II amylose-lipid complexes were observed for the maize starch pasted with stearic acid and xanthan gum. It is presumed that the xanthan gum interferes with the formation of the amylose-lipid complexes in normal maize starch. HAMS pasted alone had Type II amylose-lipid complexes. The abundant amylose content and higher pasting temperatures could be a cause for the prevalent formation of the more crystalline Type II amylose-lipid complexes. The viscosity of HAMS was too low to significantly impact the beverage, therefore it was not used in the beverage formulation.

The beverages with the modified starch had increased levels of viscosity, decreased levels of sedimentation and increased degrees of shear thinning relative to the whey beverage with no starch. The beverages with the stearic acid modified starch (with and without xanthan gum) had a lower viscosity, increased level of sedimentation and a smaller degree of shear thinning relative to the beverages with modified starch (with and without xanthan gum). The lower viscosity could be due to the lack of the retrograded starch. It is believed that the decrease in shear thinning could be attributed to the compact size of the amylose-lipid complex relative to the long uncomplexed amylose molecules. However, the beverages with stearic acid modified starch (with and without xanthan gum) showed more consistent flow properties throughout the 15 day period in relation to the beverages with modified starch (with and without xanthan gum).

Maize starch modified with stearic acid has potential as a thickener and stabiliser in a fermented whey beverage as it shows limited changes during storage and shows higher viscosity. Further research into the rheological and sensory properties of the whey beverage with starch modified lipids is recommended for a marketable product.

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LIST OF ABBREVIATIONS

BOD: Biological oxygen demand

DSC: Differential scanning calorimetry

HAMS: High amylose maize starch

LAB: Lactic acid bacteria

MPC: Milk protein concentrate

M: Pasted maize starch

MSA: Maize starch pasted with stearic acid

MXG: Maize starch pasted with xanthan gum

MSX: Maize starch pasted with stearic acid and xanthan gum

SMP: Skim milk powder

1 INTRODUCTION

Whey is a by-product of cheese manufacturing and contributes to about 85-95% of the volume of milk. Liquid whey retains approximately 55% of the nutrients contained in milk after the casein fraction has been precipitated for cheese production (Siso, 1996). The high biological oxygen demand (BOD) of whey (30–50K mg kg^{1}) (Jelen, 2011) has been attributed to the high lactose content (Mawson, 1994) that negatively affects the environment, specifically water streams. It is estimated that about 180 million tons of liquid whey is produced globally (Coetzee, 2015). Alternative ways of utilising whey have been put forward, including value addition or processing for human consumption (Jelen, 2011).

There has been a long-standing opinion that the consumption of whey has health benefits (Holsinger, Posati and DeVilbiss, 1974: Kosikowski, 1979). Recent studies have determined that whey protein isolates have bioactive peptides that may protect against cancer growth (McIntosh, Royle, Le Leu, Regester, Johnson, Grinsted, Kenward and Smithers, 1998). Whey protein has a high biological value because of the high content of branched-chained essential amino acids (Pescuma, Hébert, Mozzi and Font de Valdez, 2010). It can also be used for different purposes, being a rich source of lactose and protein.

However, only 50% of the whey produced in 1988 was utilised, of which 45% was liquid whey (Marwaha and Kennedy, 1988). Unprocessed liquid whey does not have an appealing taste (Djurić, Carić, Milanović, Tekić and Panić, 2004: Gallardo-Escamilla, Kelly and Delahunty, 2007). Product development has made whey more appealing to the consumer, by using it as an ingredient in products based on its nutritional and functional properties. Various food products utilise the functional properties of whey and whey derivatives (Huffman, 1996: Djurić *et al.*, 2004: Gulzar, 2011). A fermented whey beverage, a type of lactic acid beverage, has a poor watery mouthfeel and rapidly separates, presumably due to low solids content (Gallardo-Escamilla *et al.*, 2007).

Chemically modified starches can be used to improve the consistency of such a beverage and may prevent separation in semi-solid foods, like drinking yoghurts, by increasing the viscosity and entrapping the aqueous phase (BeMiller and Whistler, 2007). Chemically modified starches

tolerate processing conditions and low pH (< 4) better than native starches. However, there is a growing reluctance by consumers towards the use of chemically modified ingredients in food. Consumers prefer the use of clean-label ingredients (Arocas, Sanz and Fiszman, 2009).

Amylose-lipid complexes have been found to alter the functionality of native starches (D'Silva, Taylor and Emmambux, 2011: Obiro, Sinha Ray and Emmambux, 2012) and specifically, the rheological property of starch (Maphalla and Emmambux, 2015). Amylose-lipid complexes form between the lipids and the amylose fraction of the starch when pasted for long period (two hours) of time (Nelles, Dewar, van der Merwe and Taylor, 2003: D'Silva *et al.*, 2011). The amylose-lipid complex interferes with the formation of the junction zones between amylose chains, preventing gel formation (Blazek and Copeland, 2008). The amylose-lipid complex could enhance the stabilising capabilities of native starch by thickening foods without a gel forming. Furthermore, Maphalla and Emmambux (2015) showed that the addition of xanthan gum with starch and stearic acid increased the viscosity of the starch paste due to the formation of amylose-lipid complexes. Ocloo, Minnaar and Emmambux (2016) reported that amylose-lipid complexes can be produced with high amylose maize starch (HAMS) and stearic acid pasted under pressure. There is no known literature that relates to the use of HAMS with stearic acid in foods. This dissertation explores the production of a clean-label stabiliser, namely, modified maize starch and HAMS, with xanthan gum and stearic acid; and the potential of these modified starches as stabilisers in a fermented whey beverage.

2 LITERATURE REVIEW

In this chapter, literature on the chemistry and functional properties of whey, as a fermented beverage is reviewed. Furthermore, literature on amylose-lipid complexes is reviewed as a potential stabilising additive to the beverage.

2.1 Whey

As previously mentioned, whey is a by-product derived during the cheesemaking process, where whey is drained off after casein has been precipitated. The opaque raw whey can be treated in a variety of manners (Fig 2.1) to produce whey derivatives. Whey derivatives include: whey powder, lactose, demineralised whey, whey protein isolate and whey concentrates. Treating whey can increase its palatability and digestibility as well as increase the functional properties of the whey derivatives (Boland, 2011). Table 2.1 shows the various functional and nutritional applications of whey and whey derivatives (Huffman, 1996: Foegeding, Davis, Doucet and McGuffey, 2002).

The functional properties of whey makes it a versatile ingredient (Khurana and Kanawjia, 2007). Whey can be used in many different products, specifically: alcoholic beverages like whey wine and whey beer; and non-alcoholic beverages like whey fruit juice mixes and lactic acid beverages (Holsinger *et al.*, 1974: Huffman, 1996: Djurić *et al.*, 2004: Penna, Sivieri and Oliveira, 2001: Khurana and Kanawjia, 2007).

Table 2.1: Application of whey and whey derivatives for its use in foods

Product	Application
Powdered whey	Bulking agents, fermentation substrate
Lactose	Sweeteners, fermentation substrate
Whey Proteins	Gels, edible films, supplements, bulking agents
Whey protein concentrates	Emulsifiers, supplements, stabilisers
Whey protein hydrolysates	Supplements, foaming-, gelation-, and emulsifying agents

(Foegeding *et al.*, 2002: Jelen, 2011)

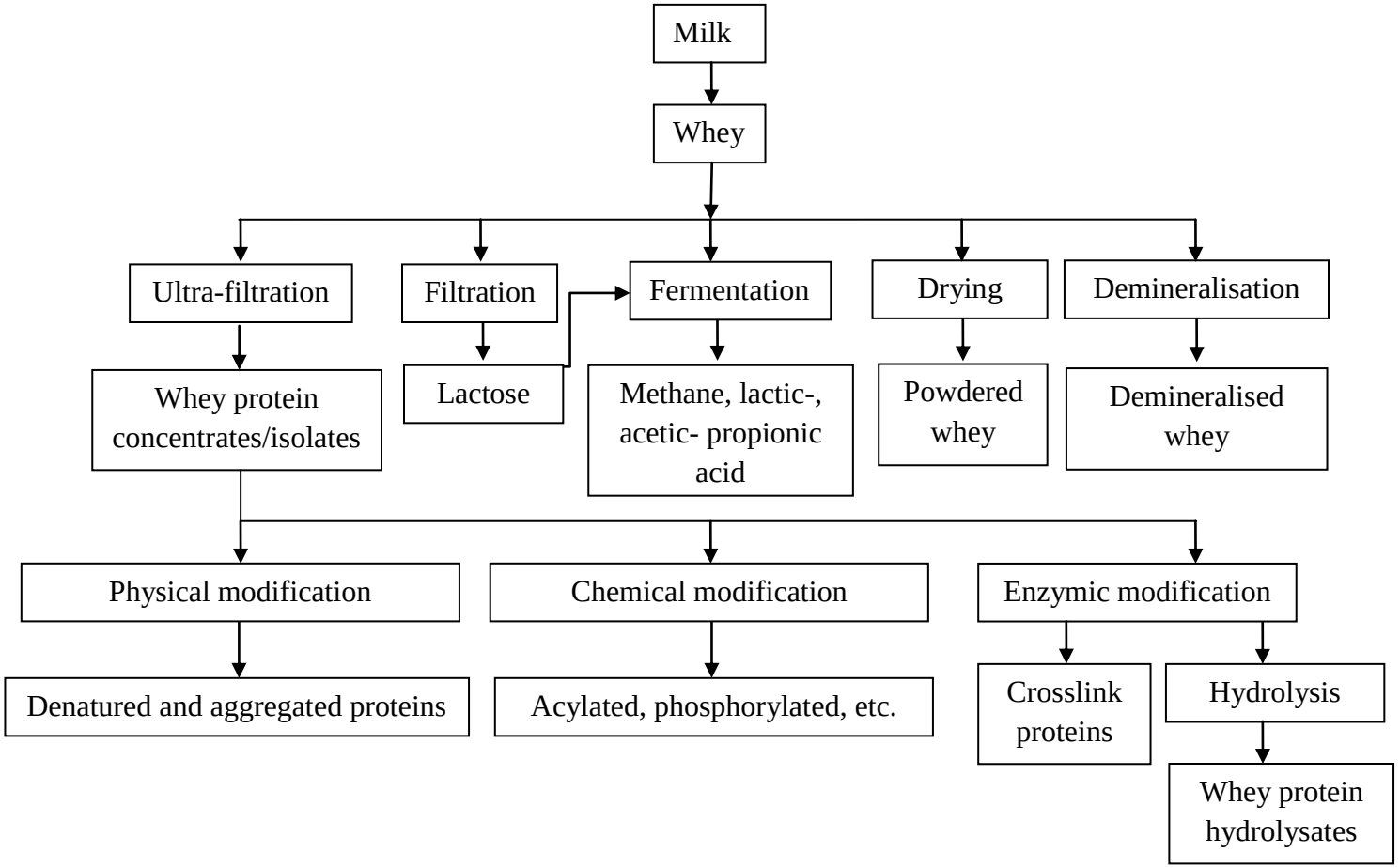

Figure 2.1: Schematic diagram of the treatments on liquid whey. Adapted from Siso (1996)

Lactic acid beverage is a growing sector in the dairy industry (Penna, Oliveira and Tamime, 2003). However, the popularity of these whey beverages have not yet been widely received (Kosikowski, 1979: Djurić *et al.*, 2004). This could be attributed to the fact that lactic acid beverages, or fermented whey beverages, separates rapidly and have a poor mouthfeel. The poor consistency of the whey beverage is as a result of its low solids content (Gallardo-Escamilla et al., 2007).

2.1.1 Whey Composition and Chemistry

Liquid whey consists of approximately 93% water, 5% lactose, 0.85% proteins, 0.36% lipids and 0.53% mineral. Other constituents present in small quantities, including: lactic acid, citric acid, non-protein nitrogen compounds and B group vitamins. Table 2.2 shows the approximate composition of sweet liquid whey and whey powders in comparison to fresh milk. Sweet whey refers to the effluent of a rennet coagulate cheese, where the pH is approximately 6.0-6.5 (Jelen, 2011). The production of cheese utilises most of the casein proteins and fat in milk, resulting in low fat and casein content in whey.

2.1.1.1 Carbohydrates

Lactose is a highly soluble sugar unique to milk. The majority of lactose is removed in from the milk into the liquid whey (Table 2.1). Lactose is not as sweet as sucrose, fructose or glucose, however, it can be described as having a sweet taste in high concentration as a 33% lactose solution has an equivalent sweet taste to a 20% sucrose solution (Walstra and Jenness, 1984).

Lactose is a reducing sugar that can undergo Maillard reactions in the presence of amino acids (e.g. lysine) upon heating. The formation of a Schiff base, between the free amino groups of amino acid and the reducing end of the sugar (aldehyde-group), reacts to form various compounds that affect the taste and colour of a product (Van Boekel, 1998). The disaccharide consists of the monosaccharide $_{D}$-galactose and $_{D}$-glucose (Figure 2.2) linked via β-1,4-glucosidic linkage.

Table 2.2: Compositional data on milk, liquid whey, and whey powder

	Unit	Milk (fresh)[a]	Liquid whey (sweet)[a]	Whey powder[b]
Dry matter	g	12.3-13	6.3-7.0	94-97
Energy	kJ	265	105	1450
Fat	g	3.1-4.0	0.05-0.4	0.8-1.2
Crude protein	g	3.1-3.6	0.85-1.15	12-13
Carbohydrate	g	4.5-4.8	4.6-5.2	71-73
Ash	g	0.7-0.8	0.5-0.6	7.5-8.5
Lactose	g	4.5-4.8	4.6-5.2	71-73
Lactic acid	g	tr	0.05-0.2	0.2-2
Citric acid	g	0.15-0.21	0.14-0.17	~2.2
Casein	g	2.3-2.8	0.04-0.05	0.60-0.65
Whey-protein	g	0.55-0.72	0.6-0.8	8.2-9.0
Na	mg	35-50	36-51	~600
K	mg	140-155	140-160	~2100
Ca	mg	115-125	40-50	600-800
Mg	mg	11-14	8-10	90-120
Fe	mg	0.03-0.1	0.1	~1.4
Cl	mg	95-110	70-120	~1500
P	mg	90-100	40-55	580-600
S	mg	30-35	~15	~200
Thiamin	mg	0.03-0.45	0.03-0.05	0.4-0.6
Riboflavin	mg	0.14-0.20	0.10-0.16	2.3-2.5
Pyridoxine	mg	0.04-0.06	0.04-0.07	0.4-0.6
Vitamin C	mg	1-2	0.9-1.5	~5
Vitamin A	mg	0.02-0.05	0.002-0.004	0.02
Carotenoids	mg	0.02-0.03	tr	tr
Density (p^{20})	$kg.m^{-3}$	1030	1025	-

The approximate composition of products per 100 grams.
a: as is basis,
b: dry basis.
Tr. is trace amounts
Walstra and Jenness (1984)

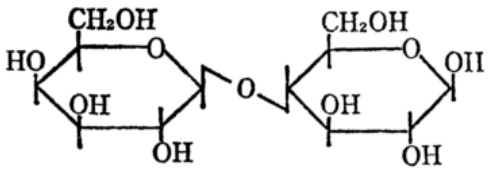

Figure 2.2: Lactose, 4-0-β-D-galactopyransosyl-D-glucopyranose (Hirotsu and Shimada, 1974)

Lactose is an important substrate for the fermentation process in various cultured dairy products. Lactose is metabolised to lactic acid through the metabolic activity of lactic acid bacteria. Other metabolic compounds derived from lactose are acetic acid, alcohol, CO_2 and intermediates like pyruvic acid, which are associated with the taste of yoghurt (Tamime and Robinson, 2007). The lactose in whey can be fermented to produce a whey beverage with a desirable sensory profile (Pescuma, Hébert, Mozzi and Font de Valdez, 2008).

2.1.1.2 Proteins

The proteins in milk are casein and whey. Whey proteins have high biological values due to the high content of essential amino acids that aids in muscle protein synthesis (Pescuma *et al.*, 2010). The whey proteins are made up of about 50% β -lactoglobulin, about 20% α-lactoalbumin, and a small amount of bovine serum albumin. The β-lactoglobulin is regarded as one of the richest known food sources of amino acids having 162 amino-acids of which 22 are Leu, 10 Ile and 9 Val (Boland, 2011). Whey proteins have an isoelectric point of about pH 4.8 and denature at temperatures higher than 70 °C. Denatured whey becomes less soluble (Smith and Campbell, 2007).

The β-lactoglobulin has a strong hydrophobic region, comprising of seven major strands of beta pleated sheet structures. The protein occurs as a non-covalently linked dimer of two monomeric subunits at neutral pH (Walstra and Jenness, 1984: Mleko and Foegeding, 1999: Boland, 2011: Gulzar, 2011). The β-lactoglobulin dissociates at a pH < 3.4 (Hambling, McAlpine and Swayer, 1992). The β-lactoglobulin contains 5 cysteine residues per mol, four of which are involved in disulphide linkages that stabilise the structure. (Demetriades, Coupland and McClements, 1997: Smith and Campbell, 2007). A single sulfhydryl group on Cys 121, is protected in an alpha helix.

The sulfhydryl group can react with other sulfhydryl groups when the Cys becomes exposed due to denaturation by heat or low pH (Boland, 2011: Smith and Campbell, 2007). This would allow for disulfide exchange and cross-linking occurring between β-lactoglobulin molecules, κ-casein and α-lactalbumin. The α-lactalbumin is also a globular protein that contains four disulphide bonds. The four disulphide bonds are responsible for the reversal in the conformational changes brought about by heat (Creamer, Bienvenue, Nilsson, Paulsson, van Wanroij, Lowe, Anema, Boland and Jiménez-Flores, 2004).

2.1.1.3 Whey protein network

Foegeding *et al.* (2002) stated that the rheological attributes of whey gels are influenced by the nature of its microstructural properties namely: fine stranded, mixed or particulate. The free thiol group of β-lactoglobulin (Cys 121) as discussed previously, is believed to react with other whey proteins during heating, resulting in the formation of a gel (Baeza, Gugliotta and Pilosof, 2003). The formation of disulfide bonds below pH 5 between whey protein aggregates, (Alting, Hamer, de Kruif and Visschers, 2000) induces a weak network formation (Ju and Kilara, 1998a) that can also result in the formation of a gel at room temperature. It is important that the whey protein should have been exposed to a heat treatment to change the globules to aggregates (Alting *et al.*2000).

The protein network of whey is generally weak compared to the network of milk due to the low protein content. Both the protein concentrations and heating time contribute to the extent of the network (Vardhanabhuti and Foegeding, 1999), which in turn contributes to the viscosity of a solution. A study by Guzmán-González, Morais, Ramos and Amigo (1999) found that the addition of whey protein concentrates to yoghurt mix, resulted in a product with lower viscosity and significantly lower level of syneresis, compared to yoghurts made with skim milk powder (SMP) and milk protein concentrates (MPC). The SMP and MPC exhibit a high degree of fused micelles when compared to the yoghurt with added whey protein concentrates. Whey protein concentrates enhance the water binding capacity of the yoghurt matrix, making it a texturally softer yoghurt with less syneresis, compared to yoghurt made with SMP.

2.1.2 Fermented whey drink

The health benefits of consuming probiotics contained in fermented dairy products, have resulted in the growth of fermented products in the dairy sector (Pescuma *et al.*, 2010). Fermented milk is defined as being prepared from 'milk' and/or 'milk products' which may include any one or combination of whole, partially or fully skimmed, concentrated or powdered milk, buttermilk powder, ***concentrated or powdered whey***, milk proteins, cream, butter or milk fat, all of which, by the action of microorganisms lead to reduction in pH and coagulation (Fuquay, Fox and McSweeney, 2011). Whey protein concentrates can be useful in drinking yoghurt production, as whey protein polymers have flow properties similar to that of food hydrocolloids, allowing for the production of a whey beverage with a drinking yoghurt like consistency (Hall and Iglesias, 1997: Mleko and Foegeding, 1999: Guzmán-González *et al.*, 1999).

Whey-based lactic acid beverages constitute an emerging segment of non-conventional dairy products (Gallardo-Escamilla *et al.*, 2007). Lactic acid beverages have gained increasing popularity in Brazil. This beverage, formulated with whey, represented approximately one-third of the market for yoghurt and dairy-based lactic acid beverages from as early as 2003 (Penna *et al.*, 2003). However, these beverages have a poor mouthfeel due to low solid content and poor protein network formation (Djurić *et al.*, 2004: Gallardo-Escamilla *et al.*, 2007). A lactic beverage should be visually and texturally as homogeneous as milk from the consumer's point of view (Gallardo-Escamilla *et al.* (2007).

Numerous factors can affect the quality of a product, including the chemical composition of the raw materials and methods of production. The process of fermenting products is subjected to a degree of variability that is ascribed to the biological activity of the lactic acid bacteria (Guzmán-González *et al.*, 1999). Lactic acid bacteria (LAB) metabolises the lactose to lactic acid. The increased concentration of lactic acid results in the decrease of the pH. This low pH of 4.5 will inhibit the growth of pathogenic microbes (Tamime and Robinson, 2007). Furthermore, the use of LAB decreases the lactose content as well as hydrolyses the β-lactoglobulin and produces other metabolites which contribute to the characteristic aroma and flavour of fermented products like yoghurt (Pescuma *et al.*, 2008).

The consistency of a whey beverage can be improved by increasing the solid content. The source of the solid content can be from a variety of sources: skim milk powder, milk powder, whey powder, whey protein concentrate and casein protein concentrate. However, this could potentially increase the production cost. The addition of more whey powder to the system has negative effects on the organoleptic properties, which causes a foul bitter taste, presumably due to the high lactose content (Djurić *et al.*, 2004) and free peptides.

2.2 Stabilisers

Stabilisers are used in the food industry to improve the textural characteristics and appearances of some food products (BeMiller and Shewry, 2008: Mason, 2009). Stabilising agents like starch and gums are examples of stabilisers that are used in yoghurts and drinking yoghurts. Hydrocolloids are stabilisers with large polymeric structures and molecular weights, which are used in foods to alter the mouthfeel by increasing the viscosity and preventing separation in food products, through associating with water and or forming structures that can hold water (BeMiller and Shewry, 2008: Mawson, 1994). Gallardo-Escamilla *et al.* (2007) have recommended the use of starch in a whey based beverage.

The most commonly used hydrocolloids are gelatine and polysaccharides (starch and gums). Starch is the predominant storage polysaccharide in plants that can be used as an ingredient in foods. Starch, as an ingredient, is accepted throughout all ethnic groups and is a relatively cheap hydrocolloid compared to gelatine. Native starch, like maize starch, has stabilising qualities whereby it can thicken, stabilise and modify the texture of food (Eliasson, Finstad and Ljunger, 1988).

The functionality of starch is limited due to its low processing tolerance that limits its industrial applications (BeMiller, 2011). These limitations have given rise to chemically modified starches. Chemically modified starches, as listed in Table 2.3, serve as important ingredients in many fabricated foods (Karim, Norziah and Seow, 2000: Copeland, Blazek, Salman and Tang, 2009).

Table 2.3: Classification of chemically modified starches

Chemically modified starches	Properties	Structural features	Viscosity
Cross-linked starch	Increase pasting temperatures and resistance to shear. Increased stability to low pH.	Diester phosphate or adipate groups	Increased paste viscosity
Stabilised/Substituted starch	Reduced pasting temperature, and improved freeze-thaw stability. Improved paste clarity	Hydroxypropyl ether, acetate ester or monoester phosphate groups	Increased paste viscosity
Cross-linked and stabilised starch	Reduced pasting temperature	Combination of above mentioned	Increased paste viscosity
Octenylsuccinylated starch	Surface activity. Used as an emulsifier. Amphiphilic nature. Can provide a fatlike mouthfeel.	Ocetenylsuccinate ester groups	Reduced paste viscosity
Oxidised starch	Reduced pasting temperature and produces soft clear gels	Hypochlorite oxidized. Carbonyl and carboxyl groups	Reduced paste viscosity
Thinned starch	Reduced pasting temperature and can produce firm gels	Lower molecular weight	Reduced paste viscosity

BeMiller and Whistler (2007), Abbas, Khalil and Hussin (2010)

There is, however, an increasing demand from consumers to consume "natural foods" that do not contain chemically modified starches (Copeland *et al.*, 2009). Consumers prefer the use of clean-label /natural ingredients, such as native starch (Arocas *et al.*, 2009: Copeland *et al.*, 2009). Physical modification processes such as heat-moisture treatment, annealing (Chung, Hoover & Liu, 2009) and pre-gelatinisation (Singh, Kaur & McCarthy, 2007) are considered modification processes that produce clean-label ingredients. Similarly, the use of food friendly chemicals such as lipids and hydrocolloids to modify starch, can be considered as clean label (Maphalla and Emmambux, 2015).

2.2.1 Amylose-lipid complex: Components, Chemistry and Application

Morrison, Law and Snape (1993) have observed that the endogenous lipids of cereal starch complex with amylose in small quantities. Amylose is capable of forming single helical inclusion complexes with a diverse range of compounds, specifically with fatty acids (Godet, Bouchet,

Colonna, Gallant and Buléon, 1996). These amylose-lipid complexes are more stable against enzymatic activity and have different properties relative to native starch (Obiro *et al.*, 2012).

2.2.1.1 Starch

Starch exists in plants in the form of discrete granules (Karim *et al.*, 2000). These granules consist of growth rings, which in turn are made up of alternating crystalline and amorphous regions out of a central point, known as the hilum (Pérez and Bertoft, 2010). It is suggested that these semi-crystalline blocklets occur in two types: a normal type, which constructs the hard shell, and the defective type, which constructs the soft shell.(Tang, Mitsunaga and Kawamura, 2006). The blocklets are made up of crystalline and amorphous growth rings (Cheetham and Tao, 1998b: Tang *et al.*, 2006) and consist of crystalline and amorphous lamellae. Amylopectin double helices form a large number of clusters that interact to build up the crystalline lamellae, and the amorphous lamellae may consist of amylose (Pérez and Bertoft, 2010).

Amylopectin is a highly branched polymer consisting of α-(1→4)-D-glucopyranosyl units and α-(1→6) units at about every 20-25 unit (Parker and Ring, 2001: Hizukuri, 1986). Amylose is an essentially linear molecule consisting of 1000-4000 α-(1→4)-D-glucopyranosyl units with random branching α-(1→6) linkages (Hizukuri, Takeda, Yasuda and Suzuki, 1981). Amylose is described as having a flexible random coil (left-handed helical arrangement) in an aqueous solution (López *et al.*, 2012) with a molecular weight in the range of 1.3-3.9 x 10^6 g/mol (Ong, Jumel, Tokarczuk, Blanshard and Harding, 1994).

2.2.1.1.1 Maize starch and high amylose maize starch

Maize starch has about 28% amylose content (Cheetham and Tao, 1998a). Native maize starch has a low swelling power that increases slowly with increase in temperature (Srichuwong, Sunarti, Mishima, Isono and Hisamatsu, 2005) with a pasting temperature of about 75 °C (Weber, Clerici, Collares-Queiroz and Chang, 2009: Doutch, Bason, Franceschini, James, Clowes and Gilbert, 2012). The average chain length of maize starch amylose is about 335 degree of polymerisation (DPn) (Takeda, Shirasaka and Hizukuri, 1984) with a molecular weight of about 2.3 x 10^6 g/mol (Bultosa, Hamaker and BeMiller, 2008).

Different varieties of maize have been cultivated with different starch contents i.e. high amylose maize starch (HAMS), which could be used in foods for its ability to form strong gels and films (Case, Capitani, Whaley, Shi, Trzasko, Jeffcoat and Goldfarb, 1998). HAMS is produced from maize with the amylose extender *ae* mutant (Shi, Capitani, Trzasko and Jeffcoat, 1998). The starch granules of HAMS require temperatures greater than 130 °C to fully disperse (Case *et al.*, 1998), possibly due to a great concentration of amylose (about 57%) in the crystalline regions (Shi *et al.*, 1998). The high processing conditions limit the commercial use of HAMS (Case *et al.*, 1998). Starches with higher amylose contents have been reported to produce more amylose-lipid complexes compared to starches with lower amylose contents (Eliasson *et al.*, 1988). The formation of amylose-lipid complexes has been reported in both maize starch (Maphalla and Emmambux, 2015) and HAMS (Ocloo *et al.*, 2016) when pasted for two hours.

2.2.1.1.2 Functional properties of starch

The conformation of the starch molecules together with the ratio of amylose to amylopectin are some of the factors that influence the properties of a starch (BeMiller and Whistler, 2007). Starch is readily used to thicken foods as it increases the viscosity in foods. In order to utilise the functionality of starch, it first needs to be gelatinised (Copeland *et al.*, 2009). Gelatinisation entails the disruption of the molecular order within the granules that results in the irreversible granule swelling, loss of birefringence and loss of crystallinity (Fennema, 1996: Jenkins and Donald, 1998). The hydrogen bonds between amylose and amylopectin molecules break, allowing for the interaction with water. Amylose leaches out of the granule and contributes to the viscosity of the solution. Fennema (1996) explains that continuous heating in excess water results in the total disruption of starch granules.

Many changes occur in the gelatinised starch on cooling. On cooling (below 60 °C) some starch molecules, specifically amylose, partially reassociate resulting in a network formation of the starch molecules (Fig 2.3) (BeMiller and Shewry, 2008). The viscosity of the pasted starch increases on cooling as the starch polymers interact with each other (molecular entanglement). HAMS forms strong gels and films that can be used in jelly-gum candies and as a coating in deep fried foods (Case *et al.*, 1998).

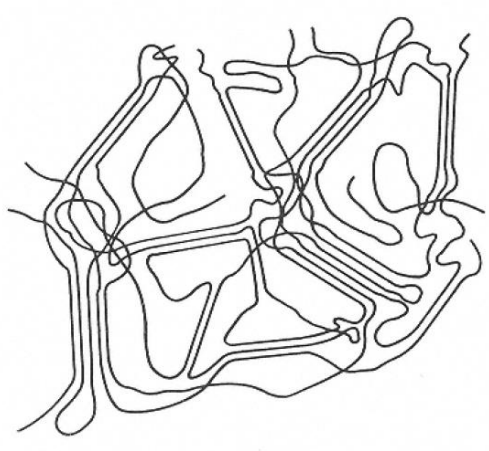

Figure 2.3: Illustration of a three-dimensional network structure found in gels. This structure is known as a fringed micelle structure, where parallel side chains depict the crystalline junction zones. The pores (large spaces) commonly contain the aqueous phase (Fennema, 1996).

A gel formation occurs when the water-starch mixture is cooled, as hydrogen bonds reform amongst adjacent starch molecules. Unbranched molecules, such as amylose, rapidly disperse in water forming gels. This occurs as segments of the long molecules collide and form intermolecular bonds over the distance of a few molecules forming junction zones (Lii, Lai and Shen, 2004). The firmness of the gel greatly depends on the level of the junction zone formation. The presence of other ingredients including fats, protein and amount of water, influences these junction zones (Fennema, 1996).

The junction zones increase with time, which increases the ordered crystalline structure that results in the displacement of the water (Fennema, 1996: BeMiller and Shewry, 2008). Crystallites form over time accompanied by an increase in rigidity and phase separation between polymer and solvent (Karim *et al.*, 2000). These changes alter the quality, acceptability, and shelf-life of foods that contain starch. These changes occur due to the process of retrogradation (Karim *et al.*, 2000). Retrogradation is a collective term used for the changes which occur in gelatinised starch from an initially amorphous state to a more ordered crystalline state (Gudmundsson, 1994).

2.2.1.2 Lipids

Fatty acyls are one of eight categorical groups of lipids. Fatty acids, a group under the fatty acyl, exist either as saturated or unsaturated, which can have between 14 – 24 carbon atoms. Fatty

acids contain an aliphatic chain with a terminal carboxylic group. Examples of lipids include palmitic acid and stearic acid.

2.2.1.3 Amylose-lipid complex Chemistry

Amylose can form a V-type helical conformation that has six to eight glucose units per turn (López *et al.*, 2012), which forms due to the addition of a complexing agent (Biliaderis, Page, Slade and Sirett, 1985: Cheetham and Tao, 1998b). The inner surface of the V-amylose complex is lined with hydrophobic methylene groups and glycosidic linkages, forming a helix with a hydrophobic cavity. The hydrophilic glycosyl hydroxyl groups are located on the outer surface area (Immel and Lichtenthaler, 2000). V-amylose co-crystallises with various compounds, in particular, fatty acids (Godet *et al.*, 1996). The hydrophobic lipid tail (aliphatic tail) of the fatty acid interacts with the hydrophobic methylene groups of the amylose.

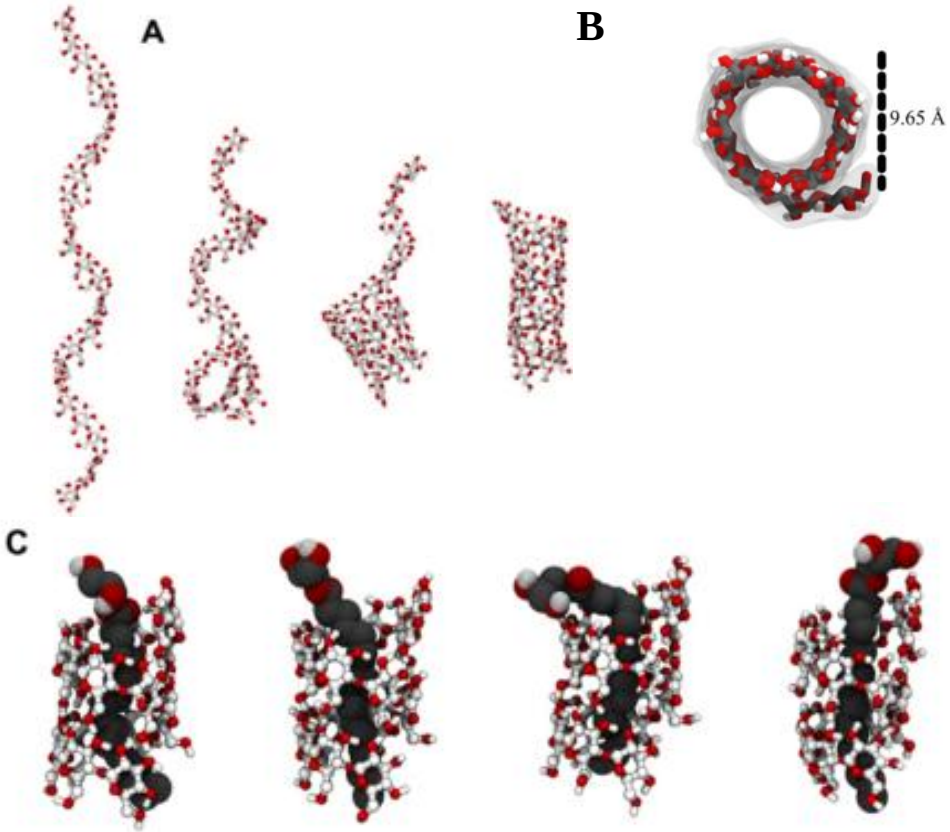

Figure 2.4: The folding of an amylose molecule into the V-amylose structure and formation of an amylose-lipid complex. (A) The folding of the amylose molecule, (B) the mean diameter of the helical conformation and, (C) the stable amylose-lipid complex (López, de Vries and Marrink, 2012).

D'Silva *et al.* (2011) observed the formation of a second viscosity (or pasting) peak with prolonged heating of native maize starch at 91 °C for 120 minutes (Fig 2.5). The second peak has been hypothesised to be the result of naturally formed amylose-lipid complexes (Nelles *et al.*, 2003: D'Silva *et al.*, 2011). Wokadala, Ray and Emmambux (2012) indicated that amylose-lipid complex formations can be enhanced by the addition of stearic acid to starch under prolonged pasting conditions at about 90 °C. There is an increased formation of amylose-lipid complexes with the incorporation of stearic acid. The increased formation of amylose-lipid complexes presumably increase the pasting viscosity beyond that of the second peak of native maize starch (D'Silva *et al.*, 2011: Wokadala, Ray and Emmambux, 2012).

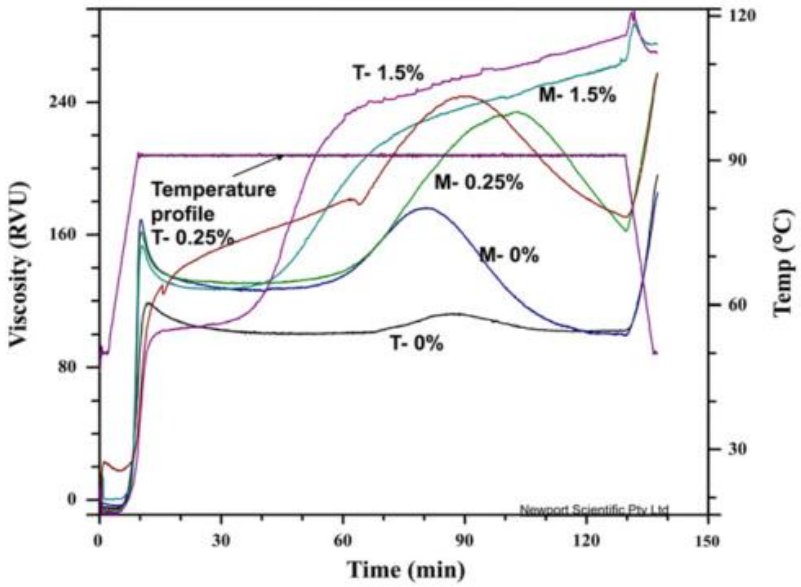

Figure 2.5: Pasting curve of stearic acid treated maize and teff starches. 'M'- 0% shows the pasting curve of native maize. M - 0.25% and M - 1.5% shows the pasting curve of maize starch modified with stearic acid modified starch. T - 0% shows the pasting curve of native teff. T - 0.25%, and T - 1.5% shows the pasting curve of teff starch with stearic acid (D'Silva *et al.*, 2011).

Cheetham and Tao (1998b) found an increased amount of V-type amylose in maize starch with amylose content in the range of 40%-84%. According to Copeland *et al.* (2009) the lipid content of the starch highly correlates to amylose content, thus the higher amylose the greater the lipid content in the specific species. This explains why amylose-lipid complexes could more readily form in HAMS. Ocloo *et al.* (2016) reported that the pasting of HAMS with stearic acid in a rheometer at 90 °C at 500 kPa also results in an increased second peak in viscosity. DSC analysis confirmed the formation of type II amylose-lipid complexes in the pasted HAMS.

2.2.1.4 Amylose-lipid complex functionality

The amylose-lipid complexes can presumably be used in a variety of food applications including: to reduce the stickiness of starchy foods, to improve freeze-thaw stability, to retard staling in bread and as dough conditioners (Copeland *et al.*, 2009) and fat replacers (Teklehaimanot, Duodu and Emmambux, 2013). V-amylose in complex with stearic acid has been demonstrated to have a reduced gel formation compared to native starch (Obiro *et al.*, 2012: D'Silva *et al.*, 2011). The amylose-lipid complexes have been shown to modify the starch's rheological properties (Maphalla and Emmambux, 2015).

Amylose-lipid complexes are not as susceptible to retrogradation as native starch (Obiro *et al.*, 2012), as amylose-lipid complexes have been reported to interfere with the realigning of amylose chains during retrogradation (Gudmundsson, 1994). The amylose-lipid complexes remain intact, thereby preventing the complex from interacting with the adjacent starch constituents.

2.2.2 Gums

Gums such as xanthan gum, guar gum, alginates and carrageenans are used in the food industry. Several proposed gums have been tested in a fermented model solution in an effort to confer stability as well as appeal to the end user (Gallardo-Escamilla *et al.*, 2007). Gallardo-Escamilla *et al.* (2007) found that xanthan gum did not interfere with the flavour release of a fermented whey beverage. Xanthan gum was an effective hydrocolloid for the enhancement of yoghurt's flavour but it did not contribute significantly to an increase in the perceived thickness when used at 0.26% in the beverage.

2.2.2.1 Xanthan gum chemistry

Xanthan gum is an extracellular polysaccharide produced by *Xanthomonas campestris* (Mali, Ferrero, Redigonda, Beleia, Grossmann and Zaritzky, 2003). Xanthan gum (Fig 2.6) can be chemically considered as an anionic polyelectrolyte, consisting of pentasaccharide subunits that form a cellulose backbone with α-1,3 linked trisaccharide side chains. The charged trisaccharide side chains consist of mannose (β1,4) glucuronic acid (β1,2) mannose and are attached to the O(3) of alternate residues (Mohammed *et al.*, 2007).

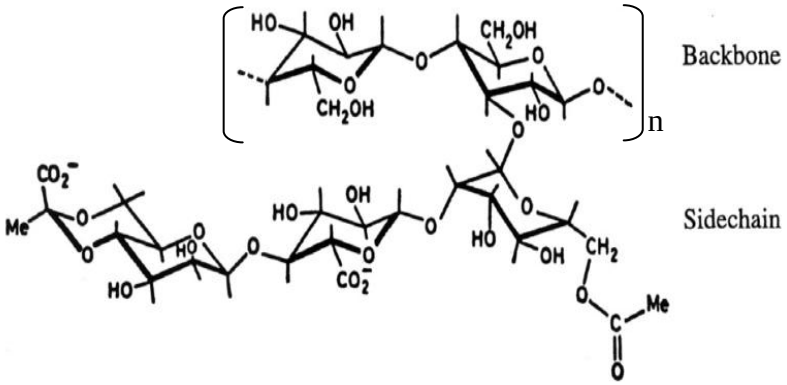

Figure 2.6: Chemical structure of xanthan gum. The side chain consists of β-D-mannopyranosyl-(1-4)-β-D-glucuronopyranosyl-(1-2)-6-*O*-acetyl-β-D-mannopyranosyl. Adapted from Mohammed, Haque, Richardson and Morris (2007)

Xanthan gum is stable at temperatures up to 90 °C and over a wide pH range (2-11) (Rosalam and England, 2006). The rheological properties of xanthan gum allow for its use as a stabiliser of emulsions and suspensions. Xanthan gum flow freely in a solution, however, the solution is capable of holding solid particles in suspension as if trapped in a gel (Mohammed *et al.*, 2007). An important characteristic of xanthan gum is its ability to create a high viscous solution at low shear forces (Rosalam and England, 2006)

2.2.3 Maize starch with xanthan gum and stearic acid

Various blends of starches and gums are used in the modern food industry. These combinations modify and control the texture of food products; improve moisture retention and control water mobility in a food product (Appelqvist and Debet, 1997). The combination of small amounts of gums (like xanthan gum and guar gum) to starch, has the potential to increase viscosity; reduce retrogradation and syneresis; and improve stability, consequently influencing the mouthfeel of a system (Barsby, Donald and Frazier, 2001: Weber *et al.*, 2009).

During pasting, amylose molecules leach out from the granules into the continuous liquid phase, where xanthan gum is located (Achayuthakan and Suphantharika, 2008). Brennan, Tan, Kuri and Tudorica (2004) hypothesised that the increase in the peak viscosity is due to the competition for available water between the starch and xanthan gum during pasting. Mandala and Bayas (2004)

reported that the addition of xanthan gum to starch results in the increased swelling of the starch granules.

The amylose and xanthan gum can interact via hydrogen bonds to form a network (Achayuthakan and Suphantharika, 2008: Weber et al., 2009). Furthermore, Maphalla and Emmambux (2015) found that the combination of xanthan gum with stearic acid in maize starch, at low concentration, resulted in an increase in the paste viscosity of starch.

2.3 Concluding remarks

- Liquid whey can be used to produce a fermented beverage. However, whey beverage lacks consumer appeal due to a poor mouthfeel. Consumers prefer whey beverages to have the same consistency as milk. A beverage should be thick enough to have an appealing mouthfeel and remain pourable.
- Native starch has the capacity to thicken food but does not have the desired stability at low pH, high temperatures and high pressure processing conditions. Chemically modified starches are readily used in industry, however consumers prefer the use of clean-label ingredients.
- Amylose-lipid complexes can be considered as a clean-label ingredient produced from pasting maize starch and HAMS. The starches can be used seperatly or in combination with xanthan gum to produce a high viscosity paste.
- The amylose-lipid complex changes the rheological properties of native starch and does not result in gelation of starch. However no known literature documents the rheological functionality of amylose-lipid complexes in a solution.

3 HYPOTHESIS AND OBJECTIVES

3.1 Hypothesis

3.1.1 High amylose maize starch (HAMS) treated with stearic acid and xanthan gum will have an increased paste viscosity. Ocloo, Minnaar and Emmambux (2013) have reported the formation of amylose-lipid complexes in HAMS pasted with stearic acid. D'Silva *et al.* (2011) and Wokadala *et al.* (2012) attributed the continuous increase in the pasting viscosity to the formation of amylose-lipid complexes. Maphalla and Emmambux (2015) have shown that the incorporation of xanthan gum to normal maize, wheat and teff starch treated with stearic acid, resulted in an increased pasting viscosity. Xanthan gum presumably interacts with the free amylose and amylose-lipid complexes through hydrogen bonding.

The combination of xanthan gum and stearic acid in HAMS will prevent gel formation on cooling. Maphalla and Emmambux (2015) have reported that the formation of amylose-lipid complexes in maize starch pasted with stearic acid, resulted in no gel formation. The amylose-lipid complex is suggested to prevent junction zone formations of amylose molecules (Blazek and Copeland, 2009) resulting in the formation of a paste rather than a gel at room temperature.

3.1.2. A fermented whey beverage with xanthan gum and stearic acid modified starch will have an increased viscosity compared to a whey beverage without starch. The addition of starch, can increase the viscosity and prevent phase separation (Lal, O'Connor and Eyres, 2006). Gallardo-Escamilla *et al.* (2007) found that xanthan gum did not significantly increase the perceived thickness of a whey beverage, which could be attributed to xanthan gum's characteristic of high zero shear viscosity and low viscosity at high shear. It is assumed that a combination of xanthan gum and starch will create an increase in viscosity (Shi and BeMiller, 2002).

Starch modified with stearic acid will be more stable over time than modified maize starch without stearic acid in a fermented whey beverage. The amylose-lipid complex has a compact physical conformation. The inability to form a gel could result in low shear values as little to no apparent starch network is formed. It has been proven that the amylose-lipid complex is resistant to hydrolysis by amylase (Putseys, Derde, Lamberts, Östman, BJOrck and Delcour, 2009: Gelders, Duyck, Goesaert and Delcour, 2005).

3.2 Objective

3.2.1 To determine the effect of xanthan gum and stearic acid addition, alone and in combination, on the pasting and textural properties of maize and HAMS, in the aim of producing a high viscosity starch.

3.2.2 To determine the rheological properties of starch modified with xanthan gum and stearic acid, used alone and in combination, on the fermentation and quality of the fermented whey beverage, with the aim of producing a stable beverage containing clean-label ingredients.

4 MATERIAL AND METHODS

4.1 Experimental design

Figure 4.1 shows the experimental design of the research. This study was conducted in two parts. The first part entailed the pasting of maize and HAMS with xanthan gum and stearic acid, alone and in combination, to determine the pasting and texturural properties. Furthermore, the effect of xanthan gum on amylose-lipid complexes in both maize and HAMS was observed and evaluated through their respective thermal properties. The second part entailed the formulation, manufacture and evaluation of a whey beverage with the addition of the modified starch. The flow properties of the beverages were determined in order to evaluate the thickening capabilities of the modified starch. The pH, percentage lactic acid and water activity (a_w) of the beverages were also determined.

4.2 Materials

Commercial yellow maize starch was obtained from Tongaat Hullett (Kliprivier South Africa). High amylose maize starch (Hylon VII) was obtained from National Starch Food Innovation Ingredion (Pty) Ltd (Gauteng, South Africa) (refer to Appendix p88). Whey powder was obtained from Clover (Pty) Ltd (Heilbron, South Africa) (Appendix p83). Stearic acid (approx. 97% GC grade) was obtained from Merck (Pty) Ltd (Modderfontein, South Africa). Xanthan gum was obtained from Chemimpo (Pty) Ltd (Randburg, South Africa).

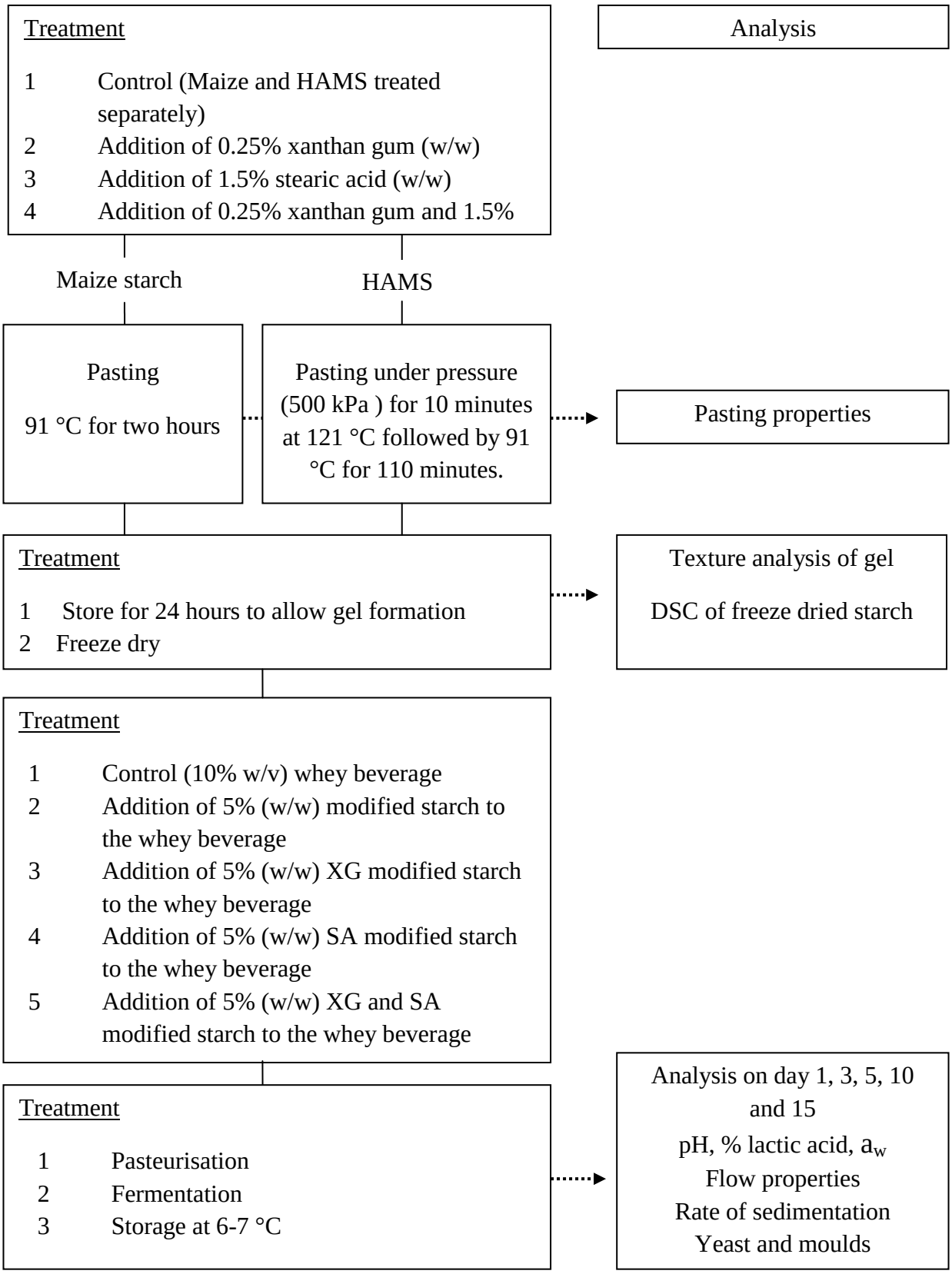

Figure 4.1: Experimental design of the research

SA, XG, HAMS and DSC denote stearic acid, xanthan gum, high amylose maize starch and differential scanning calorimetry respectively.

4.3 Methods

4.3.1 Incorporation of stearic acid

The stearic acid was incorporated into the maize starch and HAMS according to the method used by Maphalla and Emmambux (2015) at 1.5% (w/w) of the starch. The stearic acid was dissolved in absolute ethanol. The starches were added to the ethanol solution at a ratio of 1:3 (starch:absolute ethanol), and mixed. The method was modified by allowing the ethanol to evaporate over 48 hours in a fume cupboard at 25-27 °C. In upscaling, 1.5% (w/w) stearic acid was directly incorporated into the starch before pasting.

4.3.2 Pasting maize starch with the Rheometer

The rheometer (Physica MCR 101 with Rheoplus software®, Anton Paar, Ostfildern, Germany) was used for pasting and recording the pasting properties. The 10% (w/w) starch, gum and lipid mixtures were suspended in distilled water to make a total volume of 15 ml as shown in Table 4.1, similar to the method used by Maphalla and Emmambux (2015).

Table 4.1: Formulation of maize starch and high amylose maize starch with xanthan gum and stearic acid prior to pasting

Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)
Maize starch and/or HAMS	0.00	0.00
	0.00	0.25
	1.50	0.00
	1.50	0.25

The pasting cycle began with an initial stirring of 960 rpm at 50 °C for 30 seconds, followed by 160 rpm for the duration of the pasting cycle. The temperature was increased from 50 °C to 90 °C at a rate of 5.5 °C/min and kept at 90 °C for two hours to determine the pasting properties. The pasted starch was immediately removed after pasting and either frozen in liquid nitrogen for thermal property analysis or poured into cylindrical plastic moulds for texture measurements.

4.3.3 Pasting HAMS with the Rheometer

The pasting conditions were not the same for the two starch types, as a higher temperature (120 ºC) and pressure are required for the gelatinisation of the HAMS than that of maize starch (Case *et al*., 1998). The HAMS was pasted using the rheometer (Physica MCR 101 Rheometer with Rheoplus software®, Anton Paar, Ostfildern, Germany). The HAMS was pasted under 500 kPa pressure, at initial stirring of 960 rpm for 10 seconds at 50 °C, followed by a heat of 120 °C at 10 °C/min. The temperature was held at 120 °C for 10 minutes at 160 rpm, where after the paste was cooled to 90 °C and held for a further 90 minutes. Lastly the temperature was brought down to 50 °C at 160 rpm. The pasted starches were immediately removed after pasting and either frozen in liquid nitrogen for thermal property analysis or poured into moulds for texture measurements. The method was adapted from Ocloo *et al.* (2016). The method was adapted with the sample preparation. Table 4.1 shows the combination of starch, gum and lipids.

4.3.4 Pasting maize starch with the Reactor

In the upscaled production, the maize starch was pasted with a reactor vessel, IKA® LR 1000 (Staufen, Germany). The formulation as set out in Table 4.1 for the modification of the starch with stearic acid and xanthan gum alone and in combination was used. The reactor could paste an approximated volume of 1.75 *l*. The starch was pasted at max speed at 91 °C for two hours. The temperature was brought down to 50 °C and the content was immediately put in the freezer (-20 °C). The pasted starch samples were then freeze dried.

4.3.5 Formulation of the whey beverages

A beverage was prepared using a 10% (w/w) mixture of whey powder and 5% (w/w) modified starch added to filtered water. The control beverage consisted of only 10% (w/w) whey powder added to water. The amount of water was kept the same for all five samples to which the whey and modified starches were added. The four different modified starches (M, MSA, MXG, and MSX) where incorporated individually to separate mixtures that were used in the preparation of the beverages. The modified maize starches that were used are set out in Table 4.1. The beverages where homogenised using the ultra-turrax, T25 Janke and Kunkel IKA (Staufen, Germany) at approximate 1800 rpm.

4.3.6 Pasteurising and fermenting the whey beverages using the Reactor

The beverages where pasteurised using the reactor, IKA® LR 1000 (Staufen, Germany). The temperature was brought to 86 ± 1 °C and held for 30 minutes while continuously stirring at low speeds, followed by cooling at 30 °C. The beverage was then transferred to bottles for fermentation. A starter culture of lactic acid bacteria, YF-L811 (Chr, Hansen Lake Foods International, Sandton, South Africa) (Appendix p84-87) was used for fermentation. The whey beverages were inoculated with the starter cultures at 0.2% (w/v) and fermented at 42 °C for 24 hours or until target pH was reached at approximately 4.5. When the target pH was reached, the sample was mixed and allowed to rest for the first set period to commence (24 hours) at 6-7 °C.

4.4 Analyses

4.4.1 Starch gel texture

The textural properties of the pasted starches were determined according to Maphalla and Emmambux (2015). The pasted starches modified with and without xanthan gum and stearic acid (alone and in combination), were transferred into moulds (16 mm height by 37 mm diameter) directly after the pasting. The pasted starches were kept in the moulds for 24 hours at 23 ± 1 °C. The gel firmness and stickiness was analysed using the EZ-test analyser EZ-L, Shimadzu (Tokyo, Japan) with a P/20p cylinder probe (20 mm diameter). The plunger penetrated 5 mm into the starch paste, recording the maximum force and the minimum force is recorded upon retraction. This was conducted at a pre-load force of 0.02 N, pre-test speed 50 mm/min and test speed of 10 mm/min.

4.4.2 Thermal properties

The thermal properties of the freeze dried starch paste modified with and without the stearic acid and xanthan gum were determined according to Wokadala *et al.* (2012) by using a high pressure differential scanning calorimetry system, HP DSC827^{e}, Mettler Toledo (Greifensee, Switzerland). The pasted starch samples were directly transferred into liquid nitrogen after pasting and freeze dried at -40 °C for five days or until no ice crystals formed on the bottom of the pan. The freeze dried starches (7 mg) were weighed out and mixed with distilled water (21 mg). The sample was equilibrated for four hours at 23 ±1 °C prior to DSC analysis in

individually sealed pans. The analysis was conducted over a temperature range of 25 to 170 °C under 4 MPa pressure (N_2 at a rate of 10 °C/min.). Indium (T_p = 156.6 °C, 28.45 Jg^{-1}) was the standard used to calibrate the DSC and an empty pan as a reference.

4.4.3 Flow properties

The flow properties of the beverages with the different modified starches were determined according to Maphalla and Emmambux (2015) with modifications. Firstly, the flow properties of the fermented whey beverages were measured using a Vane and Cup method with a Physica MCR 101 Rheometer with Rheoplus software®, Anton Paar (Ostfildren, Germany) to limit structural damage to the matrix in the beverage. Secondly, the flow properties of beverages were measured at 5 °C to limit protein matrix disruption and to simulate viscosity at low temperatures. The power law model ($\sigma = K.\dot{\gamma}^n$) was used, where K is the consistency coefficient ($Pa.s^n$) and n is the flow behaviour index (dimensionless), to describe the flow properties. The hysteresis area was calculated by subtracting areas under the upward data point curve and under the downward data point curve during increasing and decreasing shear rates (Tárrega and Costell, 2006).

4.4.4 pH

The pH was measured using a Hanna pH 211 Microprocessor pH meter calibrated at pH 4.0 and 7.0. Aliquots of each whey beverage sample was brought to 25 ± 1 °C and the pH was measured at days 0, 1, 3, 5, 10 and 15.

4.4.5 % Lactic acid

Aliquots of the whey beverages were taken separately from each beverage after the content was mixed according to IDF Standards 150 (1991). The whey beverage was mixed, which ensured a homogenous mixture and true representation of lactic acid determination. A suspension of 10 g fermented whey was drawn and added to 10 ml water. The solution was brought to 25 °C. The sample was titrated with 0,1 M NaOH solution to an end point of pH 8,30 ± 0.01. The pH was read using a Hanna pH 211 Microprocessor pH meter. The following calculation was used to calculate the % lactic acid content. Measurements were made on days 1, 3, 5, 10 and 15.

$$\% \text{ Lactic acid} = \frac{(V \times 0.9)}{m}$$

V: volume 0.1M NaOH titrated

m: mass of sample

4.4.6 Water activity

The water activity of the whey beverages was measured using a Pawkit (Aqualab, Decagon Devices, Inc, Pullman, USA) water activity meter. The samples were brought to 25 ± 1 °C and measured on days 1, 3, 5, 10 and 15.

4.4.7 Rate of sedimentation

Measuring cylinders (50 ml) were filled separately with the fermented whey beverages and sealed with plastic wrap, 24 hours after the beverages were first stored at 6 ± 1.0 °C. This allowed for the first set. The levels of sedimentation were recorded hourly for the first five hours then daily for 15 days. The level of sedimentation was read at the interface of the clear supernatant and cloudy precipitate.

4.4.8 Yeasts and moulds

A total yeast and mould plate count was conducted using a series dilution of the fermented whey beverages on day 15 on 3M rapid Y & M petri-film. Aliquots (1 ml) of each beverage was taken separately and individually transferred to enriched serum broth. Aliquots (0.1 ml) were taken of the inoculated serum broth and aseptically plated on the petri-film. The petri-films were incubated as per instructions and measurements were taken 24 and 48 hours after incubation.

4.4.9 Statistical analysis

Firstly, the maize starch and HAMS were analysed separately and a factorial analysis of variance (ANOVA) was used to determine the difference due to added xanthan gum and stearic acid alone and in combination. The means were compared using Fisher's least significant difference test (LSD) at 5% level of significance using Statistica® version 7 software, StatSoft®, Inc (Tulsa, Oklahoma). The type of starch, concentration of stearic acid and xanthan gum were independent variables of the experiment. These experiments were repeated three times.

The second part entailed the incorporation of the modified starch to the whey beverage at 5 % w/w to the whey. The individual beverages were analysed using a repeated measures ANOVA to determine the differences due to the added modified starch treated with or without stearic acid and xanthan gum, used alone and in combination. The means were compared using LSD at 5%

level of significance also using Statistica® version 7 software, StatSoft®, Inc. The type of modified starch treated with and without xanthan gum and/or stearic acid as well as the day (1 to 15) were the independent variables of this part of the experiment. These experiments were repeated three times.

5 RESULTS

5.1 Pasting properties of maize starch and high amylose maize starch pasted with added stearic acid and xanthan gum alone and in combination

Table 5.1 and Figures 5.1-5.2 show the viscosity changes during the pasting of maize starch and HAMS, pasted with and without stearic acid and xanthan gum, alone and in combination. The pasting of maize starch (M) showed the formation of two viscosity peaks (Fig 5.1) with a highest recorded viscosity of about 2041 mPa.s at 91 ºC (Table 5.1), followed by a break down in the viscosity beyond 80 minutes. On cooling (90-50 ºC) the viscosity of the pasted maize starch increased (Fig 5.1) with a final viscosity of about 2342 mPa.s (Table 5.1). The pasting of HAMS showed the formation of three viscosity peaks (Fig 5.2), with a highest recorded viscosity of about 356 mPa.s at 91 ºC (Table 5.1), followed by a break down in the viscosity beyond 100 minutes. The viscosity of the pasted high amylose maize starch increased on cooling to about 280 mPa.s (Table 5.1).

Figure 5.1 shows that maize starch pasted with xanthan gum had two viscosity peaks, which were significantly higher ($p < 0.05$) when compared to the maize starch with no additives (Table 5.1). The highest recorded viscosity for the pasted maize starch with xanthan gum, measured at 91 ºC (2422 mPa.s), was significantly higher ($p < 0.05$) than pasted maize starch, followed by a break down in the viscosity beyond 80 minutes. The viscosity of the maize starch pasted with xanthan gum increased on cooling (about 2473 mPa.s), whilst not being significantly different ($p > 0.05$) to the final viscosity of the pasted maize starch without additives.

Figure 5.2 shows three formations of viscosity peaks for HAMS pasted with xanthan gum (HAMS-XG). The peak viscosity for the pasted HAMS-XG was significantly higher ($p < 0.05$) (~164 mPa.s) than the pasted high amylose maize starch without additives. However, the highest recorded viscosity at 91 ºC (~392 mPa.s) of the pasted starch with xanthan gum was not significantly different ($p > 0.05$) to the pasted high amylose maize starch without additives. The viscosity of the HAMS-XG

Table 5.1: Effects of stearic acid and xanthan gum alone and in combination, on the pasting properties of normal maize starch and high amylose starch

Sample composition			Peak Viscosity (mPa.s)	Highest viscosity (mPa.s) at 91 °C	Final viscosity (mPa.s) at 50 °C
Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)			
Maize	0	0	2327^{c} ±35	2041^{a} ±5	2342^{a} ±66
	0	0.25	2462^{d} ±37	2422^{b} ±75	2473^{ab} ±100
	1.5	0	2131^{a} ±73	2770^{c} ±180	2959^{c} ±167
	1.5	0.25	2251^{b} ±22	2818^{c} ±124	2536^{b} ±175
High amylose maize starch	0	0	140^{ab} ±7	356^{ab} ±19	280^{a} ±19
	0	0.25	164^{c} ±2	392^{b} ±16	238^{a} ±17
	1.5	0	131^{a} ±9	345^{a} ±24	333^{b} ±35
	1.5	0.25	149^{bc} ±20	433^{c} ±46	486^{c} ±65

Statistics were run separately for the two types of starches. Means with different superscripts differ significantly ($p \leq 0.05$).

Pasting for the normal maize starch treated sample was conducted under atmospheric pressure from 91 °C, for two hours at 160 rpm.

Pasting for high amylose maize starch samples were conducted under pressure at 120 °C for 10 minutes and 91 °C for 1,5 hours at 160 rpm.

Data derived from figures 5.1-5.2.

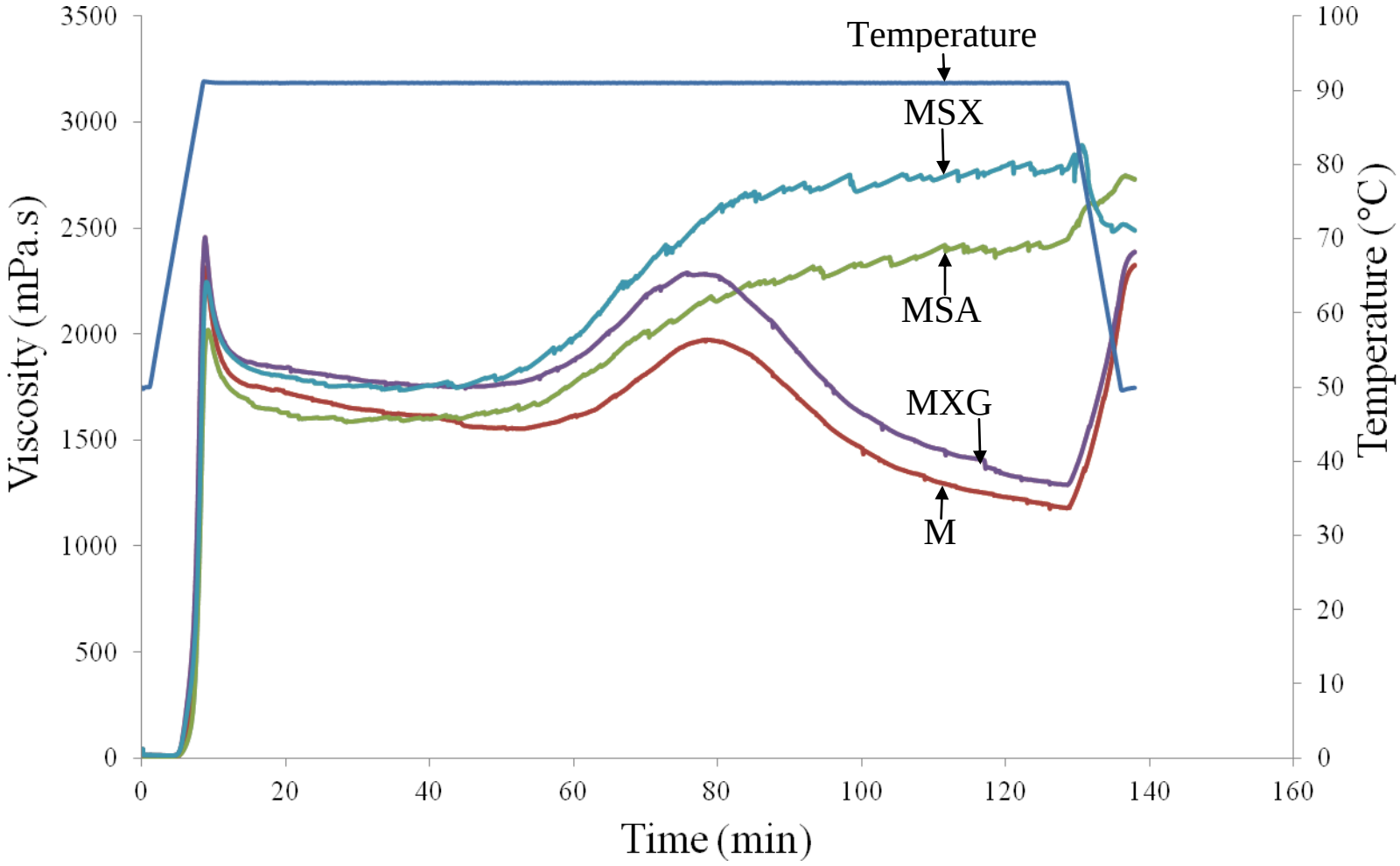

Figure 5.1: Effects of stearic acid and xanthan gum alone and in combination, on the pasting properties of normal maize starch.

M – Maize starch, MS – Maize starch with 1.5% (w/w) stearic acid, MX – Maize starch with 0.25% (w/w) xanthan gum and MSX – Maize starch with 1.5% (w/w) stearic acid and xanthan gum 0.25% (w/w).

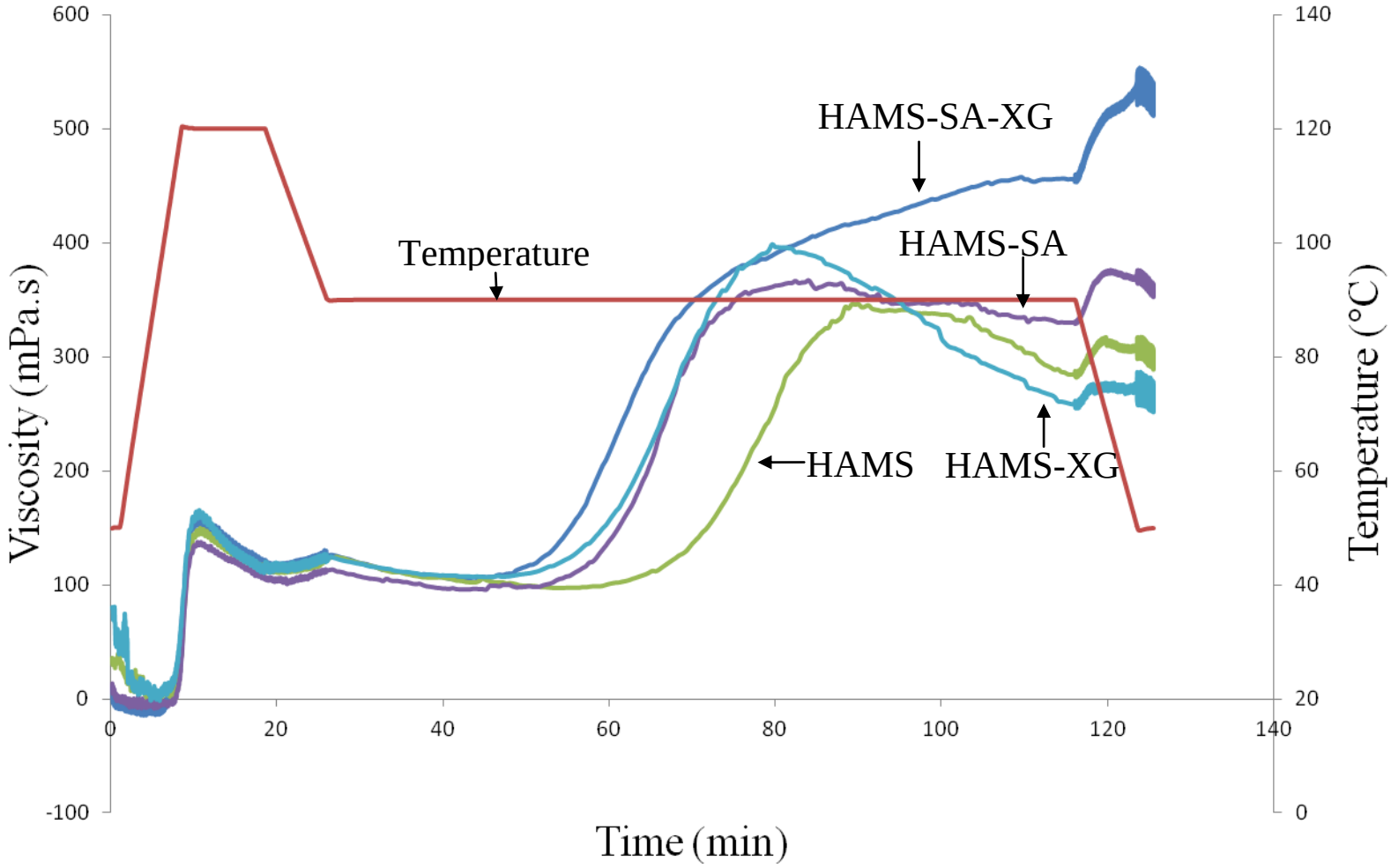

Figure 5.2: Effects of stearic acid and xanthan gum, alone and in combination, on the pasting properties of high amylose maize starch

HAMS – High amylose maize starch, HAMS-SA – High amylose maize starch with 1.5% (w/w) stearic acid, HAMS-XG – High amylose maize starch with 0.25% (w/w) xanthan gum and HAMS-SA-XG – High amylose maize starch with both 1.5% (w/w) stearic acid and xanthan gum 0.25% (w/w)

increased earlier at about 55 minutes relative to the high amylose starch pasted alone, which started at about 70 minutes. At approximately 80 minutes there was a decrease in the viscosity followed by an increase in viscosity on cooling. The final viscosity of HAMS–XG (238 mPa.s), however, being lower, was not significantly different ($p > 0.05$) to the final viscosity of the pasted HAMS (Table 5.1).

Figure 5.1 shows the formation of one viscosity peak for maize starch pasted with stearic acid (MSA). The peak viscosity for the MSA decreased significantly ($p < 0.05$) relative to the MXG. There was an increase in viscosity at about 45 minutes, which continued until about 110 minutes following a plateau in the viscosity. The high viscosity recorded at 91 °C for the MSA (about 2770 mPa.s) was significantly higher ($p < 0.05$) than the maize starch pasted alone and with xanthan gum (Table 5.1). On cooling there was a marginal increase in viscosity. The final viscosity for MSA (2959 mPa.s) was significantly higher ($p < 0.05$) than the other maize starch treatments (Table 5.1 & Fig 5.1).

Figure 5.2 shows the formation of two viscosity peaks for HAMS pasted with stearic acid (HAMS-SA). The peak viscosity and highest viscosity at 91 °C (345 mPa s) of the sample was not significantly different ($p > 0.05$) in relation to the HAMS (Table 5.1). The final viscosity (about 333 mPa s), however, was significantly ($p < 0.05$) higher when compared to the pasted HAMS and HAMS-XG (Table 5.1).

Figure 5.1 shows that maize starch pasted with stearic acid and xanthan gum (MSX) has a similar trend as the MSA during the pasting period at 91 °C but the viscosity decreased on cooling. The peak viscosity of the pasted MSX decreased significantly ($p < 0.05$) in relation to the M and MXG. The highest viscosity at 91 °C (about 2818 mPa.s) of the pasted maize starch with added stearic acid and xanthan gum was significantly higher ($p < 0.05$) than the M and MXG. The final viscosity of the MSX (about 2536 mPa.s) was significantly higher ($p < 0.05$) than the maize starch pasted without additives (Table 5.1).

Figure 5.2 shows that the viscosity of HAMS with stearic acid and xanthan gum (HAMS-SA-XG) continued to increase from about 55 minutes until about 115 minutes. The HAMS-SA-XG had the highest viscosity at 91 °C (about 433 mPa.s) and at 50 °C (about 486 mPa.s), which was significantly higher ($p < 0.05$) in relation to the other HAMS treatments (Table 5.1).

5.2 Textural properties of maize starch and high amylose maize starch with added stearic acid and xanthan gum alone and in combination

Table 5.2 shows the textural properties of maize starch and HAMS pasted with and without stearic acid and xanthan gum (separately and in combination) after storage for 24 hours at about 23 ± 1 ºC. Maize starch and HAMS, pasted with and without xanthan gum, were observed to form an opaque and rigid gel. The maize starch and HAMS with stearic acid were observed to have a paste-like appearance. These viscous pastes were pliable and malleable, unlike rigid and elastic starch gels.

Table 5.2 shows that the maize starch gel had a high maximum force of 2.41 N and that the gel of the HAMS had a maximum force of 1.65 N. The gel strength of MXG (2.25 N) was not significantly different ($p > 0.05$) to the maize starch gel. Nor was the penetration strength of the high amylose MXG (1.47 N) significantly different ($p < 0.05$) to the high amylose maize starch gel.

The penetration strength of the MSA (0.40 N) was significantly lower ($p < 0.05$) than the maize starch pasted alone (Table 5.2). The penetration strength of the HAMS-SA acid (1.53 N) was not significantly different ($p > 0.05$) to either the gels of HAMS and HAMS-XG. It was observed, however, that maximum force of the HAMS-SA was higher than the HAMS-XG. The penetration strength of the MSX (about 0.39 N) was significantly lower ($p < 0.05$) when compared to the maize starch gels without stearic acid. The penetration strength of HAMS-SA-XG (about 0.77 N) was significantly lower ($p < 0.05$) when compared to the high amylose maize starch gel.

Table 5.2: Effects of stearic acid and xanthan gum, used alone and in combination, on the texture of normal maize starch and high amylose maize pasted and stored for 24 hours at ambient conditions (~23 °C)

Sample composition			Maximum Force (N)	Minimum Force (N)	Visual appearance*
Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)			
Maize	0	0	2.41^{b} ±0.55	-0.13^{a} ±0.03	Gel
	0	0.25	2.52^{b} ±0.44	-0.14^{a} ±0.05	Gel
	1.5	0	0.40^{a} ±0.06	-0.21^{a} ±0.03	Paste
	1.5	0.25	0.39^{a} ±0.08	-0.23^{a} ±0.05	Paste
High amylose maize starch	0	0	1.65^{b} ±0.53	-0.12^{a} ±0.03	Gel
	0	0.25	1.47^{ab} ±0.15	-0.22^{a} ±0.04	Soft-Gel
	1.5	0	1.53^{ab} ±0.21	-0.22^{a} ±0.04	Paste
	1.5	0.25	0.77^{a} ±0.22	-0.22^{a} ±0.08	Paste

Statistics were run separately for the two types of starches. Means with different superscripts differ significantly ($p \leq 0.05$).

Different superscripts indicating significant differences ($p \leq 0.05$).
*Visual appearance of the pasted starch samples left for 24 hours at ~23 °C. A “starch gel” is considered as a slab that cannot be poured out freely or be spread on a surface. A “starch paste” is considered as being pourable, pliable and malleable.

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5.3 Thermal properties of maize starch and high amylose maize starch pasted with added stearic acid and xanthan gum alone and in combination

Figure 5.3-5.4 and Table 5.3-5.4 show the thermal properties of pasted maize starch and HAMS with and without stearic acid and xanthan gum, alone and in combination. Data on the endothermic peaks in Table 5.3 and 5.4 were derived from Figure 5.3 and 5.4 respectively. Type I, IIa and Iib amylose complexes have melting temperature ranges of ~98 °C, ~106 °C and ~120 °C respectively (Biliaderis and Galloway, 1989). The endotherm in the temperature range of ~137-154 °C is type III resistant starch (Shamai, Bianco-Peled and Shimoni, 2003).

The pasted maize starch had one endotherm at an approximate temperature range of 98.0-109.9 °C with a ΔH of ~0.7 Jg^{-1} (Fig 5.3 and Table 5.3 peak B). The pasted HAMS had three endotherms between ~103-112 °C, ~115-120 °C and ~137.6-151.4 °C with respective ΔH of ~0.8 Jg^{-1}, ~0.01 Jg^{-1} and ~0.2 Jg^{-1} (Fig 5.4 and Table 5.4 peak B, C and E).

The MXG had an endotherm in the temperature range of ~97.2-105.1 °C with ΔH of ~0.6 Jg^{-1} (Fig 5.3 and Table 5.3 peak B). The HAMS-XG had two visible endotherms at ~100.8-110.0 °C and ~140.3-150.6 °C with ΔH of ~0.8 and ~0.1 respectively (Fig 5.41 and Table 5.4 peaks B and E).

The MSA had four endotherms along the temperature range of ~62.3-69.3 °C, ~97.2-105.1 °C, ~107.0-114.1 °C and ~116.0-121.5 °C with a respective ΔH of ~0.3 Jg^{-1}, ~0.4 Jg^{-1}, ~1.04 Jg^{-1} and ~0.08 Jg^{-1} (Fig 5.3 and Table 5.3 peak A, B and C). The HAMS-SA had four visible endotherms with a temperature range of ~66.5-70.8 °C, ~103-112.5 °C, ~115.0-117.2 °C and ~142.5-152 °C with a respective ΔH of ~0.6 Jg^{-1}, ~1.3 Jg^{-1}, ~0.01 Jg^{-1} and 0.2 Jg^{-1} (Fig 5.4 and Table 5.4 peak A, B, C and E).

The pasted MSX sample had four endotherms, all of which correspond closely to the same temperature ranges as the MSA sample (Fig 5.3 and Table 5.3 peak A, B, C and D). However, the ΔH values for the maize starch pasted with stearic acid and xanthan gum versus the maize starch pasted with only stearic acid, were lower.

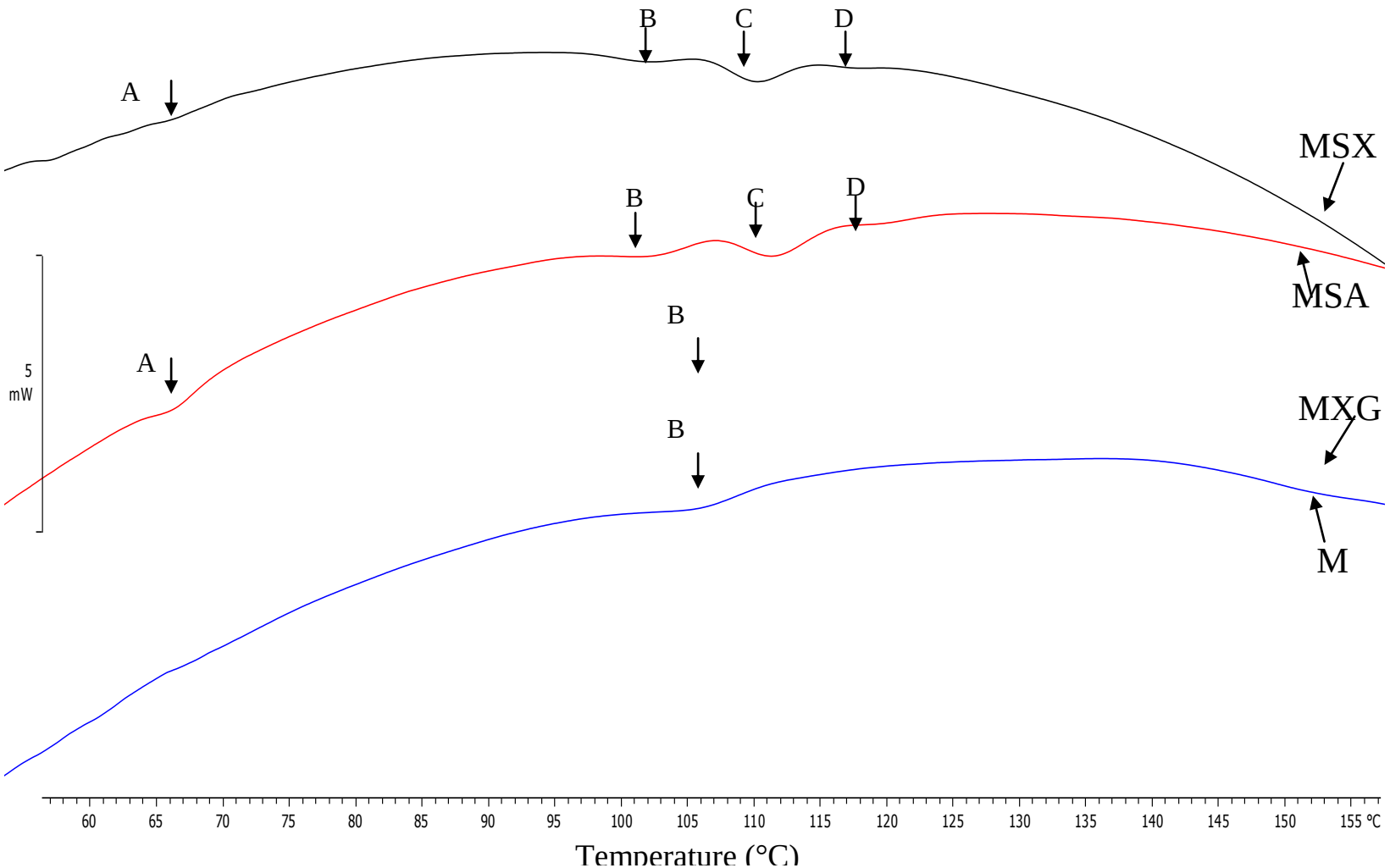

Figure 5.3: Effects of stearic acid and xanthan gum, alone and in combination, on the thermal properties of reconstituted normal maize starch after pasting. M – Maize starch, MSA – Maize starch with 1.5% (w/w) stearic acid, MXG – Maize starch with 0.25% (w/w) xanthan gum and MSX – Maize starch with both 1.5% (w/w) stearic acid and xanthan gum 0.25% (w/w). ABCD denote endothermal peaks as seen in Table 5.3.

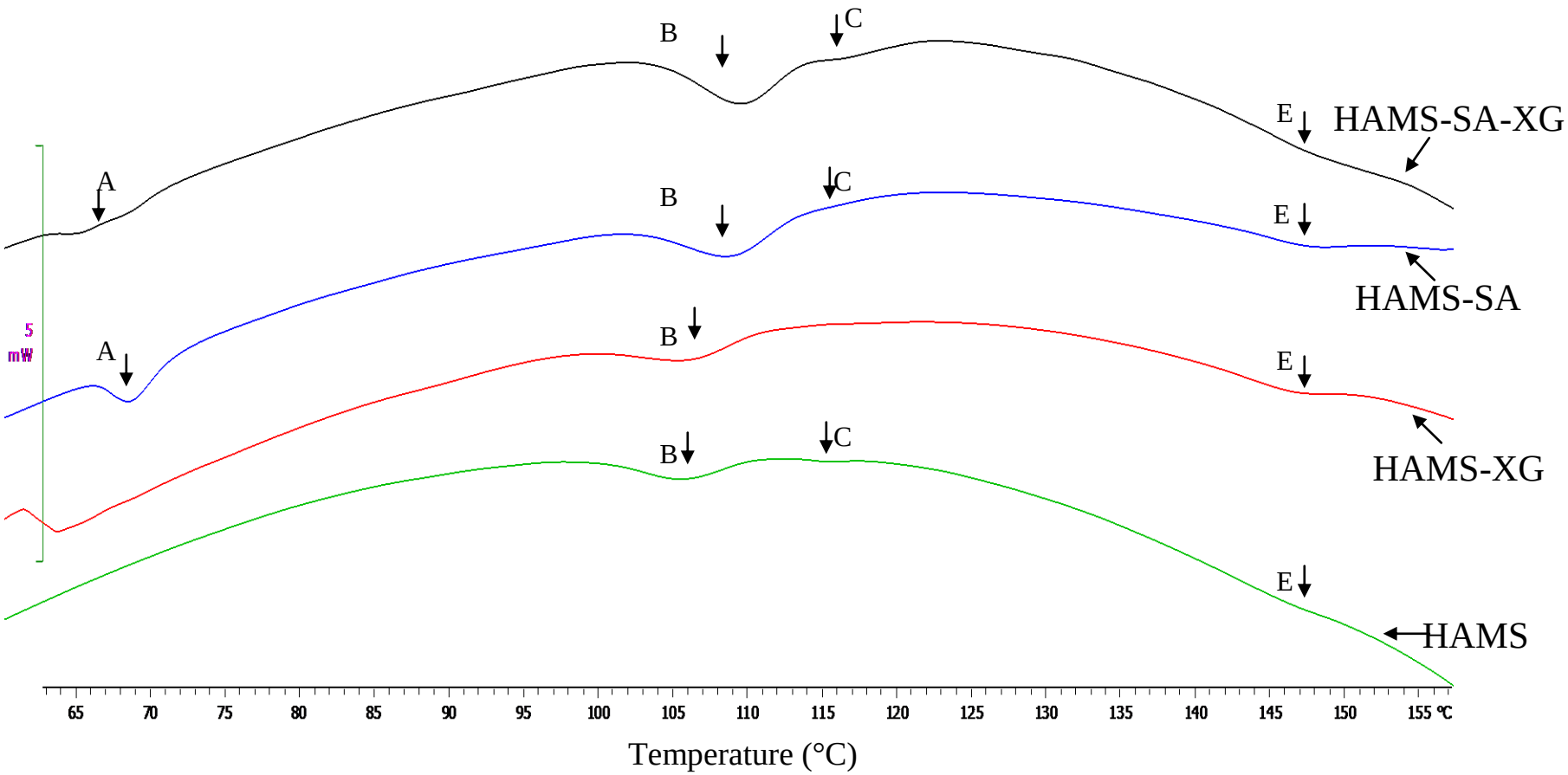

Figure 5.4 Effects of stearic acid and xanthan gum, alone and in combination, on the thermal properties of reconstituted high amylose maize starch after pasting.

HAMS – High amylose maize starch, HAMS-SA – High amylose maize starch with 1.5% (w/w) stearic acid, HAMS-XG – High amylose maize starch with 0.25% (w/w) xanthan gum and HAMS-SA-XA – High amylose maize starch with both 1.5% (w/w) stearic acid and xanthan gum 0.25% (w/w). ABCDE denote endothermal peaks as seen in Table 5.4.

Table 5.1: Thermal properties of maize starch with stearic acid and xanthan gum, used alone and in combination, two hours after pasting.

Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)	Endothermic peak A	
			Temperature range (°C)	ΔH (Jg^{-1})
Maize	0	0	ND	ND
	0	0.25	ND	ND
	1.5	0	62.3 - 69.3	0.3 ± 0.08
	1.5	0.25	62.6 - 69.9	0.1 ± 0.05
			Endothermic peak B	
Maize	0	0	98.0-109.9	0.7 ± 0.05
	0	0.25	98.4-108.9	0.6 ± 0.23
	1.5	0	97.2-105.1	0.4 ± 0.13
	1.5	0.25	97.7-104.4	0.2 ± 0.04
			Endothermic peak C	
Maize	0	0	ND	ND
	0	0.25	ND	ND
	1.5	0	107.0-114.1	1.04 ± 0.00
	1.5	0.25	106.4-113.2	0.77 ± 0.04
			Endothermic peak D	
Maize	0	0	ND	ND
	0	0.25	ND	ND
	1.5	0	116.0-121.5	0.08 ± 0.02
	1.5	0.25	114.9-119.9	0.03 ± 0.01

Mean and (±) Standard deviation.
Different superscripts indicating significant differences ($p \leq 0.05$).
ND – Not detected.

Data derived from figure 5.3.

Table 5.2: Thermal properties of high amylose maize starch with stearic acid and xanthan gum, used alone and in combination, two hours after pasting.

Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)	Endothermic peak A	
			Temperature range (°C)	ΔH (Jg^{-1})
High amylose maize starch	0	0	ND	ND
	0	0.25	ND	ND
	1.5	0	66.5-70.8	0.6 ± 0.08
	1.5	0.25	91.9-70.9	0.4 ± 0.08
			Endothermic peak B	
High amylose maize starch	0	0	99.8-109.5	0.8 ± 0.03
	0	0.25	100.8-110.0	0.8 ± 0.02
	1.5	0	103.0-112.5	1.3 ± 0.24
	1.5	0.25	104.0-113.3	1.8 ± 0.24
			Endothermic peak C	
High amylose maize starch	0	0	112.6-118.9	0.01 ± 0.01
	0	0.25	ND	ND
	1.5	0	115.0-117.2	0.01 ± 0.01
	1.5	0.25	114.2-120.6	0.04 0.01
			Endothermic peak E	
High amylose maize starch	0	0	137.6-151.4	0.2 ± 0.00
	0	0.25	140.3-150.6	0.1 ± 0.07
	1.5	0	142.5-152.0	0.2 ± 0.23
	1.5	0.25	141.0-154.0	0.2 ± 0.15

Mean and (±) Standard deviation.
Different superscripts indicating significant differences ($p \leq 0.05$)
ND – Not detected

Data derived from figure 5.4

The opposite was observed in the HAMS-SA-XG, where four endotherms were observed. These endotherms of the HAMS-SA-XG were in the same relative temperature ranges as high amylose maize starch pasted with only stearic acid but had higher ΔH for the endotherms at about 104-113 °C and ~114-120 °C.

5.4 The effects of maize starch modified with and without stearic acid and xanthan gum, alone and in combination, on the pH of fermented whey beverages

Table 5.5 shows the pH measurements of fermented whey with or without maize starch modified with stearic acid and xanthan gum, alone and in combination. The whey in solution, before fermentation, had a pH of about 6.5 (Table 4.1). The pH of the beverage after fermentation was in the range of 4.6 - 4.4 which was recorded as day 0. Following a 15 day period, all the beverages with and without additives showed a decrease in pH, with no significant difference ($p > 0.05$) between the beverages at any given day. The addition of maize starch modified with and without stearic acid and xanthan gum, alone and in combination, had no effect on the fermentation of the whey beverage.

5.5 The effects of maize starch modified with and without stearic acid and xanthan gum, alone and in combination, on the lactic acid content of fermented whey beverages

Table 5.6 shows the lactic acid content of fermented whey beverages with and without starch modified with stearic acid and xanthan gum, alone and in combination. Unfermented sweet whey in solution has an approximate content of 0.40 – 0.60% lactic acid. The lactic acid content of the fermented whey beverages was observed to have increased towards the end of the 15 day period. There were no significant differences ($p \geq 0.05$) between the different fermented whey beverages on any given day.

5.6 The effects of maize starch modified with and without stearic acid and xanthan gum, alone and in combination, on the water activity of fermented whey beverages

Table 5.7 shows the water activity (a_w) of fermented whey beverages with and without maize starch with stearic acid and xanthan gum, alone and in combination. The a_w of the fermented whey beverages with and without maize starch additives had no significant differences ($p > 0.05$) over the 15 day period.

Table 5.3: The effects of stearic acid and xanthan gum, used alone or in combination, with modified maize starch on the pH of fermented whey over a 15 day period

Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)	Day 1	Day 3	Day 5	Day 10	Day 15
No starch	0	0	4.01 ± 0.06	3.97 ± 0.06	3.92 ± 0.06	3.98 ± 0.05	3.98 ± 0.04
Modified maize starch	0	0	4.08 ± 0.13	3.93 ± 0.03	3.90 ± 0.01	3.82 ± 0.02	3.80 ± 0.01
	0	0.25	4.02 ± 0.11	3.92 ± 0.02	3.90 ± 0.01	3.79 ± 0.01	3.76 ± 0.03
	1.5	0	3.99 ± 0.11	3.91 ± 0.02	3.88 ± 0.01	3.82 ± 0.06	3.75 ± 0.02
	1.5	0.25	4.09 ± 0.14	3.95 ± 0.05	3.92 ± 0.03	3.80 ± 0.03	3.78 ± 0.06

Mean and (±) Standard deviation. Means with different superscripts differ significantly ($p \leq 0.05$).

Beverage with 5% w/v modified starch.

Table 5.6: The effects of stearic acid and xanthan gum, used alone or in combination, with modified maize starch on the % lactic acid per 100 g of fermented whey over a 15 day period

Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)	Day 1	Day 3	Day 5	Day 10	Day 15
No starch	0	0	0.76 ± 0.07	0.78 ± 0.07	0.73 ± 0.05	0.78 ± 0.05	0.83 ± 0.04
	0	0	0.65 ± 0.01	0.66 ± 0.00	0.66 ± 0.01	0.68 ± 0.02	0.70 ± 0.02
Modified maize starch	0	0.25	0.66 ± 0.03	0.67 ± 0.02	0.68 ± 0.02	0.67 ± 0.02	0.70 ± 0.02
	1.5	0	0.68 ± 0.01	0.68 ± 0.02	0.70 ± 0.02	0.70 ± 0.02	0.71 ± 0.05
	1.5	0.25	0.65 ± 0.02	0.69 ± 0.02	0.69 ± 0.04	0.72 ± 0.03	0.73 ± 0.01

Mean and (±) Standard deviation. Means with different superscripts differ significantly ($p \leq 0.05$).

Beverage with 5% w/v modified starch**.**

Table 5.7: The effects of stearic acid and xanthan gum, used alone or in combination, with modified maize starch on the water activity (a_w) of fermented whey over a 15 day period

Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)	Day 1	Day 3	Day 5	Day 10	Day 15
No starch	0	0	0.98 ±0.02	0.97 ±0.01	0.99 ±0.02	1.00 ±0.03	1.00 ±0.02
	0	0	0.99 ±0.01	0.98 ±0.01	0.98 ±0.02	0.99 ±0.01	0.98 ±0.02
Modified maize starch	0	0.25	0.99 ±0.01	1.00 ±0.01	1.00 ±0.01	1.00 ±0.02	1.00 ±0.01
	1.5	0	0.99 ±0.01	0.99 ±0.01	1.00 ±0.01	0.98 ±0.01	0.99 ±0.02
	1.5	0.25	0.99 ±0.01	0.99 ±0.01	1.00 ±0.02	1.00 ±0.01	1.00 ±0.02

Mean and (±) Standard deviation

Beverage with 5% w/v modified starch

5.7 The effects of maize starch modified with and without stearic acid and xanthan gum, alone and in combination, on the zero shear viscosity of a fermented whey beverage

Figure 5.5 shows the zero shear viscosity (viscosity at 0.001 to about 1 s^{-1}) of fermented whey beverages with and without starch modified with and without stearic acid and xanthan gum, alone and in combination, at 5 ºC over a 15 day period. The viscosity at rest, for the different fermented whey beverages, was observed to decrease towards the end of the 15 day period at different rates. The control beverage, without starch, had the lowest viscosity preceded by the beverages with added maize starch and stearic acids (MSA), maize starch stearic acid and xanthan gum (MSX), maize starch and xanthan gum (MXG), and maize starch. With the latter having the highest viscosity at rest.

The beverage with added maize starch had a significantly higher viscosity ($p < 0.05$) at rest, when compared to the beverage without starch (control). The beverage with starch, however, had a significant decrease in viscosity ($p < 0.05$) from day one (about 867 mPa.s) to day 15 (about 216 mPa.s), whilst remaining far higher in viscosity at rest, relative to the control (Table 7.1). The viscosity at rest for the fermented whey beverage with MXG was not significantly different ($p > 0.05$) to the fermented whey beverage with added starch (Table 9.1). The viscosity of the beverage with MXG decreased drastically from day one (about 978 mPa.s) to day 15 (about 215 mPa.s).

The viscosity at rest for the beverage with MSA was significantly higher ($p < 0.05$) relative to the control, yet significantly lower ($p < 0.05$) than both the beverages with starch and MXG. The viscosity at rest, for the beverage with MSA, remained relatively stable over the 15 day period with no significant differences ($p > 0.05$) between the viscosity at day one (about 86 mPa.s) and day 15 (about 24 mPa.s) (Table 9.1). The viscosity at rest for the beverage with added MSX was not significantly different ($p > 0.05$) from the beverage with added MSA. The viscosity at rest for the beverages with MSA and/or MSX remained relatively stable over the 15 day period with a slight decrease in viscosity between day one (about 293 mPa.s) and day 15 (about 30 mPa.s), when compared to the beveragemade with modified starch.

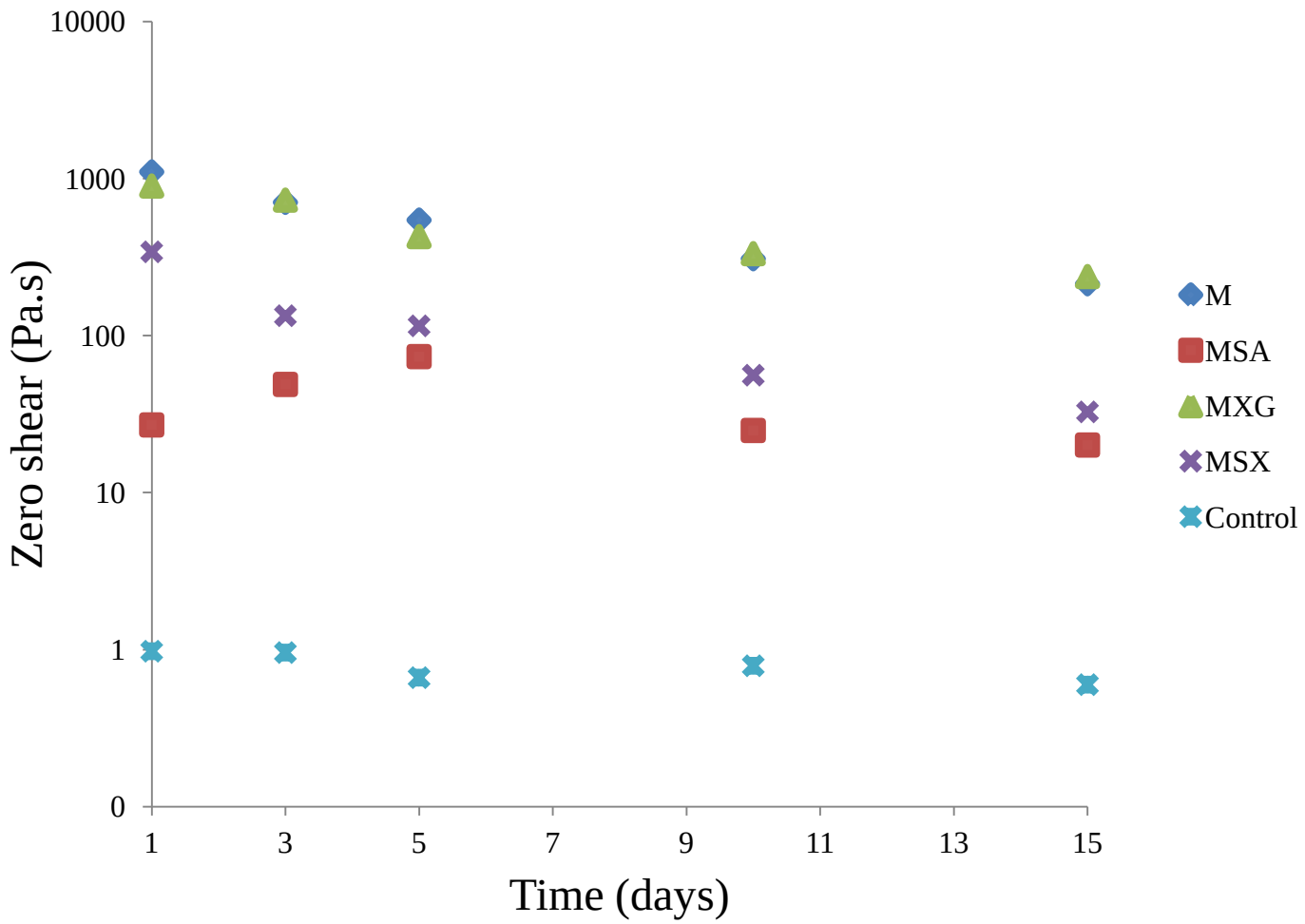

Figure 5.5: The effects of stearic acid and xanthan gum, used alone or in combination, with maize starch on the zero shear viscosity of fermented whey over a 15 day period measured at 5 °C.

Beverage with 5% w/v: M – Maize starch, MSA – Maize starch with stearic acid, MXG – Maize starch with xanthan gum, MSX – Maize starch with stearic acid and xanthan gum. Graph derived from Table 9.1 appendix p82 .

The viscosity at rest, of all the beverages with added starch, with and without stearic acid and xanthan gum, alone and in combination, at day 15, had no significant differences ($p > 0.05$) between each other, whilst all remained significantly higher ($p \leq 0.05$) than the control beverage (Fig 5.5 and Table 9.1)

5.8 The effects of maize starch modified with and without stearic acid and xanthan gum, alone and in combination, on the flow properties of a fermented whey beverage

The flow properties of a beverage can be described using the power law model: $\sigma = K\gamma^n$ where the *K*-value is the consistency coefficient (Pa.s^n), which relates to the viscosity of the product, and the *n*-value is the dimensionless flow behaviour index. A *n*-value < 1 is interpreted as shear thinning. The hysteresis area refers to the energy needed to regain an ordered state after a force is applied to disrupt the network. Figures 5.6-5.8 show the flow properties of fermented whey beverages with or without maize starch, with and without stearic acid and xanthan gum, alone and in combination.

The control beverage had the lowest *K*-value as well as the lowest degree of shear thinning. There was little change in its flow behaviour over the 15 day period (Fig 5.6 and Fig 5.7). The hysteresis of the control beverage, however, did decrease over the 15 day period from about 2394 Pa.s^{-1} to ~217 Pa.s^{-1} (Fig 5.8).

The beverage made with maize starch had a significantly higher *K*-value ($p < 0.05$) and a significantly lower *n*-value ($p < 0.05$), relative to the beverage made without starch. The *K*-value of the beverage made with maize starch significantly decreased ($p < 0.05$) over a 15 day period, from approximately 30.15 Pa.s^n to 7.18 Pa.s^n (Fig 5.6). The *n*-value of the aforementioned remained stable with no significant differences ($p > 0.05$) within the 15 day period (Fig 5.7).

The flow properties of the beverage made with MXG were not significantly different ($p > 0.05$) from the beverage made with maize starch. The *K*-value of the beverage made with maize starch and xanthan gum changed over the 15 day period from approximately 32.71 Pa.s^n to 7.17 Pa.s^n (Fig 5.6). The low *n*-value of the aforementioned remained relatively unchanged throughout the 15 day period (Fig 5.7).

The flow properties of the beverage made with maize starch and stearic acid was significantly different ($p < 0.05$) to that of the beverage made with maize starch. The *K*-value of the beverage made with MSA was significantly lower ($p < 0.05$) than the beverage made with maize starch (Fig 5.6), yet the *K*-value of the beverage made with MSA remained unchanged ($p > 0.05$) throughout the 15 day period (approx. 3.35 to approx. 1.56 $Pa.s^n$). The *n*-value of the beverage made with MSA was significantly higher ($p < 0.05$) than the beverage made with maize starch (Fig 5.7).

The flow properties of the beverage made with MSX was similar to the beverage made with MSA. The *K*-value of the beverage made with the MSX was significantly lower ($p < 0.05$) than that of the beverage made with maize starch, whilst having a significantly higher ($p < 0.05$) *n*-value (Fig 5.6 and 5.7). The *K*-value of the beverage made with MSX remained unchanged ($p > 0.05$) throughout the 15 day period.

The hysteresis of the whey beverages made with maize starch and MXG, were the highest (~22520 $Pa.s^{-1}$ and 24263 $Pa.s^{-1}$ respectively). The hysteresis of the beverages made with MSA and MSX, were significanlty smaller ($p < 0.05$) (9996 $Pa.s^{-1}$ and 11990 $Pa.s^{-1}$ respectively), relative to the beverages with pasted maize starch.

The hysteresis of the various beverages changed over the 15 day period at various rates. The hysteresis of the beverages made with maize starch and MXG changed significantly ($p < 0.05$) over the 15 day period, whereas the beverages made with MSA had no significant changes ($p > 0.05$). The beverages made with MSX had a relatively minute drop in comparison to the beverages made with maize starch.

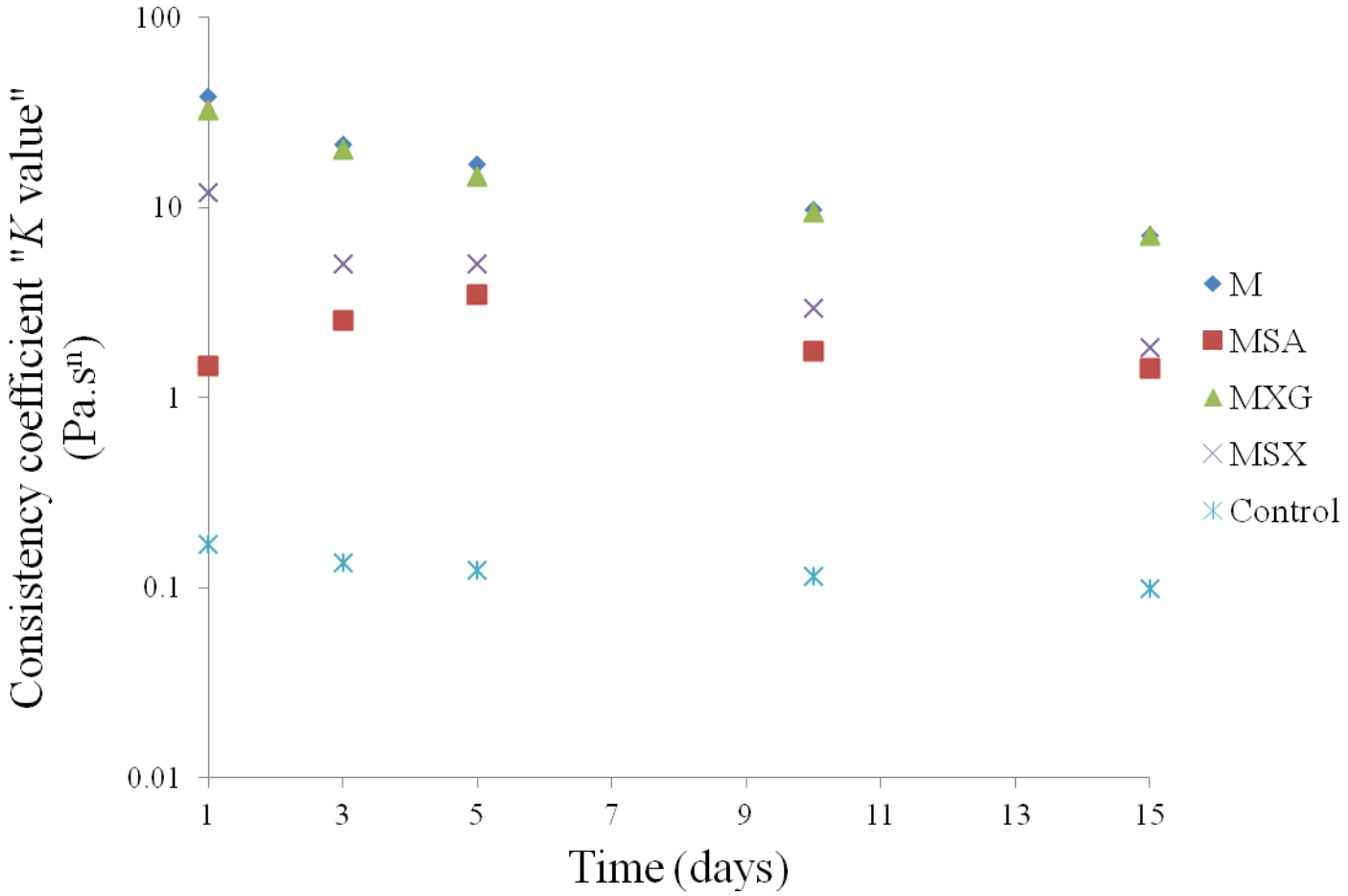

Figure 5.6: The effects of stearic acid and xanthan gum, used alone or in combination with maize starch, on the consistency coefficient of fermented whey over a 15 day period measured at 5 °C.

Beverage with 5% w/v: M – Maize starch, MSA – Maize starch with stearic acid, MXG – Maize starch with xanthan gum, MSX – Maize starch with stearic acid and xanthan gum.

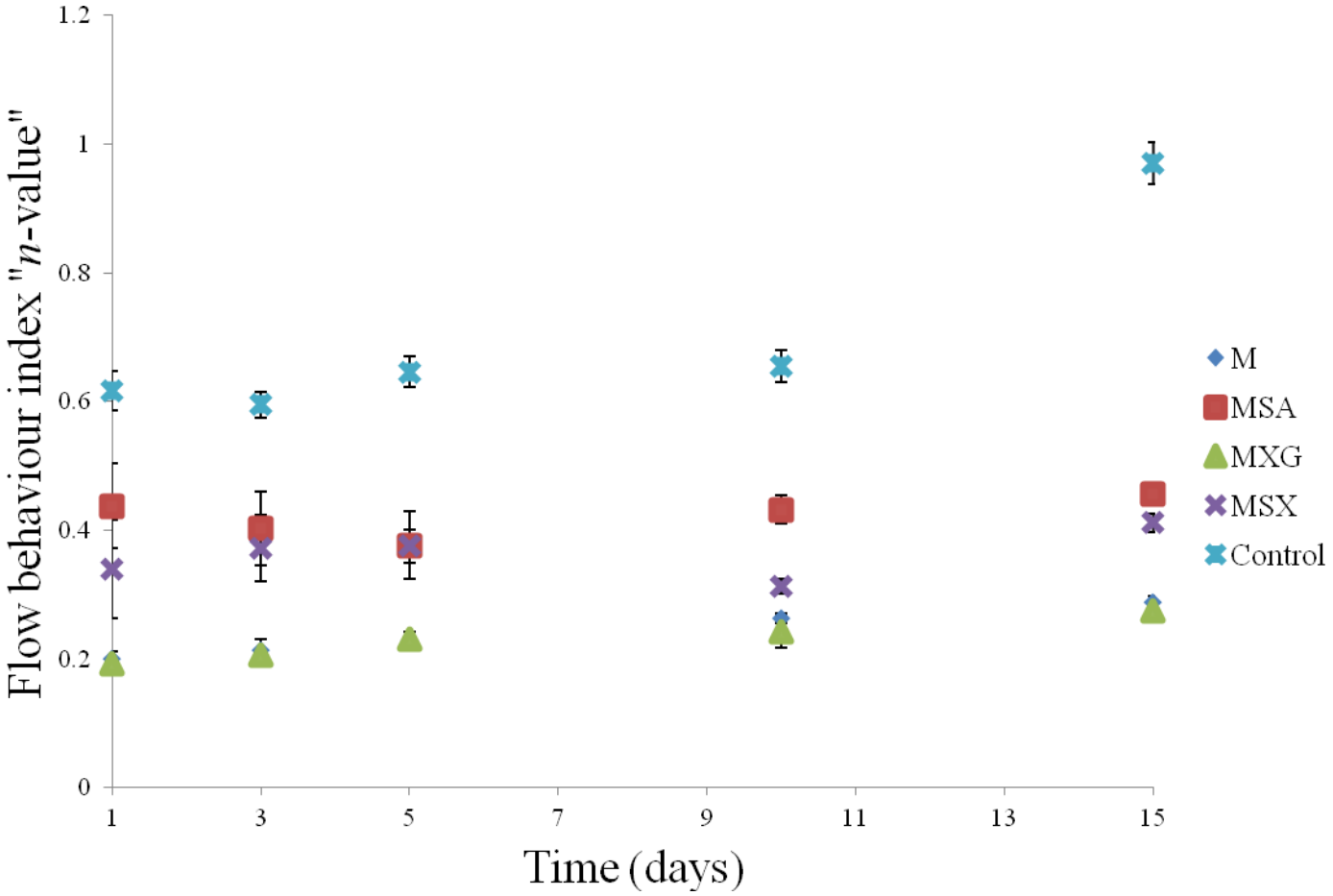

Figure 5.7: The effects of stearic acid and xanthan gum, used alone or in combination with maize starch, on the flow behaviour index of fermented whey over a 15 day period measured at 5 °C.

Beverage with 5% w/v: MSA – Maize starch with stearic acid, MXG – Maize starch with xanthan gum, MSX – Maize starch with stearic acid and xanthan gum. Error bars indicate standard deviation.

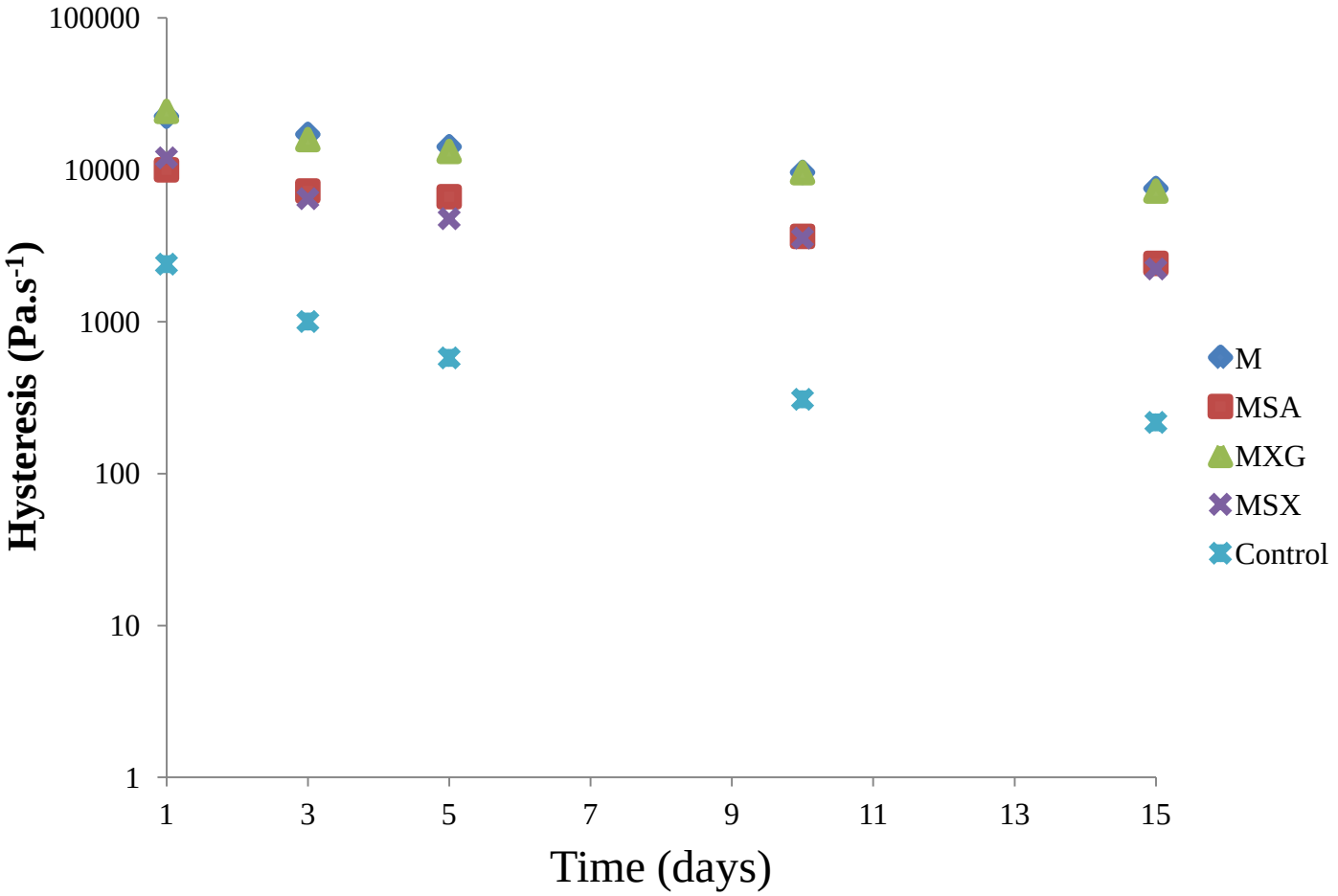

Figure 5.8: The effects of stearic acid and xanthan gum, used alone or in combination with maize starch, on the hysteresis of fermented whey over a 15 day period measured at 5 °C.

5.9 The effects of maize starch pasted with and without stearic acid and xanthan gum, alone and in combination, on the level of separation of a fermented whey beverage

Figure 5.9 shows the level of separation of the fermented whey beverages made with or without modified maize starch, with or without stearic acid and xanthan gum, alone and in combination over a 15 day period. A clear layer separated and formed on the top of each beverage at various levels. The level of the clear layer for all the beverages increased with time.

The beverage made without modified starch separated quickly relative to the other beverages with additives, achieving ~50% separation on the third day. The beverage without starch had the largest level of separation, followed by the beverage made with MSX, the beverage made with MSA and, lastly, both the beverages made with modified starch and MXG. The latter two having the lowest degree of separation (Fig 5.9). The beverages started to separate within three to four hours after mixing.

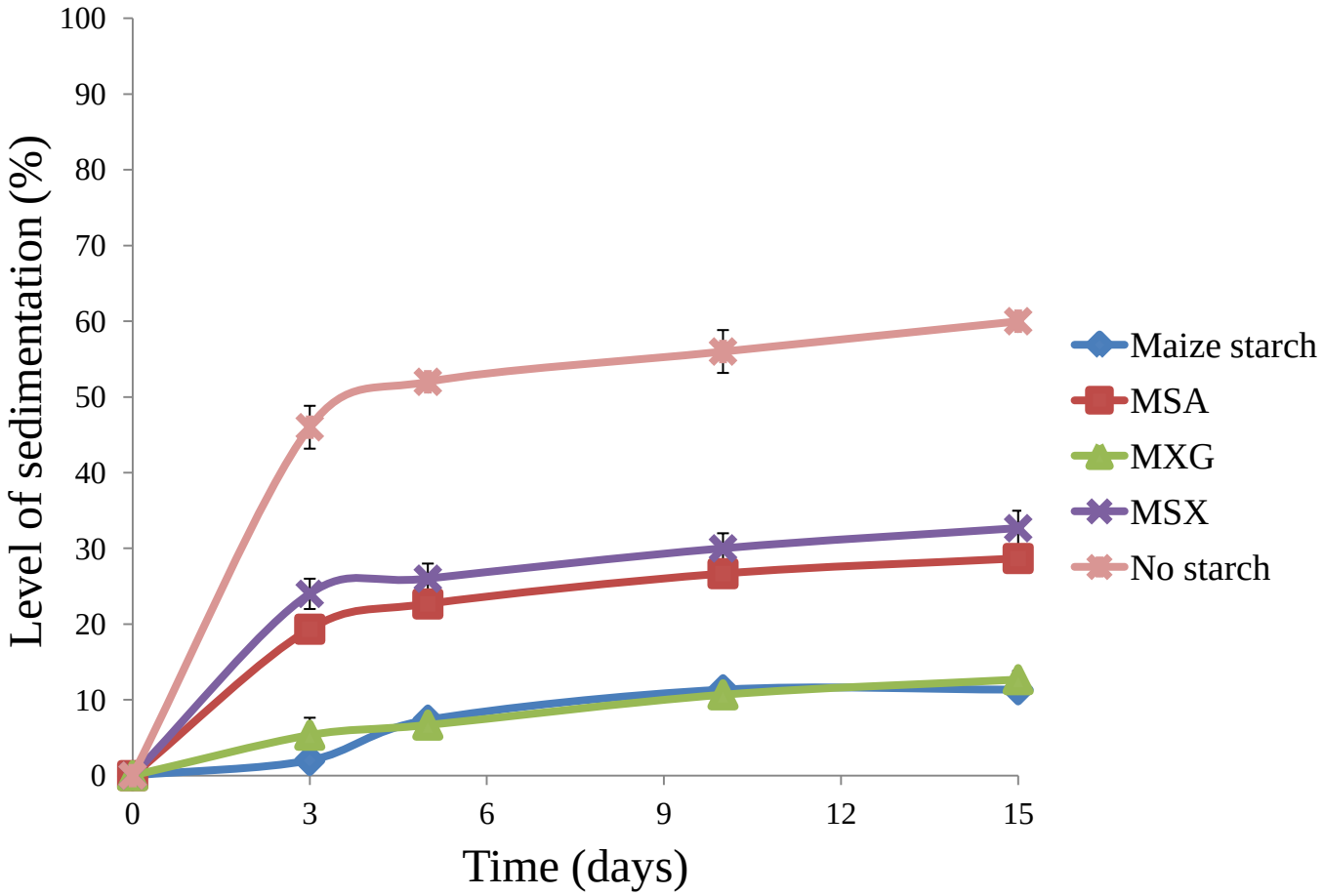

Figure 5.9: The effects of stearic acid and xanthan gum, used alone or in combination with maize starch, (5% w/v) on the level of sedimentation of fermented whey, over a 15 day period when stored at 5 °C.

Beverage with 5% w/v: M- Maize starch, MSA – Maize starch with stearic acid, MXG – Maize starch with xanthan gum, MSX – Maize starch with stearic acid and xanthan gum. Error bars indicate standard deviation.

6 DISCUSSION

A critical review of the experimental methodology used in this research project is discussed in this chapter. Furthermore, a comparative study is done on the interaction of stearic acid and xanthan gum, used alone and in combination, on the functional properties of maize starch,HAMS and the fermented beverage.,. Future consideration and possible applications for the modified starch is also presented.

6.1 Experimental methodology: Weaknesses and strengths of the experimental methodology

6.1.1 High-pressure condition for pasting of HAMS

HAMS requires high temperatures (of about 120 °C) to allow for its gelatinisation (Case *et al.*, 1998). This could possibly be as a result of the increased concentration of amylose (of more than 57%) in the crystalline regions of the HAMS granule (Shi *et al.*, 1998). In order to reach the high temperatures without excessive moisture loss, pressure of more than 400 kPa is required. High pressure reduces the boiling point of water, preventing the water from evaporating (Błaszczak, Fornal, Kiseleva, Yuryev, Sergeev and Sadowska, 2007).

The method that was used in this study was adapted from Ocloo *et al.* (2016), whereby the rheometer was used under pressure (500 kPa) to facilitate the pasting of the HAMS. The yield after the drying of the modified HAMS was about 1.5 g. HAMS was not used as a stabiliser for the fermented whey beverage because of its low viscosity (less than 1000 mPa.s) and the high pressure required for its pasting. The reactor vessel, as will be discussed, could not be used under pressure and could not reach the necessary temperatures for the gelatinisation of the HAMS.

6.1.2 Gel texture preparation and paste transference

The EZ penetration test was used to determine the textural properties of the modified starches. The method involves the deformation of a starch gel/paste and the recording of the applied force into and out of the sample. The penetration force will relate to the sample's firmness and the force required by the probe to release itself from the gel, will relate to the gel's stickiness (Liu, Ramsden and Corke, 1997) .

Any structural damage and air pockets in the gel/paste could influence the texture measurement. Caution was taken when transferring the cooled (less than 50 °C) pasted starch into the measuring moulds. The pasted starch was immediately transferred after pasting, to minimise any disruptions to the starch network structure. The pasted starch (without stearic acid) was pourable and transferred easily into the moulds. The pasted starch with added stearic acid, however, had to be removed with a spatula, as it was very thick. This is probably due to its high viscosity (section 5.1), which is attributed to the formation of amylose-lipid complexes (Wokadala *et al.*, 2012).

6.1.3 Differential scanning calorimetry

The differential scanning calorimetry (DSC) can be used to identify the presence of amylose-lipid complexes (Kugimiya, Donovan and Wong, 1980) and to differentiate between type I, type IIa or Type IIb amylose-lipid complexes (Biliaderis and Galloway, 1989). The principle of the DSC relies on the measurement of energy, in the form of heat, which is required for phase transitions and chemical reactions of a compound. The DSC measures the differences in heat flow of a sample and a reference at the same temperature.

The moisture content needs to be kept similar for all the samples during DSC analysis, as a low moisture content could result in the transitioning or melting of these complexes, at higher temperatures (Kugimiya *et al.*, 1980: Biliaderis, Page and Maurice, 1986). Moisture acts as a plasticiser and when moisture is in excess it lowers the melting temperature of the crystallite (Biliaderis *et al.*, 1986). A constant ratio of starch to water (1:3) was used. The freeze dried pre-gelatinised starch samples were rehydrated and mixed with a needle, to allow for full dispersion of the water, a method adapted from Maphalla and Emmambux (2015). The samples were left to equilibrate for a period of four hours in a sealed crucible. Amylose-lipid complexes melt at temperatures higher than 95 °C, thus to observe their endotherms, the evaporation of water had to be prevented. The use of the high-pressure DSC allowed for increase in pressure, which prevented water evaporation and facilitated in the accurate measurement of endotherms.

6.1.4 Reactor pasting and fermentation

The reactor was used to paste the maize starch (with and without stearic acid and xanthan gum) in large volumes (1.75 liters). No pressure could be generated to allow for the pasting of HAMS. The treated and untreated starch samples were pasted for the same time frame at approximately

the same temperatures. Some difficulties were experienced with the reactor as the increased volume made it harder to control the heat distribution because of the rotation speed of the arms that could not match the speed of the rheometer and thus the rate of moisture loss would be too large. The reactor was operated in a closed system to limit moisture evaporation at maximum rotation speed for each starch sample. Liquid nitrogen was not used to freeze the large amounts of starch paste but instead the pasted starch samples were stored below -20 °C immediately after pasting. The treated and untreated maize starch samples that were made with the reactor had similar gel textures to that of the samples made with the rheometer (Table 6.1). It was observed that the maize starch treated without stearic acid, formed a gel, and the maize starch treated with stearic acid, formed a paste.

6.1.5 Formulation of fermented whey beverage

The whey powder that was used was sourced locally (refer to appendix p 86). The formulation of the fermented whey beverage was conducted over a series of 1%, 5%, 10% and 20 % (w/v) trial analyses. The 3% and 5% whey formulations were observed to have a poor consistency and separated rapidly. The 10% and 20% (w/v) whey formulations had an improved consistency. The 20% (w/v) whey formulation had no apparent separation, which is probably due to the increase in the solid content (Gallardo-Escamilla *et al.*, 2007). However, the 20% (w/v) whey formulation had an acrid taste, which could probably be due to the high lactose and mineral content (Djurić *et al.*, 2004: Gallardo-Escamilla *et al.*, 2007). The 10% (w/v) whey formulation had the best overall qualities, both in texture and in taste, as was determined by the lab personnel.

The dried starch pastes were considered as being modified at molecular level and were termed as modified starch, treated with or without xanthan gum and stearic acid, alone or in combination.

Table 6.1: Effects of pasting in the reactor on the gel texture of stearic acid and xanthan gum modified maize starch

Sample composition			Maximum Force (N)	Minimum Force (N)	Visual appearance*
Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)			
Maize	0	0	2.31 ±0.76	-0.14 ±0.01	Gel
	0	0.25	2.42 ±0.66	-0.23 ±0.03	Gel
	1.5	0	0.43 ±0.02	-0.10 ±0.02	Paste
	1.5	0.25	0.44 ±0.08	-0.25 ±0.01	Paste

Means with different superscripts differ significantly ($p \leq 0.05$).

*Visual appearance of the pasted starch samples left for 24 hours at ~23 °C. A "starch gel" is considered as a slab that cannot be poured out freely or be spread on a surface. A "starch paste" is considered as being pourable, pliable and malleable.

The modified starch samples were then added to the fermented whey beverage. Separate quantities, of either 3% or 5% modified starch (w/w), were incorporated into the 10% (w/w) whey formulations prior to fermentation. The 3% modified starch mixture did not give the desired textural qualities and showed high levels of sedimentation in comparison to the 5% modified starch mixture. The beverages made with 3% modified starch reached high levels of sedimentation sooner than the beverages made with 5% modified starch. Figure 6.1 shows that the sedimentation levels on day 10 of the beverages made with 3% modified starch was similar to the sedimentation levels on day 15 of the beverages made with 5% modified starch. Singh and Byars (2009) stated that an increased content (> 3%) of a starch-lipid complexes, have shown no separation in set-style yoghurt.

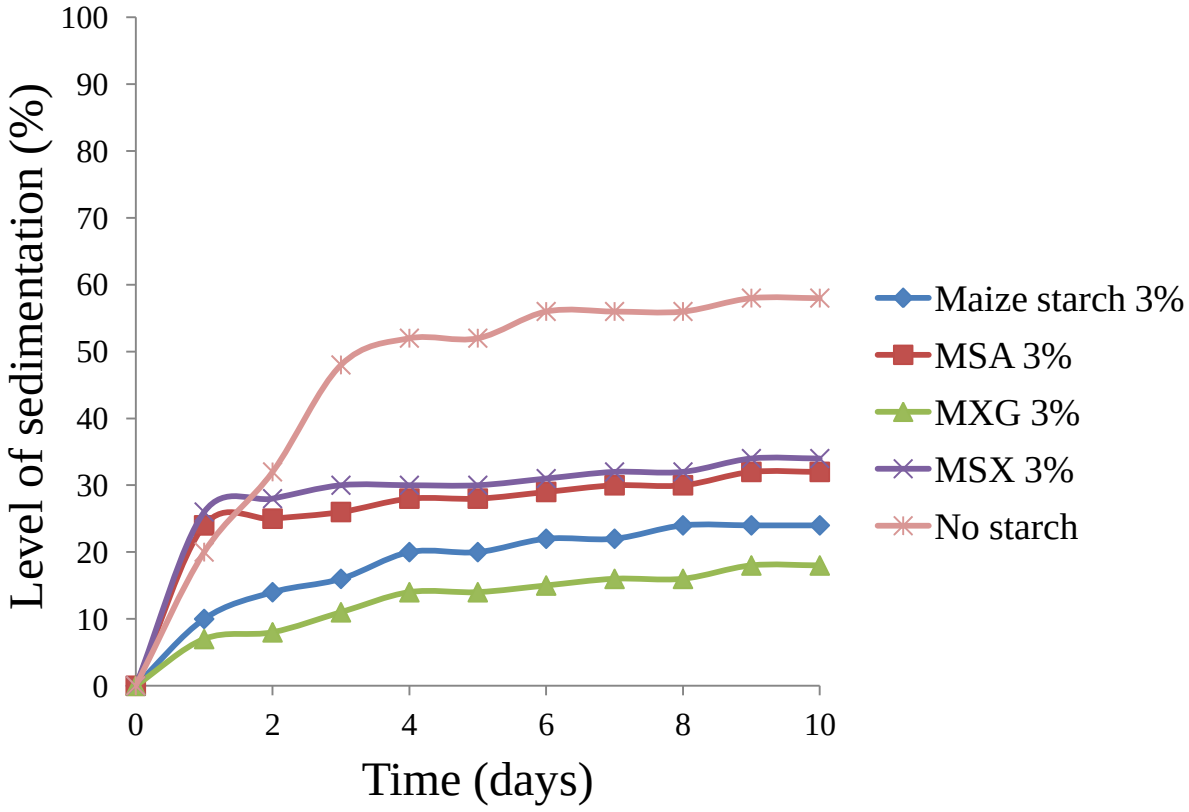

Figure 6.1: The effects of 3% (w/v) modified starch on the level of sedimentation in a fermented whey beverage MSA- maize starch with stearic acid, MXG – maize starch with xanthan gum, MSX – maize starch with stearic acid and xanthan gum.

6.2 Discussion of results

6.2.1 Functional properties of maize starch and HAMS with xanthan gum and stearic acid

6.2.1.1 Maize starch pasted with xanthan gum and stearic acid

The pasting properties of maize starch with and without xanthan gum or stearic acid, were similar to that reported by Maphalla and Emmambux (2015), with one exception as will be discussed. The increase in viscosity, at about 60 minutes for the control sample, is attributed to the formation of amylose-lipid complexes between the amylose and the endogenous lipids of the maize starch (Wokadala *et al.*, 2012). The presence of Type I amylose-lipid complexes was confirmed with DSC analysis. The endothermic peaks, with temperatures at approximately 98 °C, 106 °C and 120 °C (Table 5.1 peak B, C and D), correspond to Type I, Type IIa and Type IIb complexes respectively (Biliaderis and Galloway, 1989: Karkalas, Ma, Morrison and Pethrick, 1995). The increase in viscosity, observed at the end of pasting when the sample was cooled (below 60 °C), is attributed to the reassociation of amylose molecules (Blazek and Copeland, 2008). The starch molecules are described as becoming entangled with each other (Shi and BeMiller, 2002). Amylose will gradually reassociate with adjacent amylose molecules, forming junction zones, and eventually develop into a gel (Ferrero, Martino and Zaritzky, 1994).

The viscosity of the MXG was higher than the control, most probably due to the molecular interaction between amylose and xanthan gum (Shi and BeMiller, 2002). The hydrocolloid also increases the viscosity of the continuous phase. The gel strength of the MXG was not different to the control sample. Xanthan gum can be described as a non-gelling gum that forms weak intermolecular chain associations (Gallardo-Escamilla *et al.*, 2007).

The first viscosity peak of the MSX was lower because of the lipid, stearic acid, which interrupted granular swelling (Richardson, Langton, Bark and Hermansson, 2003) by limiting the water absorption of the granule. The continuous increase in viscosity to form a second peak, is attributed to the formation of more amylose-lipid complexes than in the control (D'Silva *et al.*, 2011: Wokadala *et al.*, 2012). The presence of Type I, Type IIa and Type IIb amylose-lipid complexes was confirmed by DSC analysis, as well as by an uncomplexed stearic acid (endotherm at about 64 °C) (Wokadala *et al.*, 2012). The increased amount of stearic acid is

available to interact with the amylose molecules, resulting in a greater formation of amylose-lipid complexes (BeMiller and Shewry, 2008). However, the formation of amylose-lipid complexes limited the gel formation, as the MSA remained paste like. The amylose-lipid complex limits junction zone formation (Blazek and Copeland, 2008), which could explain why there was little increase in the viscosity on cooling of the starch paste. Blazek and Copeland (2009) states that the amylose-lipid complex increases the space between junction zones, which could explain the soft texture of the paste. The pasting and gel properties of the maize starch pasted with xanthan gum and stearic acid were similar to that of the maize starch pasted with stearic acid alone.

The DSC results, however, were different to the results previously reported by Maphalla and Emmambux (2015). Maphalla and Emmambux (2015) suggested that xanthan gum might interfere with the formation of Type IIb amylose-lipid complexes. The endotherms of the DSC analysis in this study found that the Type I, Type IIa and Type IIb amylose-lipid complexes of maize starch pasted with xanthan gum and stearic acid were less than that of the maize starch pasted with stearic acid alone. This would suggest that xanthan gum could possibly interfere with the formation of amylose-lipid complexes when pasting under these conditions.

6.2.1.2 HAMS pasted with xanthan gum and stearic acid

The functional properties of a starch, specifically gelatinisation temperature, gelling rate, gel strength as well as its structural and thermodynamic properties, are determined by the amylose content (Shi *et al.*, 1998: Matveev, van Soest, Nieman, Wasserman, Protserov, Ezernitskaja and Yuryev, 2001). Amylose also contributes to the firmness and reduced stickiness of starch gel (Blazek and Copeland, 2008). HAMS requires higher temperatures during pasting than maize starch to allow for the gelatinisation of the amylose rich starch, as previously mentioned. The pasting properties of the HAMS pasted with and without stearic acid were similar to that reported by Ocloo *et al.* (2016). The increase in viscosity during pasting, at about 80 minutes for the HAMS, corresponds to the formation of Type IIa and Type IIb amylose-lipid complexes as shown by the DSC analysis. The endotherm at approximately 145 °C, corresponds to type III resistant starch that occurs when a starch is subjected to high temperature and pressure processing conditions (Shamai *et al.*, 2003). The pasted HAMS formed a brittle soft gel.

No previous literature was found on the pasting of HAMS-XG. HAMS-XG had a higher peak viscosity than the HAMS control and the second peak started at approximately 60 minutes. It is assumed that the increase in viscosity in the second peak can be attributed to the amylose-lipid complex rather than the presence of xanthan gum as will be discussed. The DSC reported the formation of a Type IIa amylose-lipid complex only, with no recorded ΔH at about 112 °C (Type IIb amylose-lipid complex) for the HAMS-XG. This could possibly indicate that xanthan gum interferes with the formation of Type IIb amylose-lipid complexes, as suggested previously.

High processing conditions (120 °C and 500 kPa) could possibly have dissociated and degraded the xanthan gum (Fig 6.2) (Gulrez, Al-Assaf, Fang, Phillips and Gunning, 2012). Gulrez *et al.* (2012) reported that the processing of a xanthan gum solution for two hours, even at 85 °C, reduces the molecular weight of xanthan gum by half its initial value.

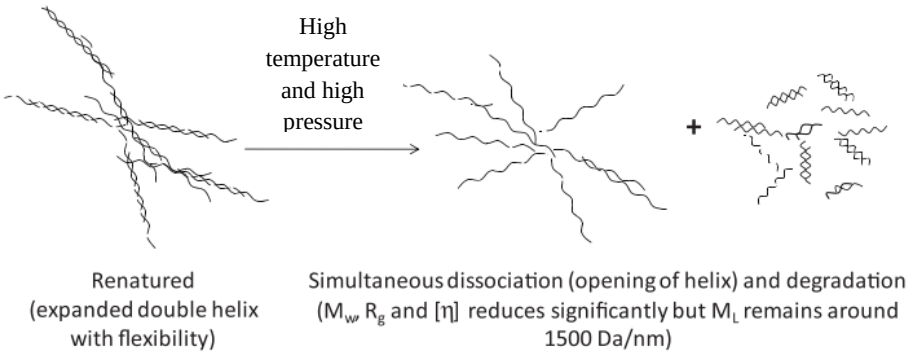

Figure 6.2: Schematic illustration of the structural and conformational changes of xanthan gum in solution when autoclaved at 120 °C (pressure not specified) (Gulrez *et al.*, 2012).

The first viscosity peak of HAMS-SA was lower than the control because the lipid presumably interrupted granular swelling (Richardson *et al.*, 2003). The increase in viscosity during pasting at about 60 minutes is attributed to the formation of amylose-lipid complexes, which were confirmed by DSC analysis. A larger ΔH was recorded at about 103 °C, which indicates a greater amount of specifically Type IIa amylose-lipid complexes, however, the same ΔH of Type IIb was recorded. Furthermore, an endotherm at about 66 °C could relate to the uncomplexed stearic acid, similar to that observed in maize starch pasted with stearic acid (Wokadala *et al.*, 2012). The increased amount of Type IIa amylose-lipid complexes appeared to affect the change in the gel structure of the HAMS, where the HAMS pasted with stearic acid remained paste like.

No previous literature was found on the pasting of HAMS with xanthan gum and stearic acid. The continuous increase in viscosity beyond approximately 60 minutes could be a result of the increased formation of both Type IIa and Type IIb amylose-lipid complexes, which was confirmed by DSC analysis. The reasons for the increase in amylose-lipid complex formations of HAMS pasted with xanthan gum and stearic acid, relative to the HAMS control and HAMS pasted with stearic acid alone, is unclear. HAMS pasted with xanthan gum and stearic acid remained paste like after 24 hours, similar to the HAMS pasted with stearic acid alone, which is likely due to the presence of the non-gelling amylose-lipid complexes. The increased number of amylose-lipid complexes in the HAMS pasted with xanthan gum and stearic acid, however, could be the reason for the lower gel strength compared to the HAMS-SA.

6.2.2 Functional properties of the fermented whey beverage

6.2.2.1 Fermentation and water activity (a_w) of the whey beverages with or without modified starch

The lactose in the whey is the main substrate for lactic acid production by the lactic acid bacteria. The increase in the concentration of lactic acid (Section 5.5) gave evidence of the metabolic activity of the microorganisms. The increase in lactic acid resulted in the subsequent decrease in pH. Fermented dairy products, such as yoghurt, generally have a pH lower than 4.5 to inhibit the growth of pathogenic microorganisms (Tamime and Robinson, 2007). The continuous drop in pH (postacidification) of the beverages (Section 5.4) during storage could be attributed to the continuous production of lactic acid by *Lactobacillus bulgaricus* (Antunes, Cazetto and Bolini, 2005). The continuous decrease in pH and increase in lactic acid, observed throughout all the beverages, suggest that the modified starch (treated and untreated) did not inhibit the metabolic activity of the starter culture.

The water activity (a_w) of the beverage relates to the partial pressure of water vapour above the beverages, over the partial pressure of water vapour above pure water. This would relate to the available water in the product. The addition of the modified starch did not decrease the a_w. BeMiller and Whistler (2007) reported that polysaccharides, like starch, generally have little effect on the a_w when used in low concentrations (less than 5%).

6.2.2.2 Flow properties of the fermented whey beverages with or without modified starch

The mouthfeel of the fermented whey beverage relates to the solid content of the product itself (Gallardo-Escamilla *et al.*, 2007). The two main contributors to the viscosity of the beverage made without modified starch would be the protein network and the exocellular polysaccharides (EPS) derived from the lactic acid bacteria (Tuinier, Zoon, Stuart, Fleer and De Kruif, 1999). The protein network consists of denatured whey protein (Appendix pp. 84) at a concentration of about 1.16 g per 100 g of the beverage, in comparison to the protein content (denatured casein, whey) of set style yoghurt, of about 5 g per 100 g (Tamime and Robinson, 2007). The extent of network formation in whey is low relative to that of casein, resulting in a weak stranded network. This lowers the expected viscosity, making whey an ideal drinking consistency.

The flow properties of the fermented whey beverage made without modified starch have not yet been documented by other researchers. The low zero shear viscosity of the beverage relates to the viscosity of the product at rest. As mentioned previously, the only components which could contribute to the product's viscosity are the whey proteins and the EPS. The low *K*-value relates to the low viscosity of the beverage.

The *n*-value of the beverage relates to the product's flow behaviour. The control beverage was observed to undergo shear thinning. The whey protein network presumably broke down and aligned with the direction of the applied shear, resulting in a decrease in the viscosity. The clockwise hysteresis value gives evidence that there was a regain of a structure, over time, which was previously deformed by a shearing action (Achayuthakan and Suphantharika, 2008). Thus, the fermented whey product can be considered as thixotropic, similar to set style and drinking yoghurt.

The flow properties of the control beverage were observed to change over the 15 day period, where there was a decrease in the viscosity. The change in viscosity could be attributed to the protein hydrolysis brought about by the residual enzymatic activity and or the low pH. The *n*-value approached 1, which suggests that there was no change in viscosity due to the total loss of structure in the product where the polymers are possibly hydrolysed into short oligomers. Short oligomers can behave similary to Newtonian fluids (Rodriguez, Cohen, Ober and Archer, 2014).

The increase in viscosity and *K*-value of the beverage made with the untreated modified starch could be attributed to the functional properties of starch. Starch forms networks with the proteins that entrap water and contributes to the solid content of the end product, thus increasing the viscosity (Tamime, Hassan, Farnworth and Toba, 2007). The *n*-value of the beverage, however, was much lower. This indicates that the beverage made with the modified starch has a large degree of shear thinning relative to the control beverage. The increased degree of shear thinning would result in the consumer perceiving a greater change in the consistency of the product when consumed. The hysteresis value of the beverage made with the modified starch showed a larger breakdown and regain of the structure, presumably derived from the starch network. The flow properties of the beverage made with MXG were similar to that of the beverage made with modified starch. Gallardo-Escamilla *et al.* (2007) reported that xanthan gum used at 0.26 % (w/v) did not significantly increase the perceived thickness of fermented whey. The flow properties of the beverage made with modified starch decreased over time presumably due to the hydrolysis of the starch and protein by the starter culture or more likely spoilage causing microorganisms. The decrease in viscosity is in response to the short polymer segments.

The lower viscosity and *K*-value of the beverage made the MSA can be attributed to the non-gelling characteristics of the amylose-lipid complex. The lower hysteresis value could be ascribed to the absence of a starch network at a low temperature (less than 25 °C). The suggestion by Maphalla and Emmambux (2015) that the amylose-lipid complex has a higher viscosity than native starch, holds true in a product that has a temperature higher than 60 °C when used at the same concentrations. The gelling properties of starch would appear to give the native starch a higher viscosity than those with amylose-lipid complexes at a temperature below 10 °C. The *n*-value, however, indicated that the beverage made with MSA had a lower shear thinning value relative to the beverage made with modified starch. The lower degree of shear thinning could be attributed to the compact size of the amylose-lipid complex, which will still be discussed (Section 6.3). Only the zero shear viscosity and *K*-value of the beverage made with xanthan gum and stearic acid modified starch were different from the MSA beverage on days one to three, where the beverage with MSX had slightly higher values but lower than the modified starch. As mentioned previously, xanthan gum has high viscosity at low shear rates.

Limited changes were observed over time in the flow properties of the beverage made with MSA (with and without xanthan gum) relative to the beverage made with untreated modified starch. The amylose-lipid complex is less susceptible to enzymatic hydrolysis (Wokadala *et al.*, 2012), which could explain why there was little change in the viscosity of the product.

6.2.2.3 Sedimentation of fermented whey beverages

The level of sedimentation for the control beverage was high, possibly due to poor network formation by the whey proteins and EPS polymers. The lack of network formation, together with the low viscosity, could result in the sedimentation of the whey proteins in the beverage. Another consideration would be the change of the charge across the whey protein with the change in pH. The isoelectric point of whey protein (β-lactoglobulin) is reached at a pH of 5.1 (Ju and Kilara, 1998b). A low charge would result in loss of water binding capacity across the protein surface, as well as a reduced repulsion between whey molecules. This could result in the whey proteins separating out of solution (Alting *et al.*, 2000).

The level of sedimentation in the beverage with the modified starch was lower than the control beverage, possibly due to the increased viscosity and starch network. The increase in viscosity and starch network could possibly keep the whey proteins in suspension. The increased level of sedimentation in the beverage treated with stearic acid and modified starch, relative to the beverage with the modified starch, could be as a result of the lack of a starch network formation. The soluble whey proteins would then have greater freedom of movement in the beverage treated with stearic acid modified starch, relative to the beverage with modified starch, resulting in the sedimentation in the beverage.

6.3 Hypothesised Model

Figures 6.3 and 6.4, are schematic illustrations highlighting the presence of amylose and amylose-lipid complex molecules in solution at various stages of shear rates. The diagram illustrates the shear aligning of the molecules in response to the applied force and the resultant shear thinning.

BeMiller and Whistler (2007) stated that the properties of polysaccharides are determined by the shape of starch molecules. The shape or conformation, in turn, is influenced by the solution or

environment including temperature. The radius of gyration (R_g) and hydrodynamic volume (V_h) are among several factors that relate to the viscosity of starch (BeMiller and Whistler, 2007). The radius of gyration describes the dimensions of a polymer chain. A linear molecule, with a greater flexibility, has a greater R_g when compared to a rigid polymer of the same molecular mass, which in turn would have a greater R_g than a branched polymer of the same molecular mass

The V-amylose will not be as flexible as the amylose molecule. Thus, it is the assumption that the amylose-lipid complex has a smaller R_g of about 9.6 nm (Zabar, Lesmes, Katz, Shimoni and Bianco-Peled, 2009) in comparison to the uncomplexed amylose molecule which is about 19.4 nm (Aberle, Burchard, Vorwerg and Radosta, 1994) because of the conformation of the V-amylose (Part E of Fig 6.2). No literature has yet been found to state the R_g of maize amylose-stearic acid complex, and more specifically if there is a difference with regards to Type I or Type II amylose-lipid complexes.

Chaplin (2016) stated that viscosity depends on the cross-sectional area (V_h) of a molecule. The molecule's orientation is dictated by the applied force. At low shear rates the polymers would be allowed to interact with each other and thus the polymer would presumably have its highest V_h However, at high shear strain rates the polymer would align to the applied force resulting in a decrease in V_h, which results in a decrease in viscosity. Chaplin (2016) further proposes that molecules of the same molecular weight but more compact in conformation, are less affected by the shear strain rate because these compact molecules are less likely to orientate themselves towards the direction of flow as a result of their smaller V_h.

It is therefore proposed that because of the smaller V_h and R_g of the amylose-lipid complexes, relative to the uncomplexed amylose, the amylose-lipid complexes presumably remained less affected by the shear strain rate resulting is a smaller degree of shear thinning (Part A to B of Fig 6.3 and 6.4). Furthermore, it may be postulated that the internal forces (hydrophobic interactions) of the amylose-lipid complex are greater than that of amylose (hydrogen bonds), which would result in the amylose-lipid complex not being deformed as easily as amylose at high temperatures (above 60 °C) and at high shear rates.

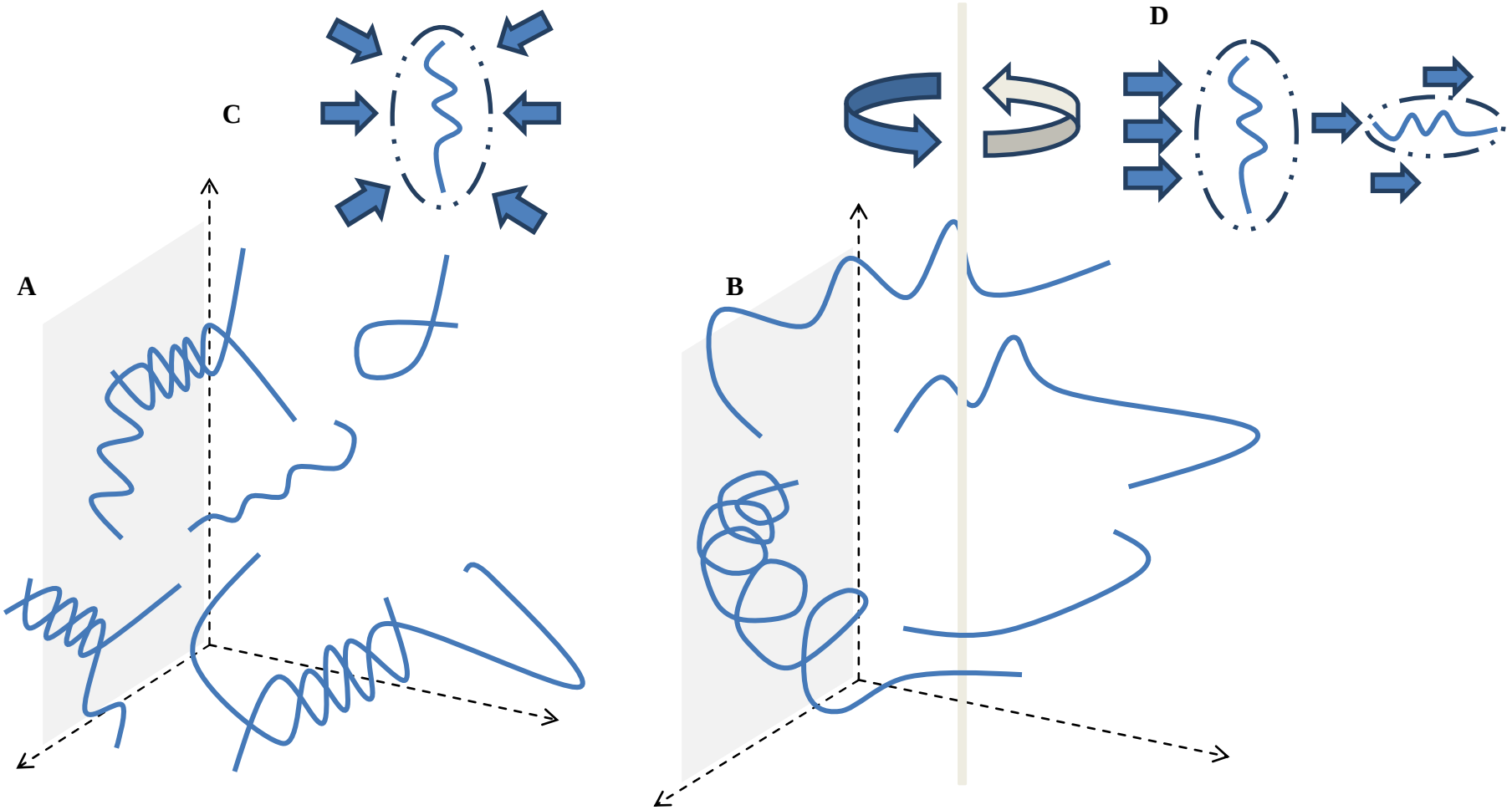

Figure 6.3: Schematic illustration of amylose in solution at A) low shear rates and B) at high shear rates. C- D) shows the force that work in on the molecules at different shear rates. Diagrams are not drawn to scale.

C **D**

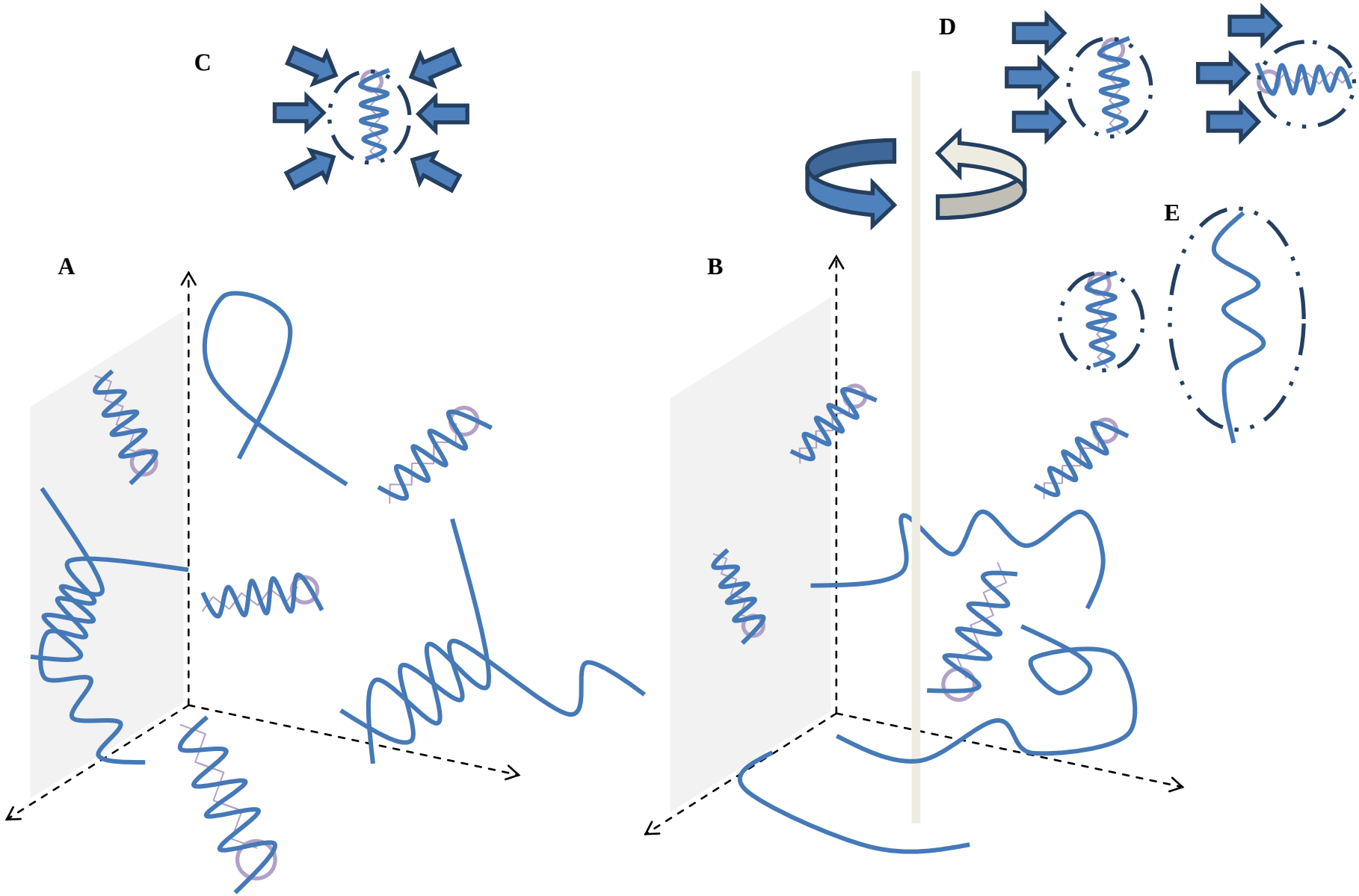

Figure 6.4: Schematic illustration of A) the in solution of amylose and amylose-lipid complexes at low shear rates and B) at high shear rates. C – D) illustrated the force on the molecules at the different shear rates and E) the cross-sectional area of amylose and amylose-lipid complex. Diagrams are not drawn to scale.

This could possibly explain why the viscosity of the amylose-lipid complex was high at high temperatures and possibly why it had a smaller degree of shear thinning relative to uncomplexed amylose under shearing conditions. Importantly, it would appear that the viscosity of the solution with amylose-lipid complexes remained (relatively) unchanged as the smaller cross-sectional area is less affected by the shear force per unit area when compared to amylose lipid complex (Part B of Fig 6.4).

6.4 Future work and possible application

6.4.1 Whey beverage

The prospects of the value addition of whey in the beverage sector will need to be assessed in order to truly evaluate the economic potential thereof. Formulation of a beverage using liquid or dried whey is one outlet that is considered as a use of whey. However, more research will be required in order to fully understand the nature of this product. Microscopy and SDS-page would give a premise to the whey protein network and structures involved in the beverage as well as changes to the protein structure over time. Product development could be used to further increase the consumer's appeal to the beverage.

6.4.2 Amylose-lipid complexes

The use of amylose-lipid complexes to increase the viscosity of a product beyond that of amylose, would only be effective at temperatures higher than 60 °C. However, the use of amylose-lipid complexes as a thickener at low temperatures (below 25 °C) is not discredited. It is proposed to investigate if an increased amount of amylose-lipid complexes in starch could contribute to an increased viscosity over time relative to that used in this study.

7 CONCLUSIONS

The addition of stearic acid and xanthan gum to maize starch and HAMS, results in an increased viscosity during pasting and produces a non-gelling starch. The non-gelling characteristic is attributed to the formation of amylose-lipid complexes, which prevent the formation of junction zones for molecular entanglement. The non-gelling characteristic and high viscosity of the starch modified with stearic acid and xanthan gum suggests that it can be used as a thickener in products where a non-gelling characteristic is required.

Starch modified with a combination of stearic acid and xanthan gum has thickening and stabilising capabilities in a fermented whey beverage. The use of modified starch in a whey beverage limits the sedimentation and maintains the viscosity of the whey beverage during storage for up to 15 days. However, this viscosity is lower than the same beverage made with starch modified without stearic acid. This suggests that starch modified with stearic acid could be used for beverages where a low viscosity is desired or where it needs to be used at higher concentrations if high viscosity is required.

8 REFERENCES

Abbas, K. A., Khalil, S. K. & Hussin, A. S. M. 2010. Modified Starches and Their Usages in Selected Food Products: A Review Study. *Journal of Agricultural Science,*2(2), 90-100.

Aberle, T., Burchard, W., Vorwerg, W. & Radosta, S. 1994. Conformational contributions of amylose and amylopectin to the structural properties of starches from various sources. *Starch-Stärke,* 46(9), 329-335.

Achayuthakan, P. & Suphantharika, M. 2008. Pasting and rheological properties of waxy corn starch as affected by guar gum and xanthan gum. *Carbohydrate Polymers,* 71(1), 9-17.

Alting, A. C., Hamer, R. J., De Kruif, C. G. & Visschers, R. W. 2000. Formation of disulfide bonds in acid-induced gels of preheated whey protein isolate. *Journal of Agricultural and Food Chemistry,* 48(10), 5001-5007.

Antunes, A. E., Cazetto, T. F. & Bolini, H. 2005. Viability of probiotic micro-organisms during storage, postacidification and sensory analysis of fat-free yogurts with added whey protein concentrate. *International Journal of Dairy Technology,* 58(3), 169-173.

Appelqvist, I. A. & Debet, M. R. 1997. Starch-biopolymer interactions—a review. *Food Reviews International,* 13(2), 163-224.

Arocas, A., Sanz, T. & Fiszman, S. 2009. Clean label starches as thickeners in white sauces. Shearing, heating and freeze/thaw stability. *Food hydrocolloids,* 23(2), 2031-2037.

Baeza, R., Gugliotta, L. M. & Pilosof, A. M. R. 2003. Gelation of β-lactoglobulin in the presence of propylene glycol alginate: kinetics and gel properties. *Colloids and Surfaces B: Biointerfaces,* 31, 81-93.

Barsby, T. L., Donald, A. M. & Frazier, P. J. 2001. *Starch: Advances in structure and function,* Cambridge, UK : Royal Society of Chemistry.

Bemiller, J. N. 2011. Pasting, paste, and gel properties of starch–hydrocolloid combinations. *Carbohydrate Polymers,* 86, 386-423.

Bemiller, J. N. & Shewry, P. R. 2008. Carbohydrate Chemistry for Food Scientists; J.N.; BeMiller; second ed.; 2007; AACC International; *Journal of Cereal Science,* 48, 879-879.

Bemiller, J. N. & Whistler, R. L. 2007. *Carbohydrate chemistry for food scientists* (2nd ed.). St. Paul, Minnesota : AACC International.

Biliaderis, C. G. & Galloway, G. 1989. Crystallization behavior of amylose-V complexes: Structure-property relationships. *Carbohydrate Research,* 189, 31-48.

Biliaderis, C. G., Page, C. M. & Maurice, T. J. 1986. Non-equilibrium melting of amylose-V complexes. *Carbohydrate Polymers,* 6(4), 269-288.

Biliaderis, C. G., Page, C. M., Slade, L. & Sirett, R. R. 1985. Thermal behavior of amylose-lipid complexes. *Carbohydrate Polymers,* 5(5), 367-389.

Błaszczak, W., Fornal, J., Kiseleva, V. I., Yuryev, V. P., Sergeev, A. I. & Sadowska, J. 2007. Effect of high pressure on thermal, structural and osmotic properties of waxy maize and Hylon VII starch blends. *Carbohydrate Polymers,* 68(3), 387-396.

Blazek, J. & Copeland, L. 2008. Pasting and swelling properties of wheat flour and starch in relation to amylose content. *Carbohydrate Polymers,* 71(3), 380-387.

Blazek, J. & Copeland, L. 2009. Effect of monopalmitin on pasting properties of wheat starches with varying amylose content. *Carbohydrate Polymers,* 78, 131-136.

Boland, M. 2011. Chemistry of Beta-Lactoglobulin,. *In:* Phillips, G. O. & Williams, P. A. (Eds.) *Handbook of Food Proteins.*, Oxford, UK : Woodhead Publishing. p. 30-55.

Bultosa, G., Hamaker, B. R. & Bemiller, J. N. 2008. An SEC− MALLS study of molecular features of water-soluble amylopectin and amylose of tef *Eragrostis tef* (Zucc.) Trotter Starches. *Starch-Stärke,* 60(1), 8-22.

Case, S. E., Capitani, T., Whaley, J. K., Shi, Y. C., Trzasko, P., Jeffcoat, R. & Goldfarb, H. B. 1998. Physical properties and gelation behavior of a low-amylopectin maize starch and other high-amylose maize Starches. *Journal of Cereal Science,* 27, 301-314.

Chaplin, M. 2016. *Water structure and Science* [Online]. London South Bank University Available: http://www1.lsbu.ac.uk/water/rheology.html [Accessed 05-05 2016].

Cheetham, N. W. & Tao, L. 1998a. Solid state NMR studies on the structural and conformational properties of natural maize starches. *Carbohydrate Polymers,* 36, 285-292.

Cheetham, N. W. H. & Tao, L. 1998b. Variation in crystalline type with amylose content in maize starch granules: an X-ray powder diffraction study. *Carbohydrate Polymers,* 36, 277-284.

Coetzee, K. 2015. Lacto Data Statistics. 18(1). 1-32, South Africa : Milk SA.

Copeland, L., Blazek, J., Salman, H. & Tang, M. C. 2009. Form and functionality of starch. *Food Hydrocolloids,* 23, 1527-1534.

Creamer, L. K., Bienvenue, A., Nilsson, H., Paulsson, M., Van Wanroij, M., Lowe, E. K., Anema, S. G., Boland, M. J. & Jiménez-Flores, R. 2004. Heat-induced redistribution of disulfide bonds in milk proteins. Bovine beta-lactoglobulin. *Journal of Agricultural and Food Chemistry,* 52, 7660-8.

D'Silva, T. V., Taylor, J. N. R. & Emmambux, M. N. 2011. Enhancement of the pasting properties of teff and maize starche through wet-heat processing with added streaic acid. *Journal of Cereal Science,* 53, 192-197.

Demetriades, K., Coupland, J. & Mcclements, D. 1997. Physical properties of whey protein stabilized emulsions as related to pH and NaCl. *Journal of Food Science,* 62(2), 342-347.

Djurić, M., Carić, M., Milanović, S., Tekić, M. & Panić, M. 2004. Development of whey-based beverages. *European Food Research and Technology,* 219(4), 321-328.

Doutch, J., Bason, M., Franceschini, F., James, K., Clowes, D. & Gilbert, E. P. 2012. Structural changes during starch pasting using simultaneous Rapid Visco Analysis and small-angle neutron scattering. *Carbohydrate Polymers,* 88(3), 1061-1071.

Eliasson, A., Finstad, H. & Ljunger, G. 1988. A study of starch-lipid interactions for some native and modified maize starches. *Starch-Stärke,* 40(3), 95-100.

Fennema, O. R. 1996. *Food Chemistry* 3rd Edition, United States of America, New York, Marcel Dekker, INC. pp. 147-221.

Ferrero, C., Martino, M. N. & Zaritzky, N. E. 1994. Corn starch-xanthan gum interaction and its effect on the stability during storage of frozen gelatinized suspension. *Starch - Stärke*, 46(8), 300-308.

Foegeding, E. A., Davis, J. P., Doucet, D. & Mcguffey, M. K. 2002. Advances in modifying and understanding whey protein functionality. *Trends in Food Science & Technology*, 13(5), 151-159.

Fuquay, J. W., Fox, P. F. & Mcsweeney, P. L. H. 2011. Encyclopedia of dairy sciences (2nd ed.). Boston : Elsevier.

Gallardo-Escamilla, F. J., Kelly, A. L. & Delahunty, C. M. 2007. Mouthfeel and flavour of fermented whey with added hydrocolloids. *International Dairy Journal*, 17(4), 308-315.

Gelders, G. G., Duyck, J. P., Goesaert, H. & Delcour, J. A. 2005. Enzyme and acid resistance of amylose-lipid complexes differing in amylose chain length, lipid and complexation temperature. *Carbohydrate Polymers*, 60(3), 379-389.

Godet, M., Bouchet, B., Colonna, P., Gallant, D. & Buléon, A. 1996. Crystalline Amylose-Fatty Acid Complexes: Morphology and Crystal Thickness. *Journal of food science*, 61(6), 1196-1201.

Gudmundsson, M. 1994. Retrogradation of starch and the role of its components. *Thermochimica Acta*, 246(2), 329-341.

Gulrez, S. K. H., Al-Assaf, S., Fang, Y., Phillips, G. O. & Gunning, A. P. 2012. Revisiting the conformation of xanthan and the effect of industrially relevant treatments. *Carbohydrate Polymers*, 90(3), 1235-1243.

Gulzar, M. 2011. Dry heating of whey protein under controlled physicochemical conditions: structures , interactions and functionalities, PhD thesis, Dept. Food Science, European University of Brittany.

Guzmán-González, M., Morais, F., Ramos, M. & Amigo, L. 1999. Influence of skimmed milk concentrate replacement by dry dairy products in a low fat set-type yoghurt model

system. I: Use of whey protein concentrates, milk protein concentrates and skimmed milk powder. *Journal of the Science of Food and Agriculture*, 79(8), 1117-1122.

Hall, G. & Iglesias, O. 1997. Functional properties of dried milk whey Propiedades funcionales de suero de leche deshidratado. *Food Science and Technology International*, 3, 381-383.

Hambling, S. G., Mcalpine, A. S. & Swayer, L. 1992. β-Lactoglobulin *In:* FOX, P. F. (ed.) *Advanced Dairy Chemistry : Proteins,* pp. 141-191, London, UK : Elsevier Applied Science.

Hirotsu, K. & Shimada, A. 1974. The crystal and molecular structure of β-lactose. *Bulletin of the Chemical Society of Japan*, 47, 1872-1879.

Hizukuri, S. 1986. Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research*, 147(8), 342-347.

Hizukuri, S., Takeda, Y., Yasuda, M. & Suzuki, A. 1981. Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydrate Research*, 94(2), 205-213.

Holsinger, V. H., Posati, L. P. & Devilbiss, E. D. 1974. Whey Beverages: A Review. *Journal of Dairy Science*, 57(8), 849-859.

Huffman, L. M. 1996. Processing whey protein for use as a food ingredient. *Food Technology*, 50(2), 49-52.

Immel, S. & Lichtenthaler, F. W. 2000. The hydrophobic topographies of amylose and its blue iodine. *Flexible and Rigid Non-glucose Cyclooligosaccharides: Synthesis, Structure, and Properties*, 52, 27.

Jelen, P. 2011. Whey Processing | Utilization and Products. *In:* Fuquay, J. W. (Ed.) *Encyclopedia of Dairy Sciences (2nd Edition)*, pp. 731-737, San Diego: Academic Press.

Jenkins, P. J. & Donald, A. M. 1998. Gelatinisation of starch: a combined SAXS/WAXS/DSC and SANS study. *Carbohydrate Research*, 308, 133-147.

Ju, Z. Y. & Kilara, A. 1998a. Effects of preheating on properties of aggregates and of cold-set gels of whey protein isolate. *Journal of Agricultural and Food Chemistry*, 46(9), 3604-3608.

Ju, Z. Y. & Kilara, A. 1998b. Gelation of pH-aggregated whey protein isolate solution induced by heat, protease, calcium salt, and acidulant. *Journal of Agricultural and Food Chemistry,* 46(5), 1830-1835.

Karim, A. A., Norziah, M. & Seow, C. 2000. Methods for the study of starch retrogradation. *Food Chemistry,* 71, 9-36.

Karkalas, J., Ma, S., Morrison, W. R. & Pethrick, R. A. 1995. Some factors determining the thermal properties of amylose inclusion complexes with fatty acids. *Carbohydrate Research,* 268(2), 233-247.

Khurana, H. K. & Kanawjia, S. K. 2007. Recent Trends in Development of Fermented Milks. *Current Nutrition & Food Science,* 3, 91-108.

Kosikowski, F. V. 1979. Whey Utilization and Whey Products1. *Journal of Dairy Science,* 62(7), 1149-1160.

Kugimiya, M., Donovan, J. W. & Wong, R. Y. 1980. Phase Transitions of Amylose-Lipid Complexes in Starches: A Calorimetric Study. *Starch - Stärke,* 32(8), 265-270.

Lal, S. N., O'connor, C. J. & Eyres, L. 2006. Application of emulsifiers/stabilizers in dairy products of high rheology. *Avances in Colloid and Interface Science,* 123-126, 433-7.

Lii, C.-Y., Lai, V. M.-F. & Shen, M.-C. 2004. Changes in retrogradation properties of rice starches with amylose content and molecular properties. *Cereal Chemistry,* 81(3), 392-398.

Liu, H., Ramsden, L. & Corke, H. 1997. Physical properties and enzymatic digestibility of acetylated ae, wx, and normal maize starch. *Carbohydrate Polymers,* 34(4), 283-289.

López, C. A., De Vries, A. H. & Marrink, S. J. 2012. Amylose folding under the influence of lipids. *Carbohydrate Research,* 364, 1-7.

Mali, S., Ferrero, C., Redigonda, V., Beleia, A., Grossmann, M. & Zaritzky, N. 2003. Influence of pH and hydrocolloids addition on yam (*Dioscorea alata*) starch pastes stability. *LWT-Food Science and Technology,* 36(5), 475-481.

Maphalla, T. G.2015. Functionality of maize, wheat, teff and cassava starches with stearic acid and xanthan gum. MSc Food Science Disertation, Dept. of Food Science, University of Pretoria.

Marwaha, S. S. & Kennedy, J. F. 1988. Whey—pollution problem and potential utilization. *International Journal of Food Science & Technology,* 23(4), 323-336.

Mason, W. R. 2009. Starch use in foods. *In:* Bemiller, J. & Whistler, R. (Eds.) *Starch (3rd Edition).* San Diego : Academic Press pp 745-795.

Matveev, Y. I., Van Soest, J. J. G., Nieman, C., Wasserman, L. A., Protserov, V. A., Ezernitskaja, M. & Yuryev, V. P. 2001. The relationship between thermodynamic and structural properties of low and high amylose maize starches. *Carbohydrate Polymers,* 44(2), 151-160.

Mawson, A. 1994. Bioconversions for whey utilization and waste abatement. *Bioresource Technology,* 47(3), 195-203.

Mcintosh, G. H., Royle, P. J., Le Leu, R. K., Regester, G. O., Johnson, M. A., Grinsted, R. L., Kenward, R. S. & Smithers, G. W. 1998. Whey proteins as functional food ingredients? *International Dairy Journal,* 8, 425-434.

Mleko, S. & Foegeding, E. A. 1999. Formation of whey protein polymers: effects of a two-step heating process on rheological properties. *Journal of Texture Studies,* 30(2), 137-149.

Mohammed, Z., Haque, A., Richardson, R. & Morris, E. 2007. Promotion and inhibition of xanthan 'weak-gel'rheology by calcium ions. *Carbohydrate Polymers,* 70, 38-45.

Morrison, W., Law, R. & Snape, C. 1993. Evidence for inclusion complexes of lipids with V-amylose in maize, rice and oat starches. *Journal of Cereal Science,* 18(2), 107-109.

Nelles, E. M., Dewar, J., Van Der Merwe, C. F. & Taylor, J. 2003. Granule integrity and starch solubility during slow, extended pasting of maize starch—the second viscosity peak. *Starch-Stärke,* 55(2), 72-79.

Obiro, W. C., Sinha Ray, S. & Emmambux, M. N. 2012. V-Amylose structural characteristics, methods of preparation, significance, and potential applications. *Food Reviews International,* 28(4), 412-438.

Ocloo, F. C. 2013. Effects of gamma irradiation and stearic acid, alone and in combination, on functional, structural, and molecular characteristics of high amylose maize starch. PhD Thesis, Dept. of Food Science, University of Pretoria.

Ocloo, F. C. K.. 2016. Effects of stearic acid and gamma irradiation, alone and in combination, on pasting properties of high amylose maize starch. *Food Chemistry,* 190, 12-19.

Ong, M. H., Jumel, K., Tokarczuk, P. F., Blanshard, J. M. V. & Harding, S. E. 1994. Simultaneous determinations of the molecular weight distributions of amyloses and the fine structures of amylopectins of native starches. *Carbohydrate Research,* 260, 99-117.

Parker, R. & Ring, S. 2001. Aspects of the physical chemistry of starch. *Journal of Cereal Science,* 34, 1-17.

Penna, A., Oliveira, M. & Tamime, A. 2003. Influence of carrageenan and total solids content on the rheological properties of lactic beverage made with yogurt and whey. *Journal of Texture Studies,* 34, 95-113.

Penna, A. L. B., Sivieri, K. & Oliveira, M. N. 2001. Relation between quality and rheological properties of lactic beverages. *Journal of Food Engineering,* 49, 7-13.

Pérez, S. & Bertoft, E. 2010. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch - Stärke,* 62(8), 389-420.

Pescuma, M., Hébert, E. M., Mozzi, F. & Font De Valdez, G. 2008. Whey fermentation by thermophilic lactic acid bacteria: Evolution of carbohydrates and protein content. *Food Microbiology,* 25(3), 442-451.

Pescuma, M., Hébert, E. M., Mozzi, F. & Font De Valdez, G. 2010. Functional fermented whey-based beverage using lactic acid bacteria. *International Journal of Food Microbiology,* 141, 73-81.

Putseys, J. A., Derde, L. J., Lamberts, L., ÖStman, E., Bjorck, I. M. & Delcour, J. A. 2009. Functionality of short chain amylose− lipid complexes in starch− water systems and their impact on in vitro starch degradation. *Journal of Agricultural and Food Chemistry,* 58(3), 1939-1945.

Richardson, G., Langton, M., Bark, A. & Hermansson, A. M. 2003. Wheat starch gelatinization—the effects of sucrose, emulsifier and the physical state of the emulsifier. *Starch-Stärke,* 55, 150-161.

Rodriguez, F., Cohen, C., Ober, C. K., Archer, L., 2014. Principles of polymer systems. New York, CRC Press pp. 278.

Rosalam, S. & England, R. 2006. Review of xanthan gum production from unmodified starches by *Xanthomonas comprestris* sp. *Enzyme and Microbial Technology,* 39(2), 197-207.

Savary, G., Handschin, S., Conde-Petit, B., Cayot, N. & Doublier, J. 2008. Structure of polysaccharide-starch composite gels by rheology and confocal laser scanning microscopy: Effect of the composition and of the preparation procedure. *Food Hydrocolloids,* 22(4), 520-530.

Shamai, K., Bianco-Peled, H. & Shimoni, E. 2003. Polymorphism of resistant starch type III. *Carbohydrate Polymers,* 54(3), 363-369.

Shi, X. & Bemiller, J. N. 2002. Effects of food gums on viscosities of starch suspensions during pasting. *Carbohydrate Polymers*, 50, 7-18.

Shi, Y.-C., Capitani, T., Trzasko, P. & Jeffcoat, R. 1998. Molecular structure of a low-amylopectin starch and other high-amylose maize starches. *Journal of Cereal Science,* 27(3), 289-299.

Singh, M. & Byars, J. A. 2009. Starch-lipid composites in plain set yogurt. *International Journal of Food Science & Technology*, 44, 106-110.

Siso, M. G. 1996. The biotechnological utilization of cheese whey: a review. *Bioresource Technology,* 57, 1-11.

Smith, A. K. & Campbell, B. E. 2007. Microstructure of milk components *In:* Tamime, A. Y. (Ed.) *Structure of Dairy Products.* Ary, UK: Blackwell Publishing. pp. 67-68.

Srichuwong, S., Sunarti, T. C., Mishima, T., Isono, N. & Hisamatsu, M. 2005. Starches from different botanical sources II: Contribution of starch structure to swelling and pasting properties. *Carbohydrate Polymers,* 62, 25-34.

Takeda, Y., Shirasaka, K. & Hizukuri, S. 1984. Examination of the purity and structure of amylose by gel-permeation chromatography. *Carbohydrate Research*, 132, 83-92.

Tamime, A. Y., Hassan, A., Farnworth, E. & Toba, T. 2007. Structure of fermented milks. *in:* Tamime, A. Y. (Ed.) *Structure of Diary Products.* Ayr, UK: Blackwell Publishing. pp. 134-169.

Tamime, A. Y. & Robinson, R. K. 2007. *Tamime and Robinson's Yoghurt - Science and Technology (3rd Edition)*, Cambridge England Woodhead Publishing. pp.13-41.

Tang, H., Mitsunaga, T. & Kawamura, Y. 2006. Molecular arrangement in blocklets and starch granule architecture. *Carbohydrate Polymers*, 63(4), 555-560.

Tárrega, A. & Costell, E. 2006. Effect of inulin addition on rheological and sensory properties of fat-free starch-based dairy desserts. *International Dairy Journal*, 16(9), 1104-1112.

Teklehaimanot, W. H., Duodu, K. G. & Emmambux, M. N. 2013. Maize and teff starches modified with stearic acid as potential fat replacer in low calorie mayonnaise-type emulsions. *Starch-Stärke*, 65, 773-781.

Tuinier, R., Zoon, P., Stuart, M. C., Fleer, G. & De Kruif, C. 1999. Concentration and shear-rate dependence of the viscosity of an exocellular polysaccharide. *Biopolymers*, 50(6), 641-646.

Van Boekel, M. A. J. S. 1998. Effect of heating on Maillard reactions in milk. *Food Chemistry*, 62(4), 403-414.

Vardhanabhuti, B. & Foegeding, E. A. 1999. Rheological properties and characterization of polymerized whey protein isolates. *Journal of Agricultural and Food Chemistry*, 47(9), 3649-3655.

Walstra, P. & Jenness, R. 1984. *Dairy Chemistry & Physics*, John Wiley & Sons, New York.

Weber, F. H., Clerici, M. T. P., Collares-Queiroz, F. P. & Chang, Y. K. 2009. Interaction of guar and xanthan gums with starch in the gels obtained from normal, waxy and high-amylose corn starches. *Starch-Stärke*, 61, 28-34.

Wokadala, O. C., Ray, S. S. & Emmambux, M. N. 2012. Occurrence of amylose–lipid complexes in teff and maize starch biphasic pastes. *Carbohydrate Polymers*, 90, 616-622.

Zabar, S., Lesmes, U., Katz, I., Shimoni, E. & Bianco-Peled, H. 2009. Studying different dimensions of amylose–long chain fatty acid complexes: Molecular, nano and micro level characteristics. *Food Hydrocolloids,* 23(7), 1918-1925.

9 APPENDIX

Table 9.1: The effects of stearic acid and xanthan gum, used alone or in combination with maize starch, on the zero shear viscosity of fermented whey over a 15 day period

Starch	Stearic acid (%w/w)	Xanthan gum (%w/w)	Day 1	Day 3	Day 5	Day 10	Day 15
No starch	0	0	1.0 ± 0.2	1.0 ± 0.0	0.7 ± 0.3	0.8 ± 0.2	0.6 ±0.4
	0	0	867.3^{gh} ± 412.4	709.0^{g} ± 10.4	509.0^{ef} ± 72.5	298.3^{de} ± 52.3	216.3^{abc} ± 16.5
Maize starch	0	0.25	85.7^{ab} ± 101.7	86.6^{ab} ± 71.3	86.7^{ab} ± 33.5	31.1^{a} ± 11.1	24.3^{a} ± 7.5
	1.5	0	978.7^{h} ± 147.4	676.0^{fg} ± 95.8	468.0^{de} ± 83.1	311.3^{abc} ± 40.4	215.3^{abc} ± 41.7
	1.5	0.25	239.1^{b} ± 180.7^{c}	99.6^{ab} ± 63.8	85.5^{ab} ± 52.1	56.7^{ab} ± 7.1	30.4^{a} ± 5.8

Mean and (±) Standard deviation. Different superscripts indicating significant differences ($p < 0.05$).
Beverage with 5% (w/v) modified starch.

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Constantia Kloof, 1709
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Clover

Certificate of Analysis

Whey Powder (3.18)

Product Description: *Certified Foodgrade - Suitable for food production*
Product Code: *NA*
Lot Number *H1410003283 H28643*

Date of Issue: *2014/10/22*
Manufactured: *2014/10/13*
Date of Expiry: *2015/10/13*

Specification	Required Value	Actual Value	Tolerance	Comment
Butterfat (%) - IDF 13:2008	≤2.50	1.26	0	passed
Moisture (%) - IDF 191:2000 PARTS 1-3	≤4.00	2.97	0	passed
Solubility Index (ml) - IDF 129:2005	≤1.5	0.1	0	passed
Sediment (mg) - Niro Method No A 4a	≤15	7.5	0	passed
pH - Niro Method No 4 2b	6 - 7	6.19	In range	passed
Protein (%) - ISO 8968-1 / IDF 20-1:2001	>0	11.6	0	passed
Lactose (%)	>0	73.1	0	passed
Total Count (/g) - ISO 4833:2003	≤30000	600	0	passed
Coli Forms (/g) - IDF 73B:1998	≤10	<1	0	passed
Mould (/g) - IDF 94B:1991	≤50	<5	0	passed
Yeast (/g) - IDF 94B:1991	≤50	<5	0	passed
Salmonella (/25g)	Neg	Neg	none	passed
Heat Treatment - Raw Milk: >72Â°/15 sec	Successful	Successful	none	passed
Heat Treatment - Final Blend: >75Â°/15 sec	Successful	Successful	none	passed

Additional Information

This product complies with the requirements of the SA Foodstuffs, Cosmetic & Disinfectants Act (Act 54 of 72 as amended)

Passed QC Test: YES

Head of Laboratory
Mariettjie Swart
Heilbron (058 853 3218)

Signed:

CHR. HANSEN
Improving food & health

FD-DVS YF-L811 Yo-Flex®

Product Information
Version: 3 PI-EU-EN 11-24-2011

Description

Thermophilic YoFlex® culture.

Taxonomy

Lactobacillus delbrueckii subsp. bulgaricus
Streptococcus thermophilus

Packaging

Material No:	Size	Type
667295	10X50 U	Pouch(es) in box

Physical Properties

Color: Off-white to slightly reddish or brown

Form: Granulate

Application

Usage
The culture will produce yoghurt with very mild flavor, very high viscosity and very low post-acidification. Suitable for cup set, stirred and drinking youghurt.

Recommended inoculation rate

Amount of milk to be inoculated	250 l/ 70 gal	1,000 l/ 250 gal	2,500 l/ 660 gal	5,000 l/ 1,300 gal	10,000 l/ 2,600 gal
Amount of DVS culture	50 U	200 U	500 U	1,000 U	2,000 U

Directions for Use
Remove cultures from the freezer just prior to use. Sanitize the top of the pouch with chlorine. Open the pouch and pour the freeze-dried granules directly into the pasteurized product using slow agitation. Agitate the mixture for 10-15 minutes to distribute the culture evenly. The recommended incubation temperature is 35-45°C (95-113°F). For more information on specific applications see our technical brochures and suggested recipes.

Range

The YoFlex® range of Direct Vat Set (DVS®) cultures spans from very mild cultures to those giving a distinct yoghurt flavor with varying viscosity profiles.

Storage and handling

< -18 °C / < 0 °F

CHR. HANSEN
Improving food & health

FD-DVS YF-L811 Yo-Flex®

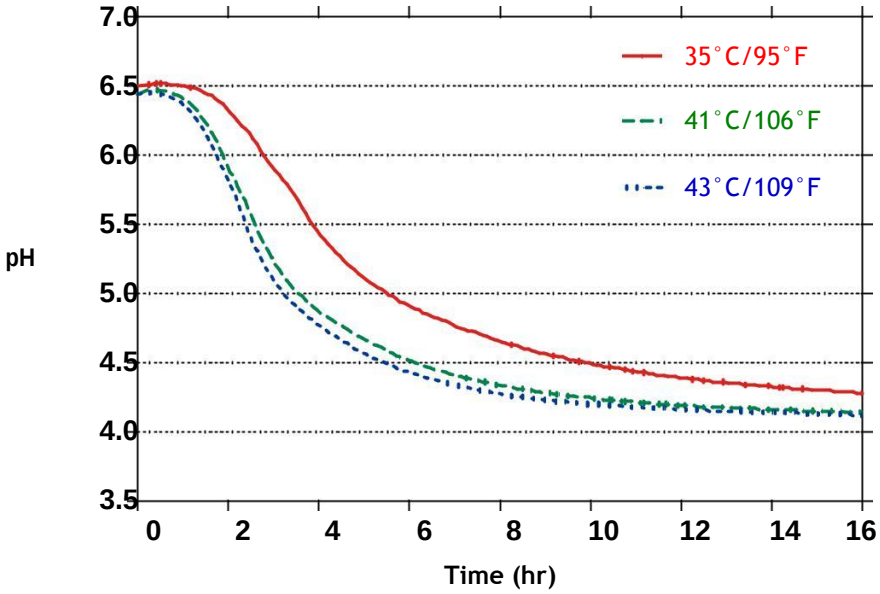
Product Information
Version: 3 PI-EU-EN 11-24-2011

Shelf life

At least 24 months from date of manufacture when stored according to recommendations.
At +5°C (41°F) the shelf life is at least 6 weeks.

Technical Data

Acidification curve



Fermentation conditions:
Whole milk +2 % skim milk powder (85°C/185°F, 30 minutes)
Inoculation: 500U/2500L

Analytical Methods
References and analytical methods are available upon request.

Legislation

Chr. Hansen's cultures comply with the general requirements on food safety laid down in Regulation 178/2002/EC. Lactic acid bacteria are generally recognized as safe and can be used in food, however, for specific applications we recommend to consult national legislation.

The product is intended for use in food.

Food Safety

No guarantee of food safety is implied or inferred should this product be used in applications other than those stated in the Usage section. Should you wish to use this product in another application, please contact your Chr. Hansen representative for assistance.

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CHR. HANSEN
Improving food & health

FD-DVS YF-L811 Yo-Flex®

Product Information
Version: 3 PI-EU-EN 11-24-2011

Labeling	Suggested labeling "lactic acid culture" or "starter culture", however, as legislation may vary, please consult national legislation.	
Trademarks	Product names, names of concepts, logos, brands and other trademarks referred to in this document, whether or not appearing in large print, bold or with the ® or TM symbol are the property of Chr. Hansen A/S or used under license. Trademarks appearing in this document might not be registered in your country, even if they are marked with an ®.	
Dietary status	**Kosher:**	Kosher Dairy Excl. Passover
	Halal:	Certified
Technical support	Chr. Hansen's Application and Product Development Laboratories and personnel are available if you need further information.	

GMO Information

In accordance with the legislation in the European Union* we can state that FD-DVS YF-L811 does not contain GMOs and does not contain GM labeled raw materials**. In accordance with European legislation on labeling of final food products** we can inform that the use of FD-DVS YF-L811 does not trigger a GM labeling of the final food product. Chr. Hansen's position on GMO can be found on: www.chr-hansen.com/About us/Policies and positions/Quality and product safety.

** Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate releas e into the environment of genetically modified organis ms and repealing Council Directive 90/220/EEC.*

*** Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.*
Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labeling of genetically modified organis ms and the traceability of food and feed products produced from genetically modified organis ms and amending Directive 2001/18/EC.

FD-DVS YF-L811 Yo-Flex®

Product Information
Version: 3 PI-EU-EN 11-24-2011

Shelf life

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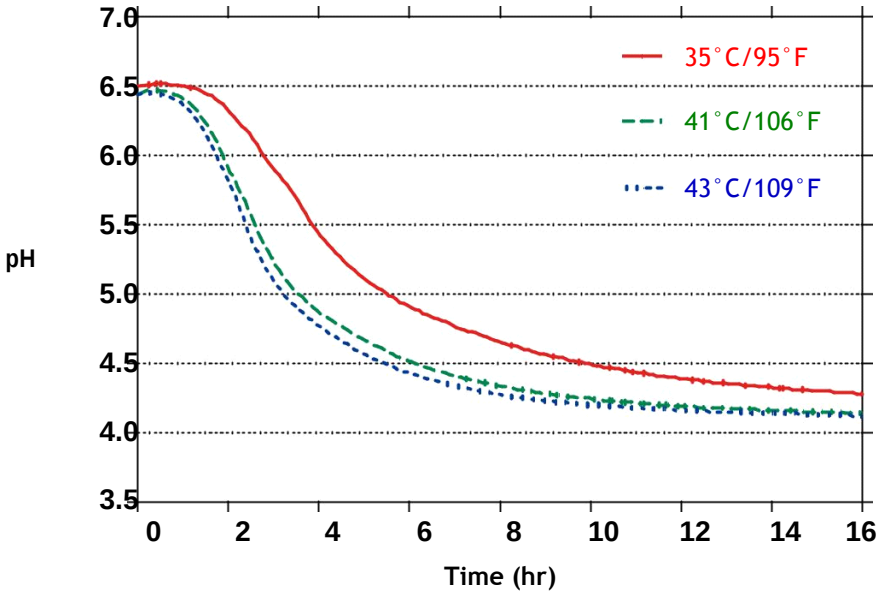

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Inoculation: 500U/2500L

Analytical Methods
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The product is intended for use in food.

Food Safety

No guarantee of food safety is implied or inferred should this product be used in applications other than those stated in the Usage section. Should you wish to use this product in another application, please contact your Chr. Hansen representative for assistance.

product data sheet confidential

Ingredion™

HYLON™ VII Starch

Label Designation	Corn Starch
Source	High Amylose Corn

PHYSICAL AND CHEMICAL CHARACTERISTICS (*):

Color		White to Off-white
Form		Fine Powder
Amylose		72% average
Granulation		
	Through USSS #100	90% minimum
	Through USSS #200	75% minimum

PHYSICAL AND CHEMICAL SPECIFICATIONS:

Moisture	14% maximum
pH (20% slurry)	4.3 – 6.7

MICROBIOLOGICAL SPECIFICATIONS:

Total Plate Count	10,000/g maximum
Yeast	200/g maximum
Mold	200/g maximum
E. coli	negative
Salmonella	negative

Meets NFPA specifications for thermophilic bacteria.

PACKAGING AND STORAGE:

HYLON VII is packaged in multi wall Kraft paper bags with a net weight of 50 lbs. We recommend that HYLON VII be stored in a clean, dry area at ambient temperature and away from heavily aromatic material. The best before date for HYLON VII is 24 months from the date of manufacture.

(*) While this information is typical of HYLON VII it should not be considered as a specification.

Effective Date: October 23, 2013

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