Stearic acid addition to maize starch and its influence on pasting viscosity behaviour

by

Robert Ernö Bajner

Submitted in partial fulfilment of the requirements for the degree

MSc (Agric) Food Science and Technology

In the

Department of Food Science
Faculty of Natural and Agricultural Sciences
University of Pretoria
South Africa

April 2002

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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DECLARATION

I declare that the dissertation herewith submitted for the degree MSc (Agric) Food Science and Technology at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

ABSTRACT

STEARIC ACID ADDITION TO MAIZE STARCH AND ITS INFLUENCE ON PASTING VISCOSITY BEHAVIOUR

by

Robert Ernö Bajner

Study leader: Mrs L. Kuyper

Co-leader: Prof. J.R.N. Taylor

Degree: MSc (Agric) Food Science and Technology

The biphasic pasting curve of maize has only recently been discovered. The cause of the second peak in the biphasic pasting curve of maize starch was not known. It was proposed to be due to formation and dissociation of amylose-lipid complexes with endogenous lipids. The poor stability of the second viscosity peak limits its potential for exploitation in practical applications.

The effects of different high holding temperatures and stearic acid addition on the biphasic pasting curve of normal maize starch were investigated using a Brabender Viskograph-E. Without added stearic acid, the first viscosity peak was unaffected whilst the second viscosity peak was the highest at a holding temperature of 82°C and decreased progressively as the holding temperature was increased to 92°C. Time of occurrence of the second peak increased as holding temperature either increased or decreased from 88°C. At a holding temperature of 90°C, when stearic acid was added up to 1.5 % (dry weight basis of starch), second peak viscosity progressively increased from 1150 to 1900 B.U. and became more stable With stearic acid concentrations of 2.0% and 3.0%, paste viscosity did not increase further during holding but viscosity decreased on cooling, instead of increasing due to setback. With 1.5% stearic acid, second peak viscosity was relatively stable during holding and on subsequent cooling to 25°C but decreased progressively as holding temperatures decreased from 90 to 82 °C. However, as holding temperature was increased above 90°C, second peak viscosity decreased progressively and became unstable, decreasing

during holding and increasing on cooling. These effects are proposed to be due to the formation and thermal stability of amylose-lipid complexes.

To investigate whether these effects were in fact due to formation of amylose-lipid complexes, the effect of increasing amylose content on the pasting of maize starch with added stearic acid was investigated. Treatment with pullulanase, a suitable amylopectin de-branching enzyme, of gelatinized maize starch with 3% stearic acid for different reaction times was investigated. As enzyme reaction time increased from 1.5 to 113.5 hours, the maximum paste viscosity decreased progressively, although the amount of amylose-lipid complexing seemed to increase. This was attributed to increased shear thinning and preferential complex formation with the linear amylopectin segments, which were too short to cause an increase in viscosity. The effect of increasing amylose content using laboratory pre-gelatinized high amylose maize starch with 3% stearic acid could not be determined due to the inability to re-hydrate the starch. This was attributed to strong association of amylose with itself, which could not be prevented during preparation of the pre-gelatinized starch. Investigations that are more conclusive are required. The use of dimethyl sulphoxide to solubilise high amylose maize starch is recommended for further investigations.

The results from this study support the hypothesis that the second viscosity peak in the biphasic pasting curve was due to amylose-lipid complexes, but do not prove it. The development of a high viscosity, shear-stable starch paste by addition of stearic acid has potential for use as a new food ingredient, as well as for non-food applications.

ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

1.1 Statement of problem

Cooking of starch in excess water to obtain a paste is the initial step in the utilization of starch in industrial manufacturing processes such as to thicken and stabilize foods (Zobel, 1988). This operation significantly alters the physical and chemical properties of the starch. Research by Nelles, Dewar, Bason and Taylor (2000) revealed that normal maize starch exhibits a biphasic pasting curve at extended holding times at high temperature, when pasted using a Rapid Visco Analyser. Such biphasic pasting behaviour of maize starch has until now not been noted, and thus presented the opportunity for research into the cause of this phenomenon. The first peak in viscosity was due to gelatinization of the starch followed by viscosity breakdown due to starch granule breakdown and shear thinning. The cause of the second viscosity peak was not known and must still be confirmed. It was proposed to be due to the formation and subsequent dissociation of amylose-lipid complexes formed with the native and other endogenous lipids of the starch. Furthermore, the time and size of second viscosity peak varied with different holding temperatures and the increased viscosity was not stable. Unless the increased viscosity due to the second viscosity peak can be stabilized and the factors causing the variation with different holding temperatures are known, the potential of this phenomenon for practical applications cannot be exploited.

1.2 Literature review

In this review, starch molecular interactions and their effects on paste properties will be discussed, with emphasis on amylose interactions with lipids.

1.2.1 Introduction

In cereals, starch occurs in the endosperm of the grain in the form of insoluble spherical granules of varying size, indicating a very complex macromolecular assembly. The starch granules consist of two main polysaccharides, essentially linear amylose and highly branched amylopectin (as reviewed by Parker and Ring, 2001). The cereal starch granules also contain native proteins and lipids (as reviewed by Kent and Evers, 1994). Starch granules naturally occur in three types, as distinguished by X-ray diffraction patterns (Figure 1), indicating their degree of crystallinity (as reviewed by Kent and Evers, 1994). Generally, cereal starches give A patterns, tubers give B patterns and certain root and seed starches give C patterns. Exceptions do occur, a notable one being high-amylose maize starch, which gives a B pattern because of its high amylose content (Zobel, 1988). The V-pattern is discussed later on.

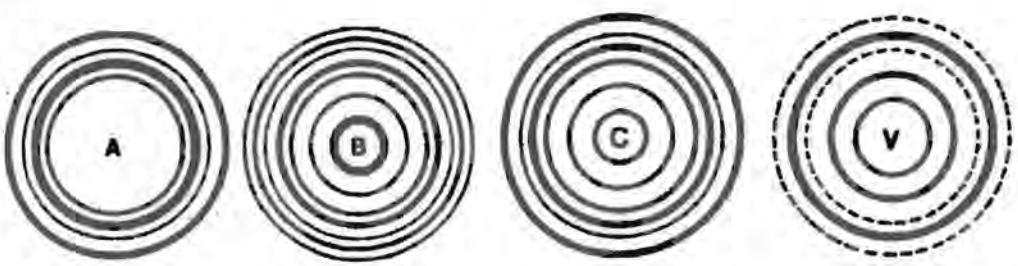


Figure 1: The A, B, C and V-type x-ray diffractions of starch granules (Zobel, 1988)

The granules are up to 30 % crystalline and 70 % amorphous. All the amylose as well as the branched segments of amylopectin are amorphous whilst the linear segments of amylopectin are crystalline (as reviewed by Kent and Evers, 1994). When the granules are viewed directly by light microscopy they exhibit shells consisting of repeating amorphous and crystalline layers, indicating the organisation of amylose

and amylopectin in the starch granule. When viewed under polarised light, the granules are birefringent, exhibiting a Maltese cross. This birefringence indicates the high level of order within the granule (as reviewed by Parker and Ring, 2001).

Starches form a paste when cooked with water. The properties of the paste are affected by various starch molecular interactions. These starch interactions and their effects shall be reviewed.

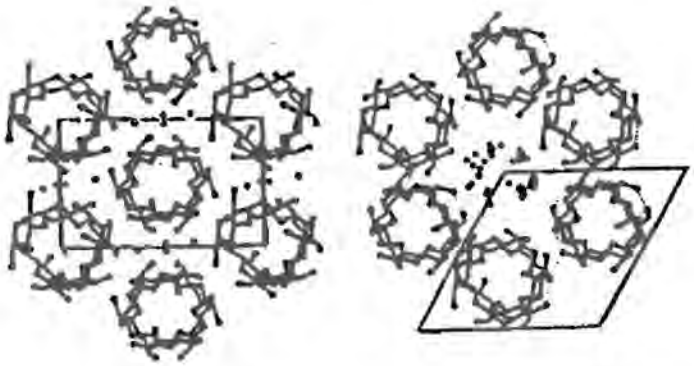
1.2.2 Composition of starch

1.2.2.1 Amylose

Amylose is an essentially linear molecule consisting of 1000 to 4000 1->4 α linked glycopyranose units and can have a molecular weight (M.W.) of 1.6×10^5 to 7.1×10^5 Da (Kent and Evers, 1994). Native amylose occurs mostly in the A and B type X-ray diffraction patterns (Zobel, Young and Rocca, 1988). Both A and B-type starches form double six-fold right-handed helices with six glycosyl residues per turn (Figure 2). This double helix consists of two amylose chains wound parallel around each other, the space between each coil of one chain filled with the coil of the other chain (Wu and Sarko 1978). In crystallised form, the helices are packed anti-parallel in a hexagonal array (Figure 3). This packing conformation leaves a central open channel in the hexagonal array (Wu and Sarko, 1978). In B-pattern starch this open channel is filled with water molecules (Wu and Sarko 1978; Zobel, 1988). The A-pattern starch is more compact with this central channel filled with another amylose double-helix (Zobel, 1988).

Figure 2: Structure of the double helix formed by amylose of A and B type starches (Zobel, 1988)

In maize starch, amylose occurs in the A pattern (cereal starches generally have the A-pattern) (Zobel, Young and Rocca, 1988; Zobel 1988). Amylose can form crystals of single helices with various polar and non-polar compounds such as organic acids, alcohols, lipids, emulsifiers and iodine if they are present during gelatinization. This type of complexing causes starch to give the V-type diffraction pattern shown in Figure 1(Rappenecker and Zugemaier, 1981; Zobel, 1988; Godet, Tran, Delage and Buleon, 1993). These V-structures have been formed and isolated only with amylose (Zobel, 1988). They will be discussed in detail under the section on amylose-lipid interactions.



A-type B-type

Figure 3: Hexagonal packing array of amylose double- helices. The central cavity is filled with another double-helix in A-type starches and with water molecules in B-type starches (Zobel, 1988)

1.2.2.2 Amylopectin

Amylopectin is a highly branched molecule consisting of glycopyranose residues with linear chain segments 1-4 α linked with branch points every 20-25 units, which are 1-6 α linked (as reviewed by Kent and Evers, 1994 and Parker and Ring, 2001). The linear chain segments of amylopectin are shorter for A-type starches than for B-type starches (Hizukuri, 1985). Current models of amylopectin (Figure 4) show the short linear chains arranged in several clusters on the longer chains (Buleon, Colonna, Planchot and Ball, 1998 according to Parker and Ring, 2001). Bands marked 1 (Figure

4) are considered to be crystalline while bands marked 2 are amorphous (Kent and Evers, 1994).

The amylopectin molecules consist of A-chains, B-chains and C-chains. A-chains are the side chains linked only via their reducing ends to the rest of the molecule. They have no other chains attaching to them. B-chains are the slightly longer chains to which the A-chains are attached. They themselves can attach to other B-chains to form several clusters. B chains are further distinguished into Ba chains, if A chains are bound, or Bb chains if A chains are not bound (Figure 5). The C-chains carry the only reducing end of the whole molecule. The inner B-chains can attach to the C-chain, of which there is only one per molecule, as shown in Figure 5 (Hizukuri and Maehara, 1990).

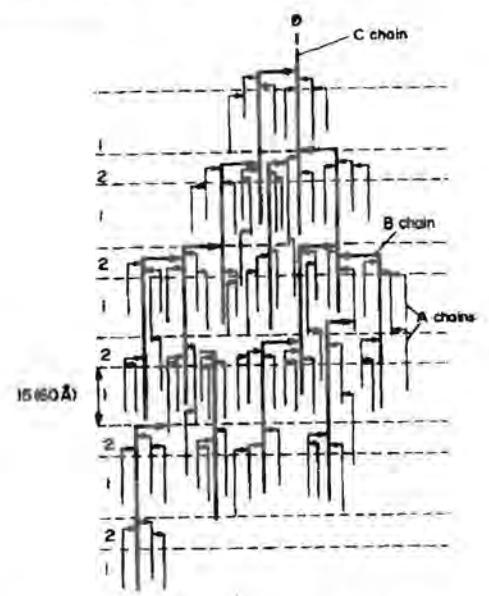


Figure 4: Amylopectin molecule (Kent and Evers, 1994).

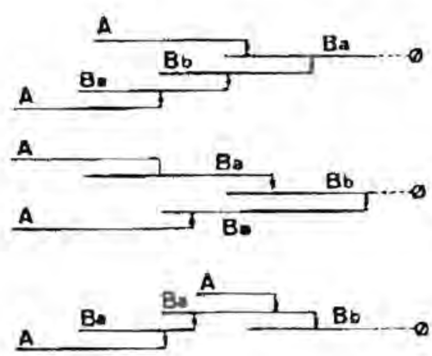


Figure 5: Linking of A, Ba and Bb chains (Hizukuri and Maehara, 1990).

De-branching amylopectin with a suitable de-branching enzyme such as pullulanase (Mercier, Frantz and Whelan, 1972; Yuan, 2000) to cleave α (1-6) branch points results in the release of short chain amylose segments (Huzikuri, 1985; Yuan, 2000). A bimodal population of chain lengths results from de-branching. The first main fraction (F1) has a degree of polymerisation (D.P.) of 12-15 and the second (F2) a

D.P. of 45-60 (Huzikuri, 1985). The F1 fraction is the most abundant by weight and number (Hizukuri, 1985) and forms double helices with each other (French, 1984; Guilbot & Mercier, 1985). This shorter F1 fraction is derived from the mostly un-branched, crystalline domain from the mid-region of the cluster. The longer F2 fraction is derived from the frequently branched, non-crystalline domain of a cluster (Hizukuri and Maehara, 1990). The distribution graphs, obtained by size-exclusion high performance liquid chromatography, of the F2 fractions of many amylopectins also have shoulders of around D.P. 18-20, indicating the presence of intermediate chain lengths (Hizukuri and Maehara, 1990; Hanashiro, Abe and Hizukuri, 1996). In general, amylopectin molecules of A-type starches have shorter chains in both of these fractions than those of the type B starches (Hizukuri, 1985). Hanashiro *et al*, (1996) found maize starch to differ slightly from other cereal amylopectins. They determined maize starch to have a F1 fraction with D.P. ~12, F2 fraction with D.P. ~30 and a shoulder to the F2 fraction with D.P. ~24 (Hanashiro *et al.*, 1996).

It is possible that on enzymic de-branching amylopectin can yield a third fraction of extra long chains (chain length 85-180) of sufficient uninterrupted length which could form a single helix, similar to V-amylose. Thus possibly forming V-helical inclusion complexes with added lipids (Takeda, Hizukuri and Juliano 1987). According to Takeda *et al.* (1987) these long chains would be derived from long Ba-chains with side-chains widely spaced and located far from the non-reducing end. Huzukuri and Maehara (1990) did find the presence of such extra long Ba chains in wheat amylopectin, which were thought to bind several A chains. However, conclusive proof remains to be established whether V-structures can form with the amylose-like segments of certain de-branched amylopectin fractions (Zobel, 1988).

1.2.3 Starch-water interactions

1.2.3.1 Gelatinization

When starch is cooked in an aqueous medium, the suspension changes markedly from a low-viscosity granular slurry to a viscous paste consisting of a dispersion of swollen granules, partially disintegrated granules and molecularly dispersed granule contents.

This transformation is due to the gelatinization of the starch suspension (as reviewed by Zobel, 1984).

When starch is heated to a characteristic gelatinization temperature range in an excess of water, the starch granules swell irreversibly to many times their original size due to the uptake of water. This swelling is accompanied by a progressive loss of the crystalline order within the granule (as reviewed by Parker and Ring, 2001). The loss of crystallinity is also detected by a loss of birefringence (Maltese crosses) under polarized light (as reviewed by Kent and Evers, 1994). Hydration of the granules begins in the amorphous regions and is strongly temperature dependant. Once this hydration is strong enough, it overcomes the H-bonds and van de Waal forces holding together the adjacent crystalline region. Disruption of a crystalline region leads to only a partial solubilisation as amylopectin molecules span several crystalline regions. As the initial solubilisation of these crystalline regions increases, the local water content of the granule increases leading to the subsequent solubilisation of more crystalline regions. Gradually the whole granule is gelatinized. During this gelatinization process, amylose is preferentially solubilised. Substantial solubilisation of high molecular weight (HMW) amylopectin does not occur although some low molecular weight (LMW) amylopectins may be solubilised (as reviewed by Parker & Ring, 2001).

As heating continues beyond the gelatinization temperature range, the granules continue to swell. This increase in volume fraction of the starch granules in suspension leads to an increase in viscosity up until the peak viscosity is reached, as seen in Figure 6 (as reviewed by Parker and Ring, 2001). Excessive swelling of the starch granule may even burst the granule, dispersing partially disintegrated granules and granule contents into the suspension (as reviewed by Zobel, 1984). Uptake of water by the granules during swelling also reduces the mobile phase surrounding the granules, and with the accompanying leaching of starch polymers into this reduced mobile phase, the viscosity of the paste is further increased (as reviewed by Kent and Evers, 1994). Thus pasting is the increased viscosity observed after gelatinization due to the combined effects of granular swelling, reduced mobile phase and leaching of granular components into the mobile phase. Lower water to starch ratio suspensions exhibit higher pasting viscosities than high water to starch

suspensions due to less mobile phase, which is also more saturated, in the suspension (as reviewed by Kent and Evers, 1994).

Furthermore, lower water content suspensions have broader gelatinization temperature ranges than high water content suspensions (Ghiasi, Hoseney and Varriano-Marston, 1982). The granules that have the easiest access to the available water gelatinize first. If the amount of available water is limited, these granules reduce the amount of water available for the hydration of the other granules, which then require higher temperatures to overcome the forces of the crystalline regions (as reviewed by Parker and Ring, 2001). The beginning of gelatinization temperature is the same, but at lower water contents the temperature required to achieve complete gelatinization is higher, thus broadening the gelatinization temperature range (Ghiasi *et al.*, 1982).

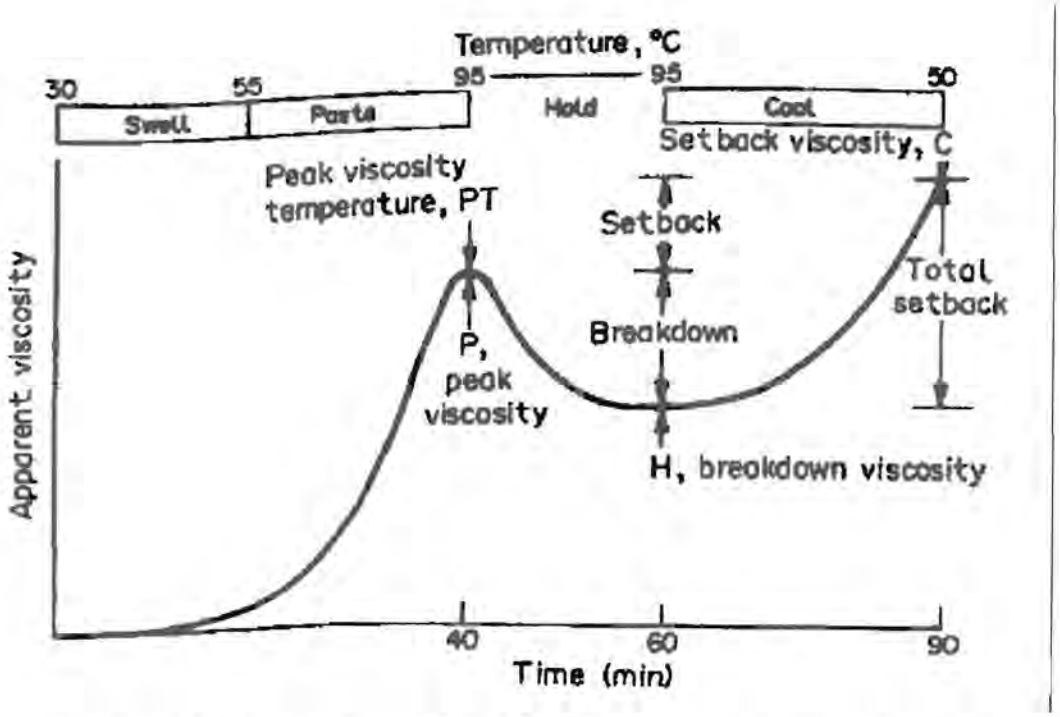


Figure 6: Chart showing characteristics of a starch paste as recorded by the Brabender Amylograph (Kent and Evers, 1994)

1.2.4 Starch-starch interactions

1.2.4.1 Entangled starch network

During pasting, amylose molecules escape from the swollen granules into the continuous phase. Some amylopectin is also released into the continuous phase by the disintegration of granules on excessive swelling (as reviewed by Kent and Evers, 1994). Together, these starch molecules form an extensive entangled polymer network in the continuous phase held together by extensive intermolecular H-bonding and electrostatic forces. This starch network traps the available water within it. Having an affinity for water, this network will absorb water and swell until the osmotic pressure generated by the network is balanced by the restorative stiffness of the network which resists swelling. Since amylose is linear, its entanglement in the network is not thought to limit swelling, but rather the entangled amylopectin is thought to limit the swelling of the starch network (as reviewed by Parker and Ring, 2001). On continuous stirring (shear action), this entangled starch network is broken up as the starch molecules, most notably linear amylose, align in direction of stirring. Known as shear thinning, this molecular alignment decreases the water holding capacity of the starch network and increases the mobility of the dispersed starch molecules (as reviewed by Kent and Evers, 1994). This effect is observed as a breakdown of paste viscosity and forms the down-side of the pasting peak of an amylograph (Figure 6).

1.2.4.2 Retrogradation

Retrogradation is observed as increasing paste viscosity (set-back) and significant firming of the starch (gel formation) on cooling (Figure 6). This is due to the re-association of the hydrated, dispersed starch molecules into an ordered three-dimensional network (Zobel, 1984). On cooling, the entangled, swollen starch network formed during pasting loses its translational motion and traps the water. Starch crystallites eventually begin to form thus increasing the paste viscosity (as reviewed by Kent and Evers, 1994). Amylose undergoes a strong retrogradation, observed as a rapid increase in paste viscosity and formation of a strong gel, due to its long linear structure promoting its crystallisation. Conversely, amylopectin does not crystallise as readily due to its highly branched structure. Compared to amylose, the

retrogradation of amylopectin is observed as a slower increase in paste viscosity and results in the formation of a less firm gel, the extent of which is proportional to the amount of short chains with D.P. 16-30 and inversely proportional to the level of short chains with D.P. 6-11 (as reviewed by Parker and Ring, 2001). Thus, retrogradation is largely due to the crystallisation of amylose and there is a strong driving force towards crystallisation into the B-structure (Zobel *et al.*, 1988).

1.2.5 Starch-lipid interactions

Cereal starches contain lipids, either native to the starch granule or accumulated from other parts of the grain (e.g. the germ) during starch extraction, with which the starch may interact (as reviewed by Parker and Ring, 2001)

1.2.5.1 Amylose – lipid interactions

1.2.5.1.1 Structure of Vh-amylose

The V-type crystallised structures are formed from collapsed A or B-type double helices, which yield a left-handed single helix with 6 glycosyl residues per turn (Figure 7). The complexing adjuncts replace the water molecules initially in the helix channel and are subsequently trapped inside the newly formed single helix (Rappenecker and Zugenmaier, 1981). The V-helix can exist with or without the complexing molecule after its initial formation. If the complexing agent is removed, or is readily soluble in water, a labile V-helix results that quickly retrogrades to the B-structure (Zobel, 1988). The V-crystals occur in a dry and hydrated state, designated as V_a and V_h respectively. (Rappenecker and Zugenmaier, 1981). The amylose complexes obtained with polar lipids yield the V_h -type x-ray diffraction pattern (Biliaderis and Galloway, 1989; Godet *et al.*, 1993). V_h-amylose is structured as a single left-handed helix consisting of six glucosyl (H_5) residues per turn and is stabilised by an intensive H-bonding network between interstitial water molecules and adjacent hydroxyl groups, on the outer surface of the helix (Carlson, Larsson, Dinh-Nguyen and Krog, 1979; Rappenecker and Zugenmaier 1981; Godet *et al.*, 1993). The helix cavity is effectively hydrophobic (Godet *et al.*, 1993) excepting for two water molecules inside the helix channel, which are H-bonded to each other but not with the

helix. They are prevented from contact with the helix by hydrophobic forces, which also trap them within the helix until they are replaced with the complexing molecule (Rappenecker and Zugenmaier, 1981).

When V_h-amylose, either still in the swollen granule or in solution, is complexed with a lipid the hydrocarbon chain of the guest lipid lies within the hydrophobic helical cavity of the V-amylose to form the amylose-lipid complex (Figure 8) (Godet *et al.*, 1993; Jovonovich and Anon, 1999). In complexes with monoacyl lipids and free fatty acids, each helical turn consists of six glycosyl residues (Godet *et al.*, 1993) but up to seven or eight glycosyl residues per turn are possible for more bulky guest molecules (as reviewed by Karkalas, Ma, Morris and Pethrick, 1995).

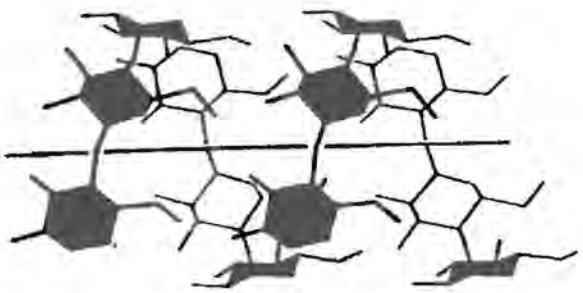


Figure 7: Two turns of a amylose six residue, single V-helix. The position of the complexing compound is indicated by the line inside the helix (Zobel, 1988)

In a monostearin-amylose complex, the hydrocarbon chain in the helix corresponds to three helical turns, as shown in Figure 8 (Carlson *et al.*, 1979). The hydrocarbon chain is stabilised within the amylose helix by hydrophobic interactions and van der Waals forces (Raphaelides and Karkalas, 1988). The H_5 atoms of each glycopyranose residue is involved with short van der Waals contacts with the two nearest CH_2 groups of the aliphatic fatty acid chain (Godet *et al.*, 1993). This is the only possible van der Waal contact between the lipid ligand and the amylose helix (Godet *et al.*, 1993). Only a very small amount of energy is required to overcome the van de Waal forces within the helix (Karkalas *et al.*, 1995). Since there is a limited number of van de Waal contacts, between the fatty acid chain and the Vh-amylose helix, and these being relatively weak forces, the major stabilizing force for the complex must be the

lower entropy of the complexed fatty acid as compared with the free state. The small differences in stability are related to the fatty acid chain length (Karkalas *et al.*, 1995).

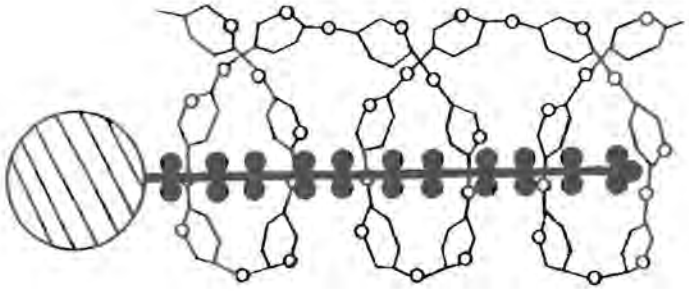


Figure 8: Schematic representation of an amylose-lipid helical complex with monostearin. The polar head of the complexing lipid is completely outside of the helix while the hydrocarbon chain occupies the helix cavity (Carlson *et al.*, 1979).

The geometries of the linear aliphatic chain and amylose helix are so complementary that very little movement is permitted in the amylose-lipid complex (Godet *et al.*, 1993). Thus, the formation of such an inclusion complex immobilises the fatty acid within the amylose helix (as reviewed by Morrison, 1995). The very tight packing and steric hindrance, provoked by the H5 atoms of the glycopyranose residues protruding into the helix, would explain why these complexes form only at high temperatures when the flexibility of the two moieties is high enough to lead to inclusion (Godet *et al.*, 1993).

The polar carboxylic group of the fatty acid is located at the helix periphery, completely outside of the helix (Carlson *et al.*, 1979; Godet *et al.*, 1993). The carboxylic group is prevented from entering the helix by steric hindrance and electrostatic repulsions (Godet *et al.*, 1993). This acts to further stabilise the complex by preventing displacement of the aliphatic chain along the axis of the helix (Godet *et al.*, 1993). Thus, the polar head acts as a "cork" after the helicoidal driving in of the aliphatic chain.

Amylose-lipid complexing can be well characterized by Differential Scanning Calorimetry (DSC), and has been extensively by Kugimiya, Donovan and Wong, (1980); Stute and Konieczny-Janda, (1983), Eliasson and Krog, (1985), Eliasson,

Finstad and Ljunger, (1988); Raphaelides and Karkalas, (1988) and Villwock, Eliasson, Silverio and BeMiller (1999).

1.2.5.1.2 Effect of lipid chain length

The acyl chain length exerts considerable influence on the properties of the amylose-lipid complexed starch paste (Raphaelides, 1992). There exists a minimum aliphatic chain length for complexing to occur. Short chain fatty acids, less than C10:0 such as caprylic acid (C8:0), do not give rise to saturation of the crystallised amylose helices, possibly due to their higher solubility in the amylose-crystallisation medium as an effect of their shorter chain lengths (Karkalas and Raphaelides, 1986; Godet, *et al.*, 1993; Godet, Tran, Lelage and Buleon, 1995).

In the model proposed for inclusion complexes by Godet *et al.* (1993) one methyl group (CH_2) on the fatty acid chain corresponds to one glucosyl residue along the helix axis. Inclusion of C8:0 inside the helix would correspond to complexation of only five glycosyl residues of the amylose, which is less than one helical turn, as determined by the stoichiometrical ratio of CH_2 groups: glucose residues by Godet *et al.* (1995). Aliphatic chains of length 12-18 C readily form the amylose-lipid complex (Yuan, 2000). Complexes with fatty acids shorter than 10:0 are rapidly transformed into B-type crystalline amylose in the presence of water (Godet *et al.*, 1995).

At low temperatures (below 40°C) longer fatty acid chains result in firmer starch gels (higher viscosity). Since fewer fatty acid molecules are required for saturation of the available amylose helices, the electrostatic forces between amylose-lipid complex helices decreases, resulting in more rigid gels. (Raphaelides, 1992).

1.2.5.1.3 Effect of unsaturated lipids

The aliphatic chain of the lipid lies inside the helical cavity in the trans conformation (Carlson *et al.*, 1979; Godet *et al.*, 1993). Inclusion of the fatty acid aliphatic chain inside the amylose helix is possible in terms of steric hindrance and interaction energy

only if the aliphatic chain is trans-conformation for unsaturated chains or fully saturated (Godet *et al.*, 1995). Unlike the trans-conformation double bonds, which maintain linear structure of the aliphatic chain, cis-conformation double bonds create a kink in the aliphatic chain that gives it a non linear structure (Eliasson and Krog, 1985; Raphaelides and Karkalas, 1988).

Contrary to the statement of Godet *et al.* (1995), cis-unsaturated fatty acids do show a limited ability to form inclusion complexes with Vh-amylose, although far less effectively than trans-unsaturated fatty acids (Raphaelides and Karkalas, 1988; Karkalas *et al.*, 1995). It is probable that there is some free rotation about the C-C bonds adjacent to the cis- C=C bonds which allows the cis-unsaturated fatty acid to adopt a quasi-linear conformation around the kink double bond which allows it to enter the Vh-amylose helix cavity (Karkalas *et al.*, 1995).

The probability of forming such a complex decreases with the increasing number of cis- C=C bonds. In order to accommodate the quasi-linear kink in the cis-unsaturated chain, the V_h-amylose helix will have to expand and increase the number of glycosyl residues per turn from six to seven or a eight, as happens with other bulky guest molecules. Thus there is a limit to the number of cis-double bonds that can be accommodated (French, 1984; Karkalas *et al.*, 1995). With low levels of cis-unsaturation the stoichiometry of the complex is not greatly affected by the expansion to a V_7 helix. A similar number of H-bonds and van der Waal forces will be involved in stabilizing the bond as in a V_6 helix. At higher levels of cis-unsaturation there will be a systematic lowering of complex stability as a result of the helix being too stretched out, significantly weakening the van der Waal forces and reducing the number of H-bonds (Raphaelides and Karkalas, 1988; Karkalas *et al.*, 1995). However, complex stability does not seem to be related to complex forming ability (Eliasson and Krog, 1985; Kaur and Singh, 2000).

1.2.5.1.4 Lipid chain orientation within the helix

Due to structural symmetry of the amylose 6_{5H} helix, three orientations of the trans aliphatic chain inside the helix are possible, at 60° rotation increments, related to the

6th order symmetry of the helix (Figure 9) (Godet *et al.*, 1993). The aliphatic hydrogen atoms tend to point towards the less crowded regions inside the helix, near the glycosidic oxygens of the amylose (Godet *et al.*, 1993). Thus any movement of the aliphatic chain within the helix is prevented by these three orientations (mean planes I, II and III): plane I prevents rotation whilst plane II and III freeze any movement in translation as well as in screwing (Godet *et al.*, 1993). This very close packing further shows the conformation symmetries of these two structures.

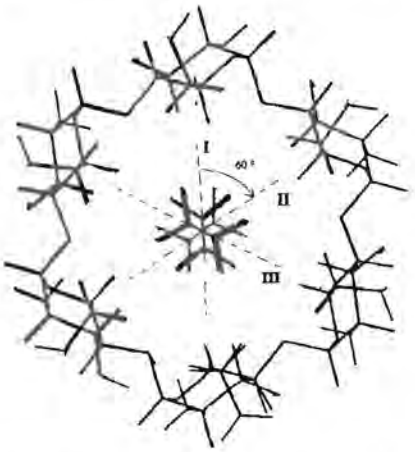


Figure 9: Schematic representation of the three possible orientations, at 60° increments, of the hydrocarbon chain within the amylose V-helix as related to the 6th order symmetry of the helix (Godet *et al.*, 1993)

1.2.5.1.5 Types of amylose-lipid complexes

Two thermally distinct forms of the amylose-lipid complex exist, namely Type I and Type II. Most amylose-lipid complexes are insoluble and amorphous Type I, but can be annealed into a crystalline form, Type II (Biliaderis and Galloway, 1989). The generalized mechanism for formation of these complexes is shown in Figure 10. These two types of complexes differ in the organisation of the helices (Biliaderis and Galloway, 1989; Morrison, 1995). The conversion of Type I into Type II requires a partial 'melting' or disaggregation to obtain sufficient mobility for crystallite formation, with the un-melted Type I helical structures acting as nuclei for crystallisation (Biliaderis and Galloway, 1989). Type I and type II complexes are present in varying ratios depending on the duration of heating (Karkalas *et al.* 1995).

As seen in Figure 10, Type I complexes consist of a random distribution of helices, which do not crystallise with each other. They are obtained from rapid nucleation (Biliaderis and Galloway, 1989). Biliaderis and Galloway (1989) postulate that Type I represents a separate thermodynamic state, with internal energy and entropy intermediate between those of a melt entity and a crystalline system. Type I complexes form below 60 °C (before gelatinization) and dissociate into helices and free lipids when heated in water in the range of 94-104 °C (Raphaelides and Karkalas, 1988; Karkalas *et al.*, 1995). The dissociation of Type I complexes involves mostly the breaking of weak intra-helical H-bonds and the few van de Waal contacts present to uncoil the helix and release the complexing lipid (Karkalas *et al.*, 1995; Jovanovich and Anon, 1999).

The Type II complex is effectively a superstructure consisting of several complexes crystallised together by inter-helical H-bonds and van de Waal contacts, embedded in and molecularly continuous with disordered chain segments (Figure 10) (Biliaderis and Galloway, 1989). Type II has a much lower internal energy and entropy than Type I (Biliaderis and Galloway, 1989). Type II complexes form above 90°C (after gelatinization) and dissociate at 100-125°C (Kugimiya *et al.*, 1980; Zobel, 1988; Karkalas *et al.*, 1995). The dissociation of Type II complexes, in addition to breaking intra-helical H-bonds and van de Waal contacts, involves true melting of crystallites into an amorphous entity, breaking inter-helical H-bonds and several van de Waal contacts on a supermolecular level (Karkalas *et al.*, 1995; Jovanovich and Anon, 1999). Type II complexes occur in two forms, type II (a) and Type II (b) (Biliaderis and Galloway, 1989; Seneviratne and Biliaderis, 1991). Type II (a) is the partially crystalline form, initially formed during gelatinization. Type II (b) is formed on further heating after gelatinization and is fully crystallised into ordered domains (Biliaderis and Galloway, 1989; Seneviratne and Biliaderis, 1991). It arises from the progressive development of larger, more perfect crystallites upon annealing and results in several metastable crystalline structures (Biliaderis and Galloway, 1989). The Type II (b) is the most stable form of the amylose-lipid complex. With less water present between crystallised helices, it has the highest dissociation energy due to the higher number of van de Waal forces between crystallised helices, which replace

many of the initial inter-helical H-bonds with interstitial water molecules (Rappenecker and Zugenmaier, 1981).

It is postulated that the relative effects on paste viscosity of these different types of amylose-lipid complexes are determined by their degree of crystallinity. Thus amorphous Type I complexes would have the lowest paste viscosity, followed by the semi-crystalline Type II (a) and the crystalline Type II (b) would have the highest paste viscosity. The Type II(b) complex is suggested to be responsible for the second peak in viscosity observed in the pasting curve of maize starch (Nelles *et al.*, 2000).

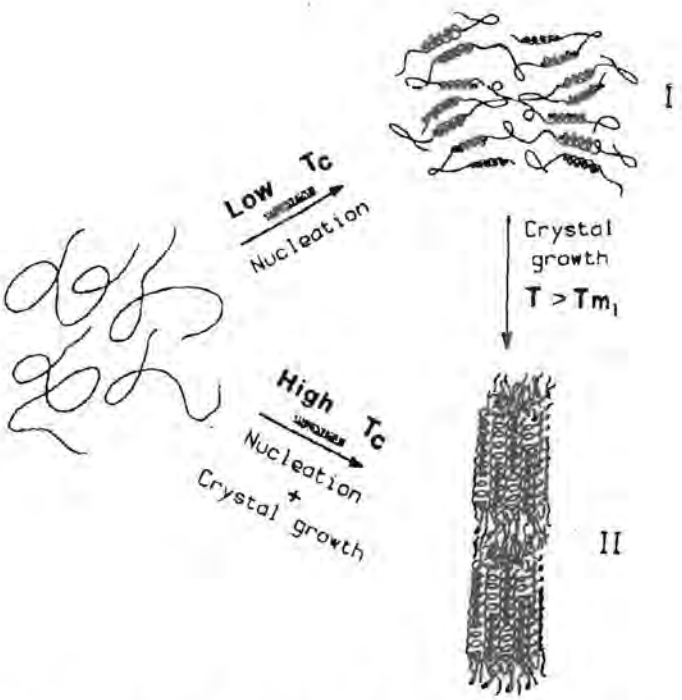


Figure 10: The generalized mechanism for amylose-lipid complex formation into type I and type II complexes (Biliaderis and Galloway, 1989)

1.2.5.1.6 Stability of amylose-lipid complexes

As previously discussed, the amylose-lipid complexes exhibit a thermal transition at high temperatures (beyond 100 °C) in excess water, which is interpreted as melting or dissociation of the complex (Kugimiya *et al.*, 1980; Raphaelides and Karkalas, 1988;

Karkalas *et al.*, 1995). However, this transition is thermo-reversible, with the amylose-lipid complex reforming on cooling (Kugimiya *et al.*, 1980). V_h-amylose complexes with free fatty acids or a hydrophobic surfactant side-chain have been found to be stable in pure water up to temperatures of 112-120 °C (Kugimiya *et al.*, 1980; Stute and Konieczny-Janda, 1983).

Ultimately the transition temperature, and thus the thermal stability of the formed complex, is determined by the properties of the complexing lipid. The transition temperature is influenced by chain length, degree of unsaturation and water content. The thermal stability of the inclusion complex was found to increase with increased chain length of the fatty acid and decreased with increasing unsaturation. Thus short chain lengths and/or unsaturated lipids are far less thermostable than long chain and/or saturated lipids. (Eliasson and Krog, 1985; Karkalas *et al.*, 1995). However it is suggested that the complexes of amylose with fatty acids of chain length C10-20 do not differ materially in their thermal stability, all having a dissociation enthalpy of ~30 J/g of complexed amylose (Eliasson and Krog, 1985; Raphaelides and Karkalas, 1988). These effects are probably related to the amount of H-bonding that can occur within the helix to stabilise it: the longer the chain and the less double bonds present, the more CH_2 groups are available for stabilisation bonds (Eliasson and Krog, 1985; Karkalas *et al.*, 1995). The influence of unsaturation is much stronger for fatty-acid complexes than for other lipid complexes (Stute and Konieczny-Janda, 1983; Elliasson and Krog, 1985).

Furthermore, the carboxyl group is sensitive to ionization by high pH and solute concentrations (Karkalas *et al.*, 1995). Low concentration salts and other low molecular weight solutes interact with the carboxyl head of the complex. Cations (e.g. Ca^{2+}) have a stabilising effect on the complex whilst anions (e.g. Cl^-, OH^-) have a destabilising effect by ionizing the complex by, thus lowering the dissociation temperature (Jovanovich and Anon, 1999). At high concentrations, both cations and anions destabilise the complex, probably due to increased electrostatic interactions in the medium (Jovanovich and Anon, 1999). Karkalas and Raphaelides (1986) found that amylose-lipid complexes precipitate from solution at pH 4.6. The insolubility of the complex at such a low pH is probably due to electrostatic repulsion in the acidic

(excess H^+) environment inhibiting the complex from H-bonding with the surrounding water molecules (Karkalas *et al.*, 1995).

1.2.5.1.7 V_h-complex superstructures

Several amylose-lipid complexes can interact via various van de Waals forces and H-bonds to agglomerate into large, ordered crystalline superstructures (Biliaderis and Galloway, 1989; Karkalas *et al.*, 1995).

Since the carboxyl group is not within the hydrophobic helix, it can interact with the polar exterior of other amylose-6_{5H} helices by H-bonding with inter-helical water molecules (Godet *et al.*, 1993) to form part of a complex superstructure. Karkalas *et al.* (1995) suggests that ionization of the carboxyl group must be suppressed or prevented in order to promote H-bonding and thus allow for flocculation of helical complexes.

Amylose helices have a tendency to align and form long helical chains consisting of several amylose molecules (Karkalas and Raphaelides, 1986; Karkalas *et al.*, 1995). The length of the adjoined helical segments is determined by the length of the lipid chain within the helix and the number of amylose molecules that lie end to end (Karkalas *et al.*, 1995). Often the amylose helix is much longer than the aliphatic chain and the helix is incompletely filled (Karkalas and Raphaelides, 1986). The long V-helical chains of the complex super-structure are thus interrupted by short segments of uncomplexed amylose (Karkalas and Raphaelides, 1986; Biliaderis and Galloway, 1989; Seneviratne and Biliaderis, 1991). These uncomplexed segments permit random orientation of helical segments in type I complexes and folding into parallel and anti-parallel arrays in type II structures (Karkalas and Raphaelides, 1986; Biliaderis and Galloway, 1989).

There are also several water molecules in the interstitial spaces between adjacent complexed helices. These are H-bonded to oxygens 2,3,4 and 6 of the glycosyl residues on the exterior of the V_h-amylose helix. These H-bonds, and several inter

helical van der Waal contacts, flocculate the amylose-lipid complexes to form crystallised superstructures (Rappenecker and Zugenmaier, 1981). The V_h structures are found to be hexagonally packed into a crystal lattice, as shown in Figure 11 (Rappenecker and Zugenmaier, 1981; Godet, 1995). The voids present in the crystal lattice are too small to accommodate fatty acid chains. Thus, these voids are more suitable for accommodating smaller molecules such as water or short-chain alcohols (Godet, 1995). Formation of such amylose-lipid complex superstructures significantly increase the peak viscosity of the resulting starch paste (Kaur and Singh, 2000).

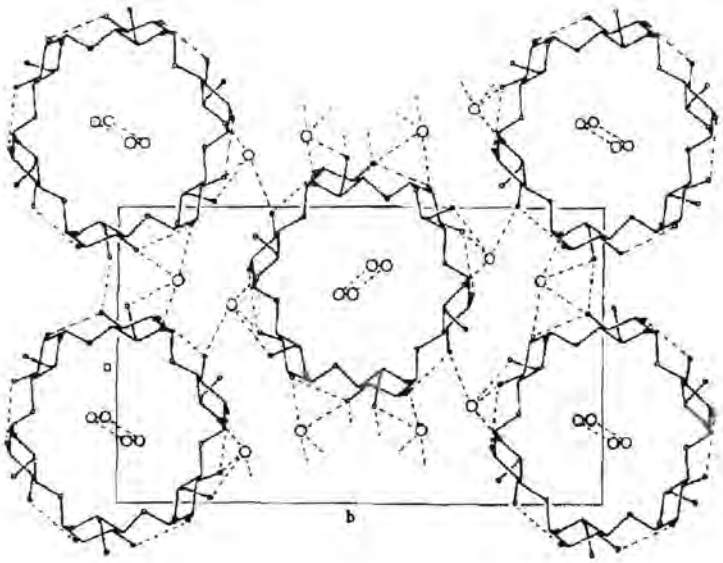


Figure 11: Hexagonal packing of V-helical amylose to form a crystal lattice. Dotted lines show the H-bonding arrangement. The helices are shown to contain the water molecules initially in the helix cavity (Rappenecker and Zugenmaier, 1981)

1.2.5.1.8 Complex forming ability of different varieties of maize starch

Different variety maize starches show different complex forming abilities. Eliasson *et al.* (1988) determined by DSC, that high-amylose maize (high-maize) starch, had the highest complex forming ability, followed by normal maize starch. Waxy maize starch showed no endotherm in the range of the amylose-lipid complex thus it can be concluded that it does not form amylose complexes (Eliasson *et al.*, 1988; Villwock,

et al., 1999). The ability to form Vh-amylose complexes is directly proportional to the amylose content of the starch (Villwock *et al.*, 1999). As expected, more amylose-lipid complexes form in starches with higher amylose content (Bhatnagar and Hanna, 1994a). High amylose maize (amylomaize) starch has an amylose content from 50 to 70% or higher, whereas normal maize starch has a amylose content of about 25 % whilst waxy maize starch has an amylose content of 1% or less, with the differences consisting of amylopectin (as reviewed by Moore, Tuschhoff, Hastings and Schanefelt, 1984).

Due to its very high amylose content, amylomaize starch granules form very tightly packed granules (due to extensive H-bonding between adjacent linear amylose molecules) that are resistant to swelling (as reviewed by Moore *et al.*, 1984). Thus, amylomaize starch cannot be gelatinized (to solubilise amylose for complexing) by cooking under normal conditions but requires the elevated temperatures of pressure cooking (Moore *et al.*, 1984). Bhatnagar and Hanna (1994a) determined that amylose-lipid complexing can also be induced in amylomaize starch (70 % amylose) by single screw extrusion, due to the high pressures and temperatures achieved during extrusion. Once gelatinized, amylomaize starch retrogrades extremely rapidly into a high-strength crystalline gel, again due to its high amylose content (as reviewed by Moore *et al.*, 1984).

1.2.5.1.9 Complexes with native lipids

Zobel *et al.*, (1988) found that heating normal maize starch with selected heat/moisture treatments caused the amylose to complex with the native granule lipids. These lipids consist mostly of free fatty acids and some phospholipids (Zobel *et al.*, 1988; Villwock *et al.*, 1999). Analysis of the composition of these native free fatty acids in normal maize starch by Villwock *et al.* (1999) showed only C16:0, C18:0, C18:1, C18:2 and C18:3 to be present in the following proportions 23 : 1,6 : 11 : 62 : 2,8 respectively. The native phospholipids are usually more complex and bulky compounds and thus do not form as stable a complex as the free fatty acids (Tomasik and Schilling, 1988).

X-ray diffraction patterns taken during the heating of maize starch showed the formation of V_h-type crystallinity (Zobel *et al.*, 1988). The ease with which these V_h-structures form suggests the amylose in maize starch to be separate from the amylopectin (Zobel, 1988). While both amylose and amylopectin can form A- and B-structures, cereal starches have native lipids (lysophospholipids and free fatty acids) associated with them that provide a mechanism for fractionating amylose from amylopectin by the formation of V_h-structures in the starch granule (Zobel, 1988; Karkalas *et al.*, 1995). Thus, in granular maize starch, the amylose probably exists to some extent as an amorphous entity associated with these native lipids.
Zobel *et al.*, (1988) determined that the V_h-type structure begins to appear just prior to the melting of the A-structure (occurs at 66-73 °C in excess water just before gelatinization). Since the granules at this stage are swollen but intact, suitable conditions are provided for effecting formation of the V_h-structure within the starch granules (Zobel *et al.*, 1988). This early appearance of a V_h-structure suggests that it is initially present as a crystal nuclei that will further develop, under favourable heat and moisture conditions, as the A-structure continues to melt. (Zobel, 1988) This initial nucleation is the limiting step in amylose-lipid complex formation (Jovanovich and Anon, 1999).

1.2.5.1.10 Complexes with added lipids

To amplify the lipid complexing effect, additional complexing agents can be added to the starch before processing under conditions conducive to complexation. For moisture contents of 18-45 % this would be heating at 90-130 °C for 1 to 16 hours, depending on the compound added (Zobel, 1988). Amylose complexing agents in various starches have been reported to result in higher gelatinization temperatures, improved dispersability in hot water, improved paste consistency, less granular swelling, less shear in the pasted state and less retrogradation on cooling (Krog, 1973 according to Kaur and Singh, 2000; Zobel, 1988). These results are due to the combined effects of both native and added lipids (Villwock *et al.*, 1999).

The amylose-lipid complex forms readily with several added lipids, such as lysophosphatidylcholine (phospholipid), monomyristin, monopalmitin and

monostearin (monoglycerides) and several types of emulsifiers (Cui and Oates, 1999; Mahr and Trueck, 1999; Fischer, Wursch and Plante, 1999; Yuan, 2000). The complexing ability differs between different lipids depending on their structure. It is the highest for saturated monoglycerides, very low for diglycerides (e.g. lecithin) and zero for triglycerides (Eliasson *et al.*, 1988). Monoglycerides and fatty acids with chain lengths from C12-C18 are very effective amylose complexing compounds in aqueous solution (Krog and Lauridsen, 1976). All these cases of lipid addition to starch generally noted an increase in viscosity of the pasted starch due to the formation of amylose-lipid complexes, relative to the complex-forming ability of the added lipids. Thus, the addition of saturated monoglycerides and fatty acids had the greatest effects of increased paste viscosity (Mahr and Trueck, 1999; Fischer *et al.*, 1999; Yuan, 2000).

The concentration of the complexing lipid has a great effect on the strength of the starch paste. At a low lipid concentration, few helices are formed and the gel is weakened since fewer junction zones exist. When the lipid concentration increases, helix formation becomes dominant until saturation level of the available amylose is reached, thus contributing to the rigidity of the gel (Raphaelides, 1992). Fischer *et al.* (1999) found the native starches they had heat-modified with monoglycerides (to induce amylose-lipid complexing) to maintain visco-stability during holding at 95 °C and subsequent cooling to 40 °C in a Brabender Viskograph. The visco-stability improved with increasing levels of monoglycerides, with the maximum reached at 2% monoglyceride level (per starch dry weight) having 97% viscostability (Fischer *et al.*, 1999).

Addition of monoglycerides to starch reduces granular swelling (water binding) during gelatinization by reducing the accessibility of water into the granule, due to their surface-activity and their hydrophobic nature, as with other surfactants. This reduces the viscosity of the gelatinized starch paste as compared to a control without surface-active monoglycerides (Roach and Hoseney, 1995). These effects appear to be related to the length of the hydrocarbon chain. Monoglycerides with longer hydrocarbon chains, being more hydrophobic, reduce granular swelling more than monoglycerides with shorter hydrocarbon chains (Osman and Dix, 1960).

Furthermore, Roach and Hoseney, (1995) found that with the addition of monoglycerides, granular swelling on heating occurs in two prominent stages of consistency, as detectable by the Brabender Viskograph. This is observed as a slightly step-like gelatinization peak on Brabender Viskograph pasting curve.

Addition of different saturated fatty acids such as myristic (C14:0), palmitic (C16:0) and stearic (C18:0) have exhibited amylose-lipid complexing to varying degrees. Myristic acid showed the highest ability to form amylose-lipid complex, followed by palmitic acid whilst stearic acid showed the lowest complexing ability (Kaur and Singh, 2000). As previously stated, complex-forming ability is not related to complex stability (Eliasson and Krog, 1985; Kaur and Singh, 2000).

Kaur and Singh (2000) found the complexing indices to increase with the increase in levels of fatty acids added. This increase in complexing indices clearly indicates the formation of amylose-lipid complexes. Pasting temperature, peak viscosity, viscosity at 95 °C, viscosity at 50 °C and paste consistency all increased with the increase in levels of fatty acids whilst water solubility progressively decreased (Kaur and Singh, 2000). These effects are attributed to the formation of amylose-lipid complexes, with the extent of these effects depending on the ability of the fatty acid to complex with amylose. Thus, it is mostly the saturated fatty acids that significantly result in these effects (Nierle and El Baya, 1990; Kaur and Singh, 2000). Nierle and El Baya (1990) found that peak viscosity was not affected by the addition of saturated fatty acids at concentrations below 0.57 % of starch, on a dry basis.

Regarding the addition of stearic acid (C18:0), Karkalas *et al.* (1995) found that the various native lipid complexes dissociated non-selectively in preference for complexing with the added free stearic acid. This suggests that stearic acid is preferentially complexed with amylose over the other mixed native lipids present in the starch. Stearic acid, with a chain length of 2.49 nm (Karkalas and Raphaelides, 1986), takes up 18.7 glycosyl residues per lipid molecule in an amylose V_6-helix having 0.8 nm spacing between coils (Raphaelides and Karkalas, 1988).Considering the molar ratio of fatty-acid/amylose for stearic acid in a V_6 amylose helix being 0.054, with 18.7 glycosyl residues per fatty-acid, it would require 9.5 mg of stearic

acid to saturate 100 mg of amylose (Karkalas and Raphaelides, 1986). The stearic acid-amylose complex is reported to have a dissociation temperature of 95-135 °C (Stute and Konieczny-Janda 1983, Eliasson and Krog, 1985; Raphaelides and Karkalas, 1988). Kaur and Singh (2000) showed that of the various fatty acids, stearic acid showed the greatest effect on increased peak viscosity of the starch paste.

Nierle and El Baya (1990) found that when stearic acid was added to wheat starch at a low concentration of 0.57% (starch dry basis) and pasted in a Brabender Viskograph, the initial gelatinized viscosity was maintained for about the first 16 minutes of the 92 °C holding period thereafter the viscosity increased again (Figure 12). This second increase in viscosity during the holding period was attributed to the formation of amylose-lipid complexes with the added stearic acid. Thus, a biphasic pasting curve was obtained, not unlike the results obtained with native maize starch by Nelles *et al*, (2000) using a Rapid Visco Analyser. However, unlike with native maize starch (Nelles *et al.*, 2000), this second increase in viscosity did not decrease during further holding at 92 °C to form a peak in the curve, but was rather stable (Figure 12).

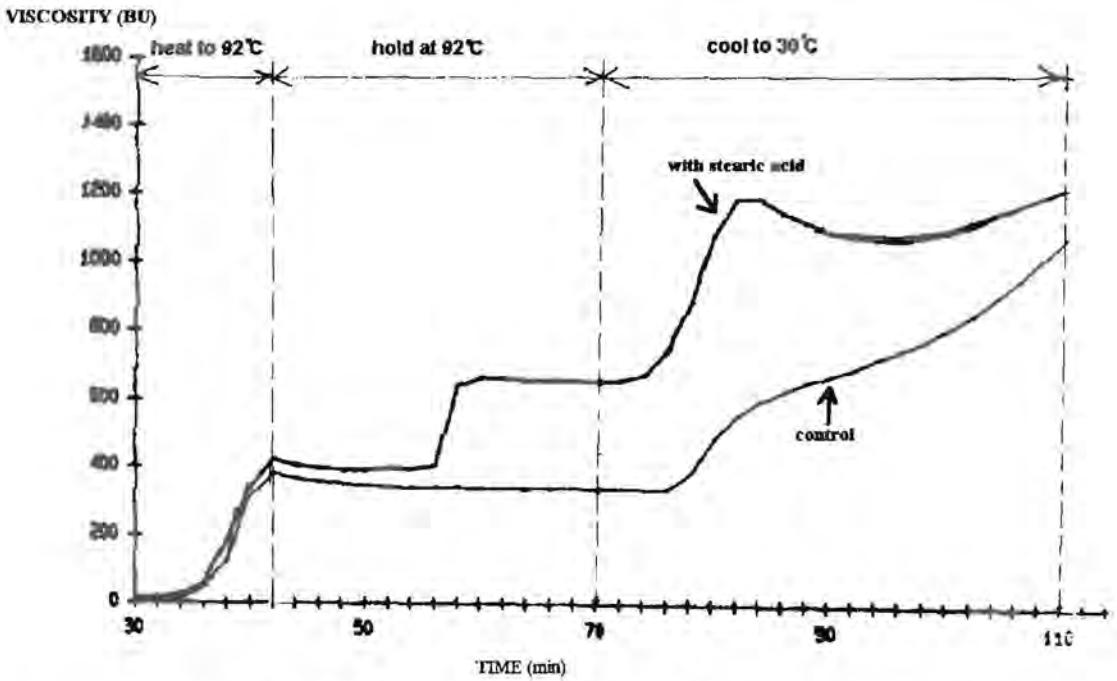


Figure 12: Brabender Viskograph pasting curve of wheat starch with added stearic acid (0.57 % of starch, dry basis). Also shown is the pasting curve of the control, plain wheat starch (Nierle and El Baya, 1990)

Bhatnagar and Hanna (1994b) investigated the effect of extruding normal maize starch with stearic acid for the large-scale production of complexed starch paste. They found maximum amylose-lipid complexing to occur at 110 – 140 °C barrel temperatures, 140 rpm screw speed and 19 % feed moisture content. Extrusion temperature was the most significant factor affecting complexing.

1.2.5.1.11 Inhibition of retrogradation by amylose-lipid complexes

Depending on the hydrophobic compound added, it is also possible to induce a conformational change to a helical form which does not readily crystallise or precipitate, thus modifying the retrogradation behaviour by favouring the formation of single helical conformations (as reviewed by Parker and Ring, 2001). This interaction is relatively strong with amylose and is expected to be much weaker with the shorter chains of amylopectin fractions (as reviewed by Parker and Ring, 2001).

The addition of a hydrophobic compound, such as fatty acids or emulsifiers, prevents the formation of the B-crystalline form (which results in the characteristic firming during retrogradation) since the single helical complex is the preferred product (Godet *et al.*, 1993; Godet *et al.*, 1995). This preference is further indicated by the observation that the amylose complex forms immediately after gelatinization (Le Bail, Bizot, Ollivon, Keller, Bourgaux and Buleon, 1999 according to Parker and Ring, 2001). Thus in bread, emulsifiers act as anti-staling agents by forming amorphous V_h-amylose complexes, which inhibit staling by retarding retrogradation (Zobel, 1988; Jovanovich and Anon, 1999). Since the retrogradation rate of amylose is so high, it is unlikely that it is responsible for the gradual staling of bread. More likely, it is the slow retrogradation of amylopectin, the larger component of starch, that is responsible for staling of bread (Tomasik and Schilling, 1988). Complexes of amylose are known to inhibit the retrogradation of amylopectin (Tomasik and Schilling, 1988). The presence of insoluble amylose-lipid complexes might indirectly influence the retrogradation of starch by altering the distribution of water between starch granules and the surrounding continuous matrix, thus disrupting the crystallisation of amylopectin (Eliasson and Krog 1985). Monoglyceride emulsifiers, with saturated

fatty acids, are more effective anti-staling agents than unsaturated ones (Tomasik and Schilling, 1988).

1.2.5.2 Amylopectin-lipid interactions

Amylopectin-lipid complexes have not been detected at all (Stute *et al.*, 1983; Tomasik and Schilling, 1988), although Villwock *et al.* (1999) did seem to find evidence to the existence of some amylopectin-lipid interactions of an unknown type.

1.2.6 Conclusions

It can be seen from this literature review that the pasting properties of all native starches are determined by the various starch-water and starch-starch interactions reviewed. However, in the case of cereal starches additional starch-lipid interactions come into play due to the presence of lipids, either native to the starch or accumulated from other parts of the grain during starch extraction, which are found only in cereal starches. Most notable is the formation of a helical amylose-lipid inclusion complex, which occurs after gelatinization, influences the pasting viscosity, and inhibits retrogradation. Thus, it seems most probable that the second peak of the biphasic pasting curve of maize starch, as observed by Nelles *et al.*, 2000, is due to the formation of such complexes with the lipids present in the starch, and not due to other starch molecular interactions. The research will thus investigate the properties of the maize starch second pasting peak by adding lipids to the starch and manipulating these starch-lipid interactions.

1.3 Objectives

To determine the effect of different holding temperatures on the biphasic pasting curve of maize starch during an extended holding period and thus determine the optimum holding temperature for formation of the second viscosity peak best suited for further investigation.

To determine the effect of stearic acid addition on the biphasic pasting curve of maize starch during an extended holding period at the optimum holding temperature. If addition of stearic acid is found to have an effect, then to further determine the effect of the addition of different concentrations of stearic acid on the pasting curve of maize starch during an extended holding period at the optimum holding temperature and thus determine the optimum stearic acid concentration.

To further determine the effect of different holding temperatures on the pasting curve of maize starch with the addition of the optimum concentration of stearic acid during an extended holding period, if an optimum stearic acid concentration can be determined.

To test the hypothesis proposed in chapter 1.1, that the biphasic pasting curve phenomenon is due to the formation of amylose-lipid complexes, by increasing the amylose content of the maize starch in the presence of excess stearic acid.

To determine the effect of different methods of increasing the amylose content of maize starch, either by treatment of the starch with a suitable amylopectin de-branching enzyme or by using a high amylose maize starch.

1.4 Hypotheses

It was proposed by Nelles *et al.* (2000) that the second viscosity peak observed in maize starch was due to amylose lipid complex formation with the endogenous lipids of maize starch. Thus it is hypothesized that the addition of stearic acid, a lipid with properties conducive to inclusion in V_h-amylose helices, to maize starch will increase the amount of amylose-lipid complexes formed in the paste, by increasing the amount of available lipids, and thus increase the viscosity and stability of the paste during extended holding at the optimum holding temperature, proportionally to the amount of stearic acid added, up to the saturation point of amylose.

It was proposed by Nelles *et al.* (2000) that the amount of amylose-lipid complexes formed during extended holding was determined by the thermal energy applied to the system at a specific holding temperature. Thus it is hypothesized that the amount of amylose-lipid complexes formed with the optimum concentration of stearic acid, will increase as the holding temperature is increased to an optimum temperature and that at holding temperatures higher than optimum, the amount of complexes formed will decrease due to thermal instability.

It is hypothesized that the treatment of gelatinized maize starch containing added stearic acid, with a suitable amylopectin de-branching enzyme will increase the "amylose" content of the starch paste by the release of linear amylopectin segments and thus further increase the paste viscosity during an extended holding time at the optimum temperature, by enabling to the formation of more amylose-lipid complexes.

It is hypothesized that the addition of stearic acid will cause a higher increase in viscosity in high amylose maize starch than in normal maize starch during an extended holding time at the optimum temperature, due to the higher amylose content enabling more amylose-lipid complexing to occur.

CHAPTER 2

RESEARCH

The following sub-sections were written in the style of articles, as required for the journal *Starch/Stärke*.

2.1 Effects of extended high temperature holding and stearic acid addition on the pasting of normal maize Starch

2.1.1 Introduction

Maize starch exhibits a biphasic pasting curve at extended holding times at high temperatures [1]. The first peak in viscosity is due to starch gelatinization. The second peak is probably due to the formation of amylose inclusion complexes with the lipids which are present in the starch [1,2]. Cereal starch granules contain native lipids, mostly free fatty acids and some phospholipids [3,4]. The starch may also contain lipids from other parts of the grain, which accumulate during the extraction process. Amorphous, Type I amylose-lipid complexes form below 60°C, before gelatinization, and dissociate above 90°C [5,6]. Semi-crystalline Type II(a) and fully crystalline Type II(b) complexes form during and after gelatinization respectively, and dissociate at 100-125°C [6-8]. The crystalline Type II(b) complex formed after gelatinization is suggested to be responsible for the second peak in viscosity observed in the pasting curve of maize starch [1].This article describes the effect of different holding temperatures on the biphasic pasting curve of maize starch, as well as the effect of adding stearic acid to induce additional amylose-lipid complexing.

2.1.2 Experimental

2.1.2.1 Materials

Low moisture white maize starch "Amyral" (approximately 26% amylose, 74% amylopectin, from African Products, Edenvale, South Africa, product code W037200); stearic acid and ethanol (99.9%).

2.1.2.2 Effect of different holding temperatures on pasting of normal maize starch

A Brabender Viskograph-E (Brabender® OHG, Duisburg, Germany) was set to a measuring range of 1000 cmg, a bowl rotation speed of 148 rpm, with a starting temperature of 50 °C, heating rate of 2.5 °C/min, holding time of 90 min, cooling rate

of 3 °C/min, final temperature of 40°C and a final holding time of 10 min. A 10% maize starch suspension (dry basis) was prepared with distilled water. Holding temperatures of 82, 84, 86, 88, 90 and 92 °C were tested. All tests were performed at least in duplicate.

2.1.2.3 Effect of stearic acid addition on pasting of normal maize starch

Stearic acid was added to the starch in the amounts of 0.5%, 0.7%, 1.0%, 1.5%, 2.0%, 3.0% and 4.0% (by weight of starch on a dry basis). The stearic acid aliquot was dissolved in ethanol in an approximately 1:100 ratio. The stearic acid-ethanol solution was stirred into the starch aliquot to ensure even dispersion of the stearic acid throughout the sample. The ethanol was evaporated off over a 50 °C water bath, thereafter the sample was dried in a 50 °C forced draught oven. Once the sample was dry, a 10% suspension (dry basis) was prepared with distilled water. The samples were pasted in the Viskograph with the profile given in section 2.1.2.2 with a holding temperature of 90°C. All tests were preformed at least in duplicate.

2.1.2.4 Effect of different holding temperatures on pasting of maize starch with 1.5% added stearic acid

A 10% suspension of maize starch with 1.5% stearic acid (by weight of starch on a dry basis) was prepared as described in section 2.1.2.3. The samples were pasted with the profile given in section 2.1.2.2., except that the sample was cooled to 25 °C instead of held at 40 °C. Holding temperatures of 82, 84, 86, 88, 90, 92 and 94°C were tested. All tests were performed at least in duplicate.

2.1.2.5 Sample characterization

Each sample was characterized in terms of time, temperature and viscosity at the following points on the viscosity curve: beginning of gelatinization, beginning of holding, 1st peak, 2nd peak, start cooling, end cooling, end of final holding.

2.1.2.6 Statistical analysis

Analysis of variance was used to determine statistical relationships in the height of the second viscosity peaks amongst the different holding temperatures and stearic acid concentrations investigated. The least significant difference for all analyses was determined at P = 0.001 using the Fisher distributions (the *F* test).

2.1.3 Results and discussion

2.1.3.1 Effect of different holding temperatures on the pasting of maize starch

As seen in Figure 13, the first viscosity peak occurred at 15 minutes with a peak viscosity just less than 1300 Brabender Units (B.U.). Since the gelatinization temperature range of maize starch is 62-80°C [9], the first peak did not vary with different holding temperatures as gelatinization occurred before the minimum holding temperature of 82°C was reached. The breakdown in viscosity after gelatinization increased proportionally as the holding temperature was increased, probably due to greater starch granule disintegration and shear thinning at higher temperatures. The second viscosity peak was significantly affected by variation in holding temperature (Figure 13 and Table 1). As the holding temperature decreased from 92 to 82°C, the second viscosity peaks were proportionally higher. The different second viscosity peak heights were determined to be significantly different ($p < 0.001$) for the different holding temperatures investigated. The second peak viscosities exceeded the first viscosity peak at holding temperatures of 88°C and lower. At a holding temperature of 82° C a viscosity of over 1600 B.U. reached. Furthermore, the relative areas of the second viscosity peaks all increased as the holding temperature was decreased to 84°C (Table 1). As the holding temperature decreased from 92 to 88 °C, viscosity increase and subsequent breakdown during holding progressively became more rapid with the result that the second viscosity peak occurred earlier. A holding temperature of 88°C resulted in the earliest second viscosity peak. As the holding temperature decreased from 88 to 82 °C, viscosity increase and subsequent breakdown were progressively later which resulted in the second viscosity peaks occurring later. There was little breakdown in the second viscosity peak during holding at 82°C. These

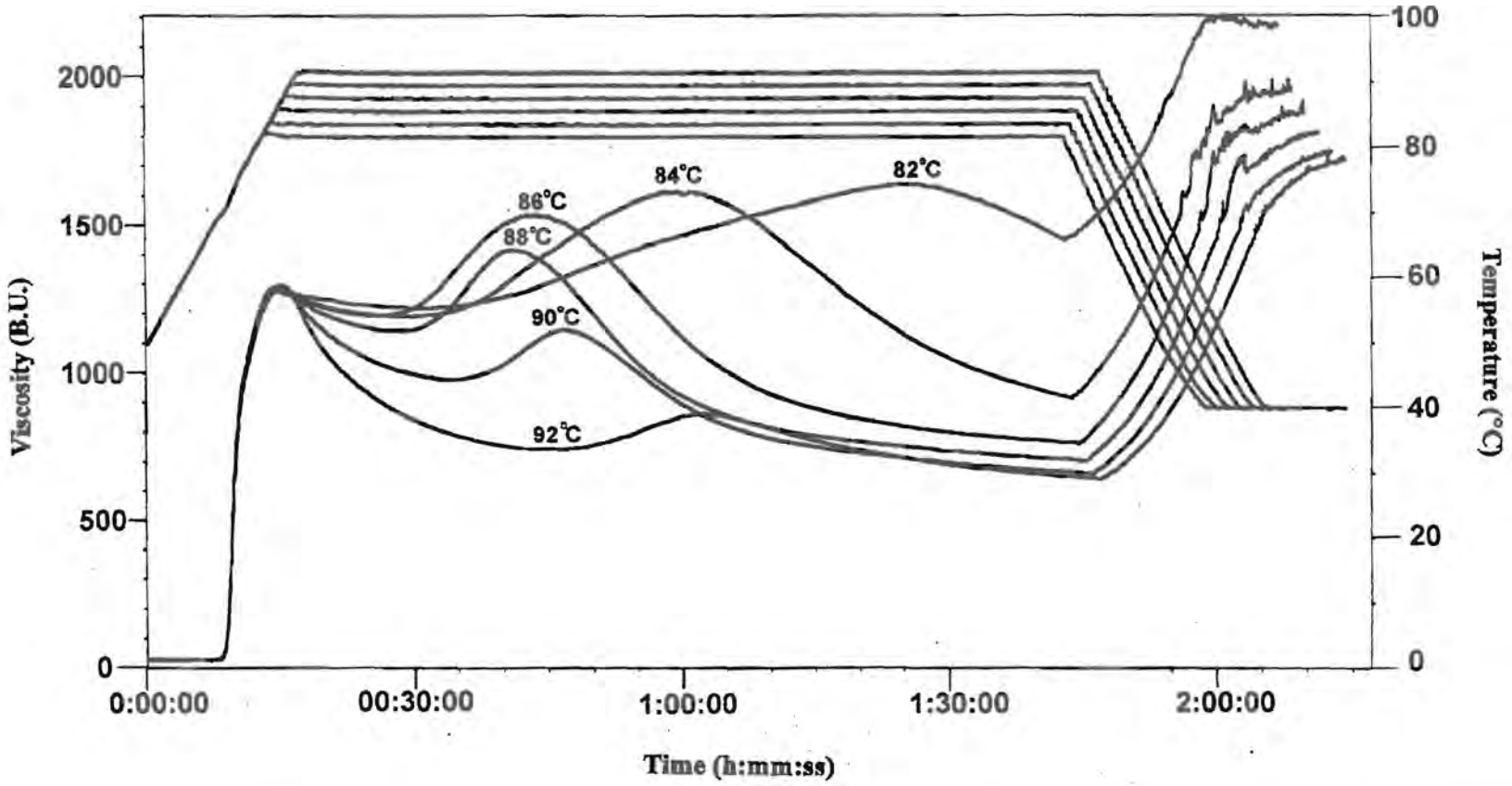


Figure 13. Pasting curves of 10% maize starch suspensions heated to and held at 82, 84, 86, 88, 90 and 92°C for 90 minutes then cooled to and held at 40°C for 10 minutes with a Brabender Viskograph-E.

Table 1. Significant points on the pasting curves of maize starch with different holding temperatures, held for 90 minutes then cooled to 40°C and held for 10 minutes.

		Holding temperature [°C]					
		82	84	86	88	90	92
1st peak	Time [hh:mm:ss]	00:14:00	00:14:00	00:15:00	00:15:00	00:15:00	00:15:15
	Viscosity [B.U.][1]	1268	1299	1295	1295	1289	1301
2nd peak	Time [hh:mm:ss]	01:26:38	00:58:20	00:45:15	00:41:12	00:46:30	01:00:00
	Viscosity [B.U.]	1640	1625	15605	1418	1150	885
	Relative area [%][2]	63	100	89	71	50	20
End of holding	Viscosity [B.U.]	1488	911	777	708	657	640
End of cooling	Viscosity [B.U.]	2126	1754	1695	1575	1510	1464
End of final holding	Viscosity [B.U]	2192	1961	1896	1824	1742	1726

[1] Means of at least two replicate experiments

[2] Calculated as the area between the line and a horizontal drawn from the lowest viscosity after the first viscosity peak to a vertical drawn at the beginning of cooling

trends correspond with the results obtained by Nelles *et al.* [1] for maize starch with holding temperatures from 98°C to 80°C using a Rapid Visco Analyser.

Amylose-lipid complexes can only form at high temperatures when the flexibilities of both the amylose and lipid are high enough to lead to inclusion [10]. According to Karkalas *et al.* [6], Kugimiya *et al.* [7] and Zobel [8], temperatures above 90°C are required to form the Type II (b) complexes, which are believed to be responsible for the second viscosity peak [1]. This temperature was not reached at most of the holding temperatures. However, the high viscosities obtained suggest that these Type II complexes were present in the paste. When the sample was held at the specified temperature, thermal energy (T.E.) was applied to the system at a rate proportional to the holding temperature. The T.E. in the system increased throughout the holding period due to the constant application of heat [1]. Since the increase in T.E. could not raise the temperature, as a specific holding temperature was maintained, it is proposed that most of the applied T.E. was taken up by the molecules themselves, causing an increase in their molecular kinetic energy (K.E.). Such an increase in K.E. could have induced the molecular flexibility required for complexing. Thus, it is hypothesised that once a sufficient level of K.E. was reached, amylose-lipid complexing was initiated, causing the increase in paste viscosity [1]. It is further hypothesised that eventually the amount of K.E. accumulated in the system was so high that it promoted the dissociation of the formed complexes, rather than further complexing. The thermal stability of the complex depends on the structural characteristics of the complexed lipid, such as fatty acid chain length, degree and type of saturation [6,11]. The less stable complexes probably dissociated first. As the level of K.E. increased, the more stable complexes also probably dissociated and the viscosity gradually decreased. Hence, there was a second peak in viscosity.

It is further hypothesised that as holding temperatures were increased, the amount of T.E. applied to the system increased proportionally to the holding temperature. As T.E. increased, proportionally less complexes were able to form which could withstand the higher K.E. levels and thus the second viscosity peak decreased proportionally in height and area. As holding temperatures increased above 88°C, inhibition by the higher K.E. caused complex formation to occur proportionally

slower. Furthermore, the higher K.E. ensured that only most stable complexes formed, which also dissociated slower due to their higher stability. Thus, the rates of viscosity increase and breakdown of the second viscosity peak were proportionally less as the holding temperature was increased above 88°C. As holding temperatures were reduced below 88°C, the rate of K.E. accumulation in the system was proportionally lower. Thus, it took longer to initiate complexing, as well to eventually dissociate the weaker complexes. These effects also reduced the rate of viscosity increase and breakdown of the second viscosity peak. At these lower temperatures, higher viscosities could be achieved, as the relatively low levels of K.E. did not inhibit complexing.

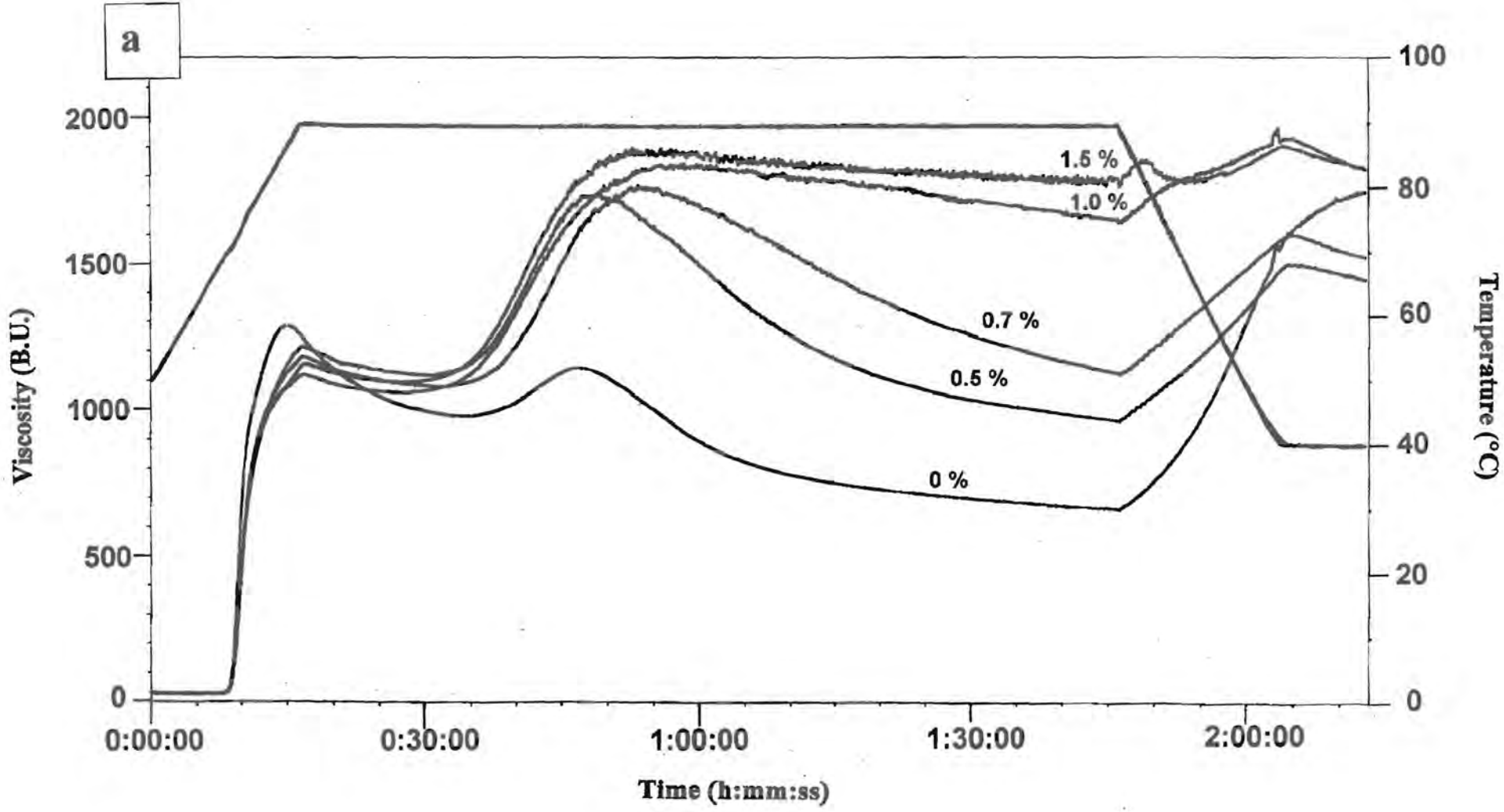
The higher the holding temperature, the more the viscosity increased on cooling and holding at 40 °C due to retrogradation. As retrogradation of both amylose and amylopectin is inhibited to a certain degree by amylose-lipid complexes [12,13], the more complexes present before cooling, the less setback occurred. At 82°C the viscosity immediately prior to cooling was still quite high, indicating a high amount of complexes still present in the paste to inhibit retrogradation. As the holding temperature was increased, the paste viscosity before cooling was proportionally lower. This indicated higher levels of uncomplexed starch, which were prone to retrogradation.

2.1.3.2 Effect of different stearic acid concentrations on maize starch pasting at a holding temperature of 90 °C

A holding temperature of 90°C was selected for investigating the effect of stearic acid addition as it resulted in the maize starch pasting curve that had both a significant breakdown in viscosity after gelatinization as well as a significant second viscosity peak (Figure 13). As seen in Figure 14a, the addition of 0.5% stearic acid lowered the first viscosity peak from just below 1300 B.U. to just above 1200 B.U. (Table 2). The first viscosity peak further decreased proportionally to 1110 B.U. as the stearic acid concentration was increased to 4.0%. The apparent temperature of gelatinization was also raised from 87°C to 90°C (the holding temperature). Amylose-complexing agents, such as endogenous lipids and added emulsifiers, have been reported to result

in higher gelatinization temperatures and less granular swelling in various starches due to their surface-activity and their hydrophobic nature, as with other surfactants [8,14]. These effects appear to be related to the length of the hydrocarbon chain [15]. It appears as if the addition of stearic acid, which has a relatively long hydrocarbon chain (C18:0), significantly reduced granular swelling during gelatinization, observed as a lower first viscosity peak. With the addition of stearic acid, the increase in viscosity of the first viscosity peak was observed to occur in two stages, which became more prominent as the stearic acid concentration was increased to 4% (Figure 14b). Granular swelling on heating apparently occurs in two prominent stages, not normally visible: the first stage is the initial granular swelling resulting from the uptake of water and the second stage involves the solubilization of starch molecules in addition to granular swelling [14]. However, the addition of lipids have been known to reduce the first stage viscosity and delay the second stage to a higher temperature to make these two stages more prominent [14], as was observed.

During gelatinization, amylose-lipid complexes initially form with the native lipids within the granule [3]. On further heating, the various native lipid complexes dissociate non-selectively in preference for complexing with added free stearic acid [6]. Stearic acid is preferentially complexed due to its linear, saturated long chain structure being more complementary for complex formation [10,11,13] than the possibly bulkier and less saturated native lipids in the granule, mostly free fatty acids and phospholipids [2,3]. If all the available stearic acid has been complexed, the remaining free amylose helices would then probably complex with the endogenous lipids present in the starch.



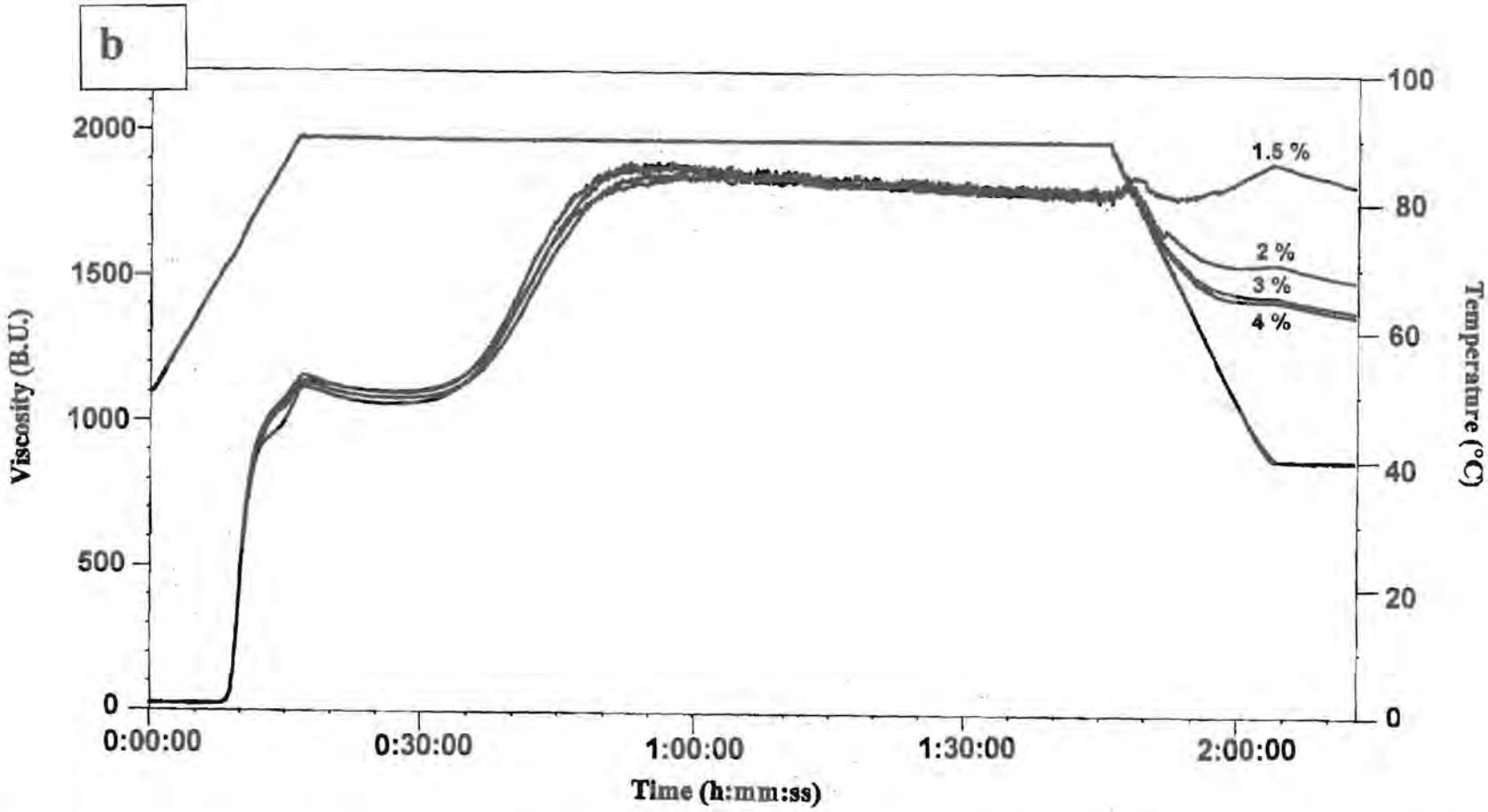


Figure 14. Pasting curves of 10% maize starch suspensions with different stearic acid concentrations (per dry weight basis of starch) heated to and held at 90°C for 90 minutes then cooled to and held at 40°C for 10 minutes with a Brabender Viskograph-E. a). 0.5%, 0.7% and 1.0% added stearic acid; b) 1.5%, 2.0%, 3.0% and 4.0% added stearic acid.

Table 2. Significant points on the pasting curves of maize starch with the addition of different concentrations of stearic acid, held at 90°C for 90 minutes then cooled to 40°C and held for 10 minutes.

		Stearic acid concentration [%]							
		0	0.5	0.7	1.0	1.5	2.0	3.0	4.0
1st peak	Time [hh:mm:ss]	00:15:00	00:17:00	00:16:30	00:17:00	00:16:00	00:17:00	00:17:00	00:17:00
	Viscosity [B.U.][1]	1289	1200	1175	1135	1145	1140	1132	1110
2nd peak	Time [hh:mm:ss]	00:46:30	00:47:38	00:52:22	00:58:10	00:55:45	00:56:32	00:59:30	01:00:48
	Viscosity [B.U.]	1150	1708	1762	1861	1899	1901	1864	1855
	Relative area [%][2]	50	46	47	85	95	97	100	100
End holding	Viscosity [B.U.]	657	956	1120	1673	1804	1798	1785	1793
End of cooling	Viscosity [B.U.]	1510	1456	1562	1955	1883	1571	1437	1438
End of final holding	Viscosity [B.U.]	1742	1453	1526	1841	1716	1517	1390	1387

[1] Means of at least two replicate experiments

[2] Calculated as the area between the line and a horizontal drawn from the lowest viscosity after the first viscosity peak to a vertical drawn at the beginning of cooling

Increasing the stearic acid concentration to 0.5%, 0.7%, 1.0% and 1.5% decreased the amount of viscosity breakdown after gelatinization and progressively increased the second peak viscosity and area during the holding period (Figure 14 and Table 2). The second viscosity peak heights were determined to be significantly different ($p < 0.001$) for stearic acid concentrations of 0 - 1.5% but not for 1.5 - 4%. It is hypothesised that at a low lipid concentration probably few complexes were formed and thus the paste was weaker (low viscosity). As the lipid concentration increased, complex formation probably became more dominant until the saturation level of the available amylose would be reached [16], thus the paste would be stronger (have a higher viscosity). This saturation point initially seems to be reached at 1.5% stearic acid concentration, as increasing the stearic acid concentration beyond 1.5% did not further increase the viscosity (Figure 14b). The molar ratio of fatty-acid/amylose for stearic acid in a V_6 amylose helix being 0.054, with 18.7 glycosyl residues per fatty-acid, it would require 9.5 mg of stearic acid to saturate 100 mg of amylose [17]. Thus the maize starch used being approximately 26% amylose, it would require, according to this relationship, approximately 2.5% stearic acid (per dry weight of starch) to completely saturate all the amylose with stearic acid.

Increasing the stearic acid concentration proportionally improved the paste stability and increased the area under the second viscosity peak (Figure 14 and Table 2). Increased paste viscosity and stability was also observed by Nierle and El Baya [2] with the addition of 0.57 % stearic acid to wheat starch. Here the most stable viscosity, maintained during holding and cooling, was observed with 1.5% stearic acid concentration. This is similar to the results obtained by Fischer *et al.* [18] for the addition of 2% monoglycerides to potato starch. The stearic acid-amylose complex is reported to have a dissociation temperature of 95-135 °C [5,11,19]. It is thus unlikely that the equivalent K.E. accumulated during the 90°C holding period. The decrease in viscosity during the holding period was probably due to the dissociation of the complexes with endogenous lipids, which would be less thermally stable due to their shape and structure being less suited for inclusion in the amylose helix. As the stearic acid concentration was increased, there would be proportionally less complexes with endogenous lipids and more complexes with stearic acid, which were probably thermally stable at 90°C. It is hypothesized that increased stability of the starch paste

with added stearic acid was due to the formation of large, crystalline, amylose-lipid complex superstructures [20], which formed a shear-resistant network in the paste.

On cooling the samples to 40 °C, samples with stearic acid concentration of 1% or less increased in viscosity (Figure 14a), whilst those with stearic acid concentration of 2% or more decreased in viscosity (Figure 14b). The sample with stearic acid concentration of 1.5% remained relatively stable, maintaining a hot paste viscosity of between 1800 and 1900 B.U. The lower the stearic acid concentration was below 1.5%, the more the viscosity decreased during the holding period indicating there was proportionally more free amylose available for setback on cooling, with only minimal inhibition by the remaining thermally stable complexes. Conversely, as the stearic acid concentration increased above 1.5%, inhibition of retrogradation became stronger with the paste viscosity actually decreasing proportionally on cooling. As the amylose-lipid complexes are thermo-reversible [7], it is hypothesised that the decrease in viscosity in the absence of retrogradation was due to the Type II crystalline complexes (high paste viscosity) transforming into to the amorphous (low paste viscosity) Type I complexes [19], which were probably favoured by the lower temperatures [6]. At 1.5% stearic acid concentration the viscosity probably remained relatively stable due to a balance of these opposing mechanisms of retrogradation and complex transformation.

On holding at 40 °C, all the samples with stearic acid similarly decreased in viscosity, in contrast to the control sample, which continued to increase in viscosity (Figure 14a). Without the strong driving force of further cooling, the few complexes present probably inhibited any further retrogradation whilst transforming into the lower viscosity amorphous Type I complexes. At 1.5% stearic acid concentration a small decrease in viscosity did occur during holding indicating that there was a small amount of free amylose available for retrogradation on cooling. However, the majority of the amylose would be still complexed with stearic acid at the end of the holding period.

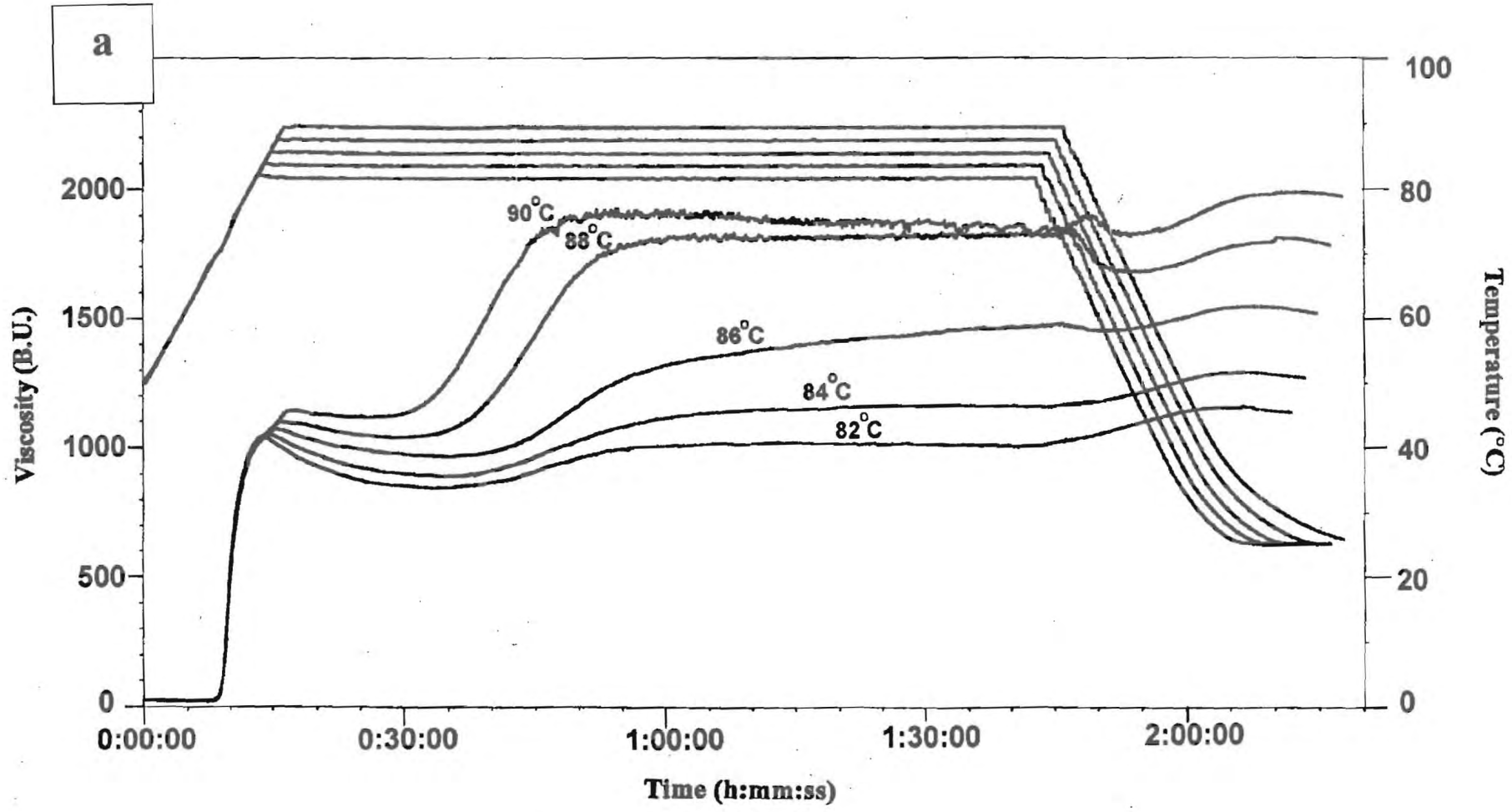
From Figure 14b it can be seen that although the viscosity on holding at 90°C did not increase further at stearic acid levels above 1.5%, on cooling the viscosity did

continue to decrease further as the stearic acid concentration increased. This increasingly stronger reversal of retrogradation is attributed to the formation of more amylose-lipid complexes at the higher stearic acid concentrations. This suggests that although not evident from the hot paste viscosity, amylose-lipid complex formation did continue above 1.5% stearic acid concentration. As retrogradation was not further reversed (observed as the extent of decreasing viscosity on cooling) by a stearic acid concentrations of 4%, it can deduced that the complete saturation of amylose with stearic acid was in fact reached at approximately 2-3% concentration, as calculated above for maize starch.

2.1.3.3 The effect of different holding temperatures on the pasting curve of maize starch with 1.5% added stearic acid

With holding temperatures 82-90°C, the first peak viscosity, due to gelatinization, was reached when the holding temperature was reached (Figure 15a). Thus, the gelatinization peak viscosity proportionally increased as the holding temperature increased. This can be attributed to reduced starch granule swelling and a higher gelatinization temperature when heated in the presence of lipids [8,14]. This effect was most evident with the sample held at 82°C, which had the lowest gelatinization peak. The highest gelatinization viscosity that was possible in the presence of 1.5% stearic acid was reached at 90°C, with higher temperatures no longer increasing the viscosity significantly further (Figure 15b and Table 3). The amount of viscosity breakdown after gelatinization was reduced as the holding temperature was increased from 82-90°C (Figure 15a) and increased as holding temperature was further increased from 90-94°C (Figure 15b). This effect was probably related to the amount of amylose complexing that occurred with stearic acid at the respective temperatures, due to the proposed stabilizing effect of the complexes.

The second viscosity peak heights were determined to all be significantly different ($p < 0.001$) for holding temperatures 82-94°C. As the holding temperature was increased from 82°C to 90°C the second viscosity peaks increased proportionally in both height and area (Figure 15a and Table 3). This indicates that complex formation was probably favoured by the higher T.E., which also probably leached more amylose



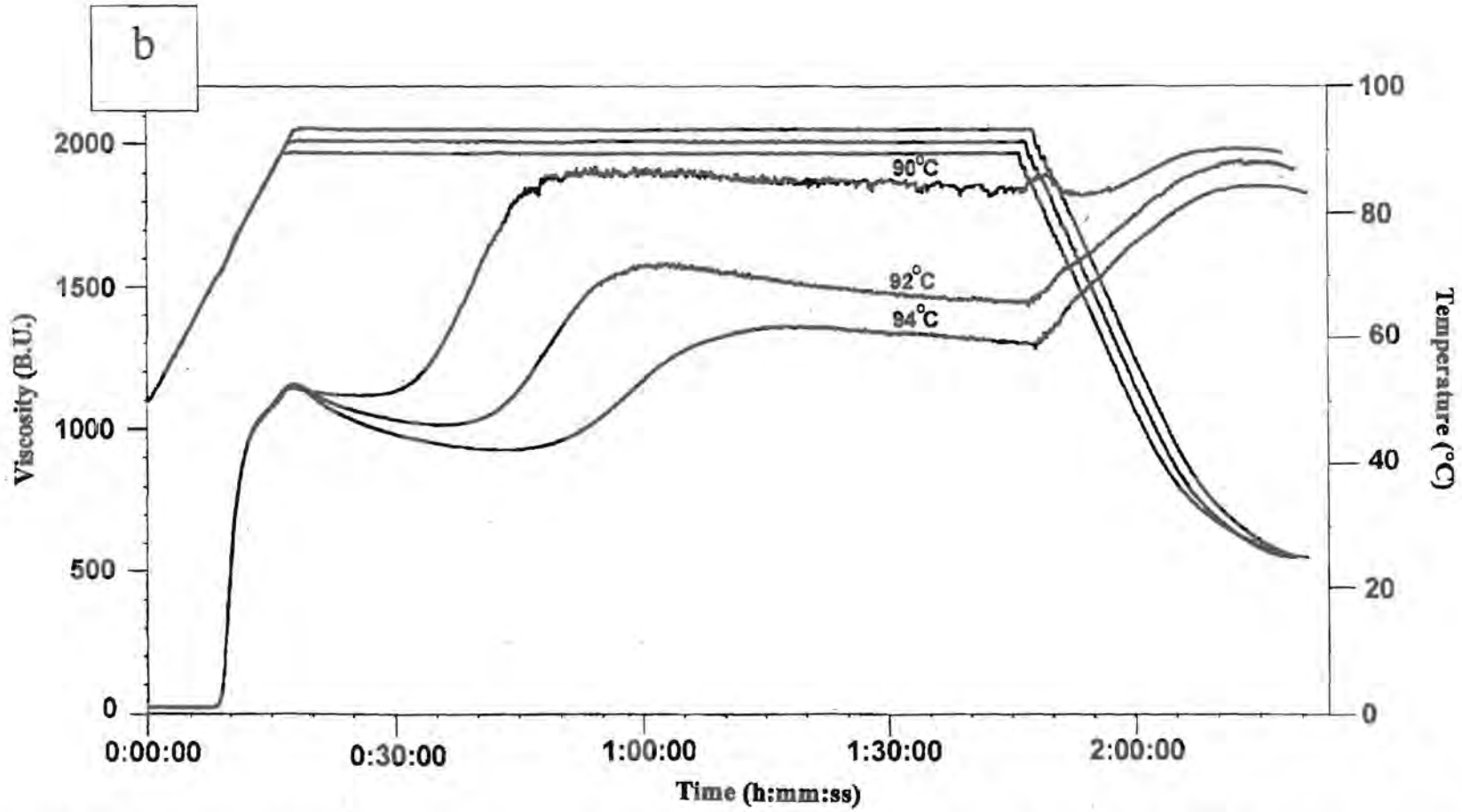


Figure 15. Pasting curves of 10% maize starch suspensions with 1.5% added stearic acid (per dry weight basis of starch) heated to different temperatures and held for 90 minutes then cooled to 25°C. a) Holding temperatures of 82, 84, 86, 88 and 90°C; b) Holding temperatures of 90, 92 and 94°C.

Table 3. Significant points on the pasting curves of maize starch with the addition of 1.5% stearic acid, held at different temperatures for 90 minutes then cooled to 25 °C.

		Holding temperature [°C]						
		82	84	86	88	90	92	94
1st peak	Time [hh:mm:ss]	00:13:35	00:14:00	00:15:00	00:16:00	00:16:00	00:16:00	00:16:00
	Viscosity [B.U.][1]	1037	1055	1068	1100	1150	1165	1150
2nd peak	Time [hh:mm:ss]	01:15:00	01:33:20	01:44:12	01:34:05	00:53:02	01:01:45	01:15:52
	Viscosity [B.U.]	1023	1178	1470	1356	1932	1638	1383
	Relative area [%][2]	18	28	48	95	100	52	37
End of holding	Viscosity [B.U.]	1008	1170	1469	1833	1846	1483	1310
End of cooling	Viscosity [B.U.]	1130	1278	1518	1764	1943	1922	1844

[1] Means of at least two replicate experiments

[2] Calculated as the area between the line and a horizontal drawn from the lowest viscosity after the first viscosity peak to a vertical drawn at the beginning of cooling

from the starch granules into the system so that more complexes could be formed as the holding temperature was increased to 90°C. As holding temperatures increased beyond 90°C, the second peak viscosity decreased proportionally in height and area (Figure 15b and Table 3). This suggests that although more amylose was probably leached from the granules, at these higher temperatures complex formation was inhibited due to excessive K.E. in the system. Thus the maximum paste viscosity was obtained at 90°C.

The samples were cooled to 25°C to investigate the effect of cooling to room temperature, as would occur in practical applications. The paste viscosities of the samples during the holding period and cooling to 25°C were generally stable for the samples held at 90°C and lower (Figure 15a). At these lower K.E. levels, the complexes that formed were presumably stable and effectively inhibited shear thinning and retrogradation of the paste. In the samples that were held at temperatures above 90°C (Figure 15b), the high amount of K.E. in the system during the holding period seemed to destabilize and dissociate the complexes that had formed. As a result, the paste viscosity gradually decreased during the 25°C holding period. Thus prior to cooling there was probably a large amount of uncomplexed amylose available for retrogradation, leading to an increase in viscosity (Figure 15b).

2.1.4 Conclusions

The addition of 1.5% stearic acid to normal maize starch can increase and stabilize the paste viscosity during extended holding at temperatures of 82 to 90°C and subsequent cooling to 25°C, under conditions of continuous high shear. Paste viscosity can be proportionally increased by increasing the holding temperature from 82 to 90°C. These effects are most likely due to the formation of amylose-lipid complexes on holding at these temperatures for extended periods of time.

2.1.5 References

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2.2 Effect of increased starch amylose content on pasting of maize starch with added stearic acid

2.2.1 Introduction

Previous investigations have shown that the addition of 1.5% stearic acid to normal maize starch increased and stabilized the paste viscosity during holding at 90°C for 90 min and subsequent cooling to 25°C, under conditions of continuous high shear (2.1). The paste viscosity increased proportionally as stearic acid concentration was increased from 0.5 to 1.5% but did not increase further as stearic acid concentrations increased from 1.5 to 4%. These effects are proposed to be due to the formation of amylose-lipid complexes [1,2] with the added stearic acid, up until the saturation point of amylose [3]. To test the hypothesis that these effects are due to amylose-lipid complexing, this work investigates whether a greater amount of amylose than present in normal maize starch will increase the paste viscosity in a sample containing 3% stearic acid beyond the viscosity limit previously observed at 1.5% stearic acid. The effects of treating the maize starch with pullulanase, a suitable amylopectin de-branching enzyme [4,5], will be described. De-branching amylopectin with pullulanase, to cleave α (1-6) branch points, results in the release of short linear segments [5,6]. It is possible that these short linear amylopectin segments will be of sufficient uninterrupted length to form amylose-lipid complexes with lipids, similar to native amylose [7]. Yuan [5] found that introducing such short chain "amylose" into maize starch by pullulanase treatment increased the amount of amylose-lipid complexing with glycerol monostearate. Whilst the effects of enzyme treatment on the opacity and rheology properties of the paste were noted [5], the effects on pasting viscosity were not. The effect of using high amylose maize starch (about 85 % amylose) instead of normal maize starch (about 25% amylose) will also be described.

2.2.2 *Materials and methods*

2.2.2.1 Materials

Unmodified high amylose maize starch ("Himaize starch 500085", minimum 85 % amylose, from African Products, Edenvale, South Africa,); pre-gelatinized maize starch ("Stygel FS", minimum 25% amylose, from African Products); low moisture white-maize starch (Amyral W037200, approximately 26% amylose, from African Products); Stearic acid; Pullulanase P 1067 (enzyme activity of 15 units per mg protein, from Sigma).

2.2.2.2 Incorporation of stearic acid

A stearic acid aliquot, determined as 3% of the respective starch aliquot (dry basis), was dissolved in warm ethanol. The stearic acid-ethanol solution was stirred into the starch aliquot to ensure a homogenous suspension. Ethanol was evaporated off over a 50°C vibrating water bath, thereafter the sample was dried in a 50°C forced draught oven.

2.2.2.3 Effect of pullulanase treatment on normal maize starch with 3% stearic acid

A Brabender Viskograph-E (Brabender® OHG, Duisburg, Germany) was set to a measuring range of 1000 cmg, a bowl rotation speed of 148 rpm, with a starting temperature of 50 °C and heating rate of 2.5 °C/min. A 10% suspension of normal maize starch with 3% stearic acid, as well as a control without added stearic acid, was prepared with distilled water. The sample was heated to 90°C in the Viskograph and held for 14 minutes, to complete gelatinization, thereafter cooled to 25°C at a rate of 3°C/minute, with a continuous stirring rate of 148 rpm. Pullulanase enzyme was prepared in a citrate-Na_2HPO_4 buffer solution of pH 5.0 [4] at a concentration of 0.153 mg solids per ml. The prepared enzyme-buffer solution was stirred into the samples to yield an enzyme activity of 0.225 units per g starch. A control sample with stearic acid was prepared with the same amount of buffer solution without enzyme. The samples were incubated at 25°C with continuous stirring for 1.5 hours, 20 hours

and 113.5 hours. A control sample with 3% stearic acid but no enzyme was incubated for 20 hours and a control sample with enzyme but no stearic acid was incubated for 1.5 hours. After incubation, the samples were pasted in the Viskograph with a starting temperature of 25°C, heating rate of 3°C/minute, holding temperature of 90°C, held for 76 minutes and thereafter cooled to 25°C, at a cooling rate of 3°C/minute, the conditions previously found to result in a high and stable second pasting peak viscosity with normal starch (2.1). The results were not statistically analysed, as there was insufficient pullulanase available to allow for enough replicate tests.

2.2.2.4 Pre-gelatinization of high amylose maize starch and the effect of 3% stearic acid addition

To gelatinize the high amylose starch, a 15% (w/v) suspension was prepared with distilled water, filled into retort pouches and sealed. The samples were pressure-cooked at 121°C for 20 minutes. Immediately on removal from the pressure cooker, the hot pouches were submerged in liquid nitrogen until frozen solid (once violent boiling over of liquid nitrogen had subsided). The frozen samples were stored at -20°C. The samples were freeze-dried and the dried samples were milled into a powder using a hammer mill. The pre-gelatinized starch powder was prepared into 10% suspensions with distilled water and samples with 3% stearic acid and without stearic acid were prepared. The samples were pasted in the Viskograph with a starting temperature of 25°C, heating rate of 3°C/minute, holding temperature of 90°C held for 76 minutes then cooled to 25°C at a rate of 3°C/minute. For comparison, pre-gelatinized normal maize starch with 0% and 3% stearic acid were similarly pasted. Each test was performed in duplicate, with good repeatability being achieved. Dilute suspensions of the pre-gelatinized normal maize starch and the pre-gelatinized high amylose maize starch were observed by light microscopy under normal and polarized light.

2.2.2.5 Sample characterization

The samples were characterized in terms of viscosity at the beginning and end of heating, at the end of the holding period, at the end of cooling, as well as at other

significant points that occurred in some samples between these phases. The relative peak areas of the pullulanase treated samples were calculated as the area between the line and a horizontal drawn from the lowest viscosity before the viscosity peak to a vertical drawn at the beginning of cooling.

2.2.3 Results and discussion

2.2.3.1 Effect of pullulanase treatment on gelatinized maize starch with 3% stearic acid

As can be seen in Figure 16, the gelatinized starch sample without (0 %) added stearic acid had a much higher initial viscosity of about 2700 B.U. than the sample with 3% added stearic acid, which had an initial viscosity of about 980 B.U. This indicates that on cooling to the enzyme incubation temperature after gelatinization, the sample without added stearic acid had a much greater viscosity setback, due to a stronger retrogradation, than the sample with 3% stearic acid. In 2.1 it was proposed that retrogradation on cooling was inhibited by the formation of amylose-lipid complexes with the added stearic acid. Thus, some amylose-lipid complexes probably already formed with stearic acid during gelatinization, which then inhibited retrogradation on cooling. Furthermore, the 20 hour control sample (no enzyme; 3% stearic acid) had an initial viscosity of 748 B.U. (Table 4, Figure 17) whilst previous investigations found that maize starch with 3% stearic acid had a viscosity of about 1000 B.U after gelatinization (chapter 2.1). It is unlikely that the addition of the buffer solution, which was less than 7.5% of the original volume of water, alone, caused such a decrease in initial viscosity of the gelatinized starch. It is more likely that this was chiefly due to molecular alignment and shear thinning of the linear amylose molecules [9] during the incubation period.

As can be seen in Figure 17 and Table 4, the initial viscosity of the 20-hour control sample (748 B.U.) was higher than that of the corresponding pullulanase treated sample (580 B.U). Thus, in addition to shear thinning, as discussed above, the pullulanase treatment further decreased the paste viscosity during the incubation period. As the enzyme reaction time increased from 1.5 to 113.5 hours, the initial paste viscosity decreased progressively from 990 to 348 B.U.

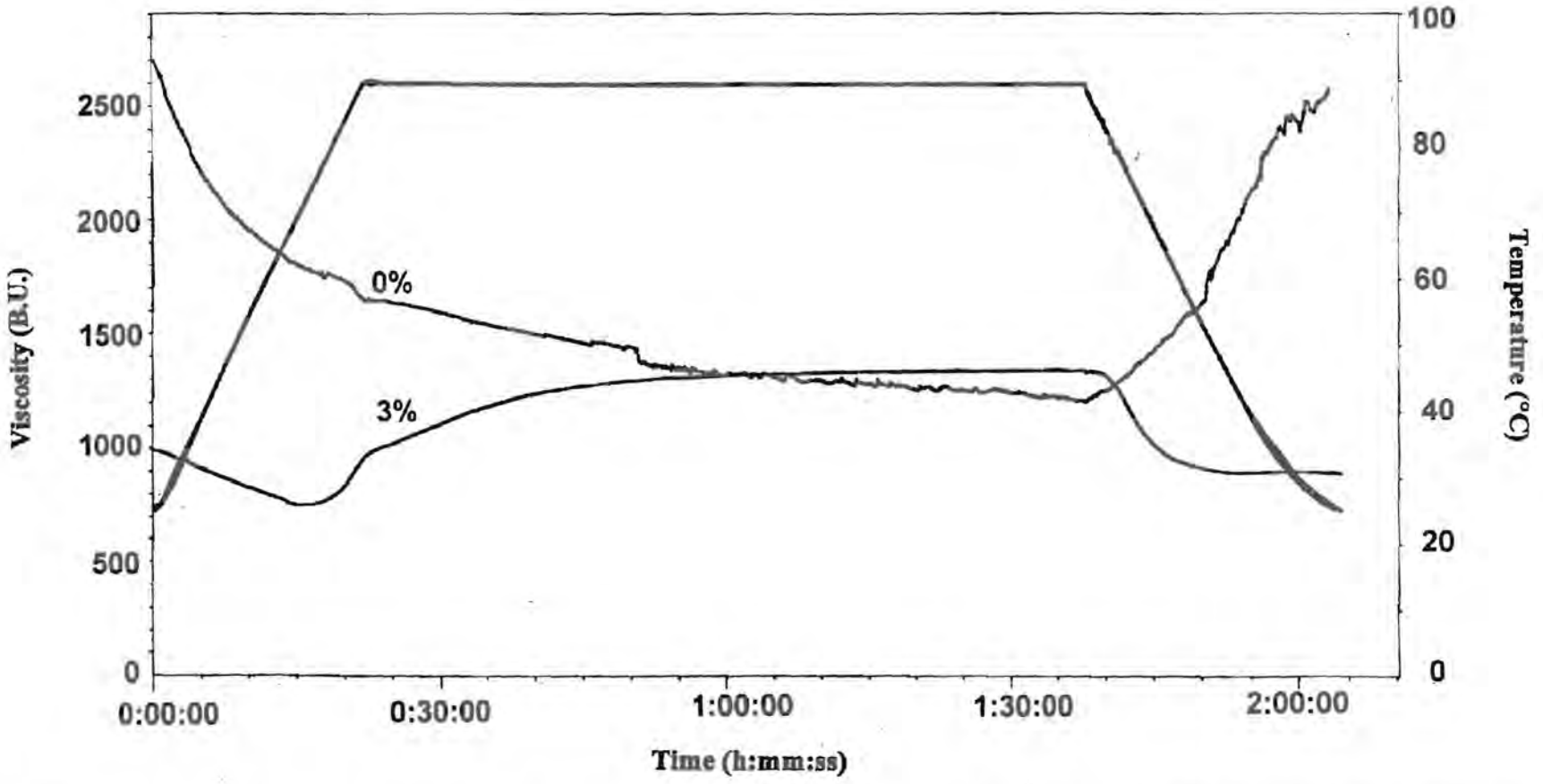

Figure 16. Pasting curves of gelatinized maize starch with 0 and 3 % stearic acid treated with pullulanase for 1.5, hours then heated from 25°C to 90°C, held for 76 minutes and cooled to 25°C in a Brabender Viskograph-E.

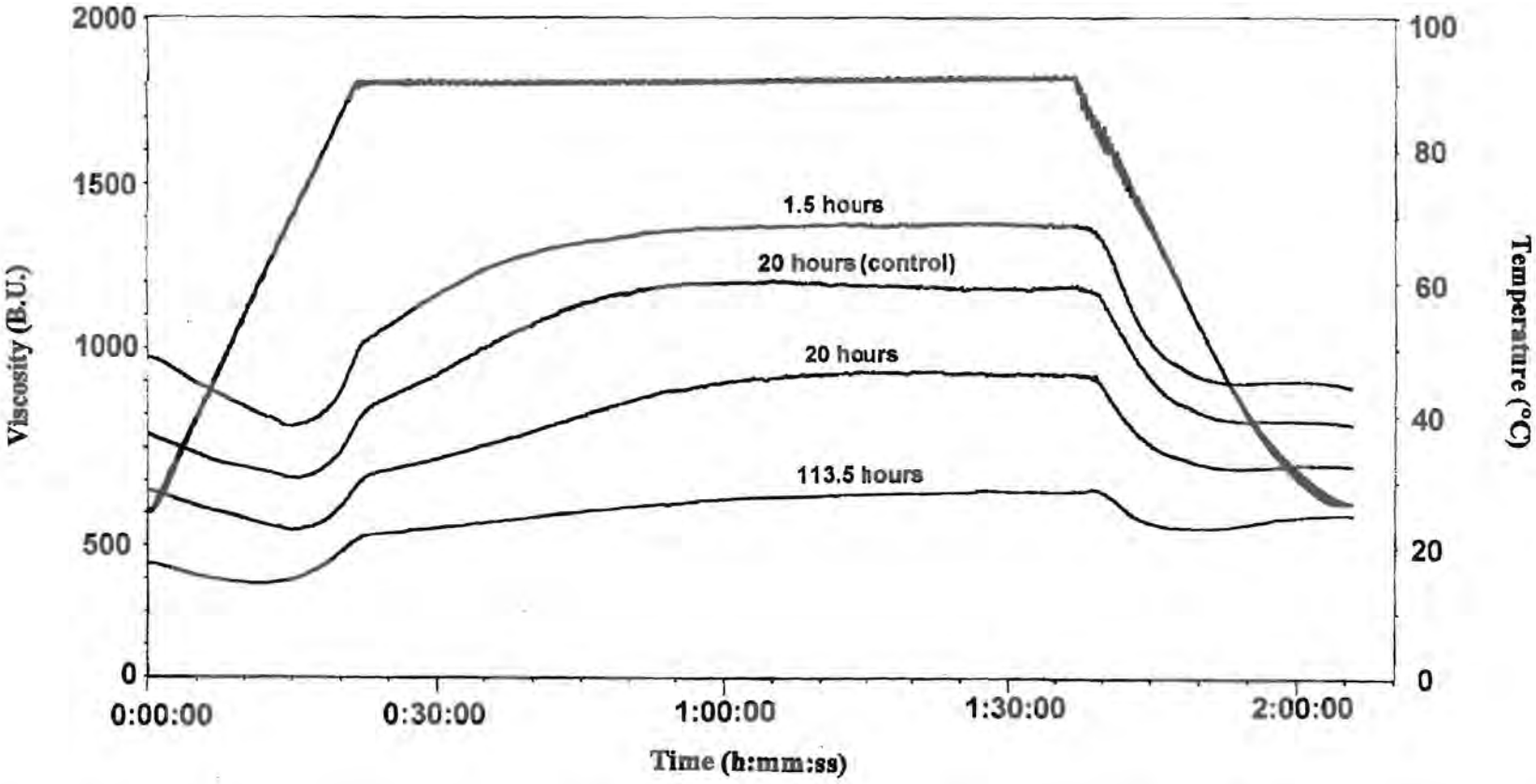

Figure 17. Pasting curves of gelatinized maize starch with 3% stearic acid treated with pullulanase for 1.5, 20 and 113.5 hours, with a control (no pullulanase) at 20 hours, then heated from 25°C to 90°C, held for 76 minutes and cooled to 25°C in a Brabender Viskograph-E.

Table 4. Significant points on the pasting curves of gelatinized maize starch with 3% stearic acid treated with pullulanase for 1.5, 20 and 113.5 hours, with a control (no pullulanase) at 20 hours, heated from 25°C to 90°C, held for 76 minutes and cooled to 25°C in a Brabender Viskograph- E.

	Units	Enzyme reaction time (hours)			
		1.5	20	20 (control)	113.5
Viscosity at beginning of heating	B.U.	990 [1]	580	748	348
Viscosity at start of peak	B.U.	766	458	613	290
Maximum viscosity	B.U.	1350	909	1186	552
Viscosity at start of cooling	B.U.	1342	896	1159	546
Viscosity at end of cooling	B.U.	846	602	753	458
Relative peak area [2]	%	100	79	98	63
Viscosity breakdown on heating	B.U.	224	122	135	58
Viscosity increase	B.U.	584	451	573	262
Viscosity breakdown on cooling	B.U.	496	294	406	88

[1] Means of at least two replicate experiments

[2] Calculated as the area between the line and a horizontal drawn from the lowest viscosity after the first viscosity peak to a vertical drawn at the beginning of cooling

The progressively lower initial viscosities probably resulted from a greater viscosity breakdown by shear-thinning during the enzyme incubation period, due to the progressively increasing amount of short linear amylopectin segments increasing molecular alignment, giving less molecular entanglement in the paste during stirring.

During heating, the paste viscosities of all the samples with added stearic acid initially decreased until about 70 °C was reached (Figure 17). The 20-hour control sample showed a slightly greater viscosity breakdown, with a decrease of 135 B.U., than the corresponding enzyme treated sample, which had a decrease of 122 B.U. As the enzyme reaction time increased from 1.5 to 113.5 hours, the breakdown in viscosity was progressively less, decreasing from 224 to 58 B.U. (Table 4). Previous results indicated that viscosity breakdown during heating after gelatinization is probably inhibited by the formation of amylose-lipid complexes (2.1). Thus, it is hypothesised that as the "amylose" content was increased in the gelatinized starch by the enzyme treatment, more amylose-lipid complexes were formed which inhibited the initial breakdown in viscosity during heating. As was to be expected, in the sample with 0% stearic acid, viscosity breakdown on heating occurred uninhibited (Figure 16).

As heating continued further to reach the holding temperature, the sample without stearic acid continued to decrease in viscosity (Figure 16), most probably due to continued uninhibited shear thinning. In contrast, the viscosities of the samples with 3% stearic acid eventually started to increase in viscosity and continued to increase to a maximum during holding at 90°C (Figure 17). The maximum viscosities reached were progressively less as the enzyme reaction time increased from 1.5 to 113.5 hours. This was probably partly due to the initial viscosities being progressively less due to enzymic de-branching of the starch, as discussed above. Thus for comparison, the amount of increase in viscosity during heating is considered more relevant than the peak viscosity attained. The viscosity increase of 573 B.U. in the 20 hour control sample without pullulanase (Table 4) was less than previously observed after gelatinization of maize starch with 3% stearic acid, which showed an increase of almost 800 B.U. on heating and holding at 90 °C (2.1). Thus cooling the sample between the end of gelatinization and the beginning of the second viscosity peak, as occurred for the enzyme incubation period, inhibited the increase in viscosity on

subsequent heating and holding at 90°C. It is proposed that this was due to retrogradation on cooling of the free amylose, which did not complex with lipids during gelatinization, and this retrogradation was not completely reversed by re-heating. Thus on re-heating, the incomplete re-solubilisation of retrograded amylose probably decreased the amount of amylose available for amylose-lipid complexing, which is hypothesised to be responsible for the increase in viscosity.

The 20 hour control (no pullulanase) showed a greater viscosity increase by 122 B.U than the corresponding sample and had an almost 20% greater relative surface area under the curve (Table 4). As the enzyme reaction time increased from 1.5 to 113.5 hours, the amount of viscosity increase progressively decreased from an increase of 584 to 262 B.U. and the relative peak surface areas progressively decreased to 63% of the largest area (Table 4, Figure 17). Native amylose molecules have a degree of polymerisation (D.P.) of 1000 to 4400 [9]. Most of the linear segments released from amylopectin have a D.P. of 12 to 60 [6]. Apart from these, only a few linear amylopectin segments have a D.P of 85 to 180, the minimum length required for forming the amylose-lipid complex [7]. Thus, only these few linear amylopectin segments were probably long enough to complex with stearic acid. Since these linear amylopectin segments are much shorter than the native amylose molecules, they were probably more mobile in the starch suspension. Thus it is proposed that the stearic acid complexed preferentially with these linear amylopectin segments, rather than with the longer native amylose molecules. In effect, the native amylose molecules probably had to unsuccessfully compete with the linear amylopectin segments to complex with the stearic acid. It is hypothesised that although the linear amylopectin segments probably complexed with the stearic acid, they were too short to effectively form the crystalline amylose-lipid complex superstructures, which are thought to increase paste viscosity and improve paste stability [1,10], as discussed (2.1). Thus, the complexes with the linear amylopectin segments probably remained in an amorphous, low viscosity state. The increase in viscosity and stability during holding was probably due to complexes formed with the native amylose molecules, which could form the crystalline amylose-lipid complex superstructures. Thus, it is proposed that as the enzyme reaction time increased from 1.5 to 113.5 hours, the amount of complexes preferentially formed with the linear amylopectin segments progressively

increased, causing the peak viscosity and relative peak area to progressively decrease, in addition to the viscosity breakdown that occurred during the enzyme reaction time due to the de-branching of amylopectin.

On cooling the gelatinized samples, no setback occurred in the samples with 3% stearic acid but rather, breakdown in viscosity occurred in all the samples with 3% stearic acid (Figure 17). As enzyme reaction time increased from 1.5 to 113.5 hours, the amount of breakdown during cooling decreased progressively from 496 to 88 B.U From previous results, the breakdown in viscosity on cooling maize starch with 3% stearic acid was hypothesised to be due to the transformation of crystalline amylose-lipid complex superstructures, which result in a high viscosity, to the amorphous state, which results in a low viscosity [2,11], as probably favoured at the lower temperatures (2.1). As discussed above, the linear amylopectin segments were probably too short to result in crystalline amylose-lipid complex superstructures. Thus, as the enzyme reaction time increased from 1.5 to 113.5 hours, the amount of crystalline complexes formed with the native amylose probably decreased, whilst the amount of amorphous complexes formed with the linear amylopectin segments increased. It is proposed that, in addition to a progressively lower peak viscosity, this resulted in progressively less viscosity breakdown on cooling due to there being fewer transformations from a crystalline form to an amorphous form. The 20-hour control (no pullulanase) showed a greater breakdown in viscosity, with a decrease of 406 B.U., than the corresponding pullulanase treated sample, which had a decrease of 294 B.U. (Table 4). This was probably due more complexes being able to form with the native amylose molecules in the absence of amylopectin linear segments, resulting in more crystalline superstructures, than in the corresponding pullulanase treated sample. As expected, the gelatinized sample without stearic acid underwent extensive setback on cooling, due to uninhibited retrogradation in the absence of amylose complexes with stearic acid (Figure 16).

2.2.3.2 Effect of 3% stearic acid addition to pre-gelatinized normal maize starch

For comparison with the effect of higher amylose content in laboratory pre-gelatinised high amylose maize starch, 3% stearic acid was added to commercial pre-gelatinised normal maize starch. As can be seen from Figure 18 and Table 5, the addition of 3% stearic acid to commercial pre-gelatinized maize starch significantly affected the pasting properties on re-hydration and subsequent heating. A first viscosity peak was observed during heating at about 50 °C. The initial increase in viscosity during heating was probably due to further swelling of the starch granules. This indicates that the commercial product was most likely not completely pre-gelatinized. The first peak was higher in the sample with 3% stearic acid (Figure 18). As the stearic acid was most likely still un-complexed at this temperature, it is proposed that the higher first viscosity peak achieved in the sample with 3% stearic acid was due to the un-complexed stearic acid having a plasticizing effect on the surface of the intact starch granules, as described by Roach and Hoseney [12], making them more flexible and thus allowing greater swelling of the granules before bursting. The viscosity decreased on further heating to form the first peak, probably due to the eventual bursting of the granules and solubilisation of amylose making the paste prone to shear thinning. In both samples, the viscosity decreased to approximately the same level by the beginning of the holding period. At that point, the 3% stearic acid sample had a viscosity of 517 B.U. and the 0% stearic acid sample had a viscosity of 531 B.U. (Table 5). Thus, the sample with 3% stearic acid showed a greater viscosity breakdown after the first peak, with a decrease of 287 B.U., than the sample without 3% stearic acid, which had a decrease of 173 B.U. (Table 5). This was probably because amylose-lipid complexing had not yet been initiated at this stage and thus there was no inhibitory effect on viscosity breakdown.

Compared to the first peak viscosity, from previous results, of almost 1300 B.U. obtained due to gelatinization of maize starch without stearic acid (2.1), the first viscosity peak of the commercial pre-gelatinized sample was much lower, 690 B.U.

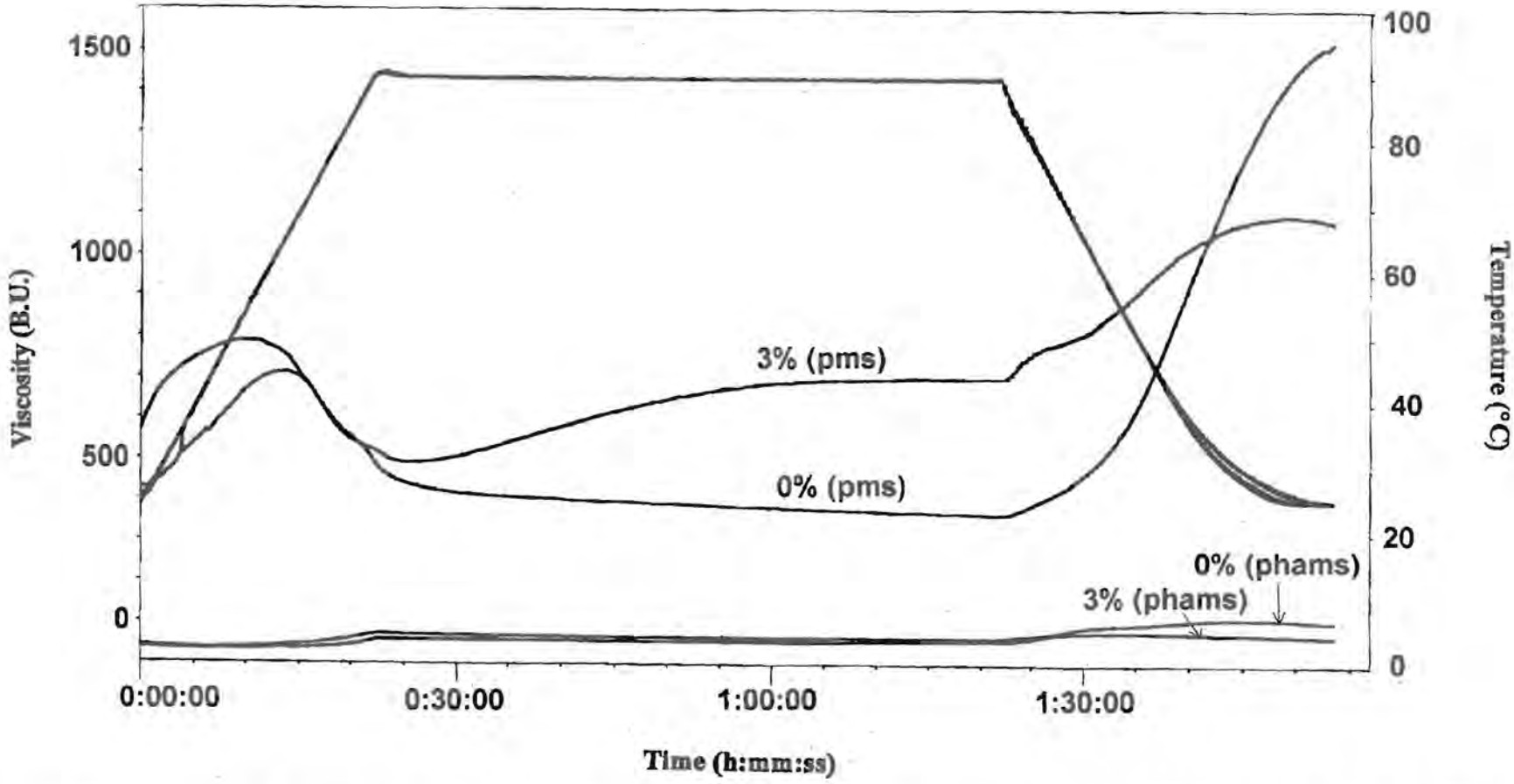

Figure 18. Pasting curves of commercial pre-gelatinized maize starch (pms) and laboratory pre-gelatinized high amylose maize starch (phams) with 0% and 3% stearic acid, heated from 25°C to 90°C, held for 76 minutes and cooled to 25°C in a Brabender Viskograph- E.

Table 5. Significant points on the pasting curves of pre-gelatinized maize and high amylose maize starches, with 0% and 3% added stearic acid, heated from 25°C to 90°C, held for 75 minutes then cooled to 25°C in a Brabender Viskograph-E.

	Units	Stearic acid concentration			
		0%	3%	0%	3%
		Normal maize starch		High amylose maize starch	
Viscosity at beginning of heating	B.U.	346 [1]	575	43	42
1st peak viscosity during heating	B.U.	690	818	- [2]	-
Viscosity beginning of holding	B.U.	517	531	68	52
Viscosity end of holding	B.U.	370	673	65	56
Viscosity end of cooling	B.U.	1509	1082	108	69
Viscosity breakdown on heating	B.U.	173	287	-	-
Viscosity change during holding	B.U.	- 147	143	- 3	4
Viscosity setback on cooling	B.U.	1139	409	43	14

[1] Means of at least two replicate experiments

[2] No increase in viscosity was observed during heating

Microscopic examination of the pre-gelatinized maize starch showed a large amount of starch granule fragments present in the sample. This high level of starch granule fragmentation, most likely resulting from the industrial post-gelatinization drying and milling processes, probably inhibited the re-hydration of the starch.

In agreement with previous investigations with maize starch (2.1), the addition of 3% stearic acid to the pre-gelatinized maize starch increased and stabilized the paste viscosity during holding at 90°C, as compared to the sample without stearic acid (Figure 18). As discussed (2.1), these effects are thought to be due to the formation of amylose lipid complexes. However, the maximum viscosity, 1082 B.U., was only reached at the end of the holding period (Table 5) and was much lower than previously observed with 3% stearic acid addition to maize starch, which reached 1864 B.U. near the middle of the holding period (2.1). This was probably partly due to the initially lower viscosity of the pre-gelatinized starch on-re-hydration, as discussed above. Comparing the amounts of viscosity increase during holding, the pre-gelatinized sample showed a lower increase in viscosity during holding of 143 B.U. (Table 5), than the increase of almost 800 B.U. previously observed with normally gelatinized starch (2.1). It is hypothesised that the poor re-hydration of the starch granules in the pre-gelatinized starch, as discussed above, reduced the amount of amylose-lipid complexes formed, as there was probably less solubilised amylose available for complexing than in normally gelatinized starch. Thus it is proposed that the lower second viscosity peak observed in the pre-gelatinized starch was due to a lower initial gelatinized viscosity, for reasons as discussed above, as well as too few amylose-lipid complexes being formed.

Retrogradation on cooling was inhibited to some extent by the addition of 3% stearic acid to the pre-gelatinized maize starch (Figure 18). As discussed (2.1), this is proposed to be due to the presence of amylose lipid complexes, which are known to inhibit retrogradation [15,16]. However, the fact that retrogradation did occur in the sample with 3% stearic acid indicates that there was some free amylose present at the end of the holding period, contrary to previous investigations with 3% stearic acid addition to normal maize starch (2.1). This supports the hypothesis that, on the

addition of 3% stearic acid, fewer amylose-lipid complexes formed in the pre-gelatinized maize starch than occurred in normally gelatinized maize starch.

2.2.3.3 Effect of 3% stearic acid addition to pre-gelatinized high amylose maize starch

To determine the effect of higher amylose content on the pasting properties of maize starch with added stearic acid, 3% stearic acid was added to laboratory pre-gelatinized high amylose maize starch. The results obtained were compared to commercial pre-gelatinized normal maize starch with 3% stearic acid. As seen in Figure 18, the pre-gelatinized high amylose starch samples exhibited no significant pasting, with their viscosities remaining below 100 B.U. during heating and holding at 90°C (Table 5). This was contrary to the pre-gelatinized normal maize starch samples with 0% and 3% stearic acid, which displayed significant pasting. This was attributed to an inability of the pre-gelatinised high amylose maize starch to re-hydrate sufficiently and thus the amylose could not be solubilised.

Due to its very high amylose content, high amylose maize starch forms very tightly packed granules that are resistant to swelling [8]. Thus, high amylose maize starch cannot be gelatinized by cooking under normal conditions but requires the elevated temperatures of pressure-cooking to gelatinize [8]. Gelatinization was further confirmed by the visible formation of a paste and rapid retrogradation into a strong gel of a sample left to cool to room temperature after pressure-cooking. This rapid retrogradation of the paste on cooling is due to its high amylose content, which undergoes a strong irreversible retrogradation due its long linear structure promoting its rapid crystallisation [17]. Rapid freezing of the hot paste in liquid nitrogen aimed to freeze the hot paste instantly before the solubilised amylose could retrograde. Microscopic observation under polarized light of the pre-gelatinized starch, taken after freeze-drying, showed the absence of starch granule birefringence (Maltese crosses). This indicates that the high amylose maize starch was gelatinized by pressure-cooking [9].

Furthermore, the pre-gelatinized starch granules, observed by microscopy under normal light after freeze-drying, were found to be intact and structurally very compact and a few compact linear structures were also observed. These compact linear structures are hypothesised to be crystallized amylose molecules.

During freeze-drying, as water was evaporated, the hydrogen groups of the amylose molecules were probably released from H-bonds with water molecules and were thus exposed to each other. Due to amylose having a strong tendency to associate with itself [18], it is proposed that the removal of water resulted in the formation of several intermolecular H-bonds between adjacent amylose molecules. It is proposed that this caused the compacting of the intact granules and crystallised the amylose molecules, which had leached from the granules during gelatinization, into the compact linear structures observed by microscopy. It is further proposed that the inability of the pre-gelatinized high amylose starch to re-hydrate effectively was due to the compact granules and linear amylose structures being mostly inaccessible to water.

Thus freeze-drying seems to have counteracted the intended effect of rapidly freezing the gelatinized hot paste in liquid nitrogen. These difficulties experienced in the development of a pre-gelatinized high amylose maize starch, which inhibit its re-hydration, probably explain why no commercial products appear to exist.

As seen in Figure 18, the pasting behaviour of the pre-gelatinized high amylose samples with 0% and 3% stearic acid only differed significantly during cooling. During heating, a very slight increase in viscosity of 10 to 25 B.U. occurred similarly in both the samples (Table 5), which was then maintained throughout the holding period. This slight increase in viscosity on heating was probably due to the intermolecular H-bonds within the amylose structures being slightly weakened with the increasing temperature thus, allowing slightly more re-hydration to occur. On cooling, the sample with 3% stearic acid had less setback, with a viscosity increase of 14 B.U., than the sample with 0% stearic acid, which setback approximately threefold as much, with a viscosity increase of 43 B.U. (Table 5). This inhibition of retrogradation in the sample with 3% stearic acid indicates that, although re-hydration was greatly inhibited, a small amount of amylose lipid complexes probably did form with the small amount of amylose that was solubilised on heating. However, as no

viscosity increase was observed during holding, the amount of amylose-lipid complexes formed was probably too few to form the crystalline amylose-lipid complex superstructures, which are thought to increase the paste viscosity [1,10]. Thus, most of the stearic acid in the suspension probably remained un-complexed.

2.2.4 Conclusions

The formation of short linear amylopectin segments by pullulanase treatment of maize starch seemed to increase the amount of amylose–lipid complexes formed with the added stearic acid. However, the maximum stable viscosity of the paste achieved during holding at 90°C was decreased by pullulanase treatment, probably due to the preferential forming of complexes with the shorter linear amylopectin segments, which were probably too short to form crystalline superstructures. For more relevant comparison of the effects of increasing the amylose content, the length of additional "amylose" segments should be similar to that of native amylose. Using high amylose-maize starch would achieve this, as its higher amylose content is all naturally occurring native amylose. Thermally pre-gelatinized high amylose maize starch could not be re-hydrated effectively, probably due the to re-compacting of the gelatinized starch granules and crystallization of the amylose during freeze-drying, making these structures inaccessible to water. Thus the effect of the higher amylose content could not be determined as the formation of amylose-lipid complexes was probably inhibited by the unavailability amylose. For further investigation into the effect of increased amylose content on the pasting properties of maize starch with added stearic acid, the use of a suitable chaotropic reagent, such as dimethyl sulphoxide or potassium hydroxide, to solubilise and actually gelatinize the high amylose starch [20] is recommended instead of attempting to thermally pre-gelatinize it.

2.2.5 References

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CHAPTER 3

DISCUSSION

The biphasic pasting curves obtained with maize starch (2.1) were similar to those observed by Nelles *et al.* (2000) and also followed a similar pattern regarding the size and time of the second viscosity peak with variation of the holding temperature. As the holding temperature decreased from 92 to 82 °C, the size of the second viscosity peak increased whilst the earliest second peak occurred at 88°C. These effects were attributed to the formation and thermal stability of amylose-lipid complexes with the endogenous lipids in the starch, as determined by the level of thermal energy applied during an extended holding period (Nelles *et al.*, 2000).

It was found that the addition of stearic acid greatly increased the second viscosity peak of maize starch during an extended holding time at 90 °C, which was determined as the optimum temperature for investigation of the second peak phenomenon (2.1.3.2). The highest second viscosity peak of almost 2000 B.U. (Table 2), which was also the most stable and maintained the increased viscosity throughout the holding period and subsequent cooling, occurred with 1.5 % stearic acid (Figure 14) and thus was the optimum stearic acid concentration.

From reviewing the literature (1.2.5.1), it was found that the presence of amylose-lipid complexes increases the viscosity of starch paste and inhibits retrogradation, due to the formation of crystalline superstructures. These effects were observed in maize starch with the addition of stearic acid and the extent of these effects increased with increasing stearic acid addition, but did not increase beyond a stearic acid concentration corresponding to the saturation point for the amount of amylose present in the starch, calculated for stearic acid as reviewed (1.2.5.1.10). Thus, the results obtained (2.1.3.2) support the hypothesis that the increase in viscosity obtained in maize starch with the addition of stearic acid was due to the formation of amylose-lipid complexes and that extent of viscosity increase was determined by the amount of amylose-lipid complexes formed. As the increase in viscosity during extended holding is due to a manipulation of the second viscosity peak by stearic acid addition, these

results also support the original hypothesis by Nelles *et al.* (2000) that the formation of the second peak in the biphasic pasting curve of maize starch is due to amylose-lipid complexing.

It was further found (2.1.3.3) that with the addition of 1.5% stearic to maize starch, if the holding temperature was varied between 82 to 90 °C, the pasting viscosity achieved during extended holding maintained its stability throughout the holding period and subsequent cooling (Figure 15a). However, the stable viscosity achieved during extended holding increased progressively as the holding temperature increased from 82 to 90 °C. The stable viscosity achieved at a holding temperature of 82 °C was approximately equal to the first viscosity peak due to gelatinization, just above 1000 B.U., whilst the stable viscosity achieved at 90 °C, slightly below 2000 B.U., was almost double that of the first viscosity peak due to gelatinization (Table 3). As the extent of viscosity increase during extended holding was probably determined by the amount of amylose-lipid complexes formed (Raphaelides, 1992; Kaur and Singh, 2000), these results support the hypothesis that the amount of amylose-lipid complexes formed during extended holding was determined by the amount of thermal energy applied to the system at a specific holding temperature.

It was found that de-branching amylopectin in gelatinized maize starch with pullulanase decreased the peak viscosity achieved during subsequent extended holding (2.2.3.1). This effect was contrary that to that proposed in the hypothesis that de-branching gelatinized maize starch will increase the "amylose" content of the starch by the release of linear amylopectin segments and thus further increase the paste viscosity by enabling the formation of more amylose-lipid complexes in maize starch with excess stearic acid. Furthermore, although the viscosity achieved during extended holding decreased as the enzyme reaction time increased, the amount of amylose-lipid complexes formed probably increased, as indicated by the progressively increasing inhibition of both breakdown during heating and retrogradation on cooling (2.2.3.1). It was proposed that this was due to preferential complexing of the lipids with the shorter linear amylopectin segments, rather than with the longer native amylose molecules, as discussed (2.2.3.1), and thus the amount of complexes with the native lipids probably progressively decreased. In effect, the experiment was un-

expectedly reversed. Instead of determining the effect of increasing "amylose" content, it determined the effect of decreasing the amount of native amylose able to complex with lipids. Thus, these results in fact support the hypothesis that increased paste viscosity was due to the complexing of native amylose with lipids. To determine the effect of increasing the "amylose" content, the original objective, the chain length of the additional "amylose" should be maintained similar to that of native amylose, as when using high amylose maize starch. Possible contamination of the pullulanase enzyme preparation with alpha-amylase, which breaks down native amylose, may have also played a role in reducing the paste viscosity and should also be considered.

It was also found that the use of a laboratory-prepared pre-gelatinized high amylose maize starch (2.2.3.3), prepared as described (2.2.2.4), could not be used to compare the effect of its higher amylose content to that of normal maize starch. This was due to difficulties experienced in preventing the strong association of amylose with itself (Hoseney, 1994) during the preparation, resulting in an inability to re-hydrate the pre-gelatinized high amylose maize starch. Thus on attempted re-hydration, the effect of stearic acid addition could not be determined, due to the amylose not being solubilised and was thus not available for complexing.

The results from this study support the original hypothesis proposed by Nelles *et al.* (2000), that the second viscosity peak in the biphasic pasting curve of maize starch was due to the formation of amylose-lipid complexes, but do not prove it. In order to prove the hypothesis, further investigation is required to determine the effect of increasing amylose content on the pasting of maize starch with added stearic acid.

The use of a suitable chaotropic reagent, such as dimethyl sulphoxide ($(CH_3)_2SO^{2-}$), or potassium hydroxide (KOH), which are solvents of starch (Hoseney, 1994), was recommended (2.2.4) to solubilise the high amylose maize starch, as an alternative to attempting to thermally pre-gelatinize it. Amylose will remain solubilised in such a solution if the anion concentration, or pH, is kept high, e.g. as with 1M KOH, due to small positive charges being induced on the hydroxyl groups of amylose, making adjacent chains repel each other (Hoseney, 1994). Thus, the amylose would presumably be available for complexing with lipids present in the solution. Furthermore, amylose can be precipitated from such a starch solution with *n*-butyl

alcohol, which forms an insoluble helical inclusion complex with the amylose (Hoseney, 1994). This presents a further possibility of first pre-preparing amylose-lipid complexes for subsequent incorporation into a pasted starch. This could possibly be achieved by precipitating the amylose out of a starch solution with *n*-butyl alcohol, as described above, and then substituting the alcohol in the helical inclusion complex with a lipid under suitable conditions.

There exists potential for the development of a natural starch with a paste high viscosity, which is relatively stable under conditions of continuous high shear, extended holding at high temperature, and subsequent cooling to room temperature, as an alternative to chemically modified starches with similar properties. For example, stearic acid addition to starch could present an inexpensive, more natural alternative to the chemical crosslinking of starches with various synthetic phosphates (e.g. phosphoryl chloride, $POCl_3$ or sodium trimetaphosphate), which are currently being used to improve paste stability (Whistler and BeMiller, 1997). Since stearic acid is a naturally occurring fatty acid, native to maize starch (Villwock, Eliasson, Silverio and BeMiller, 1999), it would be safe for use in starch to create a new food ingredient. For consumers, this would represent a more natural and thus healthier image of the food product containing this new ingredient instead of chemically modified starch.

Such a paste would be useful in applications where conditions of high temperatures, high shear and subsequent cooling are common yet a stable high viscosity is required. It could be used in products requiring a stable viscosity over various temperatures, for example prepared sauces, canned soups, custard and gravies, which may be cold or heated. Due to the increased viscosity of the lipid complexed starch paste (2.1), it would require less starch to achieve the desired viscosity in such products than with normal starches. Thus adding a very small amount of stearic acid to the starch to form such a complexed paste would reduce the amount of starch required in the product formulation for thickening. In addition to being more economic, this property could be exploited to reduce the kilojoule content of the product for the development of low-kilojoule products. As carbohydrates have a relatively high kilojoule content of approximately 17 kJ/g (Guthrie and Picciano, 1995), the lower overall starch content would significantly decrease the kilojoule content of the food product.

Furthermore, amylose-lipid complex superstructures reportedly have an increased resistance to digestion by enzymes, as the accessibility of both lipases and amylases to their respective substrates is reduced by the crystalline structure of the amylose-lipid complex superstructures (Seneviratne and Biliaderis, 1991). Thus the nutritive value of the food product containing amylose-lipid complexes is decreased (Tomasik and Schilling, 1988; Seneviratne and Biliaderis, 1991) Furthermore, a lipid complexed starch paste would behave similarly to resistant starch or dietary fibre when used as a food ingredient, as described in the patent by Mahr and Trueck (1999). This would make it suitable for use as a fat-replacer in reduced fat or fat-free products such salad creams, mayonnaise, ketchup, cheese spreads, dips, frozen desserts, peanut butter and other sandwich spreads as described in the patents by Mahr and Trueck (1999) and Yuan, (2000). The stability (shear resistance) of the starch paste makes it suitable for continuous process lines requiring the product to be pumped through long pipelines, which creates conditions of high shear. Thus food products using this new ingredient would not be limited to batch processing.

Such a lipid complexed starch could also have uses in several non-food applications requiring a stable, high viscosity. It could improve on the gelatinized starches currently being used for paper sizing and coating, textiles sizing, adhesive formulations, and drilling muds for mining (Zobel, 1988).

The production of such a paste could be achieved by the incorporation of 1.5% stearic acid into maize starch, followed by gelatinizing the paste during heating to a holding temperature of up to 90°C and holding for approximately 45 minutes, the holding time required until the increased stable viscosity was reached (Figure 15a). It is proposed that the holding temperature applied, ranging from 82 to 90°C, can be used as a variable to achieve the desired stable paste viscosity, ranging from approximately the original gelatinized viscosity to an almost doubled viscosity (Figure 15a).

On an industrial scale, the amylose-lipid complexed paste could be prepared quite simply by using existing technologies. Evaporation of the solvent, which was used to dissolve and homogenously disperse the stearic acid in the starch, could be performed by technologies used for applications involving similar large-scale evaporation and

recollection of hexane. As the solvent could be recollected and re-used, this process would be relatively cost-effective and no harmful effluent would be produced. The remaining dry starch-lipid mixture could either be directly used as a dry food ingredient, incorporated into suitable product formulations before a sufficient heating process, or first cooked with water to form the complexed paste, which can subsequently be incorporated into suitable food products. Alternatively, as reviewed from the literature (1.2.5.1.10), an extruder can be used under the processing conditions required to achieve the desired paste viscosity, as a method for the large-scale production of complexed starch paste (Bhatnagar and Hanna, 1994a, Bhatnagar and Hanna, 1994b).

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

The second viscosity peak of the biphasic pasting curve of maize starch, occurring on extended holding, varies in size and time with variation of the holding temperature. This is attributed to the extent and rate of amylose-lipid complex formation, with the endogenous lipids of the starch, and the subsequent dissociation of these complexes as determined by the level of thermal energy applied to the system during the extended holding period at a specific holding temperature.

The second viscosity peak occurring on extended holding during the pasting of maize starch can be greatly increased and stabilized by the addition of stearic acid. With 1.5% stearic acid addition, the initial viscosity after gelatinization can be almost doubled at a holding temperature of 90°C and remains relatively stable during extended holding on subsequent cooling to room temperature, under continuous conditions of high shear. These observed effects of stearic acid addition on the pasting viscosity behaviour of maize starch correspond to the reviewed effects of amylose-lipid complexes on starch pastes. This, as well as the observation that no further effects occurred beyond a stearic acid concentration that corresponded well to the calculated saturation point of amylose, for the amount present in maize starch, strongly suggests that these effects were due to the formation of amylose-lipid complexes.

Confirmation that the observed effects of stearic acid addition to maize starch increase further on increasing the amylose content of the starch, would confirm that the hypothesis that these effects are due to amylose-lipid complexes. This was not accomplished with the methods used to attempt to increase the amylose content of maize starch.

Pullulanase treatment of gelatinized maize starch was not a suitable method to determine the effect of increasing amylose content, as the resulting linear amylopectin segments were much shorter than the native amylose molecules and thus introduced a

new variable into the system. In order for only the effect of increasing amylose content to be determined, the chain length of the additional "amylose" molecules should be maintained similar to that of native amylose, such as when using high amylose maize starch. Lipids probably preferentially complexed with the linear amylopectin segments rather than with the native amylose molecules, due to their shorter length probably resulting in a greater mobility. The fact that paste viscosity decreased as the amount of complexing with native amylose probably decreased supports the hypothesis that increased paste viscosity in maize starch with stearic acid was due to amylose-lipid complexes.

Determining the effect of increasing amylose content from laboratory-prepared pre-gelatinized high amylose maize starch was also not a suitable method. The strong association of amylose with itself, which prevents it from re-hydrating, could not be prevented. Thus, it is not feasible to prepare pre-gelatinized high amylose maize starch. It is recommended that, in order to determine the effect increasing amylose content from high amylose maize starch, a suitable starch solvent such as dimethyl sulphoxide, which is known to prevent the strong association of amylose with itself, could be used to solubilise high amylose starch and maintain the availability of amylose for lipid complexing.

A natural starch paste with increased paste viscosity and relative shear stability, resulting from the addition of stearic acid and subsequent pasting, has potential for use as a new food ingredient, as well as for various non-food applications. Such a starch paste could be produced on a large-scale by existing technologies and would be suitable for continuous process lines.

CHAPTER 5

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Posters

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