Modification of the paste properties of maize and teff starches using stearic acid

by

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DECLARATION

I declare that this dissertation submitted at the University of Pretoria for the degree: MSc: Food Science has not been submitted by me for a degree at any other University or institution of higher education.

______________________
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ABSTRACT

Starch is used in many food applications as thickeners, texturisers and fat substitutes. Native starches, although useful, have low stability to conditions such as high shear, extreme pH and high temperatures encountered during food processing. Starches are modified to make them more suitable for processing conditions. The modification of starch by the use of a naturally occurring compound (for example stearic acid) may produce desirable properties and also removes the risk of a chemical residue in the starch. Starch can be from several grain sources. Teff grain is highly underutilized and under-researched. The work conducted in this project investigates the pasting properties, gelling tendencies, clarity and flow properties (using a rheometer) of teff starch pastes treated with stearic acid, in comparison to maize starch pastes. X-Ray Diffraction (XRD) and Confocal Laser Scanning Microscopy (CLSM) were also used to investigate the possible impact of stearic acid on the structure of the starch granules and pastes.

Starch suspensions containing stearic acid (0.25% - 4%) were pasted in a Rapid Visco Analyser using a short pasting cycle of approx. 30 min (held for 5 min at 91°C). Maize starch (treatments) showed a reduced peak viscosity within the holding period, while teff starch (treatments) did not. Teff starch showed increasing viscosity without reaching a peak during the holding time. The pasting cycles were then extended (holding time extended to 2 hr) to investigate the pasting behaviour of teff starch.

The extended pasting cycle resulted in a reduced first viscosity peak for maize starch with added stearic acid. Teff starch with added stearic acid showed a large increase in viscosity without the formation of the first viscosity peak. However, both starches displayed a second pasting peak. The addition of stearic acid resulted in an increase in the viscosity of the second pasting peak from about 175 Rapid Visco Units (RVU) to 228 RVU for maize starch, and from 113 RVU to 250 RVU for teff starch. The final viscosity of maize starch increased from 186 RVU to 227 RVU, while that of teff starch increased from 194 RVU to 261 RVU. The second viscosity peak was not observed with waxy
maize starch (approx. 97% amylopectin). This suggests that amylose-stearic acid complexation might have been responsible for the formation of this peak. Complexation Index (CI) values increased as the concentration of stearic acid was increased. This further suggests that some interaction between amylose and stearic acid had taken place. The pastes of maize and teff starches modified with stearic acid were more opaque and showed reduced gelling compared to their non-modified counterparts.

Maize and teff starches and their stearic acid-treated counterparts followed the Power-Law Model and were shear thinning (n < 1). However, teff starch pastes (control and treatment) seemed to be less shear thinning than their maize starch paste counterparts. An increase in consistency, k, after the extended pasting cycle was used (compared to the short pasting cycle) for the treated starches, reflects the increased viscosities obtained during extended pasting.

XRD further suggested that amylose-lipid complexes may have been present in the starch pastes (after extended pasting) due to the occurrence of the 4.4 Å and 12 Å peaks (characteristic of V-type starches). CLSM showed that stearic acid diffused into maize starch granules but not into teff starch granules. This was probably due to the pores of the surfaces of maize starch granules which may have facilitated the diffusion process. In contrast, teff starch granules do not have pores on their surfaces. This structural difference may be attributed for the pasting differences between teff and maize starches.

The effects of stearic acid on the pasting (effect on first and second peaks and final viscosity), and functional properties (reduced gelling and increased opacity of pastes) of maize and teff starches have been attributed to the formation of amylose-lipid complexes. These high viscosity and low gelling starches may be extremely useful as fat replacers. Teff starch has the added benefit of its small starch granules which may add to its ability mimic the mouthfeel of fat globules.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2. Literature Review</td>
<td>3</td>
</tr>
<tr>
<td>2.1 Starch: structure and composition</td>
<td>3</td>
</tr>
<tr>
<td>2.1.1 Starch granules</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2 Starch polymers</td>
<td>6</td>
</tr>
<tr>
<td>2.1.3 Structural organization of starch</td>
<td>9</td>
</tr>
<tr>
<td>2.2 Starch gelatinization, pasting and retrogradation</td>
<td>14</td>
</tr>
<tr>
<td>2.2.1 Gelatinization</td>
<td>15</td>
</tr>
<tr>
<td>2.2.2 Pasting</td>
<td>15</td>
</tr>
<tr>
<td>2.2.3 Retrogradation</td>
<td>16</td>
</tr>
<tr>
<td>2.3 Modification of starch</td>
<td>17</td>
</tr>
<tr>
<td>2.4 Effects of modification with fatty acid on starch functionality</td>
<td>19</td>
</tr>
<tr>
<td>2.4.1 Pasting properties</td>
<td>19</td>
</tr>
<tr>
<td>2.4.2 Retrogradation, gelling ability and syneresis</td>
<td>23</td>
</tr>
<tr>
<td>2.4.3 Starch structure</td>
<td>24</td>
</tr>
<tr>
<td>2.4.4 Flow properties at different shear rates</td>
<td>27</td>
</tr>
<tr>
<td>2.5 Factors affecting the properties of starches modified with lipid/fatty acid</td>
<td></td>
</tr>
<tr>
<td>2.5.1 Chain length</td>
<td>28</td>
</tr>
<tr>
<td>2.5.2 Fatty acid saturation level</td>
<td>29</td>
</tr>
<tr>
<td>2.5.3 Concentration of fatty acid</td>
<td>30</td>
</tr>
</tbody>
</table>
### TABLE OF CONTENTS (continued)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6 The chemistry and structure of amylose-lipid complexes</td>
<td>31</td>
</tr>
<tr>
<td>2.6.1 V-amylose</td>
<td>31</td>
</tr>
<tr>
<td>2.6.2 $V_h$-amylose</td>
<td>33</td>
</tr>
<tr>
<td>2.6.3 Orientation of the fatty acid chain within the helix</td>
<td>33</td>
</tr>
<tr>
<td>2.6.4 Type I and Type II structures</td>
<td>34</td>
</tr>
<tr>
<td>2.7 Potential applications of lipid-modified starches</td>
<td>38</td>
</tr>
<tr>
<td>2.8 Concluding remarks</td>
<td>39</td>
</tr>
<tr>
<td>3. Hypotheses and Objectives</td>
<td></td>
</tr>
<tr>
<td>3.1 Hypotheses</td>
<td>40</td>
</tr>
<tr>
<td>3.2 Objectives</td>
<td>42</td>
</tr>
<tr>
<td>4. Research</td>
<td></td>
</tr>
<tr>
<td>4.1 Paste properties of maize and teff starches modified with stearic acid</td>
<td></td>
</tr>
<tr>
<td>4.1.1 Introduction</td>
<td>44</td>
</tr>
<tr>
<td>4.1.2 Materials and Methods</td>
<td>45</td>
</tr>
<tr>
<td>4.1.2.1 Samples</td>
<td>45</td>
</tr>
<tr>
<td>4.1.2.2 Starch extraction</td>
<td>45</td>
</tr>
<tr>
<td>4.1.2.3 Proximate analyses</td>
<td>46</td>
</tr>
<tr>
<td>4.1.2.4 Amylose/amylopectin determination</td>
<td>46</td>
</tr>
<tr>
<td>4.1.2.5 Incorporation of stearic acid into starch</td>
<td>47</td>
</tr>
<tr>
<td>4.1.2.6 Starch pasting properties</td>
<td>47</td>
</tr>
<tr>
<td>4.1.2.7 Complexation Index (CI)</td>
<td>48</td>
</tr>
<tr>
<td>4.1.2.8 Starch paste clarity</td>
<td>48</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (continued)

Page No.

4.1.2.9 Starch gel texture .................................................49
4.1.2.10 Statistical analyses ..............................................49
4.1.3 Results and Discussion..................................................49
  4.1.3.1 Composition of the starches ................................49
  4.1.3.2 Pasting properties (short pasting cycle) .................50
  4.1.3.3 Pasting properties (extended pasting cycle) ..........57
  4.1.3.4 Paste clarity of starch pastes after the short and
  extended pasting cycles.............................................65
  4.1.3.5 Gel texture of starches after the short and extended
  pasting cycles..........................................................66
  4.1.3.6 Conclusions .......................................................67
  4.1.3.7 References ........................................................68

4.2 Flow properties and structure of maize and teff starch pastes modified
with stearic acid
  4.2.1 Introduction .............................................................73
  4.2.2. Materials and Methods ............................................74
    4.2.2.1 Samples ............................................................74
    4.2.2.2 Starch extraction .................................................74
    4.2.2.3 Starch composition .............................................74
    4.2.2.4 Incorporation of stearic acid into starch ..............75
    4.2.2.5 Flow properties of starch pastes at varying shear
    rates .................................................................75
    4.2.2.6 X-Ray Diffraction (XRD) .....................................76
    4.2.2.7 Confocal Laser Scanning Microscopy (CLSM) ..........76
    4.2.2.8 Statistical analyses ............................................79
  4.2.3 Results and Discussion..............................................79
TABLE OF CONTENTS (continued)

Page No.

4.2.3.1 Flow properties ......................................................79
4.2.3.2 X-Ray Diffraction (XRD) ........................................82
4.2.3.3 Confocal Laser Scanning Microscopy (CLSM) ...........86
4.2.3.4 Conclusions .........................................................94
4.2.3.5 References ..........................................................94

5. General Discussion .......................................................97
5.1 Review of Methodology ................................................97
  5.1.1 Starch extraction ..................................................97
  5.1.2 Incorporation of stearic acid into starch .....................98
  5.1.3 Flow properties and rheology of starch pastes ............98
  5.1.4 Complexation Index (CI) .......................................100
  5.1.5 Starch structure ..................................................100
5.2 Paste properties of maize and teff starches modified with stearic acid ..................................................103
5.3 Food applications of stearic acid-modified starches ..........105

6. Conclusions and Recommendations ................................109

7. References ...............................................................111
LIST OF FIGURES

Page No.

Fig. 2.1: Spherical and angular maize starch granules
(Whistler & BeMiller 1997 a) ......................................................... 4

Fig. 2.2: Individual teff starch granules
(Bultosa et al 2002) ................................................................. 4

Fig. 2.3: The compound teff starch granule as found in the endosperm
of the teff grain
(Bultosa et al 2002) ................................................................. 5

Fig. 2.4: Amylose helices
(Immel and Lichtenhalter 2000; and Zobel 1988 a) ..................... 7

Fig. 2.5 Schematic representation of the A, B and C chains; and clusters;
of amylopectin
(Kent and Evers 1994) ............................................................. 8

Fig. 2.6 The Blocklet Model - a schematic representation of the levels
of structural organisation within starch granules
(Gallant et al 1997) ................................................................. 10

Fig. 2.7: Amylopectin clusters which may contain Amylose-lipid
Complexes
(Gallant et al 1997) ................................................................. 11

Fig. 2.8: The crystal structures of A and B-type starches
(Gallant et al 1997) ................................................................. 12
LIST OF FIGURES (continued)

Fig. 2.9: X-Ray Diffraction patterns of various starches
   (Zobel 1988 a ) ................................................................. 13

Fig. 2.10: A Generalised RVA Starch Pasting Curve
   (Batey 2007) ................................................................. 14

Fig. 2.11: The structure of stearic acid ........................................ 19

Fig. 2.12: Light microscopy images of wheat starch with an added
   polyglycerol-monoglyceride (PGE/MG) mixture
   (Richardson et al 2003) ..................................................... 21

Fig. 2.13: The biphasic pasting curves for maize starch with added
   stearic acid
   (Bajner 2002) ................................................................. 22

Fig. 2.14: Effect of fatty acid chain length on gel texture
   (Whittam et al 1986) ......................................................... 23

Fig. 2.15: Large and small spherocrystals of maize starch
   (Fanta et al 2002) ............................................................. 26

Fig. 2.16: X-ray diffraction patterns of starches modified with varying
   concentrations of stearic acid
   (Tang and Copeland 2007) ................................................. 26

Fig. 2.17: Pseudoplastic behaviour
   (Mezger 2006) ................................................................. 28
LIST OF FIGURES (continued)

Fig. 2.18: The amylose-monostearin complex
   (Carlson and Larsson 1979) ....................................................32

Fig. 2.19: The dimensions of V<sub>n</sub>-amylose and the three orientations of the fatty acid chain inside the helix core
   (Godet et al 1993 b) ............................................................34

Fig. 2.20: Schematic representation of the mechanism of amylose-lipid complexation, forming Type I and Type II complexes
   (Biliaderis and Galloway 1987) .............................................35

Fig. 2.21: The arrangement of V<sub>n</sub>-amylose to form a “hexagonal” crystal lattice
   (Rappenecker and Zugenmaier 1981) .................................37

Fig. 2.22: DSC thermograms for potato amylose modified with different fatty Acids (Tufvesson et al 2003) ........................................38

Fig. 4.1.1: Effect of stearic acid concentration on the pasting properties of maize starch, for the short pasting profile ................52

Fig. 4.1.2: Effect of stearic acid concentration on the pasting properties of teff starch, for the short pasting profile .......................53

Fig. 4.1.3: Effect of stearic acid concentration on the complexation indices of maize and teff starches ...............................57

Fig. 4.1.4: Effect of stearic acid concentration on the pasting properties of maize starch, for the extended pasting cycle ...............60
LIST OF FIGURES (cont.)

Page No.

Fig. 4.1.5: Effect of stearic acid concentration on the pasting properties of teff starch for the extended holding cycle ..........59

Fig. 4.1.6: Effect of stearic acid concentration on the pasting properties of waxy maize starch for the extended pasting cycle ..........64

Fig. 4.2.1: Intervals at which samples were taken for CLSM .................78

Fig. 4.2.2: X-Ray Diffractograms of maize starch before pasting, after the short pasting cycle and after the extended pasting cycle........................................84

Fig. 4.2.3: X-Ray Diffractograms of teff starch before pasting, after the short pasting cycle and after the extended pasting cycle..........................................85

Fig. 4.2.4: CLSM images of maize starch taken at various intervals during extended holding pasting ................................88

Fig. 4.2.5: CLSM images of maize starch with stearic acid taken at various intervals during extended holding pasting ...............89

Fig. 4.2.6: CLSM images of teff starch taken at various intervals during extended holding pasting ................................90

Fig. 4.2.7: CLSM images of teff starch with stearic acid, taken at various intervals during extended holding pasting ......................91

Fig. 4.2.8: “Z slices” of maize starch granules ........................................92

Fig. 4.2.9: “Z slices” of teff starch granules ...........................................93
LIST OF TABLES

Table 2.1: Chemical modifications and their effects on starch properties ................................................................. 18

Table 2.2: Yield (%) of crystallized amylose-fatty acid complexes formed with varying Degree of Polymerization (DP) .......... 29

Table 2.3: Pasting properties of rice starch modified with different fatty acids .......................................................... 29

Table 2.4: The pasting properties of rice starch with added stearic acid ........................................................................... 31

Table 4.1.1: Chemical composition of maize and teff starch .............. 50
Table 4.1.2: Effect of stearic acid (% w/w) on the pasting properties of maize and teff starches, for the short pasting cycle ........ 54
Table 4.1.3: Effect of stearic acid concentration on the pasting properties of maize and teff starches for the extended pasting cycle ........ 60
Table 4.1.4: Complexation Indices of maize and teff starches after the extended pasting cycle .......................................... 62
Table 4.1.5: Effect of stearic acid on the paste clarity of maize and teff starch pastes using the extended pasting cycle .............. 65
Table 4.1.6: Texture of maize and teff starch pastes using the extended pasting cycle ................................................................. 67
LIST OF TABLES (continued)

Table 4.2.1: Effect of stearic acid on the flow properties in terms of the Power-Law Model coefficients for maize and teff starches during short and extended pasting ..........................80

Table 5.1.2: The overall effects of stearic acid on the paste properties of maize and teff starch pastes .........................................................103
1. INTRODUCTION

Statement of the Problem

Starch is an extremely useful food ingredient. For example, it is used as a thickener in soups, gelling aid in gums and candies, texturizer in gravies, and fat substitute in low calorie foods (reviewed by Light 1990). Starches are extracted from various sources e.g. roots, tubers and grains. Among the cereal grains, maize starch makes up to 80% of the world’s commercial starch supply (reviewed by BeMiller 2003). Another potential cereal source of starch is teff [Eragrotis tef (Zucc.) Trotter]. Teff starch granules are small (2-6 µm) and may be useful as fat mimetics and, flavour and aroma carriers (Bultosa and Taylor 2004 a). Teff starch has slightly different properties than other tropical cereal starches (e.g. maize) such as lower peak and setback viscosities. Teff starch also seems to show some resistance to breakdown. Teff is a tropical crop indigenous to Ethiopia, where its annual production is estimated at 2 million tonnes. It is a highly underutilized grain, being used for human consumption mainly in Ethiopia (Tatham et al 1994).

Native starches have low stability to the physical conditions of extreme temperatures, shear, and extreme pH used during processing (Lawton 2004). They also produce undesirable weak-bodied, cohesive and rubbery gels (reviewed by BeMiller 2003). In order to overcome the shortcomings of native starches, modified starches have been developed (reviewed by Wurzburg 1986).

Modified starches have improved functional properties and have greater resistance to heat, shear and acid, conditions that occur frequently during food processing (reviewed by BeMiller 2003). Starches may be modified chemically, by the addition of chemicals or physically, by a physical processing technique. Chemical modifications have the greatest effects on starch functionalities (reviewed by BeMiller 2003). However, added chemicals such as chlorine,
phosphates, acetyl groups, manganese may result in a residue of the chemical in the starch. This may in turn raise health concerns and/or negatively affect the sensory properties of the food product (reviewed by Lui 2005). For this reason, the United Nations Food and Agriculture Organization (FAO) and the U.S. Food and Drug Administration (FDA) have regulations regarding the nature and level of the treatments. A consumer friendly alternative to these chemicals is the use of naturally occurring food compounds e.g. fatty acids for modification.

Maize and rice starches have previously been modified with fatty acids, altering their functional properties (Bajner 2002; Kaur and Singh 2000 and Zhou et al 2007). It has been proposed that amylose forms complexes with lipid components (Biliaderis et al 1985; Karkalas and Raphaelides 1986; Biliaderis et al 1989). It is these amylose-lipid complexes that are believed to be responsible for the changes brought about in native starches. Furthermore, the formation of starch-fatty acid superstructures between these individual complexes is believed to be the cause of a second pasting peak when starch is held at high temperatures for extended periods, using a Rapid Visco Analyser (RVA) (Bajner 2002; Nelles et al 2003). Some of the properties of starches modified with fatty acids or lipids include, reduced peak viscosity and breakdown (Zhou et al 2007), a higher second viscosity peak (Bajner 2002), reduced stickiness and improved freeze-thaw stabilities (Mercier et al 1980), increased final viscosity (Zhou et al 2007) and reduced retrogradation (Richardson 2003).
2. LITERATURE REVIEW

In this chapter, the current literature on starch structure and composition, with special emphasis on maize and teff starch is first discussed. Then, the effects of starch-fatty acid interactions on starch functionality and the research on the chemistry of these interactions are reviewed.

2.1 Starch: structure and composition

Starch comprise two polymers, namely, amylose and amylpectin, which are isolated in structures known as granules, as discussed below.

2.1.1 Starch granules

Starch occurs as granules in the endosperm of the cereal grain. The form or shape of these granules is dependent on its botanical origin and may be spherical, ovoid or angular (reviewed by Thomas and Atwell 1999 a). Cereal starch granules can vary from 1 µm to 100 µm in size (reviewed by Thomas and Atwell 1999 a). Maize starch (Fig. 2.1) contains a mixture of spherical and angular granules (reviewed by Whistler and BeMiller 1997 a). Bultosa et al (2002), who used Scanning Electron Microscopy (SEM), found teff starch granules (Fig. 2.2) to be polygonal i.e. having many sides. These authors also found maize starch granules to range between 5-30 µm in size, while teff starch granules ranged between 2-6 µm. Micrographs obtained also showed that teff starch occurs as compound granules (Fig. 2.3) compared to the single granules of maize starch. In the endosperm of the grain, compound granules are formed when individual starch granules in the endosperm bind together to form a higher level of structure. Compound starch granules are also common in oats and rice (reviewed by Thomas and Atwell 1999 a).
Fig 2.1: Spherical and angular maize starch granules (Scale bar = 10 µm) (reviewed by Whistler and BeMiller 1997 a).

Fig. 2.2: Individual teff starch granules, where pg = polygonal granules and cb = cubic granules (Bultosa et al 2002).
Fannon et al (1992), showed the presence of pores on the surfaces of starch granules of maize, wheat, rye and barley, using SEM. Pore sizes range between 0.1-0.3 \( \mu m \) (reviewed by Gallant et al 1997). Fannon et al (1992) also found that granules of some starches, namely, rice and oats did not show any surface pores. Scanning Electron Microscopy (SEM) of teff starch granules showed a smooth surface with no pores (Bultosa et al 2002).

From the granule pores, channels with diameters of approximately 0.07-0.1 \( \mu m \) extend radially to the interior of the granule (reviewed by Gallant et al 1997). These channels have been proposed to facilitate enzyme attack and the entrance of small molecules such as dyes and chemical reagents into the granule matrix (reviewed by Gallant et al 1997; and Kim and Huber 2008). These channels are also responsible for controlling the degree of hydration when in an aqueous medium (Huber and BeMiller 2000).
2.1.2 Starch polymers

All starches comprise two polymers: amylose and amylopectin. Both constituents are homoglucans of α-D-glucopyranose.

**Amylose**

Amylose is an essentially linear molecule consisting of α-D-glucopyranosyl units linked via α(1-4) glycosidic linkages (reviewed by Lui 2005). The chain length (Degree of Polymerization or DP) varies between 1000–10 000, depending on the botanical source (reviewed by Lui 2005). Most starches contain between 20-30% amylose (reviewed by Kent and Evers 1994). Waxy starches contain virtually no amylose. Bultosa et al (2002) found that teff starch contained approximately 30% amylose, which is similar for maize. The molecular weight of amylose ranges between $1.6 \times 10^5$ – $7.1 \times 10^5$ (reviewed by Lui 2005). Bultosa et al (2008) found that the weight-average molar mass of teff amylose ranges between $1.5 \times 10^7$–$3.0 \times 10^7$ g/mol, and that of maize is approximately $2.3 \times 10^7$ g/mol. They also found that the amylose size of teff starch is similar to that of maize having a mean size (chain length) of 191 nm compared to 193 nm for maize.

Amylose molecules form two types of helices i.e. a single and a double helix. The single helix (Zobel 1988 a) (Fig. 2.4 a), contains six residues per turn. This helix has a characteristic hydrophobic core due to the presence of many CH groups. The exterior of the helix is hydrophilic due to the presence of many hydroxyl groups (Zobel 1988 a). The amylose molecule takes part in complexation with iodine, alcohols and fatty acids (reviewed by Eliasson and Wahlgren 2004). Amylose is considered amorphous but may be present in semi-crystalline areas since it may crystallize with amylopectin. The amylose double helix (Fig. 2.4 b), is formed when amylose molecules interact with each other. They are stabilized by hydrogen bonds and van der Waals forces (reviewed by Oates (1997). The amylose double helix is crystalline (Immel and
Lichtenthaler 2000). The formation of double helices is also partially responsible for starch retrogradation (Gudmundsson 1994).

**Fig 2.4:** (a) The amorphous amylose single helix (Immel and Lichtenthaler 2000) and (b) the crystalline amylose double helix (reviewed by Zobel 1988 a).

**Amylopectin**

Amylopectin is one of the largest biological molecules and has a molecular weight of \(7.1 \times 10^5\) (reviewed by Lui 2005). It is a highly branched polymer of \(\alpha\)-D-glucopyranosyl units linked via \(\alpha(1-4)\) glycosidic linkages, and \(\alpha(1-6)\) glycosidic linkages at branch points every 20-25 units (reviewed by Parker and
Branching of the molecule results in the formation of three chains types, namely A, B and C (Fig. 2.5) (reviewed by Kent and Evers 1994). Amylopectin is considered amorphous at branch points (reviewed by Jenkins and Donald 1995). Short amylopectin chains form crystalline double helices. These double helices occur in groups or clusters (reviewed by Parker and Ring 2001).

As reviewed by Zobel (1988 a), Lui (2005), and Parker and Ring (2001), the A Chains are the shortest chains of the molecule which contain no branch points. They are linked via their reducing ends and α(1-6) glycosidic linkages to the rest of the molecule. The B Chains are those chains to which the A chains are attached. The C Chain carries the sole reducing group of the molecule.

![Fig. 2.5: Schematic representation of the A, B and C chains and clusters of amylopectin. Bands marked ‘1’ are considered crystalline and ‘2,’ amorphous (reviewed by Kent and Evers 1994).]
Bultosa et al (2008) found teff amylopectin to have a weight-average molar mass that ranges between $10.1 \times 10^7$ – $10.5 \times 10^7$ g/mol, compared to an average of $19.6 \times 10^7$ g/mol for maize. Amylopectin molecules were also found to have a mean size (length) of 186 nm compared to 207 nm for maize.

2.1.3 Structural organisation of starch

Gallant et al (1997) proposed the Blocklet Model which describes the semi-crystalline nature of the starch granule. At the lowest level of structure (Fig. 2.6A), the alternating crystalline (hard shell) and semi-crystalline (soft shell) layers of the granule are shown as concentric rings. The hard shell is approximately 120–400 nm thick. The next level of structure (Fig. 2.6 B) shows the starch granule with its alternating crystalline and semi-crystalline layers. The granule pores are mostly contained in the crystalline layers, while the channels are contained in the amorphous areas. The second level of structure (Fig. 2.6 B) shows these layers made up of small spherical structures called ‘blocklets’. These blocklets (Fig. 2.6 C) range between 20-500 nm, depending on the botanical source. Each blocklet contains alternating crystalline and amorphous lamellae. Blockets are bigger in the hard shells than in the soft shells. The next level of structure (Fig. 2.6 D) shows the alternating crystalline and amorphous lamellae of the blocket. This is caused by the crystalline lamellae of amylopectin double helix clusters which alternate with the amorphous lamellae of amylopectin branch points. Amylose-lipid complexes (Fig.2.7) may be found with the amylopectin clusters in the crystalline lamellae.
Fig. 2.6: The Blocklet Model — a schematic representation of the levels of structural organisation within starch granules (Gallant et al 1997).
Fig. 2.7: Amylopectin clusters which may contain amylose-lipid complexes (Gallant et al 1997).

The crystalline amylopectin clusters and amylose-lipid complexes are responsible for a further level of organization, i.e. the arrangement of the starch polymers into crystal structures (Fig. 2.8) (Gallant et al 1997). Crystal structure is characteristic of the starch type (reviewed by Zobel 1988 b). A and B-type starches comprise six parallel-stranded double helices with two left-handed chains comprising each double helix (Zobel 1988 b). These helices are arranged in an anti-parallel array. This leaves a central open channel in the hexagonal array (Wu and Sarko 1978). In A-type starches this channel is filled with another double helix and in B-type starches, with water molecules (Zobel 1988 b). The A-unit cell has only 4 water molecules between the double helices. The B-unit cell has 36 water molecules of which half are tightly bound to the double helices. The helices form densely packed structures.

As reviewed by Zobel (1988 c) and Kent and Evers (1994), starches produce four distinct patterns when exposed to x-rays. These patterns are named A, B, C and V. The X-ray patterns are brought about by the organization of crystals and thus indicate the degree of crystallinity of the starch. Most native cereal starches are reported to produce the A-type pattern, while starch from tubers
and roots/seeds give B and C (a mixture of A and B) patterns, respectively. The V pattern is characteristic of starch-lipid complexes (as reviewed by Zobel 1988 c; Kent and Evers 1994; and Gallant et al 1997).

**Fig. 2.8:** The crystal structures of A and B-type starches (Gallant et al 1997).

X-ray analyses may be conducted by the Camera Method. A camera is linked to an x-ray generator. A circle of film is used to record diffraction patterns. The diffraction lines are seen as arcs on the film. The A, B, C and V patterns are observed as ‘halos’ (Fig. 2.9 (a)) on the film (reviewed by Zobel 1988 c).

Alternatively, an X-ray Diffractometer may be used for analyzing starch. A diffractometer produces data in the form of a diffractogram (Fig. 2.9 (b)), which shows peaks dependent on the size of the unit cell. Peak intensities are caused by the position of the atoms within a unit cell and their thermal vibrations. Commonly extracted data from a diffractogram are *d*-spacing, and 26 angle. *D-spacings* represent the distance between successive planes of a series and are
observed as peaks. These peaks are observed at 12 Å, 6.8 Å and 4.4 Å for hydrated V-type ($V_h$) complexes and at 11.3 Å, 6.5 Å and 4.3 Å for dry V-type ($V_a$) (Zobel 1988 c).

Fig. 2.9: a) Starch X-ray pattern designations and (b) X-ray diffractograms for different starches (Zobel 1988 c).
2.2 Starch gelatinisation, pasting and retrogradation

When starches are heated in water (typically above 60°C) they undergo physical changes that contribute to properties such as texture and mouthfeel in food products. There are three phenomena mainly responsible for this: gelatinisation, pasting and retrogradation (reviewed by Lui 2005).

These phenomena are measured using viscoamylograph-type instruments. The Rapid Visco Analyser (RVA) is one such instrument. It records viscosity of pastes with controlled temperature and stirring. The RVA can also apply a degree of shear to simulate processing conditions, making it valuable in research applications (reviewed by Booth and Bason 2007). The changes in viscosity of a starch suspension during gelatinisation, pasting and retrogradation may be understood by pasting curves (Fig. 2.10).

![Fig. 2.10: A generalised RVA starch pasting curve (reviewed by Batey 2007)](image_url)
2.2.1. Gelatinisation

Gelatinisation can be defined as the loss of molecular order within a starch granule (reviewed by Kent and Evers 1994). Molecular order may be lost when starch granules are heated in water and subsequently undergo irreversible swelling and crystalline melting (reviewed by Kent and Evers 1994). As reviewed by Parker and Ring (2001), disruption of hydrogen bonds between the polymer chains of starch takes place. This begins in the amorphous areas where the hydrogen bonds are weaker. When the level of hydration is high enough, the hydrogen bonds and van de Waals forces which hold the crystalline region together are overcome. This results in only partial solubilisation of amylopectin as these molecules extend over several crystalline regions. The solubilisation of amylopectin continues as the granules continue to take up water. The continuous uptake of water causes the granule to lose its molecular order, crystallinity and birefringence (reviewed by Kent and Evers 1994). Smaller starch granules begin to gelatinize first and are followed by the larger granules (reviewed by Thomas and Atwell 1999 b). Therefore, the entire process of gelatinization occurs over a temperature range characteristic of the particular starch (reviewed by Parker and Ring 2001). For teff starch, this gelatinisation temperature range is over the range of 68-80°C (Bultosa et al 2002). Maize starch gelatinizes between 62-80°C (reviewed by Whistler and BeMiller 1997).

2.2.2 Pasting

As reviewed by Thomas and Atwell (1999 b), starch granules continue to swell as heating is continued, causing an increase in viscosity until a maximum (peak viscosity) is reached. During this time smaller amylose molecules may leach out of the granules. Bultosa and Taylor (2004 b) found that teff starch had a greater amount of amylose leaching with time than maize starch. At the peak viscosity (Fig. 2.10) the majority of starch granules are in a swollen, but intact state (reviewed by Thomas and Atwell 1999 b). Continued heating results in
breakdown, i.e. granule rupture and starch polymers being solubilised as they leach into the solution to reduce viscosity. With continuous stirring, the polymers also align themselves in the direction of stirring to further reduce viscosity (reviewed by Whistler and BeMiller 1997 a). At this stage, non-solubilised portions of gelatinized starch granules, often referred to as ‘ghost’ granules are also present in the paste (Derek et al 1992).

2.2.3 Retrogradation

As reviewed by Whistler and BeMiller (1997 a), this stage refers to the re-association of starch polymers upon cooling of the starch paste, manifested in gel formation and increased paste opacity. The firmness of the gel is highly dependent on the formation of junction zones. Junction zones are segments of opposite starch chains that have interacted with each other and form a three-dimensional gel network structure (reviewed by Whistler and BeMiller 1997 b). Junction zone formation may either be facilitated or hindered by the presence of agents such as fats, proteins, sugars, acids and concentration of water. The formation of junction zones often indicates the attempt of starch polymers to recrystallize. As reviewed by Parker and Ring (2001), amylose pastes are known to undergo a strong retrogradation with a consequent rapid increase in paste viscosity due to their long linear nature. Amylose forms double helices which are characteristic of retrograded starch (Gudmundsson 1994). Amylopectin, shows slow and weak retrograding tendencies due to its highly branched structure. Therefore, retrogradation is largely due to the crystallization of amylose (reviewed by Zobel 1988 a). Formation of amylose double helices are characteristic of retrograded starch (reviewed by Thomas and Atwell 1999 b) Retrogradation is responsible for quality defects e.g. bread staling and precipitation in soups and sauces (reviewed by Whistler and BeMiller 1997 a).
2.3. Modification of starch

Starches are modified in order to enhance their properties for certain applications. Starches may be modified physically, by a physical processing technique, or chemically, by the addition of chemicals (reviewed by BeMiller 2003). Some physical modifications involve mechanical, radiation and/or hydrothermal treatments. A common example is the conversion of normal starch to cold water soluble starch by pre-gelatinisation (reviewed by Guilbot and Mercier 1985). Other techniques such as heat-moisture treatments, annealing and extrusion also belong to this category. Chemical modifications include crosslinking, stabilization (commonly accomplished by acetylation), acid modification and oxidation (reviewed by BeMiller 2003).

Chemical modifications (summarized in Table 2.1) have the greatest effects on starch functionality (reviewed by BeMiller 2003). Chemical modification may result in starches with slower retrogradation, reduced gelling tendencies of pastes, reduced syneresis and improved film formation (reviewed by Bao et al 2004). However, the addition of chemicals raises health concerns, thus opening the possibility for the use of naturally occurring compounds for example fatty acids.
### Table 2.1: Chemical modifications and their effects on starch properties (BeMiller 2003).

<table>
<thead>
<tr>
<th>Chemical modification</th>
<th>Effect / Change in properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crosslinking (by the use of difunctional reagents to connect OH groups on two different starch molecules)</td>
<td>Reduced rate of granule swelling and breakdown</td>
</tr>
<tr>
<td>Stabilization (by using monofunctional reagents to derivatize starch e.g. acetylation)</td>
<td>Increased peak viscosity</td>
</tr>
<tr>
<td></td>
<td>Reduced retrogradation</td>
</tr>
<tr>
<td>Addition of hydrophobic groups (Reaction with e.g. 1-octenylsuccinic anhydride)</td>
<td>Good emulsification, and emulsification stabilizing properties</td>
</tr>
<tr>
<td>Acid modification (Treatment with dilute mineral acid)</td>
<td>Increased gel strength</td>
</tr>
<tr>
<td>Oxidation (using sodium hypochlorite)</td>
<td>Reduced gelling</td>
</tr>
</tbody>
</table>

Amylose is able to form inclusion complexes with many chemical constituents such as flavour compounds, emulsifiers, fatty acids and mono- and diglycerides (reviewed by Jackson 2003 a). For the purpose of this review, only the inclusion complexes of amylose with lipid compounds such as fatty acids and emulsifiers are discussed.

Fatty acids react with the hydrophobic core of the amylose helix (Raphaelides and Karkalas 1988). Fatty acids belong to a group of lipids which consist of open-chain compounds possessing polar heads and non-polar tails, i.e they are amphipathic molecules (reviewed by Campbell and Farrell 2003). A carboxyl group serves as the hydrophilic head while a hydrocarbon chain makes up the hydrophobic tail. Fatty acids have chain lengths varying from 2-80 carbon units, with the most common being those with 12-22 carbon units (reviewed by
Christie 2003). Stearic acid (used in the research that follows in Chapter 4) is a completely saturated, 18 carbon fatty acid (Fig. 2.11).

![Figure 2.11: The structure of stearic acid showing the hydrophilic carboxyl group (head) and the hydrophobic carbon chain (tail).](image)

**2.4. Effects of modification with fatty acid on starch functionality**

Starch-lipid interactions have an impact on starchy foods, reducing granule swelling and peak viscosity (Jackson 2003a), decreasing susceptibility of the starch to alpha-amylase (α-amylase) digestion (Kaur and Singh 2000), reducing breakdown (Zhou et al. 2007), and improving freeze-thaw stabilities and retrogradation (Godet et al. 1995a). The following sections (2.4.1-2.4.4) review studies that have shown changes in starch structure and functionality after modification with lipids.

**2.4.1 Pasting Properties**

As stated, the addition of fatty acids to starches may result in altered starch properties which may be more desirable than native properties. The following describes the effect of fatty acids on starch pasting properties.

Zhou et al. (2007) used 1.0% stearic and myristic acids to modify rice starch. The RVA pasting profile for the modified starch showed reduced peak viscosity...
and breakdown; increased time to peak viscosity and final viscosity. Richardson et al (2003) suggested that the added monoglyceride forms a film around starch granules, increasing their hydrophobicity. However, there is no proof of the formation of such a layer. This layer, would reduce the ability of the granule to take up water (resulting in a reduced rate of swelling), thus the reduced peak viscosity. Reduced rate of swelling was also suggested to be responsible for the increased time taken to reach peak viscosity for maize starch (Raphaelides and Georgiadis 2006; Zhou et al 2007). The observed reduced breakdown suggests that the fatty acid may restrict granular breakdown during pasting (Zhou et al 2007). Singh et al (2002) obtained similar trends in results when working on maize starch.

In an investigation by Richardson et al (2003), a mixture of a polyglycerol ester and a monoglyceride (PGE/MG) was added to a wheat starch suspension in concentrations of 0.5% and 4.0%. After being held at various temperatures, amylose leached out from native starch (non-treated) at 70°C. With continued heating above 90°C, the granules began to fragment at the edges. With added PGE/MG mixture, starch granule swelling was almost completely restricted above 50°C and leaching was delayed until about 90°C. At 90°C some granules were still intact (Fig. 2.12). Above 90°C complete granular rupture took place. The final viscosity with added emulsifiers was higher than that of native starch (measured with a Brabender Viscoamylograph). This was attributed to the formation of complexes between amylose and the lipid fraction.
Fig. 2.12: Light microscopy images of wheat starch with an added polyglycerol-monoglyceride (PGE/MG) mixture, taken at various temperatures (Richardson et al 2003). Scale = 50 µm (shown in red).
When maize starch is held at high temperatures for an extended period of time (>90°C), a second pasting peak has been observed (Nelles et al 2000; Bajner 2002). Nelles et al (2000) attributed the presence of the second peak to the formation of amylose-lipid complexes, since this peak was not observed when the lipid fraction was removed. This second viscosity peak (Fig. 2.13) was found to be higher with the addition of fatty acids (Bajner 2002). Nelles et al (2003) found that the amount of amylose in solution decreased with pasting time from 13.1% to 6.1% with 22.5 and 50 min of pasting, respectively. This was attributed to the complexing of amylose with substances such as lipids or with other amylose molecules (retrogradation).

**Fig 2.13:** The biphasic pasting curves for maize starch with added stearic acid found using a Brabender Viscoamylograph (Bajner 2002).
2.4.2 Retrogradation, gelling ability and syneresis

Raphaelides (1992) modified potato starch with myristic, palmitic and stearic acids in an alkaline solution (0.01M potassium hydroxide). This resulted in reduced rigidity (firmness) of the starch gels. This finding was in agreement with that of Whittam et al (1986), who showed that addition of the same fatty acids resulted in lower gel rigidity (Fig. 2.14). When the amylose helices are saturated, the repulsions between adjacent helices are strong enough to completely inhibit gel formation in potato starch (Raphaelides 1992). However, of the three fatty acids used by Raphaelides (1992), the gel containing stearic acid was the most firm. One molecule of stearic acid can occupy the helical space of amylose compared to two fatty acid molecules of myristic acid. Since fewer fatty acid molecules are required to occupy the helical space of the amylose molecules, repulsive forces diminish. This possibly results in more rigid gels (Raphaelides 1992). Oleic acid (unsaturated fatty acid) gels were very susceptible to breakage (Raphaelides 1992).

![Graph](image)

**Fig. 2.14**: Effect of fatty acid chain length on gel texture, where C\(_{14}\) is myristic acid, C\(_{16}\) is palmitic acid and C\(_{18}\) is stearic acid (adapted from Whittam et al 1986).
Richardson et al (2004) found that high concentrations of an emulsifier (mixture of a polyglycerol ester and a monoglyceride) (PGE/MG) prevented potato amylose gels from forming. Instead, thick opaque pastes were formed, with a complete disappearance of the network.

Some proposed reasons for these effects on starch gelling ability are (Gudmundsson 1994):

1) The amylose-lipid complex interferes with amylopectin re-crystallization, thus retarding retrogradation.
2) The amylose-lipid complex interferes with retrogradation by retarding water distribution.
3) Co-crystallization of amylose with other compounds reduces the ability of amylose to form double helices that are characteristic of retrograded starch.

Raphaelides and Georgidis (2006) added stearic and myristic acids to 20% (w/w) maize starch suspensions in concentrations 0.5% and 1.0% (with respect to starch). An increase in the time of storage showed increased syneresis, i.e. a greater loss of water. An increase in the concentration of added fatty acid showed less syneresis. Raphaelides and Georgidis (2006) attributed this effect to the addition of fatty acids which reduce the rate of retrogradation, thus also reducing water loss during storage.

2.4.3 Effects on starch structure

Modification of starch with fatty acid and the subsequent formation of amylose-fatty acid complexes may alter the structural properties of the starch. In the two studies mentioned below, microscopy analyses were conducted to investigate starch structure after the formation of amylose-lipid complexes. Similar effects may be found with the addition of fatty acid to teff starch but is yet to be investigated.
Nelles et al (2003) conducted SEM investigations on commercial maize starch (after the native lipids were added back to the starch), at various intervals during extended pasting at 90 °C. These authors found that after the first peak, starch granules were still swollen and intact with only some leaching. Granules were still intact at the trough viscosity of the pasting trace. The trough viscosity is the lowest viscosity after the first pasting peak and before the second pasting peak. However, after the second peak, granules were burst open. They therefore concluded that the second viscosity peak coincides with the loss of granular structure. They attributed these effects to the formation of amylose-lipid complexes.

Peterson et al (2005) found that crystallization of helical inclusion complexes of amylose with native lipids resulted in the formation of crystalline aggregates after steam jet cooking. These aggregates were found to be either small and torus-shaped or larger spherical particles. Fanta et al (2002) found spherocrystals (Fig. 2.15) after jet cooking of various starches such as high amylose maize starch, rice starch and wheat starch. However, these crystals did not occur in waxy maize starch. Thus, they concluded that these spherocrystals were due to the formation of amylose inclusion complexes with native lipids. X-ray diffraction data for these spherocrystals showed d-spacings of 11.8Å, 6.86 Å and 4.48 Å. Therefore, they were classified as V-type, which is also characteristic of amylose-lipid inclusion complexes. Tang and Copeland (2007) also found that the X-ray diffraction patterns matched that of V-type starches (Fig. 2.16) after pasting with the addition of 0.05 mmol stearic acid. The 2θ angles at which peaks formed were 7.4°, 12.7°, and 19.8°. These peaks were less intense when the amount of stearic acid added was increased. Furthermore, additional peaks observed at 21.5° and 23.9° were identified as the crystalline pattern of stearic acid micelles.
**Fig. 2.15**: (a) Large spherocrystals of maize starch (b) Small spherocrystals of maize starch (Fanta et al 2002).

**Fig. 2.16**: X-ray diffraction patterns of (a) starch mixed with 0.05 mmol stearic acid (b) starch mixed with 0.14 mmol stearic acid and (c) starch containing stearic acid aggregates. The solid arrows represent peaks corresponding to amylose-lipid complexes and the open arrows represent peaks corresponding to stearic acid aggregates (Tang and Copeland 2007).
2.4.4 Flow properties at different shear rates

Starch pastes have been found to be pseudoplastic (shear-thinning) (Kaur and Singh 2000; Singh et al 2000). The Power-Law Model has been used to represent starch behaviour (Kaur & Singh 2000; Singh et al 2000). This model defines \( \tau \) as the shear stress (Pa), \( \kappa \) as the consistency coefficient, \( \dot{\gamma} \) as the shear rate (s\(^{-1}\)) and \( \eta \) as the flow behaviour index, where:

\[
\tau = \kappa \dot{\gamma}^n
\]

The Power-Law Model classifies fluids with \( n = 1 \) as Newtonian, \( n > 1 \) as dilatant and \( n < 1 \) as pseudoplastic. Pseudoplastic flow refers to flow where an increasing shear force gives a more than proportional increase in shear rate with the curve beginning at the origin as shown in (Bourne 1982). Fig. 2.17 shows two curves which demonstrate pseudoplastic behaviour.

The addition of stearic acid to rice starch was found to result in a reduced consistency coefficient (k) and flow behaviour indices (n) which were attributed to amylose-fatty acid complexation (Guraya et al 1997; Kaur and Singh 2000; Singh et al 2000). Rice starch cooked for 30, 60 and 90 minutes with added fatty acids was found to be more shear-thinning than the native starch. Rice starch was also found to be more shear thinning as the pasting time was increased (Kaur and Singh 2000). However, these results were for starches which did not show a second pasting peak. To our knowledge, shear viscosity analyses have not been conducted for starch pastes after the second viscosity peak, thus making the following research necessary (Chapter 4).
Fig. 2.17: (a) Pseudoplastic behaviour demonstrated by shear-stress ($\tau$) against shear rate ($\dot{\gamma}$) and (b) Pseudoplastic behaviour demonstrated by viscosity ($\eta$) against shear rate ($\dot{\gamma}$) (Mezger 2006).

2.5 Factors affecting the properties of starches modified with lipid/fatty acid

Various factors will affect the formation and properties of starch-fatty acid complexes. Fatty acid chain length, fatty acid saturation levels and concentrations, as well as amylose change length are among these factors.

2.5.1 Chain length

Fatty acid: Increasing chain length of the fatty acid was found to result in increased degree of complexation (Godet et al 1995 a). A minimum chain length of 10 carbon (10C) atoms is required for complexation. Complexation does not occur optimally with fatty acids having less than 10 carbon atoms since the solubility of the fatty acid in the crystallization medium increases with decreasing chain length (Karkalas and Raphaelides 1986). Tufvesson et al (2003) showed that C3 and C4 fatty acids, i.e. fatty acids containing three and four carbon atoms respectively, did not complex with amylose at all.
Amylose: Godet et al (1995a) used amylose chains of various degrees of polymerization (DP) from pea starch to crystallize with lauric (C12) and palmitic (C16) acids. The amylose:fatty acid ratio was 10:1. The yield of highly crystallized complexes obtained is shown in Table 2.2. It is clear that an increase in amylose chain length promotes greater complexation.

<table>
<thead>
<tr>
<th>Table 2.2: Yield (%) of crystallized amylose-fatty acid complexes formed with varying Degree of Polymerization (DP) (Godet et al 1995a).</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP30</td>
</tr>
<tr>
<td>Lauric acid (C12)</td>
</tr>
<tr>
<td>Palmitic acid (C16)</td>
</tr>
</tbody>
</table>

2.5.2 Fatty acid saturation level

Zhou et al (2007) showed that the effects of saturated fatty acids on the pasting properties of the starch are greater than unsaturated fatty acids. Some of the properties include lower peak viscosity, greater resistance to breakdown and reduced final viscosity (Table 2.3).

<table>
<thead>
<tr>
<th>Table 2.3: Pasting properties of rice starch modified with 1% stearic (18:0) and linoleic (18:2) acids on the basis of starch weight (adapted from Zhou et al 2007).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>PV (RVU)</td>
</tr>
<tr>
<td>BD (RVU)</td>
</tr>
<tr>
<td>FV (RVU)</td>
</tr>
<tr>
<td>TTPV (min)</td>
</tr>
</tbody>
</table>

*where PV is peak viscosity, BD is breakdown, FV is final viscosity and TTPV is the time taken to reach peak viscosity.
Tufvesson et al (2003) used Differential Scanning Calorimetry (DSC) to show fatty acid unsaturation (using a fatty acid mixture called “DHA acids” which comprised 40% ω3-docosahexaenoic acid (C22:6) reduced the ability to form Type II complexes during short heat treatment but these complexes formed during extended time treatments (Tufvesson et al 2003). In the same study, it was also found that increase in unsaturation resulted in lower stability of both complex forms (I and II) towards heat.

2.5.3 Concentration of fatty acid

The addition of fatty acids to starches have been found to give reductions in pasting peak viscosity, breakdown and increases in time taken to reach peak viscosity (Zhou et al 2007). These changes in properties were of a greater degree when fatty acid concentration is increased (Table 2.4). This also resulted in increased complexation indices (CI) i.e. the percent complexation that takes place between the amylose and the fatty acid (Kaur and Singh 2000; Zhou et al 2007). The complexation index (CI) increased when stearic acid was added at 1.5%, 3.0% and 4.5% to 13.6%, 16.90% and 21.5% (on starch weight basis), respectively (Kaur and Singh 2000). Tang and Copeland (2007) further showed that there is an optimum concentration of the fatty acid for optimal complexation, but this value differs with the fatty acid used. For stearic acid this optimum concentration is approximately 0.05 mmol per 2.5 g of starch. Above this concentration, they observed an increase in viscosity, which may be attributed to excess fatty acid molecules aggregating to form micelles.
Table 2.4: The pasting properties of rice starch with added stearic acid (1.0%) based on dry starch weight (Zhou et al 2007).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Starch 0.5%</th>
<th>Starch 1.0%</th>
<th>Starch 1.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV (RVU)</td>
<td>153.0 ± 5.1</td>
<td>134.5 ± 5.2</td>
<td>131.3 ± 5.5</td>
</tr>
<tr>
<td>BD (RVU)</td>
<td>93.8 ± 3.9</td>
<td>46.2 ± 2.0</td>
<td>8.6 ± 3.0</td>
</tr>
<tr>
<td>FV (RVU)</td>
<td>128.6 ± 4.9</td>
<td>153.2 ± 7.8</td>
<td>133.6 ± 5.5</td>
</tr>
<tr>
<td>TTPV (min)</td>
<td>4.87 ± 0.14</td>
<td>6.33 ± 0.13</td>
<td>7.93 ± 0.12</td>
</tr>
</tbody>
</table>

*where PV is peak viscosity, BD is breakdown, FV is final viscosity and TTPV is the time taken to reach peak viscosity.

2.6 The chemistry and structure of amylose-lipid complexes

Single helix amyloses that have been co-crystallized with compounds e.g. fatty acids or alcohols, are given the generic name: V-amylose (Godet et al 1993 a). These structures are formed when A or B-type double helices collapse, yielding left-handed single helices with 6 glycosyl residues per turn. V-amylose exists in dry (V\text{\textsubscript{a}}) and hydrated (V\text{\textsubscript{h}}) forms (Rappenecker and Zugenmaier 1981). These complexes are described below and any differences between the two forms are explained.

2.6.1 V-amylose

Fatty acids react with the hydrophobic core of the amylose single helix through hydrophobic interactions and van der Waals forces (Raphaelides and Karkalas 1988). The head of the fatty acid lies outside the amylose helix, while the tail (carbon chain) is completely contained within the helix (Fig. 2.18) (Godet et al 1993 a). The steric hindrance and electrostatic repulsions prevent the carboxylic head from entering the helix (Godet et al 1993 a).
Fig. 2.18: The amylose-monostearin complex showing the carbon chain of the fatty acid inside the helical space, while the head lies outside the helix (Carlson and Larsson 1979).

When amylose is complexed with a fatty acid, each helical turn is made up of 6 glycosyl residues (Godet et al 1993 a). However, for bulky guest molecules up to 8 residues per turn is possible (Karkalas et al 1995). The lipid inclusion complex typically takes up 2-3 turns of the amylose helix (Fig. 2.18) (Immel and Lichtenthaler 2000).

The hydrocarbon chain of the fatty acid is stabilized within the amylose helix by hydrophobic interactions and van der Waals forces (Raphaelides and Karkalas 1988). The hydrogen atoms which were linked to the fifth carbon atoms of the glucose residues take part in van der Waals interactions with the two hydrogen atoms linked to each carbon atom of the aliphatic chain (Godet et al 1993 a). Following the formation of the complex, the fatty acid is immobilized within the amylose helix (Morrison 1995).

Unsaturated fatty acids cannot easily access the amylose helix due to molecular rigidity about the cis -double bonds (Kaur and Singh 2000). However, the unsaturated fatty acid may take on a quasi-linear conformation around the double bond by free rotation about C—C bonds adjacent to C=C.
bonds. This results in a greater amylose chain diameter around the unsaturated chain (Karkalas et al 1995), thus facilitating complexation.

2.6.2 $V_h$-amylose

$V_h$-amylose contains two water molecules that are hydrogen bonded to each other, inside the helix. These water molecules are prevented from interacting with the interior of the helix by the hydrophobic forces present (Rappenecker and Zugenmaier 1981). In $V_a$-amylose these water molecules are not present. Lipid-complexed amylose yield $V_h$ diffraction patterns (Godet et al 1993 a) and may be either of two types: I and II. The $V_h$-amylose helix is stabilized by a strong network of hydrogen bonds between interstitial water molecules and hydroxyl groups on the outer surface of the helix (Carlson and Larsson 1979). $V_h$-amylose has an overall diameter of 13.5 Å, a 5.4 Å wide channel and an axial pitch of 8.1 Å (Eliasson and Wahlgren 2004), as shown in Figure 2.19 (a).

2.6.3 Orientation of the fatty acid chain within the amylose helix

Inside the core of the amylose helix, the fatty acid chain may be present in one of 6 positions (Godet et al 1993 b. Each position is 60° apart and corresponds to three main planes (Fig 2.19 (b)). These orientations allow for the hydrogen atoms on the fatty acid molecule to point towards the less crowded regions near the glycosidic oxygens on the amylose molecule. The complementary geometries between the amylose helix and the fatty acid chain allows for very little movement (Godet et al 1993 b; Morrison 1995).
2.6.4 Type I and Type II structures

Vₐ-amylose may be present as one of two complexes called Type I and Type II (Biliaderis and Galloway 1989). Both involve hydrogen bonds and van der Waals interactions stabilizing the helices but differ in helix organization. The mechanism for the formation of these complexes is shown in Fig. 2.20.

Type I
These complexes form below 60°C (below gelatinization) and dissociate when heated between 94-104°C (Raphaelides and Karkalas 1988). Biliaderis and Galloway (1989) suggested that helices of this type are randomly distributed and do not form superstructures. Furthermore, nucleation of these complexes is rapid. The majority of amylose-lipid complexes are of this type and known to be amorphous, but may be annealed to form the crystalline Type II. These complexes are possibly present in starch pastes pasted for a short time as in the work of Raphaelides and Georgidis 2006 and Zhou et al 2007.

Type II
These complexes form after gelatinization at temperatures above 90°C and dissociate at 100 - 125°C (Zobel 1988 b). Biliaderis and Galloway (1989)
suggested that Type II complexes are superstructures of several Type I complexes crystallized together. Non-dissociated Type I complexes serve as nuclei for crystallization. They further suggested that Type II complexes are present in two forms, namely, Type IIa and Type IIb. Type IIa is formed during gelatinization and is semi-crystalline, while Type IIb which is formed after gelatinization with continuous heating is fully crystalline and forms ordered superstructures. Rappenecker and Zugenmaier (1981) suggested that Type IIb is the most stable form of the amylose-lipid complex. These complexes are possibly present in starch pastes pasted with extended time at high temperatures as in the work of Nelles et al 2000, Bajner 2002 and Nelles et al 2003).

**Fig. 2.20:** Schematic representation of the mechanism of amylose-lipid complexation, forming Type I and Type II complexes; where T is temperature, T_c is the temperature of crystallization, and T_{m1} is the temperature of melting of Type I complexes (Biliaderis and Galloway 1989).
Amylose helices tend to form long helical chains comprising many amylose molecules (Karkalas and Raphaelides 1986). However, amylose chains are generally much longer than the complexed molecule, leaving uncomplexed segments of amylose. It is these uncomplexed segments that are responsible for random helix distribution in Type I complexes and folding into parallel and anti-parallel arrays in Type II complexes (Raphaelides and Karkalas 1988).

The formation of superstructures is facilitated by the interaction of the carboxyl group with the hydrophilic exterior of other amylose helices through hydrogen bonding (Godet et al 1993 a). In addition, water molecules present between complexed helices are hydrogen bonded to oxygens 2, 3, 4 and 6 (glycosyl residues) on the exterior of the $V_h$-amylose helix. According to Rappenecker and Zugenmaier (1981), this allows for the formation of the crystallized superstructures. These superstructures are hexagonally packed (Fig. 2.20) in a crystal lattice. The unit cell of the lattice is orthorhombic with two planes: $a = 13.65\text{Å}$ and $b = 23.70 \text{ Å}$. In addition to the two water molecules within the amylose helix, there are two additional water molecules in the interstitial spaces of the helices in the unit cell. $V_a$-amylose $V_h$-amylose transformations may take place with the number of water molecules increasing from four to sixteen, per unit cell.
**Fig. 2.21:** The arrangement of V<sub>H</sub>-amylose to form a “hexagonal” crystal lattice (showing hydrogen bonds between adjacent complexes; and 2 water molecules within each helix), where \( a = 13.65\,\text{Å} \) and \( b = 23.70\,\text{Å} \) (Rappenecker & Zugenmaier 1981). The fatty acid which is contained within the helix is not shown.

Differential Scanning Calorimetry (DSC) may be used to investigate amylose-lipid complex formation based on the heat stabilities and transition enthalpies of the complexes. The peaks on DSC thermograms correspond to endothermic processes within the sample during temperature scans (Tufvesson et al 2003). Tufvesson et al (2003) modified amylose from potato starch with different fatty acids and 1M NaOH (ratio fatty acid:amylose:1M NaOH = 0.2:1:3) and analysed for complex formation using DSC (Fig. 2.22). The addition of short chain saturated fatty acids (C3 and C4) did not form any peaks at all. Thus, no amylose complexes were detected. Longer chain fatty acids showed a single peak on the first scan which corresponded to complex transition. However, with the second scan Type II complexes were formed with saturated fatty acids having a chain length of twelve or more carbons.
Fig. 2.22: DSC thermograms for potato amylose modified with different fatty acids and 1M NaOH and heated for 24 hours at 100 °C. 1a = C16, 1b = rescan of 1a, 2a = C14, 2b = rescan of 2a, 3a = C12, 3b = rescan of 3a, 4a = C10 and 4b = rescan of 4a (Tufvesson et al 2003).

2.7 Potential applications of lipid-modified starches

Starches modified with fatty acids or lipids have many uses. The reduced peak viscosity may prove to be useful in products such as pastas and processed potatoes to prevent stickiness (Mercier et al 1980). The higher viscosity starch pastes produced after extended pasting may be used as thickeners. These starches also show reduced retrogradation tendencies (Richardson et al 2003). Therefore, they may also be used as emulsifiers to reduce retrogradation and staling in baked goods, soups and gravies. Starches modified in this way have a soft texture (softer gels) in addition to their high viscosity. Therefore, they show much potential to be used as fat replacers in low calorie foods.
Furthermore, Light (1990) suggested their use as emulsion stabilizers in beverages and salad dressings and encapsulators of vitamins and flavours.

2.8 Concluding remarks

Some information on the effect of fatty acid on the properties of starches such as that of maize, rice and potato exists. Work has not been conducted using teff starch. The changes in pasting properties, formation of the second viscosity peak during pasting and the effects on starch structure have been attributed to the formation of amylose-lipid complexes. The following research thus investigates the effect of added fatty acid (stearic acid) to teff starch in comparison to that of maize starch.
3. HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

3.1.1 Modification of maize and teff starches with stearic acid will result in starches with altered pasting properties (using the short pasting cycle) such as reduced first peak viscosities, longer time to gelatinise, increased time to peak viscosities and reduced breakdown, as was found by Zhou et al (2007) and Raphaelides and Georgidis (2006) using rice and maize starch, respectively. This is possibly due to the fatty acid forming a film around the starch granule, which reduces the rate of granule swelling and in turn reduces granule disruption (Richardson et al 2003; Zhou et al 2007).

Modification of maize and teff starches with stearic acid will result in an increased second viscosity peak when held at high temperatures for extended periods (extended pasting cycle), possibly due to the formation of complexes between amylose and stearic acid (Bajner 2002; Nelles et al 2003).

Modification of maize and teff starches with stearic acid will also result in increased final viscosity of the resulting paste due to the release of amylose exudates and in turn, folding of the starch granules (Zhou et al 2007); interaction between amylose-fatty acid complexes to form superstructures; and interaction between stearic acid molecules when in excess (Tang and Copeland 2007).

3.1.2 Modification of maize and teff starches with stearic acid will cause microstructural changes in granule structure before pasting and changes in the paste microstructure as a result of formation of starch-lipid complexes, as Nelles et al (2003) found that starch granules lost integrity as these complexes formed.
The starch X-ray Diffraction pattern will change from A-type to V-type with added stearic acid, due to the formation of complexes between amylose and stearic acid molecules. Godet et al (1995 a) and Tang and Copeland (2007) found this change in X-ray patterns with added lipids.
3.2 Objectives:

3.2.1 To determine the effect of stearic acid on the pasting properties of teff starch and commercial maize starch (as reference for comparison) using short (holding period of 5 min at 91°C) and extended (holding period of 2 hr at 91°C) pasting cycles.

3.2.2 To determine the effect of stearic acid on the structure of maize and teff starch before, during and after pasting, using X-Ray Diffraction and confocal laser scanning microscopy.

3.2.3 To determine the effect of stearic acid on the flow properties of maize and teff starches at varying shear rates (0.1 s\(^{-1}\) to 1000 s\(^{-1}\)).
4. RESEARCH

4.1 Paste properties of maize and teff starches modified with stearic acid

ABSTRACT

Native starches have low stability towards extreme temperatures, shear and pH conditions. In order to modify the properties of the starches, stearic acid was incorporated into maize and teff starches at 0.25% to 4% (w/w). Starch suspensions (10% (w/v)) were pasted for 30 min (short pasting cycle with a holding time of 5 minutes at 91°C). The maize starch showed reduced peak viscosity, breakdown and final viscosity with increasing stearic acid concentration. Teff starch with added stearic acid did not give a peak viscosity within the holding time (5 min). When the extended pasting cycle (holding time of 2 hr 91°C) was used, both starches showed the formation of a second viscosity peak. When stearic acid (0.25%) was added, the second viscosity peak increased from 175 Rapid Visco Units (RVU) to 228 RVU for maize starch. Although teff starch (with 0.25% stearic acid) did not give a first viscosity peak, a large increase in viscosity was observed. The second peak for teff starch increased from 113 RVU to 250 RVU with 0.25% stearic acid. Commercial waxy maize starch with 0.25 % added stearic acid did not produce a second pasting peak, suggesting that the peak is probably due to amylose-stearic acid complexation. The Complexation Index (which indicates the amount of amylose complexed by stearic acid) increased with pasting time (from short pasting cycle to extended pasting cycle) from 17% to 24% for maize starch and from 13% to and 24% for teff starch. The clarity of starch pastes and the firmness of starch gels decreased with pasting time when complexed with stearic acid (0.25%) for both starches. At a high concentration of stearic acid (1.5%), both starches did not form a gel. The changes in pasting and functional properties observed for both starches have been attributed to the formation of amylose-lipid complexes.
4.1.1 Introduction

Fatty acids may be added to starches to modify their properties. For example, maize and rice starches containing added fatty acid and then pasted for a short time (approx. 30 min) were found to have reduced peak viscosity, an increased time to peak viscosity and increased final viscosity (Kaur and Singh 2000; Tang and Copeland 2007; Zhou et al 2007). It is proposed that the addition of lipid forms a layer around starch granules (Richardson et al 2003). This hypothesis has not been proven. Such a layer would increase granular hydrophobicity and consequently reduce water uptake. Zhou et al (2007) also found reduced breakdown of rice starch with added fatty acid. They suggested that the fatty acid may increase the resistance of the starch granules to breakdown during pasting. The addition of fatty acid may also result in increased final viscosity during pasting (Kaur and Singh 2000; Tang and Copeland 2007; Zhou et al 2007). This may be attributed to formation of amylose-lipid complexes (Kaur and Singh 2000) and formation of micelles between fatty acid molecules when the fatty acid is in excess (Tang and Copeland 2007).

The formation of a second peak when maize starch was pasted for an extended period (> 1 hour) was attributed to the formation of amylose-lipid complexes (Nelles et al 2000). This conclusion was reached because this peak did not form when the lipid fraction was removed. Nelles et al (2003) found that most maize starch granules remained intact (although there was some swelling and leaching) until the second peak. The second peak then coincided with a loss of granular integrity. Furthermore, when the fatty acid was added to maize starch, the viscosity of the second peak increased by almost 50% and resulted in a larger area under the peak (Bajner 2002).

The effects of fatty acids on pasting properties have been studied using maize, rice and wheat starch. However, its effects have not been studied on teff starch. Teff is an indigenous African cereal. It is highly under-utilised and under-researched. The very limited research on teff starch has shown that it has some
unusual properties. The granules are small (2-6 µm) and compound (Bultosa et al 2002). Teff starch has lower peak and setback viscosities than maize starch (Bultosa et al 2002). Teff starch also shows some resistance to breakdown during pasting (Bultosa and Taylor 2004 b).

The objective of this study was to determine the effect of stearic acid on the paste properties of maize and teff starch.

4.1.2 Materials and Methods

4.1.2.1 Samples

Witkop, a white teff variety, was obtained from PANNAR, Kroonstad, South Africa. Waxy maize starch (Amioca Powder TF) was obtained from National Starch Food Innovation, Germiston, South Africa. Stearic Acid (Grade I, approx. 99% capillary GC) was obtained from Sigma-Aldrich (Product Code S4751-25G). A commercial maize starch, Amyral (from a white maize variety), was obtained from Tongaat Hulett®, Edenvale, South Africa, and was used as reference. All other chemicals were of analytical grade.

4.1.2.2 Starch extraction

Teff grain was first milled in a laboratory hammer mill to pass through an 800 µm screen. The flour was then defatted with hexane in a 1 part flour : 3 parts hexane ratio for 1 hour at 25°C. The hexane layer was then decanted and drained off. This procedure was repeated three times. The starch was then dried overnight in a fume cupboard at room temperature. Teff starch extraction was conducted by the method of Bultosa et al (2002). Flour (100 g) was suspended in 500 ml distilled water for an hour. The slurry was wet-milled in a Retsch Mill ZM 200 (Haan, Germany) with a 250 µm opening screen. The residue on the screen was discarded (to remove fibrous components), while the liquid was retained. This liquid was first sieved with a 75 µm hand sieve and
then a 38 µm hand sieve. The residue obtained on the screen was discarded and the filtrate centrifuged at 9940 g for 10 min. The supernatant was discarded and the brown protein layer was scraped off the pellet. The pellet was then re-suspended in distilled water and centrifuged again. This procedure was repeated until a white pellet was obtained. Starch samples were then freeze-dried. The purity of the starch pellet was assessed by examining it for protein bodies and fibre, using light microscopy. The purity of the starch was satisfactory since virtually no fibre or protein bodies were found.

4.1.2.3 Proximate analyses

The moisture, ash, fat and protein contents of the teff and maize starches were determined using AACC methods 44-15A, 08-01, 30-12A and 46-30 (American Association of Cereal Chemists 2000), respectively. Moisture content was determined by drying for 3 hours at 103°C. The ash content was determined by combustion of the organic components at 550°C. Crude fat content was determined by the Soxhlet method, using petroleum ether (BP 40-60°C) for fat extraction. Crude protein content (N x 6.25) was determined by the Dumas Method.

4.1.2.4 Amylose/amylopectin determination

Amylose and amylopectin were determined using the Megazyme (Bray, Ireland) Amylose/Amylopectin Assay kits. This method, developed by Gibson et al (1997), uses Concanavalin A (Con A) to precipitate amylopectin. The amylopectin is then removed by centrifugation. Amylose is expressed as a percentage of glucose released after hydrolysis, divided by the glucose released after hydrolysis of the total starch.
4.1.2.5 Incorporation of stearic acid into starch

Stearic acid was added to starch in the concentrations 0%, 0.25%, 0.5%, 1.0%, 1.5%, 2.0%, 3.0% and 4.0% (on dry weight basis of the starch). The stearic acid was first dissolved in absolute ethanol. The starch was added to this solution and covered with Parafilm and foil and placed in a shaking water bath at 50°C for 30 min. The ethanol was then evaporated off in a force draught oven at 40°C.

Before incorporation of stearic acid, commercial maize starch was freeze-dried to reach a moisture content similar to that of the teff starch.

4.1.2.6 Starch pasting properties

Both pasting cycles (short and extended holding time) were conducted using a Rapid Visco Analyser (RVA Model 3D) (Newport Scientific, Warriewood, Australia). Starch (3 g; 14% moisture) was suspended in distilled water and the weight adjusted to 28 g. The pasting profiles for both cycles are described below. The pastes were then used for the determination of complexation index and gel texture analyses.

Short pasting: This cycle began with an initial stirring of 960 rpm at 50°C for 30 s and then 160 rpm for the entire period thereafter. The temperature was increased to 91°C at a rate of 5.5°C/min and held at this temperature for 5 min. The pastes were then cooled to 50°C at a rate of 5.5°C/min.

Extended holding: This cycle began with an initial stirring of 960 rpm at 50°C for 30 s and then 160 rpm for the entire period thereafter. The temperature was increased to 91°C at a rate of 5.5°C/min and held at this temperature for 2 hr. The pastes were then cooled to 50°C at a rate of 5.5 °C/min.
4.1.2.7 Complexation Index

The Complexation Index (CI), the percentage complexation between stearic acid and starch (amylose), was determined by adapting the method of Guraya et al (1997). Starch pastes were diluted to 5% (w/v), using warm distilled water (40°C). The pastes (5 g) were placed in 40 ml centrifuge tubes. Distilled water (25 ml) was added to each tube. The tubes were vortex-mixed for 2 min. The tubes were then centrifuged at 14,000 g for 20 min. Supernatant (500 µl) was added to 2 ml iodine solution in a 20 ml test tube. This was followed by 15 ml distilled water. The absorbance of the solution was read at 690 nm. The CI was expressed as follows:

\[
CI = \frac{[\text{Absorbance(Control)} - \text{Absorbance(Sample)}]}{\text{Absorbance(Control)}} \times 100
\]

Control = the uncomplexed starch.

CI is based on the principle that iodine will bind free amylose molecules, but not those molecules that are complexed by stearic acid.

4.1.2.8 Starch paste clarity

Paste clarity was determined according to the method of Craig et al (1989), as modified by Bultosa and Taylor (2004 b). The method is based on the principle that the whiteness of a starch paste is related to the association of starch chains that reflect light. Replicate samples (50 mg ± 0.1 mg, db) for maize and teff starches, were added to 5 ml distilled water and heated to 95°C in a boiling water bath for 30 and 90 min. Each tube was vortex-mixed at 5 min intervals. The tubes were then cooled to room temperature and 1 ml of the solutions diluted to 10 ml with distilled water. The transmittance (%T) was then determined using a spectrophotometer at 650 nm. Distilled water was used as the blank.
4.1.2.9 Starch gel texture

Starch pastes (4.1.2.6) were hot-filled into small, circular plastic containers (16 mm height x 37 mm diameter). The height of the containers was raised by 1 cm by wrapping the container in aluminium foil. The starch pastes were stored in these containers for 24 hr at 22°C. The foil was then removed and the excess gel (above the rim of the container) cut off with a thread to give a flat surface with a thickness of 16 mm. Gel firmness was analysed using a TA-XT2 texture analyser (Stable Micro Systems, Godalming, England) with a P/20p cylinder probe (20 mm diameter). Gels were compressed 5 mm into the gel and the force noted. This was conducted using a pre-test speed of 2 mm/s; and test and post-test speeds of 0.2 mm/s. The test was run at 25°C.

4.1.2.10 Statistical analysis

One-way analysis of variance (ANOVA) was used to determine differences due to added stearic acid, for the starches. Means were then compared using Fischer’s Least Significant Difference Test (LSD). The differences between the various concentrations for each starch were also determined. The concentration of the added stearic acid and the different starch types were the independent variables of the experiment. The experiments were repeated three times unless stated otherwise.

4.1.3 Results and Discussion

4.1.3.1 Composition of the starches

Commercial maize starch and laboratory-extracted teff starch had similar amylose and fat contents (Table 4.1.1). The protein content of teff starch (1.58%) was higher than maize starch (0.39%). This could have been due to the different extraction method used. Teff starch was extracted using only distilled water, whereas sulphur dioxide (SO₂) is used in the production of
commercial maize starch (reviewed by Kent and Evers 1994). Sulphur dioxide promotes the breakdown of the protein matrix by the disruption of the disulphide bonds. This would have ensured that protein was more easily separated from maize starch.

**Table 4.1.1**: Chemical composition of maize and teff starch (g/100 g dry basis)

<table>
<thead>
<tr>
<th></th>
<th>Maize</th>
<th>Teff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.39 ± 0.02</td>
<td>1.58 ± 0.08</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.60 ± 0.08</td>
<td>2.38 ± 0.20</td>
</tr>
<tr>
<td>Ash</td>
<td>0.12 ± 0.06</td>
<td>0.50 ± 0.08</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>0.12 ± 0.02</td>
<td>0.15 ± 0.1</td>
</tr>
<tr>
<td>Amylose#</td>
<td>32.0 ± 3.5</td>
<td>34.6 ± 2.7</td>
</tr>
</tbody>
</table>

# % of total starch

4.1.3.2 Pasting properties (short pasting cycle)

Increasing the stearic acid concentration resulted in a decrease in the peak (viscosity at which the majority of the starch granules are intact), breakdown (the difference between the peak and trough viscosities) and final viscosities (viscosity at the end of the pasting cycle) of the maize starch, with the short pasting cycle (Fig. 4.1.1 and Table 4.1.2). Peak time (time taken to reach peak viscosity) also increased with stearic acid concentration. After holding for 5 min at 91°C, an additional peak was observed during the cooling phase for the maize starches. Teff starch showed slightly different effects with added stearic acid. Only the teff starch controls showed a peak viscosity within the holding time (5 min at 91°C), while the treatments did not. Only the teff starch control showed the cooling stage curve (Fig. 4.1.2).

The teff starch control (Fig. 4.1.2 and Table 4.1.2) showed similar behaviour to that of the maize starch control, but had a lower peak viscosity and longer peak time. This might have been due to the small size of the teff starch granules (2-6
µm) (Bultosa et al 2002). Fortuna et al (2000) found that the smaller sized granules of wheat, potato and maize starches showed greater resistance to swelling and lower peak viscosities. Furthermore, Bultosa et al (2008) attributed the lower paste viscosity of teff starches (compared to maize starch) to the smaller size of teff amylopectin molecules (weight average molar mass = 13.9 x 10^7 g/mol) compared to that of maize starch (weight average molar mass = 19.6 x 10^7 g/mol).

During the cooling phase of the short pasting cycle for maize starch, an additional peak was observed. This cooling stage curve has been attributed to a three component interaction between starch, protein and lipid (Zhang and Hamaker 2003).
Fig. 4.1.1: Effect of stearic acid concentration on the pasting properties of maize starch, for the short pasting profile (5 min holding at 91 °C).
Fig. 4.1.2: Effect of stearic acid concentration on the pasting properties of teff starch, for the short pasting profile (5 min holding at 91 °C).
**Table 4.1.2**: Effect of stearic acid (% w/w) on the pasting properties of maize and teff starches, for the short pasting cycle (5 min holding at 91 °C)

<table>
<thead>
<tr>
<th>Stearic acid (%)</th>
<th>Peak Viscosity (PV) (RVU)</th>
<th>Peak time (Pt) (min)</th>
<th>Final Viscosity (FV) (RVU)</th>
<th>Breakdown Viscosity (BD) (RVU)</th>
<th>Maximum Viscosity reached in 15 min (MV15) (RVU)</th>
<th>Peak Time (Pt) (min)</th>
<th>Final Viscosity (FV) (RVU)</th>
<th>Breakdown Viscosity (BD) (RVU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>168.6 ± 4.0</td>
<td>10.2 ± 0.1</td>
<td>150.03 ± 3.9</td>
<td>32.50 ± 2.4</td>
<td>#111.10 ± 4.2</td>
<td>12.4 ± 0.4</td>
<td>124.98 ± 5.2</td>
<td>8.55 ± 0</td>
</tr>
<tr>
<td>0.25</td>
<td>169.4 ± 3.7</td>
<td>10.3 ± 0.1</td>
<td>149.06 ± 3.7</td>
<td>26.85 ± 1.3</td>
<td>117.05 ± 4.1</td>
<td>n/a</td>
<td>105.02 ± 4.3</td>
<td>n/a</td>
</tr>
<tr>
<td>0.5</td>
<td>165.6 ± 3.7</td>
<td>10.4 ± 0.1</td>
<td>144.77 ± 3.0</td>
<td>25.26 ± 0.8</td>
<td>94.64 ± 3.4</td>
<td>n/a</td>
<td>93.31 ± 3.8</td>
<td>n/a</td>
</tr>
<tr>
<td>1.0</td>
<td>163.4 ± 1.8</td>
<td>10.5 ± 0.1</td>
<td>142.68 ± 1.8</td>
<td>24.11 ± 0.8</td>
<td>94.19 ± 9.0</td>
<td>n/a</td>
<td>92.33 ± 8.7</td>
<td>n/a</td>
</tr>
<tr>
<td>1.5</td>
<td>163.6 ± 7.4</td>
<td>10.5 ± 0.2</td>
<td>145.03 ± 5.8</td>
<td>24.28 ± 2.6</td>
<td>61.03 ± 2.4</td>
<td>n/a</td>
<td>61.08 ± 2.3</td>
<td>n/a</td>
</tr>
<tr>
<td>2.0</td>
<td>156.1 ± 4.5</td>
<td>10.6 ± 0.1</td>
<td>139.99 ± 3.8</td>
<td>22.90 ± 1.4</td>
<td>63.83 ± 2.4</td>
<td>n/a</td>
<td>63.78 ± 2.2</td>
<td>n/a</td>
</tr>
<tr>
<td>3.0</td>
<td>150.8 ± 2.8</td>
<td>10.6 ± 0.1</td>
<td>139.63 ± 2.9</td>
<td>21.35 ± 1.1</td>
<td>64.92 ± 2.3</td>
<td>n/a</td>
<td>63.54 ± 2.2</td>
<td>n/a</td>
</tr>
<tr>
<td>4.0</td>
<td>147.1 ± 3.1</td>
<td>10.7 ± 0.1</td>
<td>140.36 ± 2.5</td>
<td>19.63 ± 0.9</td>
<td>51.52 ± 2.8</td>
<td>n/a</td>
<td>51.39 ± 2.5</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Values within the same columns with different letters are significantly different (p <0.05).

# represents peak viscosity

• represents the amount of stearic acid on a dry weight basis of the starch

n/a = not applicable
The reduced peak viscosity and increased peak time observed for maize starch (Fig. 4.1.1 and Table 4.1.2) might have been due to the formation of a layer of stearic acid around the granule. Richardson et al (2004) suggested that the formation of such a layer would increase the hydrophobicity of the granule and reduce its ability to take up water. This was probably the reason for the lower peak viscosity and longer peak time.

Teff starch produced some unusual effects when stearic acid concentration was increased (Fig. 4.1.2 and Table 4.1.2). A peak viscosity was not reached within the first 15 min of pasting. Therefore the peak time and breakdown viscosity could not be determined for these treatments. Only the viscosity reached within 15 min i.e. viscosity before the cooling phase, and final viscosity are reported. During the holding time (5 min at 91°C) of pasting, teff starch pastes increased in viscosity with added stearic acid and only began to decrease as the temperature was reduced. Therefore, the recorded ‘final viscosity’ (Table 4.1.2) may not be a true representation of the final viscosity of the starch paste. However, a trend was observed. Increasing the stearic acid concentration resulted in reduced ‘final viscosity’ of teff starch pastes. A possible reason for this behaviour could be that teff starch granules (2-6 µm) are smaller than maize starch granules (5-30 µm) (Bultosa et al 2002). The small size granules may have offered a greater surface area for interaction with stearic acid. In this way, granular swelling could have been restricted and consequently delayed peak viscosity. The rather large delay in pasting with teff starch could be the reason for the general decrease in ‘final viscosity’ observed when the short pasting cycle was used. The pasting cycle was extended to understand the pasting behaviour of teff starch with added stearic acid.

Results similar to those obtained for maize starch with added stearic acid, i.e. reduced peak and breakdown viscosities (Table 4.1.2), have also been reported by Raphaelides and Georgidis (2006) who worked on maize starch and Tang and Copeland (2007) who worked on wheat starch. Zhou et al (2007) also obtained similar results with rice starch. These authors also used short
pasting cycles and the addition of stearic acid. Bajner (2002) obtained similar results with the addition of stearic acid to maize starch even though an extended pasting cycle was used. Bajner (2002) also found that the addition of stearic acid resulted in reduced peak viscosity and increased peak time. As mentioned, Richardson et al (2003) proposed that the addition of lipids to starch, may result in the lipid layer around the starch granule. The net result would be an increased hydrophobicity of the granule. This would result in a reduced ability of the granules to take up water as well as a reduced rate of swelling. This was possibly the cause for the reduced peak viscosities and increased peak times observed for maize starch.

The effects of stearic acid on pasting properties were more evident as the concentration of stearic acid was increased (Table 4.1.2). Increasing the stearic acid concentration led to lower peak, breakdown and final viscosities. This finding is in agreement with Bajner (2002), Kaur and Singh (2000) and Tang and Copeland (2007). Tang and Copeland (2007) proposed that increasing the concentration of the fatty acid would allow for a greater number of fatty acid molecules to form complexes with the amylose molecules. This could possibly be why the observed effects were more pronounced with increasing fatty acid concentration. This was further indicated by the increase in CI after the short pasting cycle (Fig. 4.1.3). The increase in CI indicates a reduced number of free amylose molecules. Thus, CI may be an indication of amylose lipid complexes. For both starches, CI increased progressively with added stearic acid, when the short pasting cycle was used. It is possible that as the concentration of stearic acid increased, a greater number of stearic acid molecules were available to interact with amylose molecules. This would result in a fewer free amylose molecules present in the paste. For maize, there was no significant difference in CI for 1.5%, 2.0% and 3.0% added stearic acid. This suggests that at approximately 1.5%, the amylose molecules were probably saturated with stearic acid. For teff starch, a saturation level could not be determined because CI continued to increase with increasing stearic acid concentration.
Fig. 4.1.3: Effect of stearic acid concentration on the complexation indices of maize and teff starches.
Key: —— maize  —— teff

4.1.3.3 Pasting properties (extended pasting cycle)

A second pasting peak was observed when maize starch was held at 91°C for 2 hours (Fig. 4.1.4 and Table 4.1.3). The viscosity of this peak increased with 0.25% stearic acid. When the stearic acid level was increased to 1.5%, the second peak did not form completely within the duration of the pasting cycle. Increasing the stearic acid concentration (0.25%) also led to increased final viscosity. A similar trend was observed with teff starch (Fig. 4.1.5). However, teff starch also did not display a first pasting peak with added stearic acid.
Fig. 4.1.4: Effect of stearic acid concentration on the pasting properties of maize starch for the extended pasting cycle (2 hours holding at 91°C), where A represents the relative area under the peak.
Fig. 4.1.5: Effect of stearic acid concentration on the pasting properties of teff starch for the extended pasting cycle (2 hours holding at 91 °C), where A represents the relative area under the peak.
Table 4.1.3: Effect of stearic acid concentration on the pasting properties of maize and teff starches for the extended pasting cycle (2 hours holding at 91 °C)

<table>
<thead>
<tr>
<th>Stearic acid (%)</th>
<th>Maize</th>
<th>Teff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Viscosity of the second peak (RVU)</td>
<td>Final Viscosity (RVU)</td>
</tr>
<tr>
<td>0</td>
<td>$175.4^{a} \pm 2.23$</td>
<td>$186.2^{a} \pm 3.20$</td>
</tr>
<tr>
<td>0.25</td>
<td>$228.2^{b} \pm 6.95$</td>
<td>$227.2^{b} \pm 11.69$</td>
</tr>
<tr>
<td>1.5</td>
<td>Second peak did not form completely</td>
<td>Second peak did not form completely</td>
</tr>
</tbody>
</table>

Values within the same columns with different letters are significantly different ($p < 0.05$)

*Represents the amount of stearic acid on a dry basis of the starch

The maize and teff starch controls displayed second pasting peaks after about 70 min of pasting. Nelles et al (2000) showed that when the lipid component was removed from maize starch, the second peak did not occur. However, the peak reappeared when the lipid component was added back to the starch. The occurrence of a second peak has been attributed to the formation of complexes between starch and lipids (Nelles et al 2000; Bajner 2002; Nelles et al 2003). These complexes occur in two forms. Type I complexes form below the gelatinization temperature and Type II at temperatures above 90 °C (Biliaderis and Galloway 1989). Therefore, it is most probably the formation of the Type II amyllose-lipid complex which was responsible for the increased viscosity. The second peak for the maize control (175 RVU) was higher than that of the teff control (113.8 RVU) (Table 4.1.3). Bultosa and Taylor (2003) showed that teff starch contained slightly less endogenous lipids than maize starch. The higher second peak viscosity for maize starch was probably due to formation of complexes with endogenous lipids. In addition, maize starch also had a higher breakdown (32 RVU) than teff starch (8 RVU). Breakdown takes place directly
after the first pasting peak is reached and is due to the rupture of starch granules (reviewed by Thomas and Atwell 1999 b). Granular breakdown probably allowed for an increased interaction between amylose and the endogenous lipids, which consequently resulted in the formation of the second peak.

The addition of 0.25% stearic acid to maize starch (Fig. 4.1.4) resulted in an increased second peak viscosity and final viscosity compared to its control. An increase in the area under the second viscosity peak was also observed. The increase in viscosity was probably due to the formation of a greater number of amylose-lipid complexes since more stearic acid molecules were present to complex with amylose. Straight chain fatty acids (such as stearic acid) are preferred over endogenous lipids for amylose complexation due to its straight chain structure (Godet et al 1995 b). Amylose complexes with endogenous lipids would be less stable because the form and structure of the lipids would not be suitable for inclusion in the amylose helix (Bajner 2002). Hence, the breakdown of viscosity after the second peak may be due to the breakdown of amylose complexes with endogenous lipids, since they would be less stable than amylose-stearic acid complexes (Bajner 2002). The subsequent increase in final viscosity could have been due to the complexes present in the starch pastes at the end of the extended pasting cycle (Nelles et al 2000). To a certain degree, the higher final viscosity observed for the treatments may also have been due to retrogradation of non-complexed amylose upon cooling of the paste.

The addition of 1.5% stearic acid resulted in a very large increase in viscosity for maize starch. The second peak did not completely form (Fig. 4.1.5 and Table 4.1.3). At this high concentration, it is possible that the second peak did not completely form because the formation of amylose-lipid complexes was still taking place. For both starches, the maximum viscosity reached was far greater than observed for the second peak when 0.25% stearic acid was added. This was probably due to a greater number of complexes formed since more stearic
Acid molecules were available for complexation. Bajner (2002) also found that the second viscosity peak increased with stearic acid concentration. Furthermore, the second peak began to form earlier with 1.5% stearic acid (after about 40 min pasting compared to 70 min observed with 0.25% stearic acid). Amylose molecules could have been saturated with stearic acid faster because it was present in a larger amount. The increase in viscosity of the starch paste could also be due to formation of micelles between stearic acid molecules which form when in excess (Tang and Copeland 2007). However, CI showed that for both starches, the addition of 1.5% stearic acid produced a greater degree of complexation than with 0.25% stearic acid during extended pasting (Table 4.1.4). This further supports the hypothesis that the increase in viscosity is probably due to amylose-stearic acid interaction.

**Table 4.1.4**: Complexation Indices of maize and teff starches after the extended pasting cycle (2 hours holding at 91 °C)

<table>
<thead>
<tr>
<th>Stearic acid (%)</th>
<th>Complexation Index (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maize</td>
</tr>
<tr>
<td>0</td>
<td>*n/a</td>
</tr>
<tr>
<td>0.25</td>
<td>24.3 ± 0.45</td>
</tr>
<tr>
<td>1.5</td>
<td>52.3 ± 0.18</td>
</tr>
</tbody>
</table>

Values within the same columns with different letters are significantly different (p <0.05) and are the means of at least 2 (n=2) repetitions.

* n/a = not applicable (0% added stearic acid taken as 0% complexation/blank
• Represents the amount of stearic acid on a dry weight basis of the starch

When the extended pasting cycle was used, teff starch displayed a similar trend to maize starch, i.e. increased second peak viscosity, area under the second viscosity peak and final viscosity (Fig. 4.1.4, Fig. 4.1.5 and Table 4.1.3). However, the teff starch did not show a first pasting peak but only an increase in viscosity. The teff starch control showed a breakdown viscosity of only 8 RVU. No breakdown viscosity was observed for 0.25% and 1.5% added stearic acid as these treatments did not form a pasting peak. Bajner (2002) and Zhou et al (2007) found that the addition of a fatty acid reduced the rate of viscosity
breakdown of maize and rice starch pastes, respectively (Bajner 2002; Zhou et al 2007). Hence, it is possible that with added stearic acid the rate of breakdown of the teff starch granules was far less than the rate of complexation between amylose and stearic acid. This could be a reason why a first viscosity peak did not form completely. The increase in the viscosity of the second peak for teff starch with 0.25% added stearic acid (increase of 136 RVU), was more than twice that of maize starch (52 RVU), when compared to their respective control (Table 4.1.3). Bultosa and Taylor (2003) found that teff starch had a greater amount of amylose leaching with time than maize starch. Therefore, more amylose would have been available for complexation with stearic acid. The addition of 1.5% stearic acid showed the same trend as maize starch, i.e. second peak began to form after about 40 min of pasting without complete formation.

To determine if it was indeed amylose-lipid interactions that were responsible for the occurrence of the second peak, waxy maize starch (97% amylopectin) and a waxy maize starch containing 0.25% stearic acid, were pasted using the extended pasting cycle. These waxy maize starches did not show a second peak (Fig. 4.1.6). This further suggests that interaction between amylose and stearic acid was probably responsible for the formation of the second peak.
Fig. 4.1.6: Effect of stearic acid concentration (0, 0.25 and 1.5%) on the pasting properties of waxy maize starch for the extended pasting cycle (2 hours at 91 °C)
4.1.3.4 Paste clarity of starch pastes after the short and extended pasting cycles

The transmittance of a starch paste is a measure of its paste clarity. The more light transmitted through a starch suspension, the less opaque the paste. Both maize and teff starch showed reduced transmittance with added stearic acid (Table 4.1.5). Transmittance was further reduced when the extended pasting cycle was used (Table 4.1.5).

**Table 4.1.5**: Effect of stearic acid on the paste clarity of maize and teff starch pastes using the extended pasting cycle

<table>
<thead>
<tr>
<th>Stearic acid (%)</th>
<th>% Transmittance</th>
<th>30 min</th>
<th>90 min</th>
<th>30 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43.2 ± 1.9</td>
<td>23.3 ± 1.6</td>
<td>38.1 ± 1.5</td>
<td>27.0 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40.4 ± 0.8</td>
<td>21.9 ± 0.7</td>
<td>39.4 ± 1.1</td>
<td>23.7 ± 1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39.3 ± 0.6</td>
<td>19.8 ± 0.6</td>
<td>34.3 ± 0.5</td>
<td>22.0 ± 0.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values within the same columns with different letters are significantly different (p < 0.05)

Only 0.25% and 1.5% stearic acid used for extended pasting
• Represents the amount of stearic acid on a dry weight basis of the starch

Craig et al (1989) suggested that lipids that reduce granular swelling of starches could reduce transmittance of starch pastes. In section 4.1.3.2 it was shown that stearic acid probably reduced granular swelling. ‘Whiteness’ or opacity is brought about by the degree of light scattered by the association of starch chains after pasting (Craig et al 1989). Since the second peak is probably an indication that amylose-stearic acid complexes had formed, the reduced transmittance obtained indicated that these complexes interfered with the association of the starch chains. This could have been the case, since amylose-lipid complexes interfere with the re-aligning of starch chains during retrogradation (Gudmundsson 1994).
The transmittance also decreased with extended pasting time (Table 4.1.5). Thus, paste opacity was further increased. The reduction in %T was much greater with extended pasting time than with added stearic acid. This was possibly due to a greater number of complexes formed with greater pasting time. This again, would be due to exposure of the amylose molecules to stearic acid for a longer period and also the formation of superstructures by the individual complexes. The clarity of a starch paste may be influenced the presence of amylose-lipid complexes (Craig et al 1989).

4.1.3.5 Gel texture of starches after the short and extended pasting cycles

Without added stearic acid, maize starch formed softer gels than teff starch (Table 4.1.6). Both starches formed gels that were short, and white in colour. This finding is in agreement with Bultosa and Taylor (2004 b). With 0.25% added stearic acid, the gels of both starches decreased in firmness. However, teff starch gels remained firmer than their maize counterparts. Similar results were obtained by Raphaelides (1992) and Richardson et al (2004), who found that the rigidity of amylose gels was reduced with the addition of fatty acids and emulsifiers, respectively. Richardson et al (2004) also found that low levels of an emulsifier (glycerol monostearate) added to potato amylose dispersions formed open structured gels with many pores. This might indicate that the addition of lipid components interfered with junction-zone formation, which is integral for gel formation. Junction zones are segments of opposite starch chains that have interacted with each other and form a three-dimensional gel network structure (Whistler and BeMiller 1997 b). Richardson et al (2004) also found that gels containing added emulsifier formed networks of which the amylose strands were thicker and longer, compared to the normally thin strands.
Table 4.1.6: Texture of maize and teff starch pastes using the extended pasting cycle

<table>
<thead>
<tr>
<th>Stearic acid (%)</th>
<th>Maize</th>
<th>Teff</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*Force (N)</td>
<td>Observations</td>
</tr>
<tr>
<td>0</td>
<td>$3.8^b \pm 0.2$</td>
<td>Short; white</td>
</tr>
<tr>
<td>0.25</td>
<td>$2.5^a \pm 0.3$</td>
<td>Softer; white</td>
</tr>
<tr>
<td>1.5</td>
<td>Did not gel</td>
<td>Did not gel</td>
</tr>
</tbody>
</table>

Values within the same columns with different letters are significantly different ($p < 0.05$)

*Force required to compress a gel by 5 mm

• Represents the amount of stearic acid on the dry weight basis of the starch

The addition of 1.5% stearic acid, resulted in no gels being formed. This finding is in agreement with that of Richardson et al (2004). These authors found that high levels of added emulsifier (as above) only resulted in the formation of a thick opaque paste, without gelling. Gel formation is largely dependent on starch retrogradation, i.e. the re-alignment of amylopectin molecules and formation of amylose double helices (network formation) (reviewed by Thomas and Atwell 1999 b). Co-crystallisation of amylose may prevent interaction between amylose and amylopectin during the recrystallisation process (Gudmundsson 1994). In addition, the amylose-lipid complex may also change or retard water distribution.

4.1.3.6 Conclusions

The increase in complexation index for the extended pasting cycle compared to the short pasting cycle for both starches and the absence of a second pasting peak when waxy maize starch was modified with stearic acid suggests that the amylose-lipid complexes are probably responsible for the formation of the second pasting peak. The changes in paste properties for both maize and teff starches, i.e. reduced viscosity of the first peak, increased viscosity of the second peak, increased final viscosity, increased paste opacity and reduced
gelling ability are attributed to the formation of amylose-lipid complexes. The absence of a first viscosity peak for teff starch during extended pasting and the consequent large increase in viscosity observed instead, is an unusual phenomenon and requires further investigation.

4.1.3.7 References


4.2 Flow properties and structure of maize and teff starch pastes modified with stearic acid

ABSTRACT

When stearic acid is added to maize and teff starches a second viscosity peak is formed during extended pasting for 2 hr at 91 °C (Section 4.1). However, teff starch does not form a first viscosity peak but a large increase in viscosity. The formation of the second peak was attributed to starch-fatty acid complexes. In this chapter, the flow properties (at varying shear rates), and structural characteristics in terms of X-Ray Diffraction (XRD) and confocal laser scanning microscopy (CLSM) were determined in order to understand the pasting behaviours of maize and teff starches observed in Section 4.1. Maize and teff starch pastes and their stearic acid treated counterparts (0.25% stearic acid) followed the Power-Law Model and were shear thinning (n < 1). Teff starch pastes seemed to be less shear thinning than maize starch pastes after both pasting cycles. There was a significant increase in k for both starches after the extended pasting cycle suggesting an increase in viscosity during pasting. XRD diffractograms showed a small peak characteristic of crystalline amylose-lipid complexes. This indicates that there may have been a change from A-type to V-type in the starch crystallinity pattern. Nile Red and Fluoroscein Isothiocyanate (FITC) were used to stain stearic acid and starch, respectively and viewed by CLSM. As such, the starch controls and their treated counterparts did not show differences at the various intervals during pasting for 2 hr. However, CLSM before pasting showed that stearic acid was inside the maize starch granules. This was not observed for teff starch granules. Stearic acid seemed to remain outside the surface of the teff starch granules. Therefore, it appears that the different pasting behaviours of the starches (Section 4.1) may be due to the mechanism of interaction between stearic acid and starch granules.
4.2.1 Introduction

With extended pasting, the addition of stearic acid to maize starch results in a reduced first viscosity peak (Section 4.1). In contrast, teff starch did not give a first viscosity peak. However, a second pasting peak was observed for maize and teff starches. The viscosities of the second peaks increased with the addition of stearic acid. This was probably due to the formation of amylose-stearic acid complexes. Similar results were found when maize starch was pasted with an extended holding time (Nelles et al 2000; Bajner 2002). In this present work, it was also found that the addition of stearic acid resulted in softer gels and more opaque pastes for both starches (See Chapter 4.1).

The viscosities reported in Section 4.1 were measured using a Rapid Visco Analyser (RVA). The RVA uses a constant shear rate of 160 rpm to measure viscosity. This shear rate is equivalent to 54 s\(^{-1}\) (Booth and Bason 2007). Starch pastes are shear thinning and follow the Power-Law Model (Guraya et al 1997; Kaur and Singh 2000; Singh et al 2000). However, viscosities measured at a constant shear rate may not fairly represent shear rates incurred during industrial processing. In the food industry, starches are exposed to various shear rates in equipment such as plate heat exchangers (50-500 s\(^{-1}\)), scrape surface cookers (10-200 s\(^{-1}\)) and jet cookers (1000-100 000 s\(^{-1}\)) (Loh 1992). Therefore, measurement of the flow properties with a rheometer, which can operate at varying shear rates, is preferred since it can simulate processing conditions better than the RVA.

Techniques such as microscopy and X-Ray Diffraction (XRD) have been widely used to study the structure of starch pastes (reviewed by Thomas and Atwell 1999 a). Savary et al (2007) used Confocal Laser Scanning Microscopy (CLSM) to investigate the structure of polysaccharide-starch composite gels. Nelles et al (2003) used Scanning Electron Microscopy (SEM) to show that maize starch granules were intact until the formation of the second viscosity peak during pasting for approximately 95 min. The second peak then coincided
with a loss of granular integrity. Fanta et al (2002) and Peterson et al (2005) used SEM to show the formation of crystalline aggregates (spherocrystals) after steam jet cooking. Fanta et al (2002) concluded that the formation of these spherocrystals was due to the formation of amylose inclusion complexes with native lipids since they did not form with waxy maize starch. Furthermore, X-ray analyses of these crystals showed a change from the A to the V pattern. Similar results were obtained by Godet et al (1995 a) using amylose and palmitic acid. Tang and Copeland (2007) also showed that starch pastes containing added fatty acid were of the V-type pattern.

The objective of this work was to determine the effects of stearic acid on the flow properties and structure of maize and teff starch pastes after short and extended pasted cycles.

**4.2.2 Materials and Methods**

**4.2.2.1 Samples**

See 4.1.2.1

**4.2.2.2 Starch extraction**

See 4.1.2.2

**4.2.2.3 Starch Composition**

See 4.1.2.3 and Table 4.1.2.1
4.2.2.4 Incorporation of stearic acid into starch

Stearic acid was added to maize and teff starches using the procedure in 4.1.2.5. However, stearic acid was added to starch in the concentrations 0% and 0.25% (relative to starch). Only one concentration of stearic acid was used because at higher concentration levels, the starches did not completely form the second peak within the holding time of 2 hr.

4.2.2.5 Flow properties of starch pastes at varying shear rates

Samples were pasted using the short and extended pasting cycles described in 4.1.2.6. However, instead of the RVA, starch pasting was conducted with a Physica MCR 301 Rheometer (Anton Paar, Ostfildern, Germany). The shear viscosity of the starch paste was then determined using the Bob and Cup Method. Measuring bob (diameter: 27 mm) was inserted into the cup (diameter: 28.9) containing the starch paste. Paraffin oil was added to the top of the starch paste and allowed to form a layer until the entire surface was covered. This was done to prevent moisture loss from the sample. The starch paste was then allowed to equilibrate at 60°C for 30 min. The starch pastes were measured at shear rates ranging between 0.1 and 800 s$^{-1}$. The Power-Law Model,

$$\tau = k \dot{\gamma}^n$$

was used to describe a flow or viscosity curve. This model describes, $\tau$ as the shear stress, $k$ as the consistency coefficient, $\dot{\gamma}$ as the shear rate and $n$ as the flow behaviour index. The shear behaviour e.g. shear thinning/thickening is given by $n$ (no unit) and the $k$ represents viscosity (Pa.s).
4.2.2.6 X-Ray diffraction

Starches were pasted using the short and extended procedures described in 4.1.2.6. The pastes from the RVA canister were immediately frozen (at -20°C) and then freeze-dried. The freeze-dried samples were milled using a mortar and pestle. The samples were prepared for X-Ray Diffraction (XRD) analysis using the back loading preparation method (the sample is pressed into the sample holder from the back). This allowed for a flat surface of the sample on the front of the holder (this side is exposed to the X-Ray beam) and minimizes preferred orientation of crystallites. The samples were analysed with a PANalytical X’Pert Pro Powder Diffractometer (Ostfildern, Germany), with an X’Celerator detector. The diffractometer was equipped with variable divergence- and receiving slits using Fe filtered Co-Kα radiation (1.78901 Å) and operated at 35 kV and 50 mA. Samples were scanned at 25 °C with 2θ in the range 2-90°. Diffractograms were interpreted using X’Pert Highscore Plus software.

4.2.2.7 Confocal laser scanning microscopy (CLSM)

Starches were pasting according to the extended pasting cycle described in 4.1.2.6. Five samples were taken at the intervals described as follows and illustrated in Fig. 4.2.1. Maize starch control: (a) before pasting; (b) before first peak (10 min); (c) after first peak (12 min); (d) before second peak (70 min); (e) after second peak (90 min); and (f) after the complete pasting cycle (2.5 hours). Maize starch with 0.25% stearic acid: (a) before pasting; (b) before first peak (10 min); (c) after first peak (12 min); (d) before second peak (70 min); (e) after second peak (110 min); and (f) after complete pasting cycle (2.5 hours). Teff starch control: (a) before pasting; (b) before first peak (10 min); (c) after first peak (12 min); (d) before second peak (70-80 min); (e) after second peak (100 min); and (f) after the complete pasting cycle (2.5 hours). Teff starch with 0.25% stearic acid: (a) before pasting; (b) after 10 min pasting; (c) after 12 min; (d) before second peak (80-90 min); (e) after second peak (100-110 min); and (f) after the complete pasting cycle (2.5 hours).
A small amount (approx. 10 mg) of the sampled starch paste was placed on a microscope slide. Fluorescent stains, Nile Red and fluorescein-isothiocyanate (FITC) were used to stain stearic acid and starch, respectively. Emmambux and Stading (2007) used Nile Red for a non-polar substance, while Savary et al (2007) used FITC to stain starch. Nile Red (1 g/L in ethanol) and FITC (0.05 g/L in 50% ethanol) were used for starch granules and for starch pastes. The stains were mixed into the sample using a spatula. A cover slip was placed over the sample and sealed with nail varnish to prevent evaporation of the solvent. Slides were placed in a dark cold room for 24 hr. These samples were then analysed on a Zeiss LSM 510 META Confocal Laser Scanning Microscope (Zeiss SMT, Jena, Germany). The excitation and emission spectra for Nile Red were 488 nm and 640-750 nm, respectively and 488 nm and 486-539 nm for FITC.
**Fig. 4.2.1:** Intervals at which samples were taken for CLSM A) maize starch and B) teff starch during the extended pasting cycle.
4.2.2.8 Statistical analysis

One-way Analysis of Variance (ANOVA) was used to determine the differences between starches (modified and unmodified) for $n$ (flow behaviour index) and $k$ (consistency coefficient). Means were compared using Fischer’s Least Significant Difference Test (LSD). The concentration of added stearic acid and the different starch types were the independent variables. The experiments were repeated three times unless stated otherwise.

4.2.3 Results and Discussion

4.2.3.1 Flow properties of starch pastes at different shear rates

The flow properties of both starch pastes and their treated counterparts followed the Power-Law Model (Table 4.2.1). The flow behaviour index ($n$) indicates the type of behaviour in response to different shear rates. A value of $n < 1$ indicates shear thinning behaviour, $n > 1$ indicates shear thickening behaviour and $n = 1$ indicates Newtonian behaviour. The $k$ value, known as the consistency coefficient is an indication of the viscous behaviour.
Table 4.2.1: Effect of stearic acid on the flow properties in terms of the Power-Law Model coefficients for maize and teff starches during short and extended pasting.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>K</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short pasting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize Control</td>
<td>0.35$^b$ ± 0.01</td>
<td>23.95$^b$ ± 0.24</td>
<td>0.98 ± 0.03</td>
</tr>
<tr>
<td>Maize + 0.25% stearic acid</td>
<td>0.33$^a$ ± 0.01</td>
<td>24.41$^b$ ± 0.64</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>Teff Control</td>
<td>0.41$^a$ ± 0.01</td>
<td>15.62$^a$ ± 0.20</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>Teff + 0.25% stearic acid</td>
<td>0.39$^{c,d}$ ± 0.01</td>
<td>17.57$^a$ ± 1.65</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td><strong>Extended pasting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize Control</td>
<td>0.40$^{c,d}$ ± 0.02</td>
<td>26.52$^c$ ± 0.16</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>Maize + 0.25% stearic acid</td>
<td>0.39$^c$ ± 0.01</td>
<td>30.40$^d$ ± 2.81</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>Teff Control</td>
<td>0.44$^d$ ± 0.01</td>
<td>29.46$^d$ ± 1.56</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>Teff + 0.25% stearic acid</td>
<td>0.40$^d$ ± 0.01</td>
<td>34.20$^d$ ± 1.21</td>
<td>0.97 ± 0.05</td>
</tr>
</tbody>
</table>

Values within columns with different letters are significantly different (p<0.05) and the pastes for the short and extended cycles are compared separately.

n is the flow behaviour index and k is the consistency coefficient from the Power-Law Model (see section 2.4.4).
After the short and extended pasting cycles, maize and teff starch pastes displayed shear thinning behaviour because they were found to have n<1. The teff starch control showed a higher n value than the maize starch control. The shear thinning behaviour of maize starch pastes has been reported by Kaur and Singh (2000) and Singh et al (2000). Shear thinning behaviour has been attributed to the aligning of starch molecules in the direction of shear (Whistler and BeMiller 1997 a). During the breakdown phase of pasting, starch granules rupture and starch polymers are free to align themselves in the direction of shear. Teff starch has shown some resistance to breakdown (Bultosa and Taylor 2004 a). This could result in starch polymers not being free to align themselves in the direction of shear as easily as maize starch polymers. This may be a possible reason why teff starch was less shear thinning than maize starch.

The addition of stearic acid seemed to reduce the Power-Law flow index (n) for both starches after the short and extended pasting cycles. This indicated that the pastes were more shear thinning. Stearic acid is widely used in the pharmaceutical industry as a lubricating agent in tablets (Husseini and Pitt 2008). This lubricating effect may have allowed starch molecules to align more easily in the direction of shear and caused the paste to shear thin more.

The Power-Law consistency coefficient, k, was higher for both starch pastes after the extended pasting cycle (2 hr holding time) compared to after the short pasting cycle (30 min holding time). An increase in viscosity was also found when the RVA was used (Section 4.1). As stated, the second peak (responsible for the high viscosity mentioned above) is believed to be due to the formation of amylose-lipid complexes and their superstructures (Section 4.1). In the control samples, these complexes were probably formed between amylose and the endogenous lipids.
The addition of stearic acid led to further increases in the consistency coefficient, k. Higher second peak and final viscosities were also observed during pasting with an RVA (See Chapter 4.1). These high viscosities were probably due to formation of amylose-stearic acid complexes and their superstructures (See Chapter 4.1).

4.2.3.2 X-Ray diffraction (XRD)

X-Ray Diffractograms of the native maize and teff starches showed peaks typical of the A-type starch pattern (Figs. 4.2.2 and 4.2.3). The peaks characteristic of this pattern are 5.8 Å, 5.2 Å and 3.8 Å (Zobel 1988 b). These peaks were observed in the native starch samples but not in the pasted samples. As these starches were pasted, the starches seemed to lose crystallinity (Figs. 4.2.2 and 4.2.3). This is a characteristic feature of gelatinisation i.e. loss of crystallinity and birefringence (reviewed by Kent and Evers 1994), which is expected to occur during cooking in the RVA.

With the short pasting cycle, the A-type starch peaks did not form (Fig. 4.2.2). Instead, the X-Ray diffractograms of the maize starch control showed a 4.4 Å peak. This peak is the first indication of the formation of amylose-lipid complexes (Zobel 1988 b). This can be attributed to the formation of complexes between amylose and endogenous lipids. The addition of stearic acid seemed to produce a larger 4.4 Å peak with the maize starch. This might indicate that a greater degree of complexation had taken place. The teff starch control and the stearic acid treatment also showed the 4.4 Å peak (Fig. 4.2.3). However, no differences in the area of the peaks were observed after the addition of stearic acid.

With the extended pasting cycle (Fig. 4.2.2), a 12 Å peak was observed for the maize starch control. The 12 Å peak is considered to be a characteristic of V-type starches with amylose-lipid complexes (Zobel 1988 b). During the extended pasting cycle with the RVA, a second pasting peak was observed
(Chapter 4.1). The addition of stearic acid to maize starch also produced the 12 Å peak (Fig. 4.2.2). However, no differences in the area of the peaks could be determined. Teff starch (Fig. 4.2.3) also did not show differences in the area of the peaks after the addition of stearic acid. The presence of the 12 Å peak on the diffractogram after the formation of the second viscosity peak during extended pasting may suggest that the V-type complexes were present. Therefore, the X-Ray Diffractograms indicate that there may have been a change from the A-type starch pattern to the V-type starch pattern. This may have been a result of the formation of amylose-lipid complexes which may have interfered with or altered the order of helices which are responsible for crystal structure (see page 12).
**Fig. 4.2.2**: X-Ray Diffractograms of maize starch before pasting, after the short and extended pasting cycles. Filled arrows show peaks characteristic of amylose-lipid complexes. SA = stearic acid.
**Fig. 4.2.3:** X-Ray Diffractograms of teff starch before pasting, after the short and extended pasting cycles. Filled arrows show peaks characteristic of amylose-lipid complexes. SA = stearic acid
4.2.3.3. Confocal laser scanning microscopy (CLSM)

Before pasting, the maize starch granules of the control sample fluoresced green with FITC and did not show any red fluorescence (Fig. 4.2.4 (a)). After the addition of stearic acid (Fig. 4.2.5 (a)) bright red areas could be seen. The red fluorescence appeared to be inside the granules (Fig. 4.2.5 (a)). The Z-slices are images at various depths into the granules, taken after the starch was complexed with stearic acid (Fig. 4.2.8 & Fig. 4.2.9). The bright red areas seen in the Z-slices for maize starch might have indicated that stearic acid had diffused into the maize starch granules (Fig. 4.2.8). Fannon et al (1992) found pores on the surface of maize starch granules (using Scanning Electron Microscopy (SEM)). Stearic acid could have entered the maize starch granules through these pores during the complexing stage. The teff starch control and the stearic acid-treated sample showed some bright red areas/spots on the surfaces of the granules (Fig. 4.2.6 (a)). The Z-slices confirmed that there was no red fluorescence from inside the granules (Fig. 4.2.9). Bultosa et al (2002) did not find pores on the surface of teff starch granules using SEM. The presence of stearic acid around the surface of the teff starch granules indicates that stearic acid did not diffuse into the teff starch granules during the complexation stage. Thus, the presence of stearic acid outside the teff starch granules may be linked to the unusual pasting behaviour, i.e. large increase in viscosity without giving a first viscosity peak, observed in Chapter 4.1. This is further discussed in Section 5.2.

The maize starch, appeared to form aggregates of swollen starch granules before the first viscosity peak (Fig. 4.2.4(b)). After the first peak, a network was observed in the background (Fig. 4.2.4(c)). This was probably formed due to breakdown of the starch granules and consequent leaching of starch polymers. Stearic acid did not appear to have any effect on the formation of the network (Fig. 4.2.5). However, for the control and treated samples, the network seemed to disappear from the formation of the second peak onwards, as pasting continued (Figs. 4.2.4 and 4.2.5). This was observed as a loss of green background in the image and was
probably due to the solubilisation of starch polymers. Teff starch (Figs. 4.2.6 and 4.2.7) also showed a loss of the green background. However, at each stage interval, the network formed (observed as green background in the image) was much lighter in colour compared to its maize starch counterpart. This might suggest that the network formed at a slower rate (Fig. 4.2.6 (d)).

Fluorescent particles were observed for both starches and their stearic acid-treated counterparts during pasting (Figs. 4.2.4 to 4.2.7). These particles could have been the stearic-acid complexes. Fanta et al (2002) found spherocrystals after jet cooking of maize starch. These spherocrystals were not found in waxy maize starch and were therefore suggested to be the amylose-lipid complexes. However, with teff starch these ‘particles’ appeared more like remnant starch granules (Figs. 4.2.6 and 4.2.7). This difference observed with teff starch pastes may have been responsible for the unusual pasting behaviour i.e. absence of a first viscosity peak (Section 4.1). However, this requires further investigation.
Fig. 4.2.4: CLSM images of maize starch taken at various intervals during extended holding pasting. (a) before pasting, (b) before 1st peak, (c) after first peak, (d) before second peak, (e) after second peak and (f) after complete pasting.
**Fig. 4.2.5**: CLSM images of maize starch with 0.25% stearic acid taken at various intervals during extended holding pasting. (a) before pasting, (b) before 1st peak, (c) after first peak, (d) before second peak, (e) after second peak, and (f) after complete pasting.
Fig. 4.2.6: CLSM images of teff starch taken at various intervals during extended holding pasting. (a) before pasting, (b) before first peak, (c) after first peak, (d) before second peak, (e) after second peak and (f) after complete pasting.
Fig. 4.2.7: CLSM images of teff starch with 0.25% stearic acid, taken at various intervals during extended holding pasting. (a) before pasting, (b) after 10 min pasting, (c) after 12 min pasting, (d) before second peak, (e) after second (b) peak (f) after complete pasting cycle.
Fig. 4.2.8: “Z slices” of maize starch granules at various depths. sg = starch granule and sa = stearic acid.
Fig. 4.2.9: “Z slices” of teff starch granules at various depths. sg = starch granule and sa = stearic acid.
4.2.3.4 Conclusions

The addition of stearic acid results in shear thinning starch pastes which follow the Power-Law Model. Teff starch pastes with added stearic acid are less shear thinning than maize starch pastes. The consistency coefficient, k, of both starch pastes and their counterparts treated with stearic acid increases after the use of the extended pasting cycle. This is probably due to amylose-lipid complexes which may form during the second peak. The formation of 4.4 Å and 12 Å on the X-Ray Diffractograms are typical of V-type starches. This indicates a change in the starch X-Ray pattern from the A-type to V-type. Stearic acid probably diffuses into maize starch granules through its pores but does not seem to diffuse into teff starch granules since they do not have granular pores.

4.2.3.5 References


5. GENERAL DISCUSSION

This chapter presents a critical review of the methodology used in the research project, discusses the interaction between starch and stearic acid and proposes some potential food applications for starches modified with stearic acid.

5.1 Review of methodology

The major methods used in the research project are critically reviewed below. Any impact of the methodology on the results is also discussed.

5.1.1 Starch extraction:

In this research, teff starch granules were extracted without any use of chemicals. Chemicals such as sodium metabisulphite and/or sodium hydroxide are commonly used in commercial starch isolation (reviewed by Kent and Evers 1994). These chemicals may alter the native state of the starch granule (Sajeev et al 2006). Therefore, only distilled water was used during the extraction process for teff starch. This, however, resulted in higher protein content of 1.5% for teff compared to 0.39% for commercial maize starch. For the commercial production of maize starch, grain is first steeped in water containing sodium metabisulphite (reviewed by Kent and Evers 1994). The sulphur dioxide liberated from metabisulphite aids in disrupting the disulphide bonds of the protein matrix and helps separate protein from starch (reviewed by Kent and Evers 1994). This increases the purity of commercial maize starches. It is therefore, important to consider the difference in protein content when explaining any differences between pasting and other functional properties of the control and stearic acid-modified maize starch and teff starches. In future work teff starch could be extracted by using sodium metabisulphite to determine the effects when modified with stearic acid. This will allow for the effect of stearic acid to be directly compared to commercial maize starch.
5.1.2 Incorporation of stearic acid into starch

Stearic acid was incorporated into the starch sample to form a homogenous mixture. The starch was added to the stearic acid-ethanol solution and placed in a shaking water bath at 50°C for 30 min. The ethanol was then evaporated off. Other scientists (Bajner 2002; Zhou et al 2007) have also used this procedure to introduce the stearic acid into the starch. However, it is not known whether stearic acid/fatty acids diffuse into the starch granule or not. In this study CLSM has indicated that some stearic acid had diffused into the maize starch granules but this did not seem to occur with the teff starch granules. Thus, the methodology of incorporating stearic acid into teff starch granules may need some development. The diffusion of stearic acid into starch granule also seems to be dependent on the nature of the starch i.e. the presence/absence of granular pores. This may have an impact on the pasting properties and is discussed in section 5.2

5.1.3 Flow properties and rheology of starch pastes:

The RVA records the viscosity changes within a starch suspension/paste as a result of heating, under stirring conditions and in the presence of water (Booth and Bason 2007). The starch was added to the water to minimize clumping and settling of starch in the edges of the canister. The paddle was then used to ‘stir’ the starch suspension by a jogging action. This is important to prevent clumping as it can result in high variability among repetitions. However, although all the precautions were taken, it is difficult to completely eliminate settling of starch in the edges of the canister. This can negatively affect the pasting curves. Thus, only repeatable measurements were taken as reliable data.

The starch samples were heated to 91°C (to prevent boiling of the sample since water boils at approx. 95°C in Pretoria) and held at this temperature for 5 min (short cycle) and 2h (long cycle) for the determining their pasting properties, using an RVA. The RVA canister is not sealed and it would be
expected that at this high temperature (91°C) some evaporation of moisture would take place. This moisture loss may be negligible for samples pasted for a short time but may be significant in samples pasted for an extended period e.g. 2 hr at 91°C used in this work. If a significant amount of water is lost, this would result in viscosity being overestimated. However, the viscosity measurements obtained for the modified starches would be relative to their respective control (non-modified) samples, which would also lose moisture. Thus, the viscosity measurements are still valid.

Another common problem in the pasting of samples such as pre-gelled and waxy starches is the formation of bubbles behind the RVA paddle blades (Booth and Bason 2007). The formation and collapse of these bubbles gives a jagged/sawtooth/noisy curve. This was observed on the pasting curves for the waxy maize starch (Fig. 4.1.6). Thus, the viscosity values should be read with care. However, the waxy maize starch sample was only used to support the hypothesis that the formation of the second peak would not occur due to the absence of amylose.

The rate of stirring with the RVA paddle was 160 rpm during pasting. This rate is equivalent to a shear rate of 54 s⁻¹ (Booth and Bason 2007). This shear rate may be insufficient to simulate most food processing conditions. Shear rates typically encountered in processing equipment range from 10-100 s⁻¹ in batch mixers and can reach values as high as 100 000 s⁻¹ in jet cookers (Loh 1992). Other commonly used shear rates in different processes and equipment include: pumping (30-500 s⁻¹), filling through nozzles (50-200 s⁻¹), plate heat exchangers (50-500 s⁻¹), scrape surface cookers (10-200 s⁻¹), jet cookers (1000-100 000 s⁻¹) and cooking extruders (500-50 000 s⁻¹) (Loh 1992). Since the RVA can function at a specific shear rate for a specific pasting profile, the viscosity values cannot be used to predict the starch performance at different shear rates. Starch pastes are Non-Newtonian i.e. viscosity decreases with increasing shear rates. Therefore, it is important to determine the stability of the starch pastes (treated and non-treated samples) at different shear rates. For
this reason, the flow properties of the starch pastes were measured at varying shear rates using a rheometer.

The flow properties of the starch pastes at varying rates of shear were determined using the Bob and Cup Method (a measuring bob is inserted into a cup containing the sample). The pastes were allowed to rest for 30 min at 60°C to achieve a thermal equilibrium. The RVA, however measured viscosity at 91°C. Temperature affects viscosity (Booth and Bason 2007). The higher the temperature, the faster starch samples would gelatinize and paste. Thus, the viscosity measurement at different temperatures between the RVA and the rheometer may suggest that the viscosity values cannot be compared. However, relative change in viscosity between samples and the trends can be compared between the RVA and the rheometer.

5.1.4 Complexation Index

As explained, this procedure is based on the ability of amylose to form a blue inclusion complex with iodine (Morrison and Laignelet 1983). Thus, amylose molecules bound to stearic acid molecules will not bind iodine. Since this method measures complexed amylose by first determining free amylose relative to the control, the amount of bound amylose may be underestimated as iodine may bind some amylopectin (Gibson et al 1997). However, the CI value obtained is relative to that obtained for the control sample, which may also bind amylopectin. Hence, this method can probably still be used to estimate a relative amount of amylose bound to stearic acid.

5.1.5 Starch structure:

X-Ray Diffraction (XRD) was used to determine if any crystalline starch-stearic acid complexes had formed during pasting. Data such as peak intensity and interplanar distance (d-spacing) describe the starch crystal type (Zobel 1988 b). For this work, a homogeneous fine powder of the freeze-dried starch paste was
analyzed. The diffractograms (Figs. 4.2.2 and 4.2.3) obtained indicate that the X-Ray pattern may have changed from A-type to V-type for the controls and stearic acid-modified starches. However, there were no significant effects for the treatments i.e. the characteristic peaks (4.4 Å, 12 Å and 6.8 Å) for V-type amylose-lipid structures did not increase when treated with stearic acid, except for the 4.4 Å peak of maize starch. This may suggest that there was no increase in the amount of crystalline amylose-stearic complexes. However, as the CI showed an increase in amylose-stearic acid complexes, it is suggested that the diffractometer may not have been sensitive enough to detect the increase in amylose-stearic acid complexes. In addition, there may have been an increase in the number of Type I and Type II(a) V-complexes. These complexes are amorphous and semi-crystalline, respectively (Biliaderis and Galloway 1989) and therefore will not be detected by diffractometers.

For the purpose of this work, starch samples were frozen overnight in a freezer at -20°C and freeze-dried for the next 3 days. This freezing rate could have been too slow and might have allowed for some retrogradation to take place. Retrogradation would allow for the formation of amylose double helices (Gudmundsson 1994). The formation of double helices would reduce the amount of amylose that is free to form complexes with stearic acid. An alternative would be to freeze the starch pastes in liquid nitrogen for immediate freezing. This would minimize retrogradation.

Moisture content has also been found to affect starch crystallinity (Svensson and Eliasson 1995). Moisture contents > 12% have been found to affect peak intensity and starch crystal type. However, when the moisture content is less than 12%, these effects are not significant (Svensson and Eliasson 1995). For the purpose of this work, dry starch of moisture contents less than 2.5% were used. This eliminated the effect of moisture. However, it may be worthwhile to conduct XRD analyses on the starch pastes (without drying) for comparison, in the future. Such an experiment would prove whether or not drying of the sample has an effect on the X-ray pattern.
CLSM is a relatively new technique to study the microstructure of food products (van de Velde et al 2003). The objective lens captures fluorescent light from the sample or stains used in a single focal plane and focuses it back into a small pinhole so eliminating the out-of-focus light (van de Velde et al 2003). CLSM was used to determine any structural changes after the starch was modified with stearic acid and pasted. Micrometer sized crystals have been reported by Fanta et al (2002) and Peterson et al (2005) who used SEM and XRD. These crystals have been suggested to be the amylose-lipid complexes. However, in this work, no complexes could be distinguished by CLSM. This could be because the Confocal Laser Scanning Microscope has a micrometer size resolution and complexes may be only a few nanometers in size. In fact, using Transmission Electron Microscopy (TEM), Kim and Lim (2009) found nano-sized (10–20 nm) particles believed to be complexes between amylose and n-butanol. Hence, TEM may prove to be a useful tool to view amylose-stearic acid crystals. There is potential for further research in this area. Perhaps, the use of Small Angle X-Ray Scattering (SAXS) may provide additional information on the size and structure of amylose-stearic acid complexes. SAXS has been used successfully in determining the spacing between lamellae of starch growth rings (9 nm) and the width of amylopectin superhelices (Waigh et al 1999). Such a study may also be combined with Wide Angle X-Ray Scattering, which has been used to study crystal structure (Donald et al 2001).
5.2 Paste properties of maize and teff starches modified with stearic acid

A comparative summary of the effects of stearic acid on maize and teff starch pastes based on the results in Chapter 4, is given in Table 5.1.2.

Table 5.1.2: The overall effects of stearic acid on the paste properties of maize and teff starch pastes.

<table>
<thead>
<tr>
<th>Effect of added stearic acid on:</th>
<th>Maize starch pastes</th>
<th>Teff starch pastes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity of the first peak</td>
<td>Decreased</td>
<td>Viscosity increased steadily but did not form a peak.</td>
</tr>
<tr>
<td>Viscosity of the second peak</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Final viscosity</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Complexation Index</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Opacity of Pastes</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Gel Texture</td>
<td>Reduced firmness</td>
<td>Reduced firmness</td>
</tr>
<tr>
<td></td>
<td>Reduced gelling ability</td>
<td>Reduced gelling ability</td>
</tr>
<tr>
<td></td>
<td>with the addition of 1.5%</td>
<td>with the addition of 1.5%</td>
</tr>
<tr>
<td>Flow behaviour index (n)</td>
<td>Shear thinning (n &lt; 1)</td>
<td>Shear thinning (n &lt; 1) but more shear stable than maize starch</td>
</tr>
<tr>
<td>Viscosity coefficient (k)</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>X-ray pattern</td>
<td>Indicates formation of the V-type pattern</td>
<td>Indicates formation of the V-type pattern</td>
</tr>
</tbody>
</table>
Maize starch seemed to have a delayed and reduced first peak viscosity for the short (5 min holding at 91 °C) and extended (2 hr holding at 91 °C) pasting cycles. However, teff starch did not display a first viscosity peak or breakdown viscosity when stearic acid was added (Chapter 4.1). Instead, it displayed a steady increase in viscosity (Fig. 4.1.3.5). These different pasting patterns may be explained by structural differences between the modified teff and maize starches as shown by CLSM (Fig. 4.2.5(a)). CLSM indicated that stearic acid was present inside the maize starch granules (Fig. 4.2.5(a)). Maize starch granules have been reported to have pores on their surfaces (Fannon et al 1992). These pores lead to channels that extend into the granules (Gallant et al 1997). Diffusion of stearic acid into the granules may have been facilitated by the pores and channels of the maize starch granules. However, from the CLSM micrographs of teff starch, stearic acid does not appear to be inside the granule but more concentrated around the surface of the granule (Fig. 4.2.7(a)). This may be because teff starch granules do not have surface pores (Bultosa et al 2002).

The presence of stearic acid inside the maize starch granules (Fig. 4.2.10) could have restricted water uptake by the granules because stearic acid is partly hydrophobic. This would explain the reduced peak viscosity and longer peak time for maize starch (Table 4.1.2). Porosity of starch granules also aids in water uptake during pasting (Booth and Bason 2007). Furthermore, the presence of surface pores might have served as sites for granular rupture and consequent breakdown. Thus, the control and stearic acid-treated maize starches showed peak and breakdown viscosities (Table 4.1.2).

The presence of stearic acid around the teff starch granules (Fig. 4.2.7(a)), may have created a hydrophobic layer around the granules. The formation of this hydrophobic layer around teff starch granules could have restricted granular swelling, which would in turn delay granular rupture. This may have been the cause of the absence of first peak and breakdown viscosities (Figs. 4.1.2 and 4.1.2).
The formation of the second viscosity peak led to higher viscosities for both starches. As stated, the second peak has been attributed to the formation of amylose-lipid complexes (Chapter 4.1). The increases in CI for both starches also support this hypothesis as it indicates that some interaction between amylose and stearic acid had taken place. X-Ray Diffraction data also indicated that the formation of these complexes was probable since the d-spacings obtained matched those of amylose-lipid complexes (Figs. 4.2.3 and 4.2.4). Nelles et al (2003) also found that the second viscosity peak coincided with a decrease of starch content in solution, which was attributed to amylose going out of solution. Amylose going out of solution could increase its potential to form complexes with lipids.

Godet et al (1995 a) used a water/DMSO (dimethyl sulfoxide) mixture to isolate amylose-lipid complexes. In future studies, complexes could be isolated using this method. The crystals could then be further analysed using XRD, TEM, Small angle x-ray scattering (SAXS) and Wide angle x-ray scattering (WAXS) to elucidate information on their structure and properties.

5.3 Food applications of stearic acid-modified starches

The unique properties of stearic acid-modified starches are the high viscosities with the addition of only a small amount of stearic acid (0.25% dry starch weight basis) and the consequent reduction in gelling ability. Starches with these properties may have potential to be used as fat replacers/mimetics.

Fat mimetics are tailored to mimic the fat-like character of foods without sacrificing the desirable qualities associated with the product. This has become necessary as there is a general consumer trend towards healthier foods. In a patent by Mahr and Treuck (1999), the production of a starch modified with lipid was reported to possess a creamy and smooth texture. The high viscosity and reduced gelling nature of starch-fatty acid pastes may suggest that the complexes have these characteristics. Amylose-lipid complexes are believed to
form crystals that are spherical in shape (Fanta et al 2002). In addition, these authors also found amylose-lipid complexes to be very small in size, ranging between (10-20 nm). Thus, their small size and shape may be able to mimic those of small fat globules.

The inclusion of stearic acid into the starch results in a higher viscosity. Therefore, less starch is required to produce the desired viscosity. This is another factor that would reduce the total kilojoule content of a food. Teff starch may be preferred over maize starch. A small amount of added stearic acid can result in larger increases in viscosity with teff starch. Stearic acid-modified starches would be useful in fat-free or low-fat cakes, puddings, salad creams and margarines.

Fat mimetics can also be used in high-fat foods such as mayonnaise, salad dressing or margarines. Mayonnaise, for example, is an emulsified viscous food prepared from vegetable oil (Quesada and Clark 1993). Commercial mayonnaise contains 52% oil (according to South African Legislation). Mayonnaise is known for its characteristic viscosity, mouthfeel and taste (Quesada and Clark 1993). Stearic acid modified starches can be used as a fat replacer in these high fat products.

Small, intact, swollen starch granules can mimic the mouth sensation of fat (Oreopoulou 2006). Teff starch may be more preferred over maize starch. This is because its comprises small starch granules (Bultosa et al 2002). Product development may be conducted by adding stearic acid to teff starch and cooking the starch until the starch granules are swollen. The swollen state of the granules could be confirmed by light microscopy. The suspension of swollen granules could then be used to substitute part of the fat content in a mayonnaise (Quesada and Clark 1993). Research that will have to be conducted should include tests to determine if the stability of the emulsion of the reduced-calorie product (containing stearic acid-modified starch) is of a satisfactory standard when compared to a reduce-calorie mayonnaise already
available on the market. This may be accomplished by refrigerating the product. During refrigeration some of the fat can crystallize and separate from the emulsion. A comparison of the sensory properties of the reduced-calorie mayonnaise (containing stearic acid-modified starch) and the reduced-calorie mayonnaise already available on the market, will have to be conducted. This could be accomplished by a descriptive sensory panel who will aid in determining if significant differences in the sensory attributes of the reduced-calorie product (containing the fat replacer) can be perceived.

It is also important that the reduced-calorie mayonnaise (containing the fat replacer) maintains the desired viscosity and is stable to shear forces that may be brought about by stirring and changes in pH. It is also important that the mayonnaise does not show syneresis. Syneresis refers to the stage when water molecules are squeezed out as starch polymers realign with each other (Thomas and Atwell 1999 b). The degree of syneresis can be determined by freezing a sample of mayonnaise and thawing it. The thawed product is then centrifuged and the mass of the supernatant used to produce a percentage of water. Ma et al (2005) conducted this type of trial on mayonnaise that contained starch-based fat mimetics. In addition, the viscosity of the mayonnaise can be tested at varying shear rates, using a rheometer. These data can then be compared to that of the reduced-calorie mayonnaise already on the market, by subjecting it to the same procedure.

Also of relevance is that Seneviratne and Biliaderis (1991) found that amylose-lipid complex superstructures had an increased resistance to digestive enzymes (lipases and amylases). This is due to the crystalline structure of the complex which limits accessibility of the enzyme to the substrate. The available energy of the product containing these complexes, in terms of available nutrients, would be reduced. Mahr and Treuck (1999) described the behaviour of lipid-complexed starches as being similar to resistant starch or dietary fibre, when used as an ingredient. In some reduced-calorie breads about 25 to 30% of the fat is removed and replaced with fibre (Vetter 1993). Therefore, the
stearic acid-modified starch may be added to these low-calorie breads as ‘fibre’. However, this will require further investigation and product development trials.
6. CONCLUSIONS AND RECOMMENDATIONS

Both teff and maize starch produce a higher second peak viscosity during extended pasting for two hours when pasted with 0.25 % stearic acid. The high second peak viscosity can be attributed as a result of amylose-lipid interaction. This is supported by the pasting curve of waxy maize starch, CI data and XRD. In future trials, it is important to ensure that endogenous lipids are removed so as to determine the effect of the added fatty acid alone.

Teff starch (modified with SA) did not show a first viscosity peak during extended pasting in comparison to maize starch. This could have been because stearic acid might have from a layer on the surface of teff starch granules and greatly delayed their rupture. An experiment that would provide visual data during pasting of teff starch (SEM or TEM) would generate more knowledge of the pasting behaviour of teff starch.

X-Ray Diffraction data suggests the formation of amylose-stearic acid complexes. The formation of amylose-stearic acid complexes is probably responsible for the lower first viscosity peak for maize starch and increase in the second viscosity peak for maize and teff starches. These complexes are also probably the reason why teff starch does not form a first viscosity peak during the holding time but increases in viscosity steadily. It is possible that the formation of these complexes is aided by the pores on the maize starch granule surfaces, which may allow for easy diffusion into the granules. The absence of pores of the teff starch granule surface may prevent or delay diffusion into the granule, therefore resulting in the delay in the formation of the first viscosity peak. The pasting of teff starch is unusual and requires further investigation.

The high viscosities and the low gelling tendencies suggest that these complexes can be useful as fat replacers/mimetics in reduced-calorie or fat free foods. Such starches may provide the mouthfeel associated with fatty foods. Furthermore, the stearic acid-modified starches seem to be less shear thinning
than their controls and may be useful in products processed at high shear rates, for example, in batch mixers, pasteurisers and jet cookers. Teff starch may be more preferred in this area as it seems to be less shear thinning (due to its resistance to breakdown) than maize starch. Future research can be conducted by increasing the level of added stearic acid and determining the shear stability of starch pastes at higher shear rates.
7. REFERENCES


