Occurrence of amylose-lipid complexes during maize and teff starch biphasic pasting

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ABSTRACT

The occurrence of amylose-lipid complexes was determined during maize and teff starch biphasic pasting i.e. peak paste viscosities at short and prolonged pasting times. Maize and teff starches were pasted for 11.5 and 130 min with or without added stearic acid followed by thermo-stable alpha-amylase hydrolysis in a rapid visco-analyzer. X-ray diffraction analysis of pastes before and residues after hydrolysis showed crystalline V-amylose diffraction patterns for the starches pasted for a prolonged time with added stearic acid while less distinct V-amylose patterns with non-complexed stearic acid peaks were observed with a short pasting time. Differential scanning calorimetry of pastes before and residues paste hydrolysis showed that Type I amylose-lipid complexes were formed after pasting for the short duration with added stearic acid, while Type II complexes are formed after pasting for the prolonged time. The present research provides evidence that amylose-lipid complexes play an important role in starch biphasic pasting.

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Abbreviations used:

RVA, Rapid Visco-analyzer; XRD, X-ray Diffraction; DSC, Differential Scanning Calorimetry; Rpm, Revolutions per minute; RVU, Rapid Visco Analyzer Units.

1. Introduction

Starch is widely used as an ingredient that improves the texture of various processed foods due to the viscous properties of starch pastes. The presence of added fatty acids during wet heat processing of starch has been shown to alter the starch pasting properties (Raphaelides & Georgiadis, 2006; Tang & Copeland, 2007; Zhou Robards, Helliwell, & Blanchard, 2007). A biphasic starch pasting phenomenon is observed when non-defatted commercial maize starch (Nelles, Dewar, Bason & Taylor, 2000; Nelles, Dewar, Van der Merwe & Taylor, 2003) or defatted maize starch with added stearic acid (fatty acid) (D'Silva, Taylor, & Emmambux, 2011) is pasted for a prolonged time (> 30 minutes).

The biphasic pasting phenomenon is characterized by the commonly observed paste peak viscosity after pasting for a short time (<15 min) and a second higher peak paste viscosity after pasting for a longer time (> 30 min) (D'Silva et al., 2011; Nelles et al., 2000, 2003). Biphasic pasting has also been observed when starch from teff is pasted with added stearic acid for a prolonged time (D'Silva et al., 2011). Teff is an under researched indigenous African cereal. Teff starch has been reported to have different pasting properties compared to maize starch. It shows a lower peak viscosity, setback viscosity and is more resistance to break down than maize starch (Bultosa, Hall & Taylor, 2002).

During biphasic starch pasting, the paste viscosity after prolonged pasting can be up to three times that of the commonly observed paste viscosity after pasting for a short time (D'Silva et al., 2011). The higher paste viscosity after prolonged pasting during biphasic starch pasting may hence provide a new avenue for utilization of starch through use of lower starch concentrations to attain particular viscosities or consistencies in foods (D'Silva et al., 2011).

It has been suggested that the starch biphasic pasting phenomenon results from the presence of endogenous (Nelles et al., 2000, 2003) and/or added (D'Silva et al., 2011) fatty acids since it does not occur when defatted maize starch or teff starch is pasted alone. The increased paste viscosity after prolonged pasting during the biphasic pasting phenomenon is accompanied by loss of starch granule integrity and decreased soluble amylose content (Nelles et al., 2003). The peak paste viscosity after pasting maize or teff starch for a short time (≈ 15 min) decreases with increased amounts of added fatty acid (stearic) while the paste viscosity after prolonged pasting (120 min) increases with increased fatty acid addition (D'Silva et al., 2011). These observations suggest an interaction of endogenous lipids and/or added fatty acid (stearic) with starch during the biphasic pasting. Although this interaction has been hypothesised to occur through amylose-lipid complex formation (D'Silva et al., 2011; Nelles et al., 2003), the nature and occurrence of amylose-lipid complexes during the biphasic starch pasting is still not clear. Understanding the occurrence and nature of amylose-lipid complexes during biphasic pasting could facilitate utilization of the highly viscous second biphasic paste in products that use starch for improving texture.

The aim of this study therefore is to determine the occurrence and nature of amylose-lipid complexes in teff and maize starch during biphasic starch pasting. Maize and teff starches pasted for a short and prolonged time with added stearic acid were analyzed based on their resistance to enzymatic (α -amylase) hydrolysis and

using more refined techniques such as X-ray diffraction and differential scanning calorimetry

2. Materials and Methods

2.1. Materials

Teff Starch was extracted from South African white teff (Whitkop, Pannar, Kronstad, South Africa) according to the method used by D'Silva et al., (2011). Commercial white normal maize starch (Amyral[®]) was obtained from Tongaat Hulett (Edenvale, South Africa). The protein content of the teff and maize starch were 1.6% and 0.6% w/w (db) ($N \times 6.25$), respectively. The starches were defatted according to a method by Nelles et al., (2000) using a mixture of chloroform and methanol (3: 1). Thermally stable alpha-amylase from *Bacillus licheniformis* (EC.3.2.1.1, 3000 U/ml) was obtained from Megazyme Ltd (Bray, Ireland). Stearic acid (analytical grade) was obtained from Sigma–Aldrich Company (St. Louis, MO, USA).

2.2. Methods

2.2.1 Starch pasting using the RVA

A rapid visco analyzer unit (RVA, Model 3D, Newport Scientific-Warriewood, Australia) was used for pasting and to record viscosity. Stearic acid was incorporated into the starch according to D'Silva et al., (2011) at 1.5% w/w of the starch (db). This stearic acid level was also shown by to give a high and optimal second biphasic paste viscosity (D'Silva et al., 2011). The defatted maize and teff starch (10% w/w) with or without added stearic acid was pasted up to 11.5 min or for a prolonged time 130 min. The RVA condition 960 rpm and 50 °C for 10 sec, then heated to 90 °C at a rate of 10 °C/min with 160 rpm stirring then held for the required time.

2.2.2 Thermostable alpha-amylase hydrolysis of first and second peak viscosity pastes

In order to assess the resistance to hydrolysis of the starch pastes from the first and second peak viscosity for the starches pasted with or without added stearic acid, thermostable alpha-amylase from *Bacillus licheniformis* (EC.3.2.1.1, 0.375 U) was immediately added to the paste at the first peak paste viscosity (11.5 min) or second biphasic high viscosity (130 min) at 75 °C. The paste viscosity was then monitored using the RVA thermocline software in order estimate the extent of hydrolysis. The paste was hydrolyzed until there was minimal change in viscosity (10 min) and then the resultant material (unhydrolyzed residue) was washed with distilled water and freeze dried. The final viscosity was obtained and the rate of starch paste hydrolysis was quantitatively estimated by plotting the viscosity as a percentage of the initial paste viscosity and then fitting the data to a first order exponential decay equation using the Levenberg-Marquardt iteration for minimization of the chi-square values with OriginPro v8 software (OriginLab Corporation-MA, USA). The decay constant parameter was obtained as an estimate of the rate of hydrolysis. The experiments were repeated at least three times.

2.2.3. X-ray diffraction

X-ray diffraction (XRD) study was conducted using an X'Pert PANalytical diffractometer (Eindhoven, Netherlands) on freeze dried teff and maize starch pastes obtained after pasting for a short (11.5 min) and prolonged (130 min) time with or without added stearic acid and on the residues obtained after thermostable alpha-amylase hydrolysis. The samples were gently ground to a fine powder and then the moisture content equilibrated at 95% relative humidity to approximately 25% w/w. The XRD operating conditions were; 45 kV, 40 mA and CuK α 1 (0.154 nm). Scanning was done from 5 to 30° (2 θ) with an exposure time of 16 min 14 s, step size of 0.026° and a time/step ratio of 229.5 s. The degree of crystallinity was determined as the

percent integrated area of crystalline peaks to the total integrated area above a straight baseline (Cheetham & Tao, 1998).

2.2.4. Differential scanning calorimetry (DSC)

The thermal properties were assessed using a high pressure DSC system with STARE[®] software (HPDSC-827, Mettler Toledo, Greifensee, Switzerland). Analyses were conducted on both freeze dried teff and maize starch pastes obtained after pasting for a short (11.5 min) and prolonged (130 min) time with or without added stearic acid, and on the residues obtained after thermostable alpha-amylase hydrolysis. The starch powder (10 mg) was mixed with distilled water (30 mg), and then equilibrated for at least 2 h at room temperature. Scanning was done from 40 to 125 °C at a rate of 10°/ min. Indium (Tp = 156.61 °C, 28.45 J/g) was used as a standard to calibrate DSC and an empty pan as a reference.

2.4. Statistical analysis

Data was analysed using one way ANOVA using SAS v8 software (SAS Institute Inc, Cary, NC). The means of the values obtained from the analyses of the starches pasted with or without added stearic acid for a short or prolonged time and the residues after alpha-amylase hydrolysis were compared using Duncan's multiple range test with a $p \leq 0.05$ separation limit.

3. Results and Discussion

3.1. Pasting of teff and maize starch with or without added stearic acid

Maize starch pasted with added stearic acid had a significantly ($p < 0.05$) reduced and delayed paste peak viscosity of about 218.9 RVU at 11.1 min while that pasted without added stearic acid had a paste peak viscosity of about 257.0 RVU at 10.2 min (see Fig.1). Teff starch pasted with added stearic acid did not show a peak viscosity but had plateau viscosity of about 111.9 RVU starting at 13.0 min (see Fig.

1). Teff starch pasted without added stearic acid on the other hand had a paste peak viscosity of about 135.4 at 11.1 min (see Fig. 1).

Two paste peak viscosities (biphasic pasting) were observed when teff or maize starches with added stearic acid were pasted for a prolonged time (130 min) (see Fig. 1). The defatted teff starch samples pasted without added stearic acid on the other hand mainly showed a single paste peak viscosity after pasting for a short time (11.5 min), and the viscosity decreased with prolonged pasting time (see Fig. 1). The maize starch pasted without added stearic acid showed a small paste peak viscosity at about 30–60 min of pasting and the viscosity also decreased with prolonged pasting time (see Fig. 1). After prolonged pasting, teff starch pasted with added stearic acid had a viscosity of about 255.8 RVU while the sample pasted without added stearic acid had a viscosity of about 58.7 RVU. Maize starch pasted with added stearic acid had a paste viscosity of about 361.0 RVU after prolonged pasting while that pasted without added stearic acid had a viscosity of about 83.0 RVU.

A reduction and delay in paste peak viscosity after pasting of cereal starches for a short time (< 15 min) with added fatty acids has also been reported for rice starch pasted with added stearic or linoleic acid (Zhou et al., 2007); maize starch pasted with added myristic, palmitic (Raphaelides & Georgiadis, 2006) or stearic acid (D'Silva et al., 2011; Raphaelides & Georgiadis, 2006); wheat starch pasted added with caprylic acid, lauric acid, myristic acid, palmitic, stearic, oleic or linolenic acid (Tang & Copeland, 2007). The reduced and delayed paste peak viscosity after pasting cereal starches with added fatty acids for a short time was hypothesised to result from interaction of the added lipids with starch granules through: formation of a layer of amylose-lipid complexes on the granule surface (Eliasson, Finstad, & Ljunger, 1988); formation of a rigid network of intragranular amylose-lipid complex structures (Becker, Hill & Mitchell, 2001); or formation of a lipid layer on the granule surface

which hinders water uptake through increased hydrophobicity (Richardson, Langton, Bark & Hermansson, 2003).

The second paste peak viscosity observed after prolonged pasting of teff and maize starches with added stearic acid in the present study is in accordance with results reported by D'Silva et al., (2011). It has also been reported that a more pronounced biphasic pasting (higher second paste peak viscosity) occurs when teff or maize starch is pasted with increased amounts of added stearic acid. Non-defatted commercial maize starch pasted for a prolonged time also gives the second paste peak viscosity, while the defatted form does not (Nelles et al., 2000). The small peak at about 30-60 minutes for the defatted maize control sample in the present work (Fig. 1) could possibly be due to residual endogenous lipids (D'Silva et al., 2011). It has also been reported that the formation of the second paste peak viscosity is accompanied by loss of amylose from solution, and disintegration of the maize starch granules (Nelles et al., 2003). The formation of the second paste peak in the presence of added stearic acid suggests that after prolonged pasting of the teff or maize starch with added stearic acid, there maybe further interaction between the added stearic acid the starch probably involving amylose-stearic acid complexes.

3.2. Thermostable alpha-amylase hydrolysis of teff and maize starch pastes from the first and second viscosity peaks

The viscosity change during thermostable alpha-amylase hydrolysis of the maize and teff starches that were pasted with or without added stearic acid is shown in Figure 2.

Teff and maize starches pasted for prolonged time (130 min) with added stearic acid had significantly ($p \leq 0.05$) higher final viscosities values of 45.4 and 38.5 RVU, respectively after paste alpha-amylase hydrolysis compared to those pasted for short time (11.5 min) which had values of 27.3 and 29.4 RVU, respectively (Fig. 2).

After alpha-amylase hydrolysis, the starches pasted without added stearic acid for both 11.5 min and 130 min had significantly ($p \leq 0.05$) lower final viscosity with values of 11.1 and 17.2 RVU respectively for teff, plus 20.9 and 17.0 RVU respectively for maize compared to those pasted with added stearic acid (Fig. 2). Higher final viscosities for samples pasted with added stearic acid probably suggests that resistance to alpha-amylase hydrolysis increases when teff and maize starches are pasted with added stearic acid.

In order to analyze the hydrolysis kinetics in terms of paste viscosity values recorded during hydrolysis, Levenberg-Marquadt non-linear least-squares curve fitting of the values to a first order exponential decay equation carried out. Exponential equations have been used by other researchers to estimate the rates of hydrolysis for starches containing amylose-lipid complexes (Gelders, Duyck, Goesart & Delcour, 2005). The relative rates of paste hydrolysis were estimated from the rate of paste viscosity reduction during hydrolysis according to the equation:

$y = y_0 + Ae^{-t/n}$, where y is percent viscosity (viscosity at time (t)/initial viscosity), y_0 is the estimated initial percent viscosity at given time (t), A is the amplitude and n the decay constant. The data fit to the equation with R^2 values of at least 0.99 for all the samples.

Teff and maize starch pasted for 130 min with added stearic acid had the lower decay constants (n) values of -3.0 and -3.9, respectively, while those pasted for 11.5 min which had values of -2.7 and -2.9, respectively. The starch samples pasted without added stearic acid for 11.5 min and 130 min gave higher decay constant values of -1.4 and -0.9 respectively for teff starch and values of -2.4 and -1.3 respectively for maize starch.

The lower decay constants (n) probably suggest increased hydrolytic resistance of the teff and maize starch pasted with added stearic acid for 130 min

compared to the starches pasted for 11.5 min and those pasted without added stearic acid. Starch has been shown to become more resistant to enzymatic or acid hydrolysis with the formation of amylose-lipid complexes (Gelders et al., 2005; Guraya, Kadan & Champagne, 1997). The observed increase in hydrolytic resistance therefore could have been due to the formation of teff and maize amylose-stearic acid complexes in the first and second peak viscosity pastes when the starch is pasted with added stearic acid.

3.3. WAXS of teff and maize starch from the first and second peak viscosity pastes and the residues after alpha-amylase hydrolysis.

The WAXS patterns of teff and maize starches from the first and second peak viscosity pastes before and after thermostable alpha-amylase hydrolysis are shown in Fig 3. When teff or maize starch was pasted with added stearic acid for a short time (11.5 min) or for a prolonged time (130 min), it showed XRD peaks at $2\theta = 7.5, 12.7$ and 19.9° before and after alpha-amylase hydrolysis (see Fig 3a and b, respectively). These peaks have been assigned to V-type crystallinity that results from presence of amylose-lipid complexes (Brisson, Chanzy & Winter, 1991). On the other hand, teff or maize starches pasted for 11.5 and 130 min without added stearic acid had major XRD peaks at about $2\theta = 13.0, 17.0$ and 19.9° both before and after alpha-amylase hydrolysis. These samples also showed minor XRD peaks at $2\theta = 14.8, 20.6, 22.4, 23.3$ and 26.6° . Starches with A-type crystallinity may show XRD peaks at $2\theta = 15, 17, 18, 20^\circ$ with minor peaks at $2\theta = 22, 23$ and 26 (Cheetham & Tao, 1998; Imberty, Chanzy, Perez, Bule'on & Tran, 1988). The diffraction patterns of the samples pasted without added stearic acid were therefore probably due to mostly amorphous starch (relatively smooth bell-shaped curves) and a small amount of starch with mixtures of V and A-type crystallinity. In Fig. 3 (a and b), maize starch pasted without added stearic acid for 130 min showed XRD peaks with relatively high intensity at 13.0 and

19.9°. These peaks which are characteristic of amylose-lipid complexes (Brisson et al., 1991) were probably due to amylose-lipid complexes formed as a result of amylose complexing residual endogenous lipids in the starches.

Although V-amylose diffraction patterns were observed for the starches pasted with added stearic acid for both short and prolonged times, teff and maize starch pasted with added stearic acid for a short time showed an additional diffraction peak at $2\theta = 21.5^\circ$. The additional peak was at the same location (20 degrees) as the highest intensity peak for pure stearic acid (Figs. 3a and b) suggesting the presence of uncomplexed stearic acid. V-amylose diffraction patterns with such an additional peak that indicated the presence of non-complexed (or non-specifically bound) fatty acids were demonstrated by Biais, Le Bail, Robert, Pontoire & Bule'on (2006). In the present study, after the short pasting time (11.5 min), the stearic acid is probably not entirely complexed with the amylose molecules. The stearic acid on the other hand is probably entirely complexed with the teff and maize starch after prolonged pasting (130 min) which corresponds to the second high paste viscosity.

The teff and maize starches pasted with added stearic acid for a prolonged time (130 min) showed sharper peaks with significantly ($p < 0.05$) higher relative crystallinity of 35.2% and 45.6%, respectively, before alpha-amylase hydrolysis compared to those pasted for a short time (11.5 min) which had relative crystallinity of 31.6% and 24.4 %, respectively (see Fig. 3a). The relative crystallinity of teff and maize starches pasted with added stearic acid for a short time increased from 31.6% and 24.4%, to 38.5% and 26.7% respectively after thermostable alpha-amylase hydrolysis (see Fig. 3b). The relative crystallinity of teff and maize starches pasted with added stearic acid for a prolonged time (130 min) also increased after alpha-amylase hydrolysis from 35.2% and 45.6%, to 55.3 % and 58.5%, respectively (Fig. 3b).

Increased crystallinity of the starches on hydrolysis is probably due to preferred hydrolysis of the amorphous component of the starches hence leaving a greater proportion of crystalline V-amylose components. These V-amylose components are more resistant to hydrolysis (Gelders et al., 2005).

3.4. DSC of teff and maize starch from the first and second peak viscosity pastes

The DSC patterns of teff and maize starches from the first peak viscosity (short pasting time, 11.5 min) and second high viscosity (prolonged pasting, 130 min) pastes plus their controls are before and after hydrolysis are shown in Fig. 4(a) and 4(b) respectively.

The starches pasted for a short time (11.5) with added stearic acid had an endotherm with onset (T_o), peak (T_p), and endset (T_e) temperatures of 66.1, 68.6, 71.8 °C, respectively with a ΔH of about 1.9 J.g⁻¹ for maize and 67.2, 68.9, 72.4 °C, respectively with a ΔH value of about 1.5 J.g⁻¹ for teff both before and after amylase-hydrolysis (see Fig 4a and 4b). This endotherm was absent in teff and maize starches pasted with added stearic acid for a prolonged time (130 min) (see Fig. 4a and b). The endotherm was probably due to non-specifically bound (non-complexed) stearic acid as observed in the WAXS patterns of the starches pasted with added stearic acid before and after hydrolysis (see Fig. 3) since the melting endotherm peak temperature (T_p) of pure stearic acid is about 69 °C. Endotherms within this range have also been ascribed to non-complexed fatty acids by other researchers (Biliaderis, Page & Maurice, 1986; Raphaelides & Karkalas, 1988).

The starches pasted for a short or prolonged time with added stearic acid before and after amylase-hydrolysis showed a second endotherm at $T_p \approx 100$ °C (see Fig. 4a and 4b). For this endotherm, the teff and maize starch samples pasted for a short time had T_o , T_p , T_e values of 94.2, 101.8, 107.8 °C and 94.8, 100.3, 104.5 °C,

respectively before and after alpha-amylase hydrolysis. The corresponding ΔH values were about 1.58 and 0.85 J.g⁻¹ for maize and teff starch, respectively, before and after hydrolysis. For the second ($T_p \approx 100$ °C) endotherm, starch pasted for a prolonged time with added stearic acid had T_o , T_p and T_e values of 96.5, 101.3 and 105.8 °C, respectively with ΔH value of about 0.76 J.g⁻¹ while teff starch had values of 95.4, 100.6 and 104.6 °C, respectively with a ΔH value of about 0.87 J.g⁻¹ before and after alpha-amylase hydrolysis. This second endotherm ($T_p \approx 100$ °C) is characteristic of Type I amylose-lipid complexes (Biliaderis et al., 1986; Raphaelides & Karkalas, 1988).

Teff and maize starches pasted with added stearic acid for a prolonged time exhibited an additional endotherm at higher temperatures both before and after amylase hydrolysis (see Fig 4a and 4b). For maize starch, the third endotherm had T_o , T_p and T_e values of 107.1, 110.7 and 114.7 °C, respectively while for teff starch the T_o , T_p and T_e values were 106.6, 109.4 and 112.4 °C, respectively before alpha-amylase hydrolysis. After alpha-amylase hydrolysis, the ΔH values of the third endotherms ($T_p: \approx 110$ °C) increased to from 0.44 and 0.53 J.g⁻¹ to 1.36 and 1.49 J.g⁻¹ for maize and teff starch, respectively (see Fig 4b). This third endotherm ($T_p \approx 110$ °C) has been assigned to result from Type II amylose-stearic acid complexes (Biliaderis et al., 1986; Raphaelides & Karkalas, 1988). The increase in ΔH values after alpha-amylase hydrolysis could have been due to the fact that hydrolysis removed the more amorphous components of the samples (Gelders et al., 2005; Seneviratne & Biliaderis, 1991).

Amylose-lipid complexes may show three endotherms with T_p values at <80 °C, 80-104 °C, and/or at > 104°C. These endotherms correspond to; non-complexed lipids, Type I complexes and Type II complexes, respectively (Raphaelides & Karkalas, 1988). Fig 4 suggests that Type I amylose-lipid complexes were observed in

the teff and maize starch pasted for a short time with added stearic acid (first biphasic paste) while Type II V-amylose was mainly observed in the starches pasted for a prolonged time (second biphasic pastes) with added stearic acid. On the other hand, the starches pasted without added stearic acid for a short or prolonged time showed neither clear Type I nor Type II amylose-lipid complex endotherms (see Fig. 4a). This is in accordance with WAXS results in section 3.3.

Type I starch contains randomly oriented amylose-lipid complex helices which when heated at high temperature ($> 90\text{ }^{\circ}\text{C}$) convert to regularly organized Type II amylose-lipid complex helices probably through lamellae thickening (Biliaderis et al., 1986). Such organized crystalline amylose-lipid complexes form an extensive network of hydrogen bonds involving water-water, helix-helix, and helix-water molecule interactions (Rappenecker & Zugenmaier, 1981). This extensive network of hydrogen bonds could be responsible for the relatively high viscosity of the second biphasic paste compared to the first one.

4. Conclusions

It is concluded that amylose-lipid complexes play an important role in the maize and teff starch biphasic pasting phenomenon that involves paste peak viscosities at short and prolonged pasting times in the presence of added stearic acid (fatty acid). The first biphasic pastes of maize and teff starch that occur after pasting for a short time (11.5 min) contain mostly Type I V-amylose (amylose-lipid complexes) while the second biphasic pastes that occur after pasting for a prolonged time (130 min) contain Type II V-amylose. Biphasic starch pasting is associated with the formation of regularly organized crystalline amylose-lipid complexes after a prolonged pasting time. These results may facilitate utilization of the relatively high viscosity of the second biphasic paste that occurs after prolonged pasting through

application of lower starch concentrations to improve consistency and viscosity in foods.

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Figure captions

Fig. 1. Effect of added stearic acid* on the paste viscosity of teff and maize starch pasted for a prolonged time (130 min) at 90 °C in a Rapid Visco-analyzer (RVA).

*Stearic acid was added at 1.5 % w/w of the starch.

Fig. 2. Effect of thermal stable alpha-amylase hydrolysis on viscosity of teff and maize starches pasted for 11.5 and 130 minutes at 90 °C with or without added stearic acid*.

*Stearic acid was added at 1.5 % w/w of the starch.

Fig. 3. Effect of added stearic acid on the X-ray diffractograms of teff and maize starches pasted for 11.5 and 130 minutes at 90 °C before (a) and after (b) thermostable alpha-amylase hydrolysis.

Hydrolysis was done at 75 °C for 10 minutes with thermal stable alpha-amylase

Presented data was normalized and vertically offset for clarity.

Fig. 4. Effect of added stearic acid on the thermal properties of teff and maize starch pasted for 11.5 and 130 minutes at 90 °C before (a) and after (b) thermostable alpha-amylase hydrolysis.

Figure 1

Figure 2

Figure 3a

Figure 3b

Figure 4a

Figure 4b