Occurrence of amylose–lipid complexes in teff and maize starch biphasic pastes

Obiro Cuthbert Wokadala [a], Suprakas Sinha Ray [b], Mohammad Naushad Emmambux [a],*

[a] Department of Food Science, University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa
[b] DST/CSIR Nanotechnology Innovation Centre, National Centre for Nano-Structured Materials, Council for Scientific and Industrial Research (CSIR), Meiring Naude Road, Pretoria 0001, South Africa

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ABSTRACT

The occurrence of amylose–lipid complexes was determined in maize and teff starch biphasic pastes i.e. peak viscosity pastes 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 after 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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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 min).

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 ($\approx$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 hypothesized to occur through amylose–lipid complex formation (D'Silva et al., 2011; Nelles et al., 2003), the nature and occurrence of amylose–lipid complexes in the biphasic starch pastes 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 biphasic starch pastes. Maize and teff starches pasted for a short and prolonged time with added stearic acid were analyzed based on their resistance to enzymatic (alpha-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 × 6.25), respectively. The amylose content of the teff and maize starches determined according to the method by Chrastil (1987) was 28.5% and 28.9%, 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 s, 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 *B. 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 PANanalytical 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 CuKα1 (0.154 nm). Scanning was done from 5° to 30° (2θ) 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 (T_p = 156.61 °C, 28.45 J g^{-1}) was used as a standard to calibrate DSC and an empty pan as a reference.

2.3. Statistical analysis

Data was analyzed 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