

Effect of steeping additives on tef starch extraction and its quality

Tatenda Nyakabau, Obiro Cuthbert Wokadala and Mohammad Naushad Emmambux

Department of Food Science, University of Pretoria, Pretoria, Republic of South Africa

Tef is an indigenous African cereal and considered as lost crop of Africa. There is no research on the effect of steeping additives on the quality of isolated tef starch. A white tef grain was milled and steeped in distilled water, sodium hydroxide, lactic acid, metabisulfite, and a combination of lactic acid and metabisulfite. A combination of metabisulfite and lactic acid improves the starch yield, but steeping with sodium hydroxide produces the highest starch purity (lowest protein content). Both the Rapid Visco Analyzer and a Rheometer showed similar pasting properties of starches independent of the treatment. The different steeping additives showed differences in pasting properties. Sodium hydroxide showed the highest peak, breakdown and set back viscosity compared to the other steeping additives. Extracted tef starches using sodium hydroxide as steeping additives also showed higher gelatinization temperatures. According to principle component analysis (PCA), the different properties of tef starch extracted with sodium hydroxide steeping is apparently due to its lower protein content. SEM and CLSM indicated that sodium hydroxide is a very effective solvent to solubilize the protein matrix surrounding the compound starch granule to produce more single starch granule during extraction. The low protein content probably result in faster water absorption and higher peak viscosity and lower pasting temperature. Different steeping additives can affect the functionality of tef starches and this need to be considered when comparing properties of starches extracted with different additives.

Keywords:

Gelatinization / Starch pasting / Starch quality / Steeping additives / Tef starch extraction

1 Introduction

Starch is a very versatile food ingredient with many applications in the food as well as the non-food systems. Commercially, starch is mainly extracted from cereal grains (e.g., maize), tubers and roots (e.g., cassava) and stems and piths (e.g., sago). Among the cereal grains, tef (*Eragrostis tef*) is an indigenous crop to Africa and is one of the underutilized “lost crops” of Africa tef [1, 2]. Tef is cultivated in South Africa and Ethiopia. It

is mainly used as flour for *Injera* making in Ethiopia [2]. There is limited cultivation in South Africa and tef flour is sold at a premium price and mainly used in gluten free formulation. Tef (also written as Teff) has a great potential as a source of starch that could replace commercial starches in various industrial applications. Limited studies by Bultosa et al. [3] suggest that tef starch may perform better under high shear processing, in frozen and refrigerated foods compared to native cereal starches extracted from maize, wheat, sorghum, and rice. Due to the small size (2–6 μm in diameter) of its starch granules [3], tef starch can offer applications as a fat mimetic in food, and as flavor and aroma carrier [2] similar to other small starches like rice and amaranthus. The tiny granules of tef starch are also probably suited for use in the cosmetic, pharmaceutical, paper, textile, and photographic industries [4].

Tef is considered as the smallest cereal grain in the world [2] measuring 1.0–1.7 mm in length and 0.6–1.0 mm

Correspondence: Dr. Mohammad Naushad Emmambux, Department of Food Science, University of Pretoria, Old Agricultural Building (Building 46), Lynwood Road, Private bag \times 20 Hatfield, 0028 Pretoria, Republic of South Africa
E-mail: naushad.emmambux@up.ac.za
Fax: +27-12-420-2839

Abbreviations: **AACC**, American Association of Cereal Chemists; **ANOVA**, analysis of variance; **CLSM**, confocal laser microscopy; **RVA**, Rapid Visco Analyzer

diameter with a 1000 seed weight of only around 0.2 g [5]. The grain is oval in shape and its color ranges from milky white to almost black, but its most common colors are white, red, and brown [3]. In terms of the structure, the central endosperm of the tef grain is floury and the outer cells of the endosperm are horny [3]. The hard endosperm comprises relatively larger proportion of the kernel than in maize and results in much greater problems during starch extraction.

Limited studies by Bultosa et al. [3] have demonstrated that tef starch exists as compound granules located in the central endosperm. These starch granules are embedded in a protein matrix and this also makes starch extraction difficult [6]. Therefore, the proteins surrounding the starch granules must be degraded to fully recover starch granules from the endosperm. Previous investigations have shown that treatments of cereal grains such as maize and sorghum with metabisulfite (0.2% w/w) [7–9], lactic acid (0.5% w/w) [10–13], or sodium hydroxide (0.2% w/w) [14] prior to wet milling, disperses the protein matrix surrounding starch granules, facilitating their release from the protein matrix. Several researchers have reported improved starch yield and purity from steeping maize or sorghum with metabisulfite [8], lactic acid [6, 10, 11], or sodium hydroxide [14] prior to wet milling.

Although there is substantial information available on starch extraction from commercial sources of starch such as maize and sorghum, information on extraction of starch from tef is not available. Recently, D’Silva et al. [15] extracted tef starch using distilled water only and obtained starch with a protein content of 1.5% which was higher than that of a commercial maize starch (0.39%), hence the need to investigate starch extraction from tef using steeping additives. The high residual protein may affect the starch properties. The objective of this research is therefore, to determine the effects of adding metabisulfite, lactic acid, sodium hydroxide, and a combination of lactic acid and metabisulfite to steep water during starch extraction from tef grain on starch yield, purity, and pasting properties.

2 Materials and methods

2.1 Materials

The tef grain sample used in this research was a South African variety, Wit kop (white in color) obtained from PANNAR (Kroonstad, South Africa). Hexane, sodium hydroxide (1 N), lactic acid (88%), and sodium metabisulfite (~65.5% SO₂) were purchased from Merck Chemicals Ltd and were of analytical grade.

2.2 Starch extraction method

It is noted that due to the small size of the tef grain, it is not really possible to remove the outer pericarp and germ from

the grain. Hence, tef grain was first milled using a hammer mill to pass through a sieve size of 500 μm to produce a whole grain meal. The whole grain meal obtained from the mill was defatted as described by D’Silva et al. [15]. Defatted tef flour (125 g, as-is) was then steeped in five different solutions (625 mL) that have been used in the literature and reported to be optimal: distilled water (control); 0.2% w/v sodium metabisulfite [8, 10]; 0.5% v/v lactic acid [6]; 0.2% w/v sodium metabisulfite + 0.5% v/v lactic acid [12, 16, 17]; 0.5% v/v sodium hydroxide [14, 18] in a shaking water bath at 40 ± 1°C for 2 h. Defatting of the tef was done in order to remove lipids which have been shown to significantly affect the pasting properties of cereal starches including tef [15, 22]. The starch was then extracted based on procedure by Bultosa et al. [3] and D’Silva et al. [15]. The presence of the lipids can lead to delayed and reduced peak paste viscosity, and an increased final viscosity [15, 20]. Defatting was also done as it is difficult to degermed small grain like tef as compared to maize for example. The extracted starches were freeze dried (5 days at –80 kPa, –45°C), weighed and stored in sealed plastic jars at room temperature.

2.3 Analyses

2.3.1 Basic starch characterization

Solid yield was expressed as percentage of the total starch content of flour and expressed on a dry matter basis. Solid yield and starch recovery were calculated using the following equations:

$$\% \text{Solid yield} = \frac{\text{extracted starch weight (db)} \times 100}{\text{defatted flour weight (db)}} \quad (1)$$

$$\% \text{Starch recovery} = \frac{\text{starch weight (db)} \times 100}{\text{grain starch weight (db)}} \quad (2)$$

Moisture, protein crude fat, and ash were determined according to American Association of Cereal Chemists (AACC) [21] methods 44-15A, 46-30, 30-12A, and 08-01, respectively. Total starch content was determined enzymatically following the megazyme amyloglucosidase/α-amylase AACC Method 76-13 [21].

2.3.2 Pasting properties

The pasting properties of the extracted tef starches were determined with a Rapid Visco Analyzer (RVA model 3D, Newport Scientific, Sydney, Australia) as well as a Rheometer (Physica MCR 301 Rheometer (Anton Paar, Ostfildern, Germany)). The RVA is an approved AACC method (Method number 61-02) [21]. Tef starch was mixed with distilled water to 10% w/w in an aluminum canister and the slurry subjected to RVA or Rheometer pasting. The starch

slurry was held at 50°C for 2 min, heated to 91°C in 7 min, held at 91°C for 5 min, cooled to 50°C in 7 min, and then held at 50°C for 2 min. Stirring speed was 960 rpm for the 10 initial seconds, then 160 rpm throughout while the rate of heating and cooling was at a constant rate of 5.5°C per min. Peak viscosity, time to peak viscosity, trough, breakdown, final, setback viscosities were determined from the pasting curve for each starch sample.

2.3.3 Differential scanning calorimetry (DSC)

Thermal properties of extracted starches were determined using a high pressure differential scanning system with Stare[®] software (HPDSC-827, Mettler Toledo, Greifensee, Switzerland) according to Wokadala et al. [22]. Approximately 10 mg of tef starch samples were weighed into aluminum DSC pans and distilled water (30 mg) was added. Samples were allowed to equilibrate at room temperature for 2 h before analyzing in the DSC. Sample pans were heated from 30 to 100°C at a rate of 10°C/min. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of gelatinization (ΔH_{gel}) were calculated.

2.3.4 Scanning electron microscopy (SEM)

SEM was done according to Bultosa et al. [3]. Freeze dried starch and the defatted tef flour were mounted on aluminum stubs covered with double sided conductive carbon tape. The stubs were sputter-coated with gold in an Olaron E 5200 coating unit to a thickness of about ± 20 nm. Prepared samples were then viewed using a JEOL JSM 840 (Tokyo, Japan) scanning electron microscope operated at 5 kV. The samples were observed at low ($\times 500$) and high magnification ($\times 2500$ – 3500). The images obtained were analyzed for granule size determination based on Ferret's distance using ImageJ[®] software (National Institute of Health, Maryland, USA).

2.3.5 Confocal laser scanning microscopy (CLSM)

Texas red was used to stain protein in the extracted starches. Texas red is a fluorescent dye which selectively stains proteins through conjugating with protein residues such as tyrosine, serine, and histidine [19] hence enabling the proteins associated with the starches to be identified. Texas red dye (5 mg) was dissolved in a 5 mL ethanol solution (50% v/v). The prepared dye was diluted by 200 \times using a 30% glycerol solution. The diluted dye (0.2 mL) was added to the extracted starch (0.2 mg) in eppendorf centrifuge tube (2 mL) then vortexed for 5 s. The tubes were left to stand for 1 h in at room temperature. The stained starch was washed twice with excess distilled water then mounted on a slide for microscopic observation. During washing, centrifugation was done at

5000 rpm for 1 min. The starches were then viewed with a Zeiss LSM 510 META Confocal Laser Scanning Microscope (Zeiss SMT, Jena, Germany). The samples observed with an excitation and emission wavelengths of 480 and 640–750 nm, respectively.

2.4 Statistical analysis

All experiments were repeated three times and the data obtained subjected to analysis of variance (ANOVA) procedures to test for significant differences between the different steeping conditions with a 95% confidence interval using Statistica version 10 software (StatSoft, Tulsa, OK). Differences in means were compared using Fischer's least significance difference test. Correlations between the RVA and the Rheometer pasting data were determined with the Pearson's correlation coefficient (r) using Microsoft excel. The experiments were repeated three times. A principle component analysis (PCA) was performed to assess the relationship between the physical, chemical, and pasting properties of the extracted starch using the different steeping agents.

3 Results and discussion

3.1 Effect of steeping additives on the chemical composition of tef starch

The chemical composition of starch indicates the purity of the starch extracts, whereby higher starch and lower protein, fat, and ash contents are mostly desirable. The composition; solid and starch yield of tef starch extracted using distilled water (control); 0.2% (w/v) metabisulfite; 0.5% v/v lactic acid; 0.2% w/v metabisulfite + 0.5% v/v lactic acid; 0.5% v/v sodium hydroxide solutions as steeping additives are shown in Table 1.

The protein content of laboratory isolated or commercially wet milled maize starches generally ranges from 0.3 to 1.0% [23]. In the current research the mean protein content of extracted starches was significant ($p < 0.05$) between the steeping treatments. Starch obtained with the sodium hydroxide treatment had the lowest protein content (0.58%, db); and the highest protein content (2.71%, db) was from the metabisulfite treatment. There were no significant differences ($p < 0.05$) in ash content of tef starch extracted by the different steeping treatments, except for sodium hydroxide treatment which had a higher ash content of about 0.60% (db; Table 1). Low and non-significant ($p > 0.05$) values were obtained for the fat content of starch extracted by the different steeping treatments with a range of 0.09–0.12% (db; results not reported in Table 1). The extracted starches showed a purity of about 88.2–95.8% (db). The lowest starch purity 88.2% and the highest starch (95.8%, db) were from distilled water and metabisulfite treatment + lactic acid treatment, respectively (Table 1).

Table 1. Effect of steeping additives on composition properties of tef starch^{a)} (db)

Treatment ^{a)}	Moisture [%]	Protein [%]	Ash (%)	Starch [%]	Solid yield (%)	Starch recovery (%)
Distilled water	2.4 ± 0.56	1.28 ± 0.1b	0.27 ± 0.04a	88.2 ± 2.8a	43.76 ± 1.07bc	55.0 ± 1.95bc
Sulfur dioxide (SO ₂)	1.8 ± 0.29	2.71 ± 0.2d	0.32 ± 0.04a	91.8 ± 0.5b	40.60 ± 1.35b	53.1 ± 1.56b
Lactic acid (LA)	1.6 ± 0.20	1.10 ± 0.1b	0.30 ± 0.04a	95.2 ± 0.8bc	45.04 ± 1.57c	61.1 ± 2.7c
SO ₂ + LA	1.4 ± 0.37	2.25 ± 0.3d	0.30 ± 0.04a	95.8 ± 0.5c	54.45 ± 1.34d	74.3 ± 1.52d
Sodium hydroxide	1.5 ± 0.45	0.58 ± 0.1a	0.60 ± 0.01b	95.0 ± 2.8bc	30.96 ± 0.49a	42.0 ± 0.66a

Values are means ± of three independent experiments and all values are as dry basis except the moisture content.

Mean values in the same column with different letters are significantly different ($p < 0.05$).

a) Tef starch extracted by steeping defatted tef flour in five different solutions at 40°C for 2 h: distilled water (control); 0.2% w/v sulfur dioxide; 0.5% v/v lactic acid; 0.2% w/v sulfur dioxide + 0.5% v/v lactic acid; 0.5% v/v sodium hydroxide.

The solid yields from tef starch steeped in the five steeping solutions varied from about 31 to 55% (Table 1). Lactic acid treatment significantly improved the solid yield from the control of about 44–45%. Furthermore, addition of lactic acid and metabisulfite in combination resulted in a substantial increase in solid yield to about 55% (db), which was significantly higher than that by lactic acid or metabisulfite alone. In contrast, steeping with sodium hydroxide did not improve solid yield. The sodium hydroxide treatment decreased yield to about 31%. The starch recoveries (Table 1) from tef during wet milling were significantly different ($p < 0.05$) for all treatments with a range of 42–74% (db). The use of lactic acid and metabisulfite in combination produced the highest starch recoveries (74%, db). This is in agreement with several reports [6, 10, 12, 24–27]. Ruan et al. [27] and Singh and Johnston [28], suggested that there is some synergy between lactic acid and metabisulfite. Lactic acid probably increased the porosity of the cellular membranes and softened the protein matrix in the vitreous regions of the tef kernel, which promoted absorption of metabisulfite [29]. Metabisulfite has been shown to have capacity to improve starch yields through disruption of protein matrixes as it can break disulphide bonds [13, 30]. Similarly lactic acid can also disrupt protein matrix to release starch surrounded by the protein matrix [12]. The low solid yield of starch extracted with sodium hydroxide suggests that it can effectively disrupt the proteins matrix by partial solubilization (ref). Sodium hydroxide can also partially solubilize starches [14] and it can be postulated that these partially solubilized starches were removed during centrifugation and scraping of the brown layer to give a lower solid yield. Although the yield was higher for metabisulfite and metabisulfite + lactic acid as steeping additives, the protein content for the starches extracted in these additives was higher compared to control. Dailey et al. [12] found a lower protein solubilization with metabisulfite compared to lactic acid. In the current research, it is possible that this difference may be because tef protein is different from other cereals. Limited research has suggested that tef protein is mainly composed of prolamin, but there are some difference to sorghum and maize proteins [31]. The major protein in tef is prolamin, similar to maize and sorghum. However this

prolamin is far less polymerized and has a lower free energy of hydration compared to sorghum, and has more basic polypeptides than maize.

3.2 Pasting, thermal, and microstructure properties of extracted starch

The pasting properties for tef starches extracted using different steeping treatments determined by the RVA and Rheometer are shown in Figs. 1 and 2, respectively. Both the RVA and Rheometer exhibited similar trends in pasting properties of extracted tef starches (Figs. 1 and 2). There was significantly ($p < 0.01$) high positive correlation coefficient between the RVA and Rheometer. The correlation coefficients were between 0.97 and 0.99 for pasting temperature, peak time, final viscosity, and trough viscosity.

Starch extracted with sodium hydroxide as steeping additive had different pasting properties compared to the control and the rest of the starch samples from different steeping

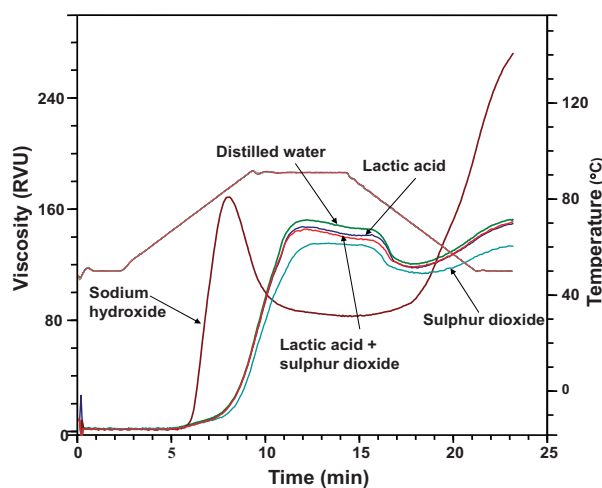


Figure 1. Effect of steeping additives on the pasting profiles of tef starch determined using the Rapid ViscoAnalyser. Defatted tef flour steeped at 40°C for 2 h using five different steeping solutions: distilled water (control); 0.2% w/v sulfur dioxide; 0.5% v/v lactic acid; 0.2% w/v sulfur dioxide + 0.5% v/v lactic acid; 0.5% v/v sodium hydroxide.

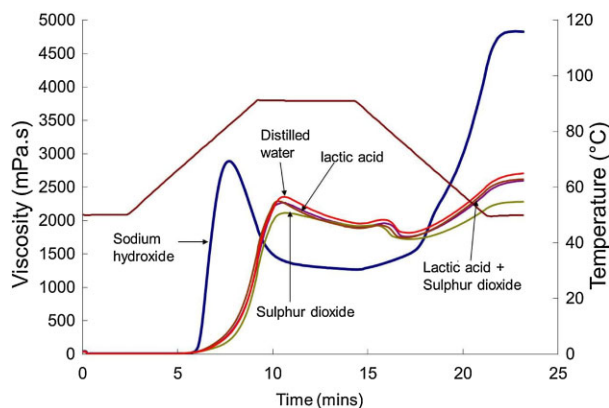


Figure 2. Effect of steeping additives on the pasting profiles of teff starch determined using a Rheometer. Defatted teff flour steeped at 40°C for 2 h using five different steeping solutions: distilled water (control); 0.2% w/v sulfur dioxide; 0.5% v/v lactic acid; 0.2% w/v sulfur dioxide + 0.5% v/v lactic acid; 0.5% v/v sodium hydroxide solution were used to extract teff starch at 40°C for 2 h.

treatments (Figs. 1 and 2). Starch samples from the sodium hydroxide treatment had significantly ($p < 0.05$) lower pasting temperature, peak time, and trough viscosity but, significantly higher peak, breakdown, final, and setback viscosities compared to the control (Figs. 1 and 2). There were no significant ($p < 0.05$) differences in the pasting properties of teff starches extracted with the other steeping additives (lactic acid, metabisulfite, and lactic acid in combination with metabisulfite). The pasting properties of these starches (except the ones steeped with sodium hydroxide) were similar to those reported by Bultosa et al. [3]. These starches have better paste stability indicated by their low breakdown and set back viscosities compared to the sodium hydroxide treatment.

Similar results for the RVA curve characterized by a faster peak paste viscosity for starches extracted with alkaline (NaOH) medium have been reported by other researchers using sago [32, 33], potato, maize [33], and rice starch [34–36]. Two main mechanisms have been proposed by the various researchers. Based on studies involving sago starches [32, 33], maize, potato, and rice [37], it has been suggested that the alkaline treatment leads to increased and faster swelling of starch granules due to possible disruption internal granules structure [32, 33]. Other researchers based on studies involving rice starch [34, 36, 38] have suggested that the observed changes resulted from the disruption of the protein matrix surrounding rice starch granules by the alkaline treatment. According to Tanaka et al. [39], rice starch granules are compound granule and are also surrounded by protein bodies which are a very difficult to remove. This is further shown with the microscopy results below. Alkaline steeping at high temperature (40°C) can also cause starch damage and this can reduce the pasting temperature and affect the pasting properties [40]. It is important as future studies to determine the

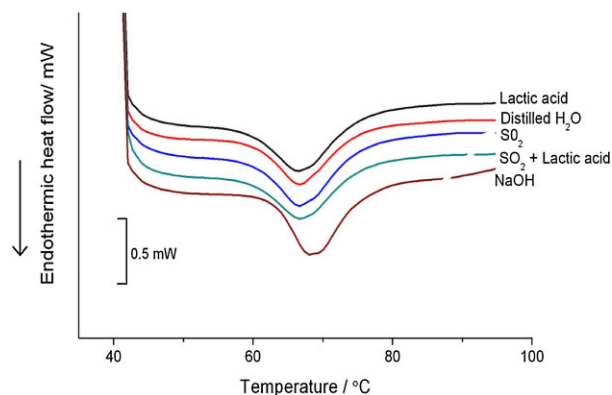


Figure 3. Effect of steeping additives* on the thermal properties of isolated teff starches. *Additives used were distilled water (control); 0.2% w/v sulfur dioxide; 0.5% v/v lactic acid; 0.2% w/v sulfur dioxide + 0.5% v/v lactic acid; 0.5% v/v sodium hydroxide.

starch damage of the teff starches extracted with different additives.

Figure 3 shows the thermal properties of the teff starch extracted with different additives. All the extracted teff starches showed endotherm at about 57–75°C. The starches extracted with the different steeping additive showed similar endotherm except for sodium hydroxide as steeping additive. Teff starch extracted with sodium hydroxide as steeping additive showed higher onset of 63°C, and end set temperature of 81.5°C. These endotherms correspond to the gelatinization temperature of the teff starches and were within the range of values reported by Bultosa and Taylor [37]. Starches extracted by alkaline treatment have been recently shown by other researchers to give increased onset, peak, and endset gelatinization temperatures compared to those pasted without the alkaline steeping [31, 32, 41, 42]. It has been suggested that the increased gelatinization temperatures results from annealing of starch due to the effects of the alkaline on the internal structure of the starch granules which leads to rearrangement of starch chains thereby increasing the gelatinization temperatures [41, 42]. Other researchers suggested that higher gelatinization temperatures could also result from a stabilizing effect on the internal starch granule structure due to imbibed Na⁺ ions through electrostatic interaction between the Na⁺ ions and the hydroxyl groups of starch [31, 42, 43]. The enthalpy of gelatinization values for control (distilled water), lactic acid, metabisulfite, and lactic acid + metabisulfite steeped starches were 5.4 ± 0.6 , 5.5 ± 0.1 , 5.4 ± 0.9 , and 5.8 ± 0.8 J/g, respectively. The starch extracted with steeping in NaOH on the other hand had a value of 6.8 ± 0.1 which was significantly ($p > 0.05$) higher than that of the starches extracted with distilled water, lactic acid, and metabisulfite but was not significantly different from that extracted with a combination of lactic acid and metabisulfite. The increase in enthalpy of gelatinization due the alkaline (NaOH) steeping were also reported by

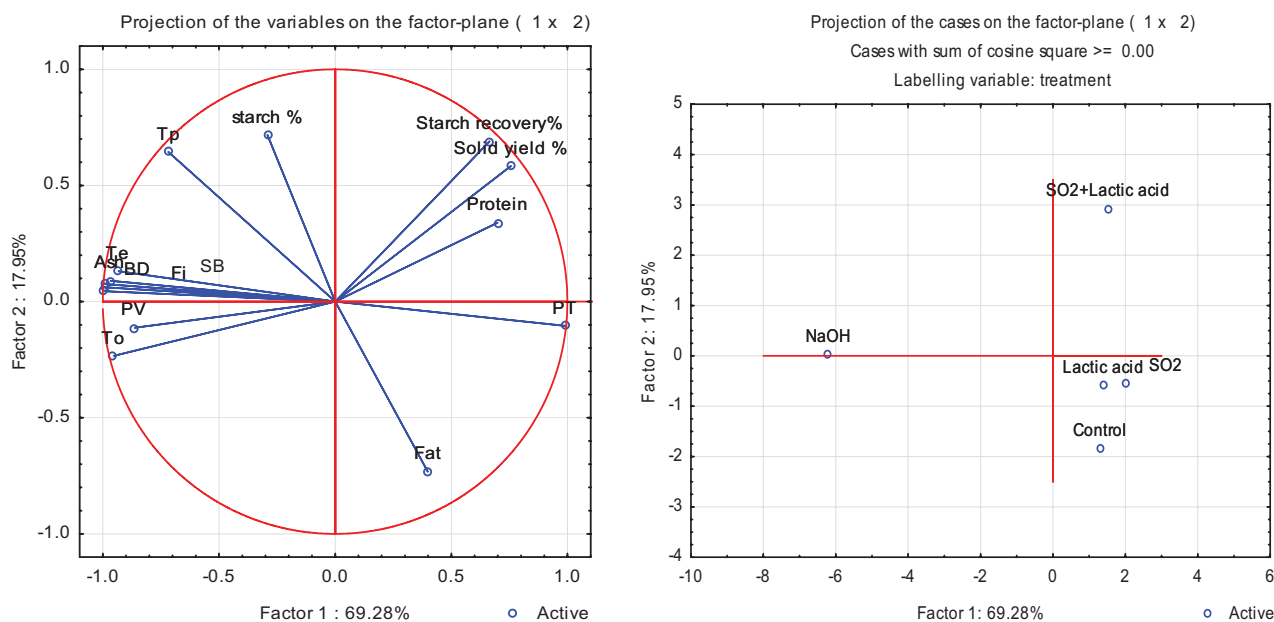


Figure 4. Principal component analysis of the physical, chemical, pasting properties data of extracted tef starches using different steeping additives.

Puchongkavarin et al. [35]. This increase can be due to the annealing effects [41, 42] or possibly due to stabilization effect of Na^+ ions [32, 42, 43] as discussed above. Several other researchers on the other hand dealing with maize [41, 42] and sago starch [32, 33] have shown no significant difference in the gelatinization enthalpy on alkaline treatment although the gelatinization temperatures increased.

In order to assess the relationship of the low protein content in the starch extracted with alkaline (NaOH) treatment, a PCA was performed on the data. Figure 4 shows the relationship between the different treatments, the chemicals and pasting properties in terms of principal component analysis. The PCA in Fig. 4 shows that factor 1 accounts for about 70% of the variation and factor 2 accounts for about 18%. The PCA distinguished the starch extracted with sodium hydroxide as steeping additives from the other steeping treatments; and the sodium hydroxide treatment is associated with lower protein content, higher starch purity, higher peak viscosity, final viscosity, and higher gelatinization temperature as stated before.

Sodium hydroxide is considered as a good solvent and it can solubilise the major protein encapsulating the starch [44]. Thus, in the current study, starch from the sodium hydroxide steep had the lowest protein content resulting in a higher rate of swelling and hence the highest peak viscosity. During wet milling (the centrifugation and scraping steps), it was also observed that the protein layer was easy to scrape off from the starch for samples steeped in sodium hydroxide. Lim et al. [45] and Debet and Gidley [46], have reported that water uptake by starch granules were inhibited by the presence

of protein. The difference in pasting viscosity has been reported to be due to the differences in residual protein and perhaps damaged starch [45, 47, 48]. Thus, the protein content of starch plays an important role in determining the pasting properties of starch, though protein content of starch may not be wholly responsible for the pasting property changes [45, 47, 48]. Pasting properties can be affected by the starch granules and molecules. The molecular aspects have been described above, and microscopy was done to evaluate the microstructure of the granules.

The SEM of all the starch extracted with the different additives mainly consisted of individual granules (Fig. 5). The individual granules were smooth on the surfaces without any indication of granule damage such as pits and striations. This probably indicated that the extent of starch damage that occurred in the starches was limited. However, as stated earlier, future studies should probably focus on the potential starch damage that can occur during the steeping with increased steeping time in order to optimize the yield and quality of the extracted starch.

Larger bodies which were either compound granules or protein bodies with the starches are also observed with SEM. The individual tef starch granules appear as polygonal bodies of 2–6 μm . The protein bodies seem to form a network around compound starch granules with individual protein bodies of about 1–2 μm diameter (Fig. 5f) with the compound starch granules seems to be about 10–30 μm . Similar sizes and shapes of the individual starches, compound granules, and the protein bodies have been reported by Bultosa et al. [3]. The individual starch granules in the compound granule are

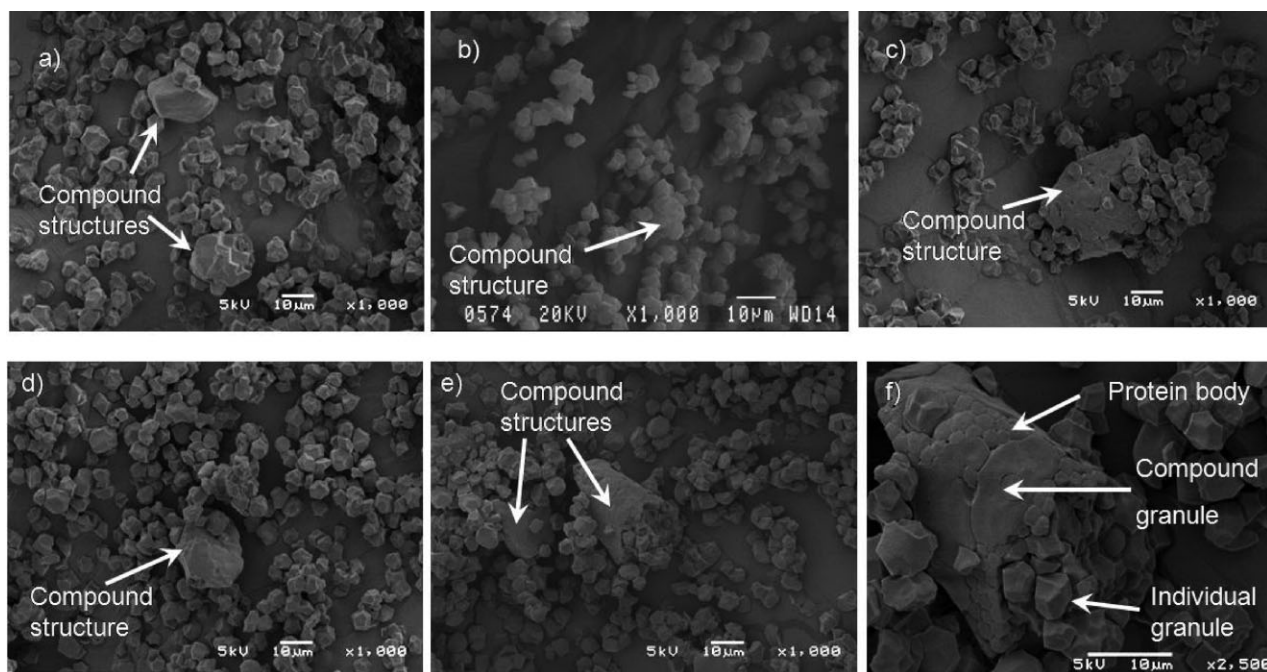


Figure 5. Scanning electron micrographs of tef starch granules of starches extracted with distilled sulfur dioxide (a), sodium hydroxide (b), lactic acid (c), and sulfur dioxide/lactic acid (d), water (e), and granule-protein body compound structure at high magnification for distilled water (f). Arrows in a, c, and e indicate larger bodies that were either granule-protein body compound structures.

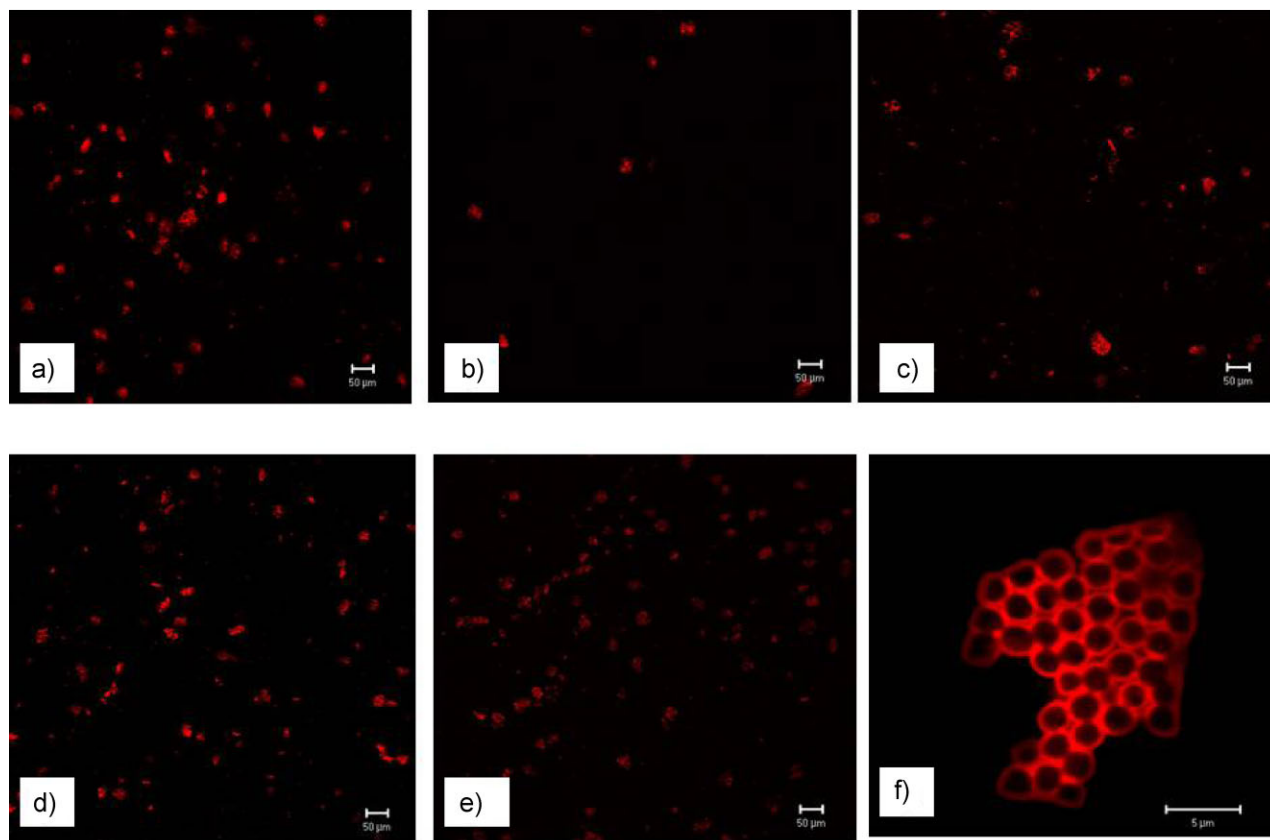


Figure 6. Confocal laser scanning micrographs of protein bodies in extracted starch using distilled water (a), sodium hydroxide (b), sulfur dioxide (c), lactic acid (d), and sulfur dioxide/lactic acid (e), and protein body structure (f).

in direct contact with each other within the compound structure (Fig. 5f). Their relative concentration of the individual, compound, and protein bodies between extracted starches with different steeping additives were not clear with SEM; but it seems that sodium hydroxide has fewer compound granules.

Texas red dye selectively stained protein bodies present in the starch [19] in CLSM. Fewer protein bodies were observed in tef starch extracted by steeping in sodium hydroxide (Fig. 6b) compared to that extracted using the other steeping additives (Fig. 5a–e) as starch from the sodium hydroxide steep showed less red color. The other additives seems to have a protein body concentration similar to that of distilled water thereby indicating that their effect on the protein bodies in the starch maybe similar to that of distilled water. The protein appeared as brightly colored irregular masses of about 30 μm that consisted of about 1–2 μm protein bodies. This result is similar to the SEM micrographs (Fig. 5) and suggests that the extracted starches had a larger proportion of compound granules when steeped in distilled water, metabisulfite, lactic acid, and metabisulfite + lactic acid compared to sodium hydroxide.

Sodium hydroxide steeping for tef starch extraction hence seemed to relatively encourage protein network disintegration to release individual starch granule from the compound starch granule. As stated before sodium hydroxide can solubilize protein effectively. These micrographs support the earlier explanation that protein may form a layer around starch granules and this will delay pasting and will produce a lower pasting viscosity and a lower break down viscosity. Bultosa et al. [3] suggested that tef starches has a lower breakdown and is less shear thinning compared to other starches. However this seems to be dependent on the presence of compound starch granules surrounded by protein network to reduce swelling and breakdown. This may suggest that extracted compound starch granules surrounded by a protein matrix from tef may have different pasting properties compared to individual starch granules.

4 Conclusions

The present study shows that both the Rapid Visco Analyzer and the Rheometer can be used interchangeably to determine the pasting properties of tef starches. Among the five treatments sodium hydroxide steeps gives starch with lower protein content, higher starch purity, higher peak viscosity, final viscosity, and higher gelatinization temperature compared to distilled water, metabisulfite, lactic acid, and metabisulfite + lactic acid. The results of this investigation indicates that NaOH (alkaline) is potentially a viable steeping agent for extraction tef starch with regard to high purity. It appears that NaOH is able to remove protein bodies encapsulating the tef starch granules and thereby probably

improved the properties. NaOH however modifies the pasting properties of the extracted starch leading to lower paste stability. In order to optimize functional properties and yield, further studies need to be conducted to assess the potential role of starch damage that may occur with NaOH (alkaline) treatment especially with increased steeping time on modification of the pasting and thermal properties of the isolated tef starch. The low retrogradation and low breakdown of the extracted starches (except the one with NaOH) suggest that it can be used in pies and filling where low retrogradation is required and perhaps in high shear application. The single granule obtained from NaOH can be used as fat mimetics due to the small granule size.

We are grateful to the National Research Foundation of South Africa for funding this research.

The authors have declared no conflict of interest.

5 References

- [1] National Research Council, *Lost Crops of Africa. Vol. 1: Grains*, National Academy Press, Washington, DC 1996.
- [2] Bultosa, G., Taylor, J. R. N., in: Colin, W. (Ed.), *Encyclopedia of Grain Science*, Elsevier, Oxford 2004, pp. 281–290.
- [3] Bultosa, G., Hall, A. N., Taylor, J., Physico-chemical characterization of grain tef [*Eragrostis tef* (Zucc.) Trotter] starch. *Starch/Stärke* 2002, 54, 461–468.
- [4] Lindeboom, N., Chang, P. R., Tyler, R. T., Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: A review. *Starch/Stärke* 2004, 56, 89–99.
- [5] Bultosa, G., Physicochemical characteristics of grain and flour in 13 tef [*Eragrostis tef* (Zucc.) Trotter] grain varieties. *J. Appl. Sci. Res.* 2007, 3, 2042–2051.
- [6] Singh, V., Haken, A., Niu, Y., Zou, S., Eckhoff, S., Hybrid-dependent effect of lactic acid on corn starch yields. *Cereal Chem.* 1997, 74, 249–253.
- [7] Watson, S., in: Whistler, R. L., Bemiller, J. W., Paschal, E. F. (Eds.), *Starch: Chemistry and Technology*, Academic Press, New York 1984, pp. 417–464.
- [8] Watson, S., Sanders, E., Steeping studies with corn endosperm sections. *Cereal Chem.* 1961, 38, 22–33.
- [9] Wall, J. S., Paulis, J. W., in: Pomeranz, Y. (Ed.), *Advances in Cereal Science and Technology*, American Association of Cereal Chemists, St. Paul, MN 1978.
- [10] Du, L., Li, B., Lopes-Filho, J., Daniels, C., Eckhoff, S., Effect of selected organic and inorganic acids on corn wet-milling yields. *Cereal Chem.* 1996, 73, 96–98.
- [11] Haros, M., Suarez, C., Effect of chemical pretreatments and lactic acid on the rate of water absorption and starch yield in corn wet-milling. *Cereal Chem.* 1999, 76, 783–787.
- [12] Dailey, O. D., Jr., Dowd, M. K., Mayorga, J. C., Influence of lactic acid on the solubilization of protein during corn steeping. *J. Agric. Food Chem.* 2000, 48, 1352–1357.

- [13] Dailey, O. D., Jr., Effect of lactic acid on protein solubilization and starch yield in corn wet-mill steeping: A study of hybrid effects. *Cereal Chem.* 2002, *79*, 257–260.
- [14] Eckhoff, S., Du, L., Yang, P., Rausch, K. et al., Comparison between alkali and conventional corn wet-milling: 100-g procedures. *Cereal Chem.* 1999, *76*, 96–99.
- [15] D’Silva, T. V., Taylor, J., Emmambux, M. N., Enhancement of the pasting properties of teff and maize starches through wet–heat processing with added stearic acid. *J. Cereal Sci.* 2011, *53*, 192–197.
- [16] Eckhoff, S., Singh, S., Zehr, B., Rausch, K. et al., A 100-g laboratory corn wet-milling procedure. *Cereal Chem.* 1996, *73*, 54–57.
- [17] Wang, F., Chung, D., Seib, P., Kim, Y., Optimum steeping process for wet milling of sorghum 1. *Cereal Chem.* 2000, *77*, 478–483.
- [18] Yang, P., Du, L., Wang, D., Li, B. et al., Effects of alkali debranning, roller mill cracking and gap setting, and alkali steeping conditions on milling yields from a dent corn hybrid. *Cereal Chem.* 2000, *77*, 128–132.
- [19] Titus, J. A., Haugland, R., Sharrow, S. O., Segal, D. M., Texas red, a hydrophilic, red-emitting fluorophore for use with fluorescein in dual parameter flow micro-fluorometric and fluorescence microscopic studies. *J. Immunol. Methods* 1982, *50*, 193.
- [20] Tang, M. C., Copeland, L., Analysis of complexes between lipids and wheat starch. *Carbohydr. Polym.* 2007, *67*, 80–85.
- [21] American Association of Cereal Chemists (AACC), *Approved Methods of the American Association of Cereal Chemists*, 10th edn., American Association of Cereal Chemists, St. Paul, MN 2002.
- [22] Wokadala, O. C., Ray, S. S., Emmambux, M. N., Occurrence of amylose–lipid complexes in teff and maize starch biphasic pastes. *Carbohydr. Polym.* 2012, *90*, 616–622.
- [23] Hosene, R. C., *Principles of Cereal Science and Technology*, American Association of Cereal Chemists, St. Paul 1994.
- [24] Yang, P., Eckhoff, S., Reducing steep time by adding lactic acid during countercurrent steeping of corn with different initial moisture contents. *Cereal Chem.* 2000, *77*, 529–534.
- [25] Perez, O., Haros, M., Suarez, C., Corn steeping: Influence of time and lactic acid on isolation and thermal properties of starch. *J. Food Eng.* 2001, *48*, 251–256.
- [26] Haros, M., Perez, O. E., Rosell, C. M., Effect of steeping corn with lactic acid on starch properties. *Cereal Chem.* 2004, *81*, 10–14.
- [27] Ruan, R., Lei, H., Chen, P., Deng, S. et al., Ozone-aided corn steeping process. *Cereal Chem.* 2004, *81*, 182–187.
- [28] Singh, V., Johnston, D., An enzymatic process for corn wet milling. *Adv. Food Nutr. Res.* 2004, *48*, 151–171.
- [29] Shandera, D., Parkhurst, A., Jackson, D., Interactions of sulfur dioxide, lactic acid, and temperature during simulated corn wet milling. *Cereal Chem.* 1995, *72*, 371–378.
- [30] Adebowale, A. A., Emmambux, M. N., Beukes, M., Taylor, J. R. N., Fractionation and characterization of teff proteins. *J. Cereal Sci.* 2011, *54*, 380–386.
- [31] Perez, O. E., Haros, M., Suarez, C., Rosell, C. M., Effect of steeping time on the starch properties from ground whole corn. *J. Food Eng.* 2003, *60*, 281–287.
- [32] Karim, A., Nadiha, M., Chen, F., Phuah, Y. et al., Pasting and retrogradation properties of alkali-treated sago [*Metroxylon sagu*] starch. *Food Hydrocolloids* 2008, *22*, 1044–1053.
- [33] Nor Nadiha, M., Fazilah, A., Bhat, R., Karim, A. A., Comparative susceptibilities of sago, potato and corn starches to alkali treatment. *Food Chem.* 2010, *121*, 1053–1059.
- [34] Lim, S.-T., Lee, J.-H., Shin, D.-H., Lim, H. S., Comparison of protein extraction solutions for rice starch isolation and effects of residual protein content on starch pasting properties. *Starch/Stärke* 1999, *51*, 120–125.
- [35] Puchongkavarin, H., Varavinit, S., Bergthaller, W., Comparative study of pilot scale rice starch production by an alkaline and an enzymatic process. *Starch/Stärke* 2005, *57*, 134–144.
- [36] Hamaker, B. R., Griffin, V. K., Effect of disulfide bond-containing protein on rice starch gelatinization and pasting. *Cereal Chem.* 1993, *70*, 377.
- [37] Bultosa, G., Taylor, J., Chemical and physical characterisation of grain Tef [*Eragrostis tef* (Zucc.) Trotter] starch granule composition. *Starch/Stärke* 2003, *55*, 304–312.
- [38] Hamaker, B. R., Griffin, V. K., Changing the viscoelastic properties of cooked rice through protein disruption. *Cereal Chem.* 1990, *67*, 261–264.
- [39] Tanaka, K., Sugimoto, T., Ogawa, M., Kasai, Z., Isolation and characterization of two types of protein bodies in the rice endosperm [*Oryza sativa*]. *Agric. Biol. Chem.* 1980, *44*, 1633–1639.
- [40] Lee, H. C., Htoon, A. K., Paterson, J. L., Alkaline extraction of starch from Australian lentil cultivars Matilda and Digger optimised for starch yield and starch and protein quality. *Food Chem.* 2007, *102*, 551–559.
- [41] Mendez-Montealvo, G., Sánchez-Rivera, M., Paredes-López, O., Bello-Pérez, L., Thermal and rheological properties of nixtamalized maize starch. *Int. Biol. Macromol.* 2006, *40*, 59–63.
- [42] Méndez-Montealvo, G., Trejo-Espino, J. L., Paredes-López, O., Bello-Pérez, L. A., Physicochemical and morphological characteristics of nixtamalized maize starch. *Starch/Stärke* 2007, *59*, 277–283.
- [43] Bryant, C. M., Hamaker, B. R., Effect of lime on gelatinization of corn flour and starch 1. *Cereal Chem.* 1997, *74*, 171–175.
- [44] Lásztity, R., Oat grain – A wonderful reservoir of natural nutrients and biologically active substances. *Food Rev. Int.* 1998, *14*, 99–119.
- [45] Lim, S.-T., Lee, J.-H., Shin, D.-H., Lim, H. S., Comparison of protein extraction solutions for rice starch isolation and effects of residual protein content on starch pasting properties. *Starch/Stärke* 1999, *51*, 120–125.
- [46] Debet, M. R., Gidley, M. J., Three classes of starch granule swelling: Influence of surface proteins and lipids. *Carbohydr. Polym.* 2006, *64*, 452–465.
- [47] Wang, L., Wang, Y. J., Comparison of protease digestion at neutral pH with alkaline steeping method for rice starch isolation. *Cereal Chem.* 2001, *78*, 690–692.
- [48] Wang, L., Wang, Y. J., Rice starch isolation by neutral protease and high-intensity ultrasound. *J. Cereal Sci.* 2004, *39*, 291–296.