University of Pretoria etd – Bultosa Beri G 2003

4. GENERAL DISCUSSION

4.1. Methodologies - a critical review

Starch isolation: In this work tef starch granules were extracted by dry milling of the grain, soaking of the milled products in distilled water and then wet milling of the slurry as described by Taylor et al. (1997). The dry milling will separate the grain into its anatomical parts: flour, bran and shorts, whereas the wet milling separates into various chemical components: oil, fibre, protein and starch (Kent and Evers, 1994). The fibre was separated from protein and starch by a screening through a 100 µm screen. The starch was separated from protein by repeated suspending into distilled water, centrifugation and scraping off the protein layer. The other possibility of starch granule extraction is by wet milling via soaking the whole grain in distilled water without prior dry milling of the grain to flour (Grant, 1998). In wheat, the wet milling method was reported to cause lower starch granule damage, lower water absorption index, higher peak viscosity, lower breakdown viscosity and lower cold paste viscosity than the former method (Grant, 1998). The SEM (Figures 3.1 and 3.3) of tef starch granules and light microscopy (data not shown) indicated that damage of the isolated granules was not evident. This is because in part tef starch granules are small size in nature (2-6 µm in diameter) and possibly they are less prone to damage while dry milling of the grain to flour. In other starch isolation methods, chemicals dilute alkali (NaOH) (Mistry and Eckhoff, 1992), sulphur dioxide (0.1-0.2 %) (Watson, 1984), protease enzyme digestion (Lumdubwong and Seib, 2000) and washing with detergent (Baldwin, 2001) are used to reduce residual protein associated with starch granules and thereby also non–starch lipids of the lipo-protein matrix. Gradient centrifugation with cesium chloride solution (80 %, v/v) has also been used to purify wheat starch granules prepared conventionally (Sulaiman and Morrison, 1990). The tef starch protein content (mean 0.19 %) was higher than pure maize starch (*ca.* 0.06 %) (Morrison et al. 1980) but similar to pure wheat starch (*ca.* 0.16–0.19 %) (Morrison et al. 1980), a value recommended as an index to indicate negligible non–starch lipid in the starch granules. Thus, the tef starch used in this study can be regarded as pure as wheat starch.

University of Pretoria etd – Bultosa Beri G 2003

Amylose/amylopectin ratio: Amylose can be determined by: (1) iodine binding with amylose (Morrison and Laignelet, 1983) (2) selective quantitative precipitation reaction of amylopectin with the lectin concanavalin A (Con A) (Gibson et al. 1997); (3) high performance size exclusion chromatography (HPSEC) (Kobayashi, Schwartz and Lineback, 1985) and (4) differential scanning calorimetry (Kugimiya and Donovan, 1981).

The basis of the iodine binding method is the capacity of amylose to form a helical blue inclusion complex with iodine (Morrison and Laignelet, 1983). The method can be affected by: starch dispersion procedures, methods used to generate I_3^- that initiate the reaction, starch lipids that potentially complex with amylose and residual starch proteins (Knutson, 1986). The amylose assay is conducted either via colorimetric (Morrison and Laignelet, 1983), potentiometric (Banks, Greenwood and Muir, 1971) or amperometric (Larson, Gilles and Jenness, 1953) procedures. Starches are usually dispersed by the use of dimethyl sulphoxide (DMSO) or dilute alkali (*ca.* 1 M NaOH or KOH). In this work, starch was dispersed using 1 M NaOH and a protein precipitant trichloroacetic acid (TCA) was used to suppress the protein interference with the colour reaction (Chrastil, 1987).

In the colorimetric procedure the absorbance of the blue colour of the amylose iodine complex is measured at *ca.* 620 nm (maximum absorbance) (Chrastil, 1987). Amylose binds iodine to the extent of *ca.* 20 mg iodine/100 mg amylose (Morrison and Laignelet, 1983). Amylopectin can also weakly bind iodine (*ca.* 1 mg iodine/100 mg amylopectin) giving a characteristic pink red colour with absorbance maxima at *ca.* 540 nm (Morrison and Laignelet, 1983). Because of a distinct absorbance maxima difference, the interference of amylopectin in the amylose determination is usually regarded as negligible. According to a review by Gibson et al. (1997) due to the possibility of a little iodine binding to amylopectin, the method will over estimate the amount of amylose. There have been efforts to modify the Morrison and Laignelet (1983) method. Knutson and Grove (1994) have reported the use of 3 M $CaCl_2$ in combination with 15–30 min sonication to enhance starch dispersion at relatively low temperature (65–70 ^{O}C). Knutson (1986) used water (10 %) and DMSO (90 %) mixtures to generate I_3^- to eliminate the necessity for the preparation of buffered

University of Pretoria etd – Bultosa Beri G 2003

solutions of iodine in potassium iodide. Martinez and Prodolliet (1996) have reported the use of continuous stirring while cooling the dispersed starch to room temperature before allowed to react with iodine to suppress the gelation of amylose.

In the potentiometric method, electromotive potential difference due to iodine activity between the sample half–cell and control half-cell is measured by titrating with standard iodine solution (Banks et al. 1971). The amount of iodine bound to amylose is determined from a plot of bound iodine versus free iodine. Free iodine is obtained from the amount of iodine added to the control half–cell, whereas bound iodine is obtained from difference of original amount of iodine added to sample half cell and free iodine. In the amperometric method current change due to activity of iodine is measured by titrating with standard iodine solution (Larson et al. 1953). Iodine binding to amylose is then estimated from a plot of current (μA) versus standard iodine solution directly. Both methods were described as precise but less convenient than the colorimetric method (Knutson, 1986). Determination of amylose by the iodine binding method is still regarded as standard, cheap, simple and reliable (Gérard, Barron, Colonna and Planchot, 2001).

In the Con A method, the starch sample is dispersed by DMSO (Gibson et al. 1997). The dispersed starch is precipitated in ethanol, while extracting the lipids. The precipitated starch is re–dispersed by DMSO in acetate/salt solution. Con A under defined conditions of pH, temperature and ionic strength (Megazyme International, 1996) specifically precipitates the branched fraction (amylopectin) of the starch. The precipitate formed is removed by centrifugation. Amylose in the supernatant of the centrifugation is hydrolysed to glucose by α-amylase and amyloglucosidase. The glucose generated is measured colorimetrically at 510 nm by reacting with glucose oxidase peroxidase reagent (GOPOD). Total starch from DMSO acetate/salt solution prior to Con A treatment is also hydrolysed by α-amylase and amyloglucosidase to glucose and the glucose generated is measured at 510 nm after reacting the same way with the GOPOD reagent. The amount of amylose is then determined from absorbance ratio of the supernatant to that of the total starch. This method ignores the possibility of a few intermediate branched amylose (IBA) (intermediate between liner amylose and branched amylopectin) molecules that can potentially interact with Con

University of Pretoria etd – Bultosa Beri G 2003

A (Gérard et al. 2001). If the IBA will interact with Con A, it is removed as an amylopectin fraction and thereby leads to underestimation of the amylose. Preparation of a calibration curve is not required for this method. Another advantage is that total starch can be determined concurrently. The method is not as cheap as the iodine binding method.

In the HPSEC method, after dispersion of starch samples, amylose and amylopectin are separated on a SEC column such as the TSK HW75 S gel column (2.6 x 200 cm, TosoHaas, Germany) (Gérard et al. 2001). The amylose/amylopectin ratio is estimated from peak area ratio measurements (Kobayashi et al. 1985). HPSEC will also separate IBA molecules that are present in some starches (Gérard et al. 2001). IBA can bind iodine, form a complex with free fatty acid and react with Con A. This can lead to an overestimation of amylose by iodine binding and DSC methods as compared to Con A and HPSEC (Gérard et al. 2001).

In the DSC method the starch sample, lysolecithin and water after hermetically sealing in the DSC pan are heated above the gelatinisation temperature of the starch (Kugimiya and Donovan, 1981). On gelatinisation and cooling, an amylose-lysolecithin complex is formed and the melt enthalpy of the complex is used to estimate the amylose content (Kugimiya and Donovan, 1981).

Gérard et al. (2001) compared amylose determination by iodine binding, Con A, HPSEC and DSC methods in potato, normal maize, wheat, smooth pea, wrinkled pea and in maize mutant starches. The DSC method gave large discrepancies compared to other methods and was regarded as less accurate. The Con A and iodine binding gave erroneous values for maize mutant starches but almost similar values to the SEC values for other starches. Amylose in the tef starches determined by Con A and iodine binding methods in this study gave almost similar values. The accuracy of amylose analysis by HPSEC, Con A and iodine binding methods are in part influenced by the IBA fraction (i.e. whether to consider it as amylose or amylopectin), amylopectin chain lengths and lipids. The HPSEC and Con A methods do not measure IBA molecules as amylose, whereas in the iodine binding it is measured as amylose. It is suggested that in the future IBA will require some sort of standardisation whether to describe it as amylose or not.

University of Pretoria etd – Bultosa Beri G 2003

Starch lipids: Tef starch lipids reported in this work were determined by: (1) the soxhlet method using petroleum ether (boiling point 40-60 ^{O}C), (2) hydrolysis of the starch granules with 24 % HCl at *ca.* 78 ^{O}C, followed extraction with n–hexane and *in situ* generated ethyl formate, (3) extraction with CM (2:1, v/v) at *ca.* 28 ^{O}C and (4) extraction with WSB (1:5, v/v) at *ca.* 90 ^{O}C. Lipid extraction from starch is influenced by: solvent polarity, lipid nature (i.e. whether free or bound to carbohydrate and/or proteins) and the procedures used (Morrison, 1988). In the soxhlet method, a low temperature boiling non-polar solvent (such as petroleum ether or hexane) is heated continuously, allowed to come into contact with the sample held in a porous thimble and while the solvent mixes with the sample, lipids are dissolved into the solvent (James, 1996). The non-polar solvents are effective for extracting non-polar lipids, commonly triacyl lipids, followed by diacyl lipids, monoacyl lipids and free fatty acids (Morrison, 1988). Lipids extracted from starch by this method are few because the extraction condition will not lead to disintegration of starch granules and the solvent is also less efficient for extracting bound lipids (Morrison, 1988).

Heating of starch granules with 24 % HCl at *ca.* 78 ^{O}C for 30 min will hydrolyse starch, destroying the granules to release bound lipids (James, 1996). Because of this lipids extracted by this method are regarded as total starch lipids (Morrison, 1988). During starch acid hydrolysis, lysophospholipids are also hydrolysed (Morrison and Coventry, 1985). This can slightly affect the results by this method. After starch granule destruction the quantity of lipids extracted can be also affected by solvent type, extraction duration and filtration procedures (James, 1996). The use of n–hexane for extraction has been frequently reported (Vasanthan and Hoover, 1992 a and b; Hoover et al. 1996 b). However, due to the non-polar nature of n-hexane, it might not exhaustively extract all lipids released by hydrolysis (FAO, 1986). Because of this, to dissolve more lipids, the use of *in situ* generated ethyl formate along with n-hexane is recommended (FAO, 1986). This approach was used in this study.

In the cold (*ca.* 28 ^{O}C) CM (2:1, v/v) solvent lipid extraction, methanol is required to disrupt hydrogen bonds to release lipids that are bound to carbohydrate and proteins, whereas chloroform is required to dissolve the lipids (Folch, Lees and Stanley, 1957).

University of Pretoria etd – Bultosa Beri G 2003

This procedure is often used to quantify starch granule surface lipids and residual non–starch lipids (Morrison, 1988; Norja et al. 1997). By the use of suitable starch granule clean-up procedures, it is possible to reduce to negligible the interference of non-starch lipids in the determination of starch surface lipids (Morrison, 1995). The method will not lead to disintegration of starch granules and is thus often used as a precursor for the determination of internal starch lipids (Morrison, 1988). This approach was used in this work. At ambient temperature, the use of other solvents such as water saturated n–butanol (WSB) (1:5, v/v) (Vasanthan and Hoover, 1992 a) and n-propanol water (n-PW) (3:1, v/v) (Liukkonen and Laakso, 1992) to extract starch granule surface lipids was also reported. According to Vasanthan and Hoover (1992 a) at ambient temperature, extraction with CM is slightly superior than to the use of WSB.

By the use of CM most lipid classes (polar and non-polar) can be extracted (Vasanthan and Hoover, 1992 a). Extraction of neutral lipids (NL), phospholipids (PL) and glycolipids (GL) from wheat, maize, rice, potato, cassava, lentil and fababean starches has been reported (Vasanthan and Hoover, 1992 a). From each class separation of different lipids by the use of silica gel G thin layer chromatography (TLC) (Merck, Darmstadt, Germany) was reported.

In the extraction of internal starch lipids, hot water alcohol mixtures are used (Morrison and Coventry, 1985). The internal tef starch granule lipids reported in this work were extracted with hot (*ca.* 90 ^{O}C) WSB (1:5, v/v) (Morrison et al 1980). Water is required for starch granule swelling and gelatinisation (Morrison, 1995). Thus, with the use of a hot water–alcohol mixture more lipids are extracted than with cold solvent extraction because in the hot solvent extraction starch granules are disrupted (Vasanthan and Hoover, 1992 a). In this work, the quantity of lipids extracted by hot solvent was found to be larger than the quantity extracted with the cold solvent (Table 3.3). The internal starch lipids are specifically LPL and free fatty acid in nature and are believed to possibly exist as lipid–amylose complexes (Morrison, 1995).

University of Pretoria etd – Bultosa Beri G 2003

Comparative studies of the efficiency of internal starch lipid extraction by hot water alcohol mixtures by different alcohols (methanol, ethanol, n-propanol, *iso*-propanol and n–butanol) have been undertaken (Morrison and Coventry, 1985). The proportion of water in the mixture for swelling and disruption of the starch crystallites was found to play a critical role and it was markedly different for different alcohols to exhibit maximum efficiency. Hot ethanol water (75 %, v/v) mixture was least efficient of all. Hot methanol water (75-85 %, v/v) was as efficient as hot n–propanol water (74-78 %, v/v) and as hot *iso*–propanol water (74-78%, v/v), but the high vapour pressure of methanol makes the use of methanol unattractive. Hot n-butanol water (84 %, v/v ≈ WSB, 1: 5, v/v) was as efficient as n–propanol water (74-78 %, v/v). However, the amount of water to be used along with n–butanol is limited in proportion. The hot water alcohol solvent mixture can also extract prolamin proteins (Morrison and Coventry, 1985). Where a prolamin is expected as a protein residue associated to the starch granules, the use of n-butanol water mixture is recommended, since the use of n-propanol can also extract prolamins (Morrison and Coventry, 1985).

In all the gravimetric methods of lipid determination described above, the polar solvents used to extract starch lipids can also slightly extract carbohydrates and this can slightly affect the reliability of the results (Morrison, 1995). Total starch lipids can be also determined by gas chromatography (GC) with a polar GC column (30 m x 0.25 mm) like Supleco SP 2330 from direct acid hydrolysis or from different solvent fractionated lipids after converting the fatty acids to fatty acid methyl esters (Vasanthan and Hoover, 1992 a).

University of Pretoria etd – Bultosa Beri G 2003

Starch gelatinisation: Tef starch gelatinisation was studied by birefringence loss using the *Kofler* hot stage method and DSC. In a *Kofler* hot stage, gelatinisation temperatures (^{O}C): onset (To), peak (Tp) and conclusion (Tc) are measured at *ca.* 2 %, 50 % and 98 %, respectively of starch granules birefringence loss by visual observation (Atwell et al. 1988). In the DSC gelatinisation endotherm study, the heat flow difference between sample and reference as a function of temperature is recorded to generate the DSC thermogram peak (Biliaderis, 1983). From the DSC thermogram, the gelatinisation temperatures: To, Tp and Tc in ^{O}C are measured at peak onset, maximum peak height and peak conclusion temperatures, respectively. The gelatinisation enthalpy (Δ H) is recorded from the peak area integration.

By birefringence loss measurement, the tef starch gelatinisation temperature range was 68–80 ^{O}C, whereas with DSC the range was 64–82 ^{O}C. The range appeared relatively wider with the DSC than *Kofler* hot stage method. This is in part because the melt of double helices and crystalline order measured with the DSC are known to commence before birefringence loss starts and to finish after the loss of birefringence (Liu et al. 1991). The structural manifestation for birefringence loss of starch granules is known to be mainly related to changes in starch molecular chain organisation in the amorphous portion of the starch granules (Zobel, 1988 b). Thus, the two techniques are not measuring exactly the same structural changes in the starch granules.

DSC gelatinisation and other endotherms. The gelatinisation endotherm is particularly sensitive to the availability of water for starch granule hydration (Sahai and Jackson, 1999). For tef starch a moisture of 79 % was used and the data were evaluated from single symmetrical endotherms. This is because at moisture > 60 % a single symmetrical DSC endotherm for starch gelatinisation is observed, due to cooperative melting of crystallites and double helices (Donovan, 1979). At low moisture content (< 25 %), starches show a high temperature endotherm in the range 140-180 ^{O}C, due to the melt and uncoiling of double helices (Sahai and Jackson, 1999). In this work also for dry maize, DZ-Cr-37 and South African Brown starches high temperature endotherm of Tp in the range 152–161 ^{O}C was observed. When the moisture content is limited (*ca.* 60-30 %) starch gelatinisation will show a bi–phasic

University of Pretoria etd – Bultosa Beri G 2003

endotherm transition with the second transition taking place at high temperature (Donovan, 1979). This was indicated that the starch granules have responded differently (Biliaderis et al. 1986; Sahai and Jackson, 1999).

The DSC starch endotherms can be also affected by sample preparation techniques (methods of water addition to starch sample), sample mass, heating rate and moisture equilibration times (Yu and Christie, 2001). Due to the poor thermal conductivity of starch and poorly uniform packing nature, efficient heat transfer throughout the sample is not uniform and this can adversely affect reproducibility of starch DSC endotherms (Yu and Christie, 2001). Methods of water addition to the starch granules (placing starch sample into humid environments to absorb moisture, sampling from pre-mixed water and starch in bulk or directly adding water into the starch sample in the DSC pan) can affect the homogeneity of starch granule hydration (Yu and Christie, 2001). Sample mass can affect resolution and sensitivity (Yu and Christie, 2001). With small sample mass (3-10 mg) resolution is good but sensitivity is poor, whereas for large sample mass (> 30 mg) resolution is poor and sensitivity is good. Because of this, the use of *ca.* 10-20 mg is recommended (Yu and Christie, 2001) and in this work for gelatinisation endotherm study a similar mass range was used. Heating rate can affect kinetics of starch granule structural changes and thereby starch DSC endotherms (Yu and Christie, 2001). The heating rate can be influenced by sample mass and DSC pan type. With aluminum pans, a heating rate of 10 ^{O}C/ min is normally used, as used in this work. Equilibration time is required to attain homogeneous starch granule hydration (Yu and Christie, 2001). For starch gelatinisation an equilibration time of more than one h. to attain homogeneous granule hydration was recommended (Beta et al. 2000) and similar approach was used in this work.

X-ray diffraction: Starch granule crystal type and degree of crystallinity are usually elucidated by X-ray diffraction (Zobel, 1988 b). The positions of the diffraction peaks that depend on interplanar crystal spacing ($n\lambda = 2d \sin \theta$) and peak intensities that depend on number and type of reflection from each set of planes are used to describe starch crystal types (Zobel, 1988 b). The degree of starch granule crystallinity is determined from area prescribed under crystalline and amorphous portions of the diffractogram (Komiya and Nara, 1986). In a semicrystalline material like starch, the diffractogram is considered to be composed of crystalline diffraction peaks superimposed on an amorphous background. To separate the two regions a line is drawn that connects the peak baselines. The area above the line is the crystalline region and the lower area is the amorphous region. The ratio of upper area to the total diffraction area is taken as the degree of crystallinity. In this work the crystallinity of tef starches were also evaluated using this principle.

For an X-ray diffraction study, homogeneous fine powder is prepared as thin–walled in a glass to allow as much as possible the granule crystallites to orient in every direction to X-ray beam (Skoog, Holler and Nieman, 1998). Starch crystallinity can be affected by moisture and temperature changes (Svensson and Eliasson, 1995). With moisture > 12 % and a temperature increase of > 40 ^{O}C both the intensity and starch crystal type can be changed. However, when the moisture content of starch is ≤ 12 %, crystallinity of a starch granule is essentially not affected by moisture (Hartley, Chevance, Hill, Mitchell and Blanshard, 1995). In this work to avoid the influence of moisture, X-ray diffractograms were taken from dry starch (6-12 %) at ambient temperature (25 ^{O}C). According to Hartley et al. (1995) for X-ray diffraction study, storing of the starch sample for one week over saturated salt solution, e.g. P_2O_5, LiCl, K_2CO_3, $CaCl_2$ or $(NH_4)_2SO_4$ to equilibrate starch to specified relative humidity is recommended.

University of Pretoria etd – Bultosa Beri G 2003

4.2. Physico - chemical properties of tef starch and potential applications

A summary of the physico–chemical properties of tef starches determined in this work is given in Table 4.1. Comparison was made to the physico–chemical properties of other starches summarised in Tables 2.2 and 2.3. The tef starch granule size (2–6 μm in diameter) is as small as that of rice starch granules but is slightly larger than amaranthus and quinoa starch granules. Tef starch has compound type starch granules like amaranthus, quinoa, rice and oat starch granules. Because of their very small granule size and very white colour, native commercial rice starches are used as a cosmetic dusting powder, laundry stiffening agent, paper and photographic paper powder, sugar coating in confectionery and as carrying agent in the formulation of pharmaceutical tablets (Champagne, 1996). In this line, on the basis of granule size similarity, the native tef starch is expected to offer a similar functionality as that of native rice starch.

Starch granules of small size like amaranthus (Zhao and Whistler, 1994 a), rice (Champagne, 1996) and quinoa (Koziol, 1993) are recommended for use as fat substitutes (mimetics). This is because their granule size is almost the same as that of homogenised fat globules. Thus they can provide a texture perception similar to that of fat globules. For tef starch a similar application is expected. When small sized starch granules (amaranthus 1–2 μm, rice 3–8 μm and small size (B) wheat 2–8 μm) are spray–dried with a small amount of bonding agents (0.1–1.0 % proteins or water soluble polysaccharide) they will form an aggregate with extensive bulk pores and/or cavities that can serve as a carriers of flavours and other small bioactive compounds (Zhao and Whistler, 1994 a). In this regard tef starch is also expected to offer a similar functionality.

The amylose/amylopectin ratio in the five tef varieties studied is similar to normal maize and wheat (Morrison and Laignelet, 1983), sorghum (Beta et al. 2000) and rice starches (25 - 30 %) (Juliano, 1971). The waxy type or amylo type starch functionality is therefore not expected. However, for different botanical sources of starch the amylose and amylopectin molecular weights, *dp* and degree of branching are different (Swinkels, 1985). They can also slightly vary within the same botanical source of the starch (Shibanuma, Takeda and Hizukuri, 1996). For example, in terms of average *dp*,

Table 4.1 Physico-chemical properties of grain tef starch (db) determined in this research

Property	Mean and standard deviation for five tef varieties	Remark
Individual granule size (diameter in μm)	2-6 and majority are 3-5	Individuals are from compound granules
Compound granule size (diameter in μm)	*ca.* 23	Measured from Figure 3.2
Shape	Polygonal sides of smooth surface	
Ash (%)	0.16 ± 0.04	
Protein (%) (N x 6.25)	0.19 ± 0.03	
Crude fat (%)	0.29 ± 0.03	Petroleum ether extract
Lipids: 24 % HCl (mg/g)	8.9 ± 0.7	n-Hexane and *in situ* generated ethyl formate extract
CM (mg/g)	4.3 ± 0.3	Cold (28 ^{O}C) chloroform methanol extract
WSB (mg/g)	7.8 ± 0.4	Hot (90 ^{O}C) water saturated butanol extract
Amylose (%)	28.4 ± 2.8	Concanavalin A method
Amylose (%)	28.2 ± 0.8	Iodine binding method
Phosphorus (mg/g)	0.65 ± 0.08	
Lysophospholipid (mg/g)	10.6	Phosphorus x 16.3*
Potassium (μg/g)	21.5 ± 1.7	
Sodium (μg/g)	21.6 ± 2.2	
Calcium (μg/g)	93.1 ± 13.9	
Magnesium (μg/g)	44.5 ± 11.0	
***Kofler* gelatinisation temp. range (^{O}C): To-Tp-Tc**	68.0-74.0-80.0	
DSC gelatinisation endotherms range: To, Tp and Tc in ^{O}C, and ΔH in J/g	63.8-65.4, 70.2-71.3, 81.3-81.5 and 2.28-7.22	

Table 4.1. Continued

Pasting properties: Ti (^{O}C), PV (RVU), Pt (min), HPV (RVU), BV (RVU), Rst (RVU/min), CPV (RVU), SBV (RVU)	74.0 ± 1.1, 269 ± 13, 4.19 ± 0.62, 190 ± 13, 79 ± 17, 8.4 ± 1.8, 292 ± 14, 101 ± 11	Where: Ti, PV, Pt, HPV, BV, Rst, CPV, and SBV are pasting temperature, peak viscosity, time to peak viscosity, hot paste viscosity, breakdown viscosity, rate of shear thinning, cold paste viscosity and setback viscosity, respectively
Peak viscosity	Medium–low	
Resistance to shear	Medium–high	
Retrogradation tendency	Medium–low	
Crystallinity (%)	37	
X-ray diffraction: *d* value (Å) and intensity (%)	5.85 (83.8), 5.16 (97.0), 4.89 (99.4), 4.41 (36.4) and 3.84 (80.2)	A type starch crystalline polymorph
Water absorption index (%)	108 ± 4	
Water solubility index (%)	0.34 ± 0.08	
Swelling factor (SF): 50, 55, 60, 65, 70, 75, 80, 85 and 90 ^{O}C	1.07 ± 0.20, 1.22 ± 0.08, 1.48 ± 0.05, 3.24 ± 0.37, 5.97 ± 0.15, 7.48 ± 0.44, 8.66 ± 0.10, 8.16 ± 0.12, 7.54 ± 0.02	SF by Blue Dextran method
Amylose leaching (AML) (%): 50, 55, 60, 65, 70, 75, 80, 85, 90 and 95 ^{O}C	0.06 ± 0.02, 0.07 ± 0.02, 0.11 ± 0.01, 1.32 ± 0.22, 2.62 ± 0.18, 5.09 ± 0.35, 6.31 ± 0.29, 6.99 ± 0.15, 7.86 ± 0.11, 9.72 ± 0.21	AML by iodine binding method
Paste clarity. Transmittance (%) of 0.1 % starch	27.5 ± 2.4 (opaque)	In glass cuvettes using Perkin Elmer Lambda EZ 150 spectrophotometer
Paste texture	Short	
Gel texture: Firmness in grams (g)	724.6 ± 93.3	
: Adhesiveness in negative force area (g.s)	31.8 ± 0.7	

Table 4.1. Continued

Gel syneresis (%) at 4 ^{O}C storage of 3, 7, 10 and 21 days	6.6 ± 0.2, 12.9 ± 0.4, 21.0 ± 0.5, 31.5 ± 0.7	Lower syneresis than maize starch
Gel syneresis (%) at -18 ^{O}C storage of 3, 7, 10 and 21 days	3.9 ± 0.3, 5.9 ± 0.3, 9.3 ± 0.4, 14.2 ± 0.4	Lower syneresis than maize starch
Freeze–thaw stability (%) (1st, 2nd and 3rd cycle)	3.9 ± 0.2, 6.9 ± 0.3, 11.9 ± 0.5	More stable than maize starch
***In vitro* digestibility (%) with Porcine pancreatic α-amylase enzyme at : 0.5, 1, 2, 3, 4 and 5 h.**	4.7 ± 0.7, 10.1 ± 1.3, 16.3 ± 1.1, 22.6 ± 1.5, 29.5 ± 0.9, 32.6 ± 2.2	Surface erosion and endocorrosion in nature.
Acid hydrolysis with 2.2 M HCl at: 1, 2, 3, 4, 8, 12, 16, 20 and 24 days	2.0 ± 0.0, 2.6 ± 0.2, 3.6 ± 0.2, 4.8 ± 0.2, 7.9 ± 0.1, 11.3 ± 0.4, 13.8 ± 0.4, 18.3 ± 0.6, 22.0 ± 1.1	Slightly more etched than maize starch

Where: To is onset, Tp is peak, Tc is conclusion gelatinisation temperatures in ^{O}C, and ΔH is gelatinisation enthalpy in J/g.
* Assuming that tef starches are weakly phosphorylated.

University of Pretoria etd – Bultosa Beri G 2003

amylose of potato > cassava > wheat > rice > maize, whereas amylopectin of cassava > rice (japonica) > potato > maize > wheat > rice (indica) (Swinkels 1985; Champagne, 1996). These differences can fundamentally influence the amylose and amylopectin to display different starch functionality (Fredriksson et al. 1998). For instance, due to the smaller *dp* of amylose in maize starch, it is more prone to gelation than other cereal starches (Swinkels, 1985). The tendency of tef starch to gelation is less than maize starch and this suggests that the *dp* of amylose in tef starch is probably larger than the maize starch. The smaller swelling factor, smaller proportion of % crystallinity, and smaller gelatinisation enthalpy of tef starches than the maize starch are indicative of higher proportion of short A chain amylopectin in the tef starches than the maize starch. Thus, this may in part have contributed to the observed small swelling power, low thickening power and slow retrogradation functional properties of the tef starch.

The high level of lipids in tef starches could affect the degree of swelling, gelatinisation, pasting and retrogradation properties, since such physico–chemical properties in other cereal starches are influenced by their starch lipids, presumably due to amylose–lipid complex formation (Vasanthan and Hoover, 1992 b). Lipids can also act as barriers in starch chemical modification reactions (Baldwin, Melia and Davies, 1997). On processing and storage of a product where tef starch was an ingredient, contribution to rancid flavour may take place, most probably due to oxidation of the unsaturated fatty acid moiety of surface starch lipids, since internal starch lipids are resistant to oxidation (Swinkels, 1985). This can make the usage of tef starches in delicately flavoured foods such as dairy products or in lightly flavoured puddings less attractive because such products demand the use of bland tasting starches like cassava (Thomas and Atwell, 1999). When lipid containing starches like wheat and maize are hydrolysed for starch hydrolysates (maltodextrins, glucose, maltose and fructose syrups) they demand lipid filtration to purify the hydrolysates (Galliard and Bowler, 1987). If tef starch is considered for such applications the same may hold true. However, lipids can also play a positive role by reducing shear induced starch breakdown during pasting (Vasanthan and Hoover, 1992 b) and by slowing starch retrogradation tendencies (Gudmundsson, 1994). In this study, similar effects were also observed, i.e. tef starch breakdown and retrogradation tendencies were low.

University of Pretoria etd – Bultosa Beri G 2003

SEM of isolated tef starch granules showed that essentially all protein bodies had been removed during extraction, which suggests that the majority of proteins determined as crude protein (mean 0.19 %) are most probably contributed by SGAP (starch granule-intrinsic proteins). As reviewed by Baldwin (2001) the SGAP are known to affect the swelling, gelatinisation and pasting properties of starch. The SGAP were also reported to reduce shear induced starch breakdown and to support the structure of gelatinised starch (Han et al. 2002 a). In starch hydrolysate syrups, the amino acids of SGAP may react via Maillard reaction with reducing sugars to impart unwanted flavour, colour and odour to the syrups (Baldwin, 2001). If in future, tef starch is processed for such products it will probably face the same challenge.

During the baking of tef *injera,* tef starch is reported to be completely gelatinised, forming a spongy texture matrix (Parker et al. 1989). The tef SGAP can probably, through close association with amylopectin, participate in the spongy starch matrix texture of *injera*. This is because it was suggested that, in other starches, the SGAP will support the structure of gelatinised starches through possible peptide cross links with amylopectin fraction (Han et al. 2002 a). In the air dried *injera* called *dirqoosha* (a traditional Ethiopian food usually prepared for travelers with no access to fresh *injera*), the tef starch looks like pregelatinised starch (personal observation). This is because *dirqoosha* can be preserved for a long time and when wetted in hot or cold water it will almost attain the sensory qualities of the original *injera*. The tef SGAP may also support the gelatinised structure of tef starch in *dirqoosha*. However, at present there is no literature describing the details of the properties of tef starch in *dirqoosha* and the role of the tef SGAP.

The functionality of tef starches for a given food product and related application can be predicted from its paste, gel and retrogradation properties. The thickening power (peak viscosity) of tef starch is lower than maize starch and appears to be slightly higher than reported for rice starch (Tan and Corke, 2002), but the viscosity breakdown and setback are smaller than that of maize and rice starches. When starches are selected for different food applications (e.g. cakes, sauces, soups, custards, pie fillings, deserts, dairy products), in addition to their qualities of thickening power, their suitability to high temperature, high shear and high acid food

University of Pretoria etd – Bultosa Beri G 2003

processing conditions must be considered (Thomas and Atwell, 1999). The stability of the starch towards distribution and storage conditions is also important (Thomas and Atwell, 1999). Comparing the data of Deffenbaugh and Walker (1989) and Beta et al. (2000), starch cooking duration can be ordered as: wheat > maize > sorghum > potato > cassava starches. This will place the cooking duration of tef starch between wheat and maize starches. The cooking duration can affect the starch gelatinisation (Thomas and Atwell, 1999). Such information is useful for example in tef cake preparation, since the cake cell structures are in part influenced by the extent of starch gelatinisation (Thomas and Atwell, 1999). The tef starch paste/gel is opaque and slightly less white than the maize starch paste. This makes tef starch a possible candidate for spoonable salad dressing applications, since the starch used in a spoonable salad dressing should be opaque (Thomas and Atwell, 1999). However, in for example thickened fruit pie– filling, tef starch would be less preferred since starches used in such a product should be translucent as the colour of the product is preferred to be the colour coming from the fruit (Thomas and Atwell, 1999). Based on comparison with data given in Kuhn and Schlauch (1994), Walker, Ross, Wrigley and McMaster (1988), and Tan and Corke (2002) and the results of this work, the breakdown viscosity of native starch can be ordered as: potato < waxy maize < cassava < wheat < maize < rice< tef. In this aspect tef starch looks more suitable for use in food products processed under high shear conditions like in a salad dressings (Thomas and Atwell, 1999) than many other starches.

Tef starch paste can be regarded as a gel forming system rather than a close-packed system. This is because tef starch granule swelling capacity is small and in low swelling capacity starches like wheat the system is regarded as gel forming, whereas in high swelling capacity, starches like potato the system is regarded as a close-packed system (Hermansson and Svegmark, 1996). In a starch paste of a gel forming system amylose leaching is not restrained and the leached amylose can form a continuous gel phase outside of the granule, which on shearing of such system a short consistency is achieved, contrary to long consistency for close-packed systems (Hermansson and Svegmark, 1996). As the consistency of tef starch paste was found to be short and smooth, it makes it a suitable candidate as a thickening agent in puddings and sauces (Thomas and Atwell, 1999). The leached amylose in a gel

University of Pretoria etd – Bultosa Beri G 2003

forming system can also interact with aroma compounds, emulsifiers, lipids, proteins and other polysaccharides. This can influence the stability and sensory perception of a food product (Hermansson and Svegmark, 1996). Thus, in the tef starch applications such impact is expected.

The low syneresis of tef starches under refrigeration, freeze and freeze-thaw treatments indicate a slower retrogradation than maize starch. Since tef starch retrogradation is slow, its application in the production of type III resistant starch (Alonso et al. 1999) might not be attractive. The slow retrogradation makes tef starch desirable for use in products such as cream soups, sauces, pie-fillings and baked products where staling is a problem (Whistler and BeMiller, 1997).

4.3. Future research needs

In this thesis characterisation of the starches of five grain tef varieties in terms of morphology, composition (amylose/amylopectin ratio, lipids, proteins, phosphorus and trace microelements), physico-chemical proprieties (X-ray diffraction, swelling, amylose leaching, gelatinisation, pasting, paste clarity, gel texture, retrogradation) and modification of native starch granules with α-amylase and mild acid treatments has been reported.

The amylose and amylopectin molecular size and *dp* influence the fundamental physico-chemical properties of a starch to a large extent. Thus, future work on the structural characterisation of amylose (molecular size, *dp*, degree of branching) and amylopectin (molecular size, chain length distribution and its *dp*) could give a better understanding of the links between structure, physico-chemical properties and functionality. Lipids reported in this work were determined gravimetrically. Because of this, further characterisation of the nature of free fatty acids and phospholipids by other complementary techniques e.g. the use of thin layer chromatography (TLC) and gas chromatography (GC) is required to give a more comprehensive picture as to the nature of tef starch lipids. The SGAP are implied as one factor that influences properties of gelatinised starches. Thus future work on tef SGAP identity, structure and functionality is required. The nature of phosphorus (whether it is as phospholipid, phosphorylated to glucose moieties and/or inorganic phosphate) in the starch can

University of Pretoria etd – Bultosa Beri G 2003

influence swelling, paste clarity and degree of starch crystallinity. Determination of the nature of phosphorus in tef starch by the use of ^{31}P nuclear magnetic resonance spectroscopy is required.

Starches can offer functionality to food and in non–food applications as: granular, gelatinised, paste, gel, retrograded, enzyme– and acid–modified, heat and moisture treated forms. The morphology and physico-chemical properties characterised can give directions where potential utilisation of tef starch seems better than the use of other native starches. The tef starch granule size is very small, of smooth shape and gives short and smooth paste texture. Relative to the maize starch, it is shear tolerant and has slow retrogradation tendency. Thus, application study in food: as fat mimetic (in baked products, cheese sauces, frozen desserts and mayonnaise like products), in spoonable salad dressings, sugar coating in confectionery and encapsulating agents (flavours, aromas and vitamin enrichments) should be investigated. In non–food industries, study for application like as cosmetic dusting agent, carrying agent for pharmaceutical tablets and as a laundry stiffening agent, paper and photographic paper powder will also be important.

Similar starch characterisation of some other grain tef varieties, at least on those varieties intensively cultivated for food usage in Ethiopia is important to get more information on the range of properties of tef starch. This is important because by investigating the properties of different tef varieties, discovery of waxy– or amylo–type starches in tef varieties may be possible.

Research toward an appropriate technology for tef starch extraction (starch recovery, negligible granule damage and commercially acceptable purity) to optimise the economic utility of tef starch extraction is also important.