# 3. RESEARCH

## 3.1. Physico-chemical Characterisation of Grain Tef [*Eragrostis tef* (Zucc.) Trotter] Starch

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## *Abstract*

Starch isolated from five grain tef (*Eragrostis tef*) varieties was characterised and compared with commercial maize starch. The granules are compound comprising many polygonal shape (2-6 μm in diameter) simple granules. The crude composition is similar to the normal native cereal starches. The starch amylose content range from 24.9-31.7 %. Gelatinisation temperature range was 68.0-74.0-80.0 $^{o}$C, typical of tropical cereal starches, and resembling rice. The mean intrinsic peak viscosity (269 RVU), breakdown viscosity (79 RVU), cold paste viscosity (292 RVU) and setback viscosity (101 RVU) determined were considerably lower than that of maize starch. Tef starch has higher water absorption index (WAI) (mean 108 %) and lower water solubility index (WSI) (mean 0.34 %) than the maize starch.

### 3.1.1. Introduction

Tef [*Eragrostis tef* (Zucc.) Trotter] is a $C_4$ tropical cereal (Ketema, 1997). It is cultivated as a major cereal in Ethiopia and represents 20 % (two million tons) of the total cereal production of the country (Central Statistical Authority, 1997). Foods from grain tef are staples for the majority of Ethiopians (Ketema, 1997). Tef flour is widely used for making *injera* (fermented, pancake-like sour bread), traditional alcoholic drinks like *tella* (opaque beer) and *katikalla* (local spirit), *kitta* (sweet dry unleavened bread), *muk* (gruel) and porridge.

The grain tef is very small and uniform in size (1.0-1.2 mm in length) (Umeta and Parker, 1996). It comes in a range of colours from milky white to almost dark brown. The most common colours are very white, white, light brown and dark brown. A complete nutritional composition, including vitamins, essential amino acids and micronutrients of the grain is given in a publication of the National Research Council of the USA (NRC) (1996). Protein, fat, ash and carbohydrate are given as 9.6 %, 2.0 %, 2.9 % and 73.0 %, respectively. The amino acid composition of grain tef is reported to be comparable to that of egg, except for its lower lysine content (Jansen, Dimaio and Hause, 1962). The micro- and macronutrients of grain tef are apparently higher than barley, wheat and sorghum (Mengesha, 1966). The nutrient composition of grain tef indicates that it has good potential to be used in other foods and beverages worldwide.

Starch is the largest proportion in the carbohydrate of grain tef (Umeta and Faulks, 1988). Matrix change of starch was reported to be a major contributor to the texture of *injera* (Parker, Umeta and Faulks, 1989). During the baking of *injera*, starch is completely gelatinised to form a steam–leavened, spongy matrix, in which fragments of bran, embryo, micro–organisms and organelles are embedded. An anatomical study of tef grain has revealed that it contains compound starch granules (Umeta and Parker, 1996), similar to those of rice (Juliano, 1984) and amaranthus (Zhao and Whistler, 1994 b). The pericarp of the grain also contains starch granules like sorghum (Umeta and Parker, 1996). From the compound starch granules, individual starch granules of size between 2–6 μm in diameter are released on milling (Umeta and Parker, 1996). To date, information on the physico–chemical properties of grain tef starch is very

limited. Therefore, in this work, starch composition, granule morphology, gelatinisation temperature, pasting properties, water absorption and water solubility indexes are reported.

### 3.1.2. Materials and Methods

#### 3.1.2.1. Samples

Four Ethiopian grain tef varieties were obtained from 1999 harvest at tef improvement programme of Debre Zeit Agricultural Research Centre, Debre Zeit, Ethiopia: DZ-01-196 (very white in colour and fairly large grain), DZ-01-99 (deep brown in colour), DZ-Cr-37 (white in colour and a three way cross breed) and DZ-01-1681 (light brown in colour). One South African grain tef variety, South African Brown (deep brown in colour) produced in South Africa, ex. grain trader was obtained from Agricultural Research Council (ARC) of South Africa, Pretoria, South Africa. Maize starch (Merck UniLAB, code: 587 14 00) was analysed for comparison.

#### 3.1.2.2. Starch granule extraction

Starch granules were extracted according to Taylor Dewar, Taylor and von Ascheraden (1997) using only distilled water. The grain was sieved to remove extraneous foreign material. It was then milled in a laboratory hammer mill fitted with 800 μm screen. Flour (20 g) was mixed with 100 mL distilled water to form a slurry. This was stirred at intervals for over one h., then passed through a wet mill (Retsch, Haan, Germany) with a 250 μm screen. The liquid containing the starch was retained and the fibrous residue on the screen was discarded. The resulting liquid suspension was filtered through a 100 μm sieve. The throughs were centrifuged in 100 mL glass centrifuge tubes at 800 x g for 2 min. After the supernatant was decanted off, the brown protein layer was scraped off. The starch pellet obtained was re-suspended in distilled water and centrifuged again. After decanting off the supernatant, the protein layer was again scraped off. This procedure was repeated until apparently pure starch pellet (white colour) was obtained. The purity was checked by light microscopy. The starch pellet was then dried at 50 $^{o}$C in a vacuum oven. The dried starch mass was reduced gently using mortar and pestle to avoid granule damage and the milled sample was characterised.

### 3.1.2.3. Scanning electron microscopy (SEM)

Whole grains were plunge–frozen in liquid nitrogen, fractured, and freeze dried at -80 $^{O}$C. Dried samples were mounted and sputter–coated with gold in a Polaron E 5200 coating unit (Polaron Equipment, Watford, England) to a thickness of ± 20 nm. Starch granules were mounted on aluminum stubs covered with double–sided tape. Mounted starch granules were sputter–coated as above. Prepared samples were then viewed in a JEOL JSM 840 scanning electron microscope (JEOL, Tokyo, Japan) operated at an accelerating voltage of 5 kV. Starch granules were measured with the use of the calibrated scale bar on the micrograph.

### 3.1. 2.4. Proximate analysis

Moisture, ash and crude fat contents were determined according to Approved Methods: 44-15A, 08-17 and 30-25, respectively of the American Association of Cereal Chemists (1983). Crude protein (N x 6.25) was determined by the Dumas method.

### 3.1.2.5. Amylose/amylopectin ratio

Amylose/amylopectin ratio was determined using a Megazyme amylose/amylopectin assay kit (Megazyme International Ireland, Bray Business Park, Bray, Ireland) of selective quantitative precipitation reaction of concanavalin A (Con A) with amylopectin according to Gibson, Solah and McCleary (1997) and by colorimetric method of iodine binding with amylose according to Chrastil (1987).

### 3.1.2.6. Gelatinisation temperature

A monolayer starch slurry (1 % in glycerol: water, 1:5) was prepared on a microscope slide. Starch gelatinisation temperature range: To (onset), Tp (peak) and Tc (conclusion) was measured by birefringence loss according to Atwell, Hood, Lineback, Varriano–Marston and Zobel (1988) using a hot stage on a polarised light microscope system.

### 3.1.2.7. Pasting properties

Starch pasting properties were evaluated using a Rapid Visco–Analyser (RVA model 3D, Newport Scientific, Sydney, Australia). Starch (2.8 g, db) was suspended in

distilled water and adjusted to a total weight of 28 g. The sample was equilibrated at 50 $^{O}$C for 1 min, heated to 93 $^{O}$C in 7.5 min at a rate of 5.7 $^{O}$C/min, held at 93 $^{O}$C for 5 min, cooled to 50 $^{O}$C in 7.5 min at a rate of 5.7 $^{O}$C/min. From the resulting pasting curve, temperature at initial viscosity increase ($T_i$), peak viscosity (PV), time to peak viscosity ($P_t$ ) (time at PV - time at initial viscosity increase), hot paste viscosity (HPV), breakdown viscosity (BV) (PV-HPV), rate of shear thinning ($R_{st}$) (PV-HPV)/13.5 min- $P_t$), cooled paste viscosity (CPV), and setback viscosity (SBV) (CPV-HPV) were calculated.

#### 3.1.2.8. Water absorption index (WAI)

Water absorption index was determined according to Anderson, Conway, Pfeifer and Griffin (1969).

#### 3.1.2.9. Water solubility index (WSI)

The supernatant from WAI measurement evaporated at 105 $^{O}$C overnight, and the WSI value was calculated as dry residue divided by the total substance in the original 2.5 g WAI sample and expressed as a percentage (Anderson et al. 1969).

#### 3.1.2.10. Statistical analysis

Data were analysed using Statistica for Windows (Statsoft, Tulsa, USA, 1995). One–way analysis of variance (ANOVA) was performed to determine the difference in properties among the starch varieties. Means were then compared at $p < 0.05$ using Fisher's least significant difference (LSD) test.

### 3.1.3. Results and Discussion

#### 3.1.3.1. Starch granule structure

Scanning electron micrographs of individual tef starch granules and compound starch granules are shown in Figures 3.1 and 3.2, respectively. The individual tef starch granules are packed as a compound starch granules (Figure 3. 2) and are polygonal in shape (2–6 μm in diameter) (Figure 3.1) as reported by Umeta and Parker (1996). Most common sizes are of 3 - 5 μm.

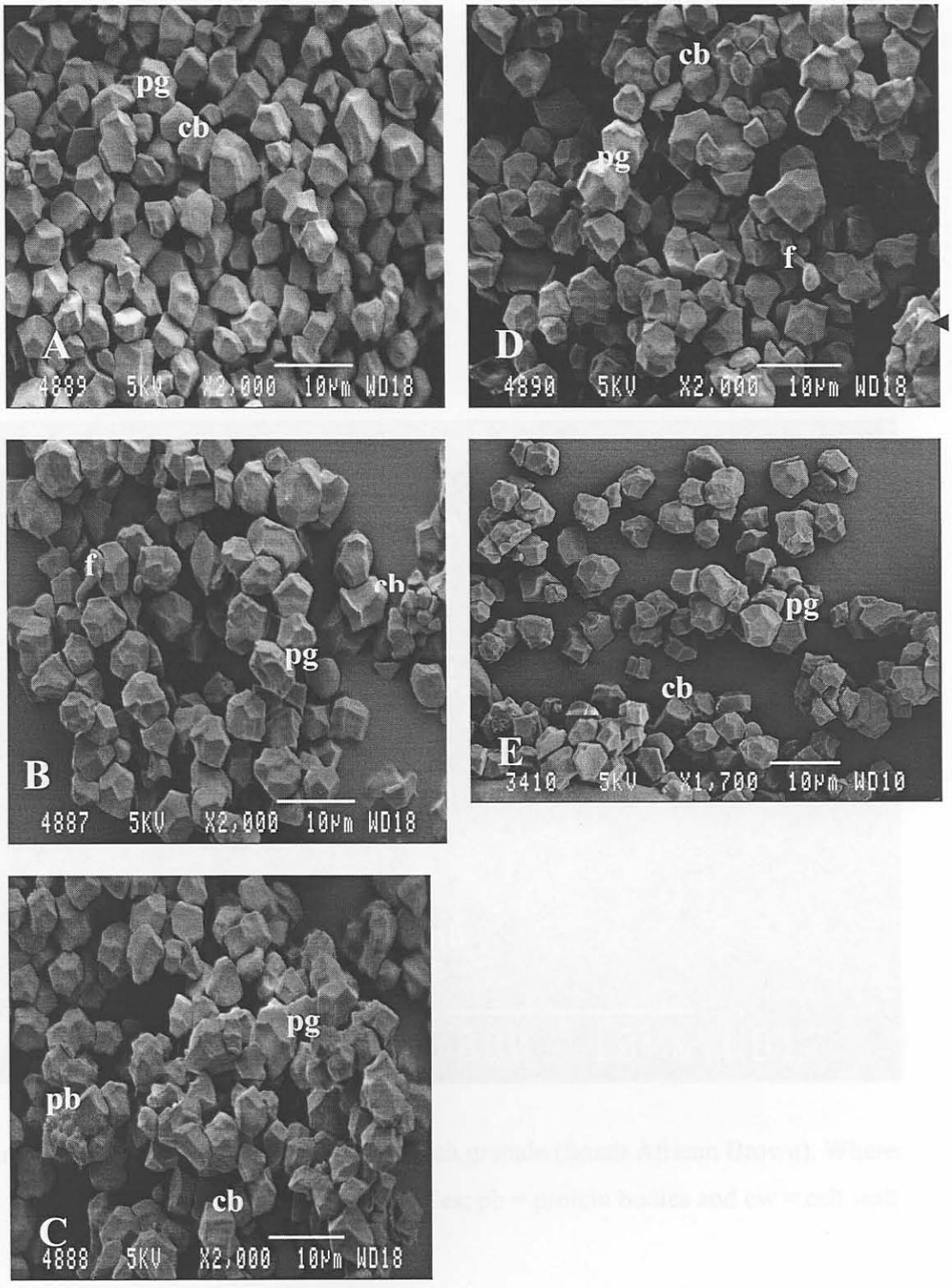


Figure 3.1. SEM of individual starch granules from different tef varieties (A = DZ-Cr-37, B = DZ-01-1681, C = DZ-01-196, D = DZ-01-99 and E = South African Brown). Where: pg = polygonal shape starch granules with a number of sides; cb = cubic shape starch granules; pb = protein bodies and f = fibre

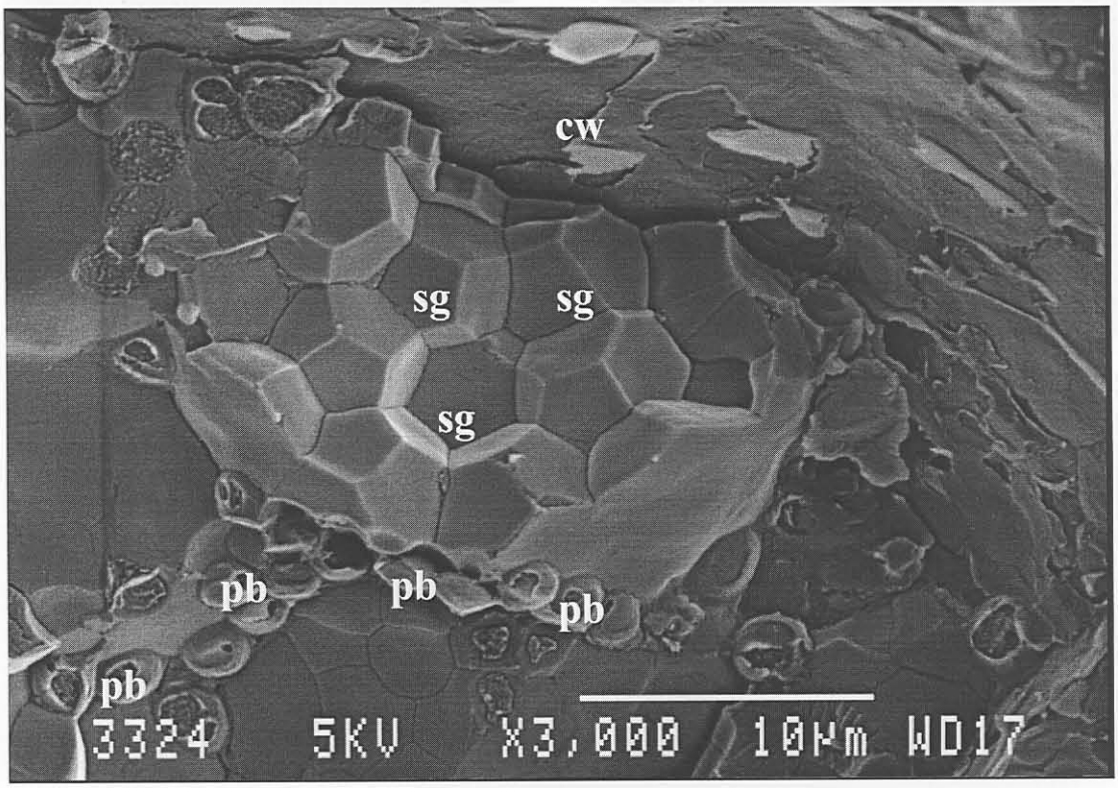


Figure 3.2. SEM of a tef compound starch granule (South African Brown). Where: sg is individual starch granules; pb = protein bodies and cw = cell wall

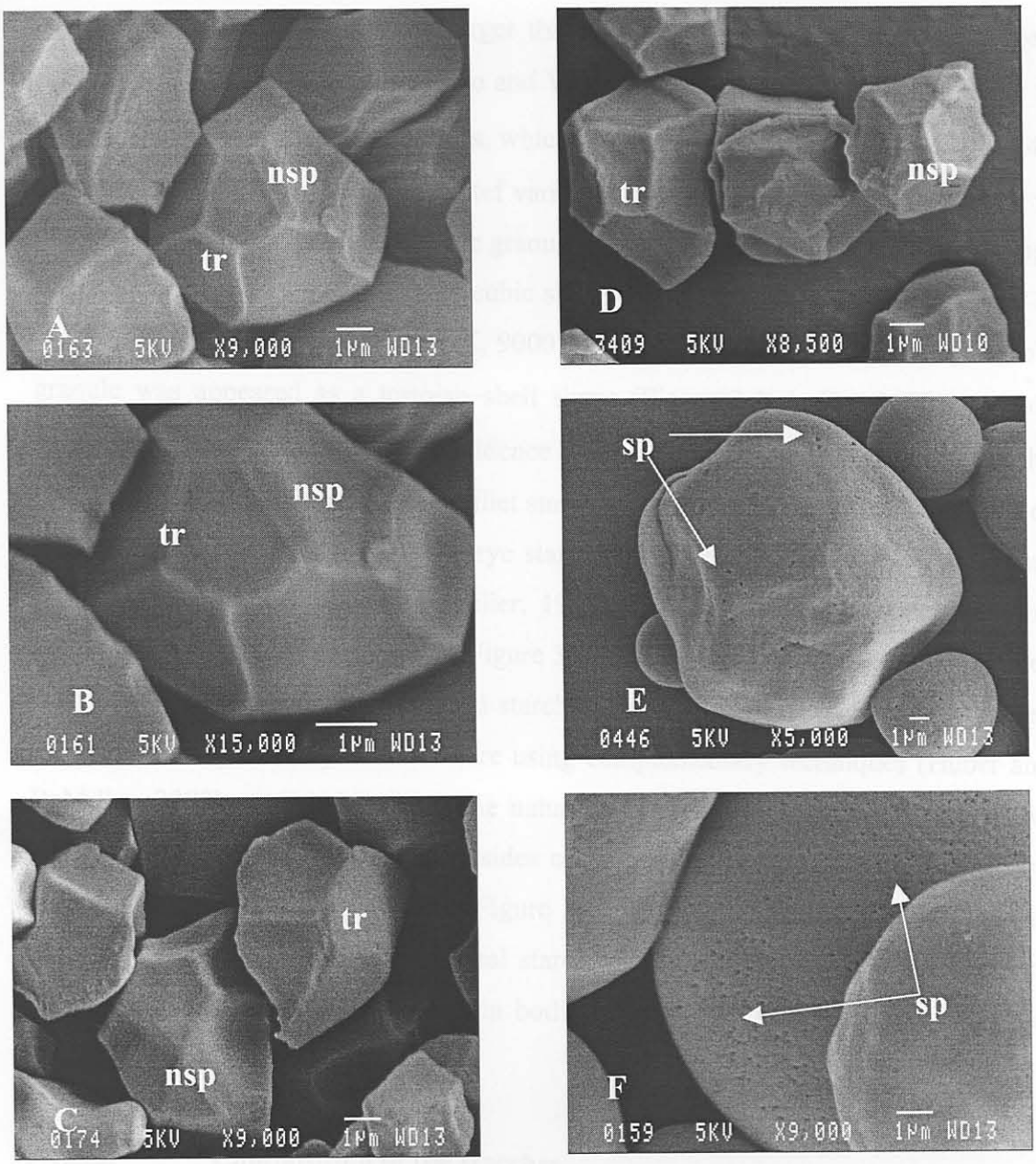


Figure 3.3. SEM of starches from different tef varieties and maize at different magnification ranges (5000 X–15000 X). A and B = DZ-01-196, C and D = South African Brown, E and F = Maize. Where: tr = tortoise-shell shape, nsp = no surface pores and sp = surface pores

The granule size is thus slightly larger than individual amaranthus starch granules, which are 1-2 μm in diameter (Zhao and Whistler, 1994 b). They are comparable in size to individual rice starch granules, which are 3-5 μm in diameter (Juliano, 1984). The starch granules in the different tef varieties appeared morphologically similar to one another (Figure 3.1). Most of the granules had a number of sides (Figure 3.1. pg) while a few of them had essentially cubic shape (Figure 3. 1.cb). In some granules at higher SEM magnifications (8500 X, 9000 X and 15000 X) the shape of the whole granule was appeared as a tortoise–shell shape (Figure 3.3 A–D, tr). The granule surface appeared smooth with no evidence of pores (Figures 3.3 A–D, nsp). On the surface of maize, sorghum and the millet starches, and along the equatorial grooves of large granules of wheat, barley and rye starches the presence of surface pores were reported (Fannon, Hauber and BeMiller, 1992). In this work also surface pores on maize starch granules were evident (Figure 3.3 E and F, sp). The presence of cavities at the hilum of potato, rice and wheat starch granules was reported (Baldwin, Adler, Davies and Melia, 1994). In the future using complementary techniques (Huber and BeMiller, 2000) more evidence on the nature of tef starches surface, which support this finding might be achieved. The sides of the starch granules where other starch granules packed were well formed (Figure 3.2. sg). There is no evidence of strong attachment between adjacent individual starch granules within the compound starch granule (Figure 3.2. sg). Most protein bodies are located outside of the compound starch granules (Figure 3.2. pb).

### 3.1.3.2. Composition of the starches

The composition (ash, protein, fat and amylose), WAI and WSI of the starches of the different varieties are shown in Table 3.1. The starches had ash contents in the range of 0.13-0.23 %. With the exception of DZ-01-196 starch, there was no significant difference ($P > 0.05$) in their ash contents. Cereal starches have a lower amount of minerals (determined as ash) bound to the phosphate ester group when compared to potato starch (Swinkels, 1985; Whistler and BeMiller, 1997). The ash content determined was comparable to that of typical cereal starch ash (0.1-0.2 %) as reported by Swinkels (1985).

The mean protein content of the tef starches (0.19 %) was higher than that of the

maize starch (0.07 %). This is probably because in commercial maize starch extraction, $SO_2$ is used, which breaks disulphide bonds solubilising protein (Watson, 1984). Tef starches had protein contents in the range of 0.16-0.23 %. The highest protein was recorded for South African Brown starch. The lowest was recorded for DZ-01-196. Protein in the range of 0.06-0.40 % has been reported (Watson, 1984; Swinkels, 1985; Whistler and BeMiller, 1997) for other cereal starches. The protein content among the tef varieties probably varied depending upon the degree of contamination of the starch by the proteins of the endosperm and by the proteins of a residual starch synthase enzyme (Whistler and BeMiller, 1997).

The crude fat (ether extract) content of the tef starches (mean 0.29 %) was relatively low as compared to that of the maize starch (0.34 %). The crude fat of grain maize is around 4.45 % (db) (Watson, 1984), higher than that of grain tef which is around 2.00 % (db) NRC (1996). Crude fat (petroleum ether extract) is mostly non–starch lipid i.e., not endogenous to the starch (Morrison, Tan and Hargin, 1980). The low crude fat content in tef starch is most probably related to the low crude fat content of the grain.

The mean amylose content of the tef starches was 28.4 % when analysed by the Con A method of Gibson et al. (1997) and was 28.2 % when analysed by iodine binding of Chrastil (1987). For the maize starch it was 29.5 % and 27.8 %, respectively. The amylose content of DZ-01-196 was the highest by both methods (Table 3.1). The lowest amylose content was for DZ-Cr-37 by both methods. South African Brown starch contained virtually the same amylose content as that of DZ-01-196 when analysed by iodine binding. The amylose contents of tef starch varieties DZ-Cr-37 and South African Brown of relatively higher protein contents were relatively higher when analysed by method of Chrastil (1987) than Gibson et al. (1997). This is possibly because trichloroacetic acid (a protein precipitant) is used in the method of Chrastil (1987) and thereby it suppressed the protein interference of amylose determination. Both methods showed that amylose content of the tef varieties studied to be typical of normal native cereal starches like maize, sorghum and wheat (Morrison and Laignelet, 1983; Beta et al. 2000) with no waxy– or amylo– type starches. Properties such as gelatinisation and gelation characteristics (Leloup, Colonna and Buléon, 1991),

solubility and the formation of resistant starch (Sievert and Pomeranz, 1989) are dependent on amylose/amylopectin ratios. Thus, the small variations in the amylose content among tef varieties may influence also the starches to have slightly different properties.

### 3.1.3.3. Gelatinisation temperature

Mean onset (To), peak (Tp) and conclusion (Tc) gelatinisation temperatures of tef starch were 68.0-74.0-80.0 $^{O}$C, respectively (Table 3.1). For the maize starch the values were 65.0-73.0-80.0 $^{O}$C, respectively. Tef starch gelatinisation temperature is thus similar to tropical cereal starches and resembling most closely rice starch 68.0-74.5-78.0 $^{O}$C (Snyder, 1984). The range is somewhat narrower when compared to that for the maize starch 65.0-73.0-80.0 $^{O}$C. Starch gelatinisation is an irreversible process and includes granule swelling, native crystallite melting, loss of birefringence and starch solubilisation (Atwell et al. 1988). Under uniform experimental conditions, point of initial gelatinisaton and the range it occurred is governed by the strength of the miceller networks and heterogeneities within the granule population (Atwell et al. 1988). The narrow gelatinisation temperature range observed for tef starch as compared to the maize starch is probably related in part to its relatively more uniform granule size distribution (2–6 μm in diameter) than the maize starch granules (5–30 μm in diameter) (Whistler and BeMiller, 1997). Because in wide size range granules like wheat (2–55 μm in diameter, 52–85 $^{O}$C) (Whistler and BeMiller, 1997) and barley (0.9–44.9 μm in diameter, 52.0–69.7 $^{O}$C) (Tang, Ando, Watanabe, Takeda and Mitsunaga, 2001) the range of gelatinisation temperature is also wide.

**Table 3. 1. Composition (ash, protein, crude fat and amylose), gelatinisation temperature, water absorption index (WAI) and water solubility index (WSI) of starches from different tef varieties and maize**

| Variety | DZ-01-99 | DZ-01-196 | DZ-01-1681 | DZ-Cr-37 | South African Brown | Mean (Tef) | Maize |
|---|---|---|---|---|---|---|---|
| Granule size [μm] | 2–6 | 2–6 | 2–6 | 2–6 | 2–6 | 2–6 | 5–30 |
| Ash [%] (db) | $0.16^{a}$±0.02 | $0.23^{b}$ ± 0.01 | $0.16^{a}$±0.00 | $0.13^{a}$±0.02 | $0.13^{a}$±0.02 | 0.16±0.04 | $0.12^{a}$±0.03 |
| Protein [%](N x 6.25) (db) | $0.17^{b}$± 0.01 | $0.16^{b}$± 0.01 | $0.19^{c}$± 0.00 | $0.22^{d}$± 0.01 | $0.23^{d}$± 0.01 | 0.19±0.03 | $0.07^{a}$± 0.01 |
| Crude fat [%](db) | $0.32^{bc}$± 0.01 | $0.31^{b}$± 0.01 | $0.25^{a}$± 0.03 | $0.29^{b}$± 0.02 | $0.26^{a}$± 0.02 | 0.29±0.03 | $0.34^{c}$± 0.01 |
| Amylose [%][1] | 30.1 $^{bc}$± 0.6 | $31.7^{c}$ ± 1.6 | $28.8^{b}$± 0.5 | $24.9^{a}$± 0.8 | $26.3^{a}$± 1.1 | 28.4±2.8 | $29.5^{c}$± 2.1 |
| Amylose [%][2] | $28.6^{a}$±1.7 | $28.8^{a}$±1.2 | $27.4^{a}$±0.7 | $27.2^{a}$±2.2 | $28.8^{a}$±0.5 | 28.2±0.8 | $27.8^{a}$±1.4 |
| Gelatinisation temp.[°C](To–Tp–Tc) | 67.0–73.5–80.0 | 67.0–73.5–80.0 | 68.0–74.0–80.0 | 68.0–74.0–80.0 | 68.0–73.5–79.0 | 68.0–74.0–80.0 | 65.0–73.0–80.0 |
| WAI [%](db) | $103^{b}$± 3 | $109^{bcd}$± 4 | $114^{d}$± 7 | $110^{cd}$± 4 | $105^{bc}$± 2 | 108±4 | $86^{a}$± 2 |
| WSI [%](db) | $0.25^{a}$± 0.03 | $0.23^{a}$± 0.03 | $0.37^{b}$± 0.02 | $0.45^{c}$± 0.05 | $0.39^{bc}$± 0.02 | 0.34±0.08 | $0.98^{d}$± 0.06 |

Values within the same row with different letters are significantly different ($p < 0.05$) and are means of 3 determinations. Where: To is onset, Tp is peak and Tc is a conclusion gelatinisation temperatures; amylose [%][1] by the Con A method of Gibson et al. (1997) and amylose [%][2] by the iodine binding of Chrastil (1987).

### 3.1.3.4. Pasting properties

The RVA pasting curves of tef starches and maize starch are given in Figure 3.4. The viscosity parameters evaluated are shown in Table 3.2. The mean initial swelling temperature (Ti) for tef starches (74.0 $^{O}$C) was virtually identical to that of the maize starch (74.1 $^{O}$C) (Table 3.2). The different tef varieties showed no significant difference ($p > 0.05$) in their Ti. Beta et al. (2000) reported the Ti of maize starch as 73.6 $^{O}$C and for ten sorghum starches as 69.4 $^{O}$C (mean). Initial swelling temperature indicates the minimum temperature required to cook a starch (Newport Scientific, 1995). Thus, for the tef starch, this temperature (Ti) is similar to that of the maize starch, but apparently higher than sorghum starch.

The mean peak viscosity (PV) (269 RVU) of the tef starches was considerably lower than for the maize starch (313 RVU). The highest PV among tef starches was recorded for DZ-01-196 (291 RVU), and the lowest was recorded for DZ-01-99 (256 RVU). Peak viscosity indicates water-holding capacity of the starch (Newport Scientific, 1995). Peak viscosity can be affected by granule size (Fortuna, Januszewska, Juszczak, Kielski and Palasinski, 2000), molecular structure of amylopectin (Shibanuma, Takeda and Hizukuri, 1996), cross–linking, starch water concentration, lipids, residual proteins (Whistler and BeMiller, 1997) and RVA operating conditions (Batey and Curtin, 2000). Small granule size correlated positively with resistance to swelling, less swelling and less peak viscosity in wheat, potato and maize native starches (Fortuna et al. 2000; Li and Yeh, 2001) and this may apply to the case of tef starch. Tef starches took a longer time (mean Pt 4.19 min.) to reach PV than the maize starch (mean 2.90). The longest time was recorded for South African Brown starch (5.10), and the shortest was recorded for DZ-Cr-37 starch (3.43).

The mean breakdown viscosity (BV) for tef starch pastes (79 RVU) was considerably lower than for the maize starch paste (129 RVU). This is because of the small PV value of the tef starch since the hot paste viscosity (HPV) of tef starches and maize starch are approximately the same. At BV, the swollen granules disrupt further and amylose molecules will generally leach out into the solution and align in the direction of the shear (Newport Scientific, 1995; Whistler and BeMiller, 1997).

The rate of shear thinning (Rst) for all the tef starches (mean 8.4 RVU/min.) was lower than for the maize starch (12.2 RVU/min.). The highest Rst was recorded for DZ-01-1681 (10.5 RVU/min.) and the lowest was recorded for DZ-01-99 (6.6 RVU/min.). The degree of Rst is reported to be influenced by the structural network of starch molecules, morphology and rigidity of the swollen starch granules (Subramanian, Hoseney and Bramel–Cox, 1994), and starch granule associated proteins (Han, Campanella, Guan, Keeling and Hamaker, 2002 a). Higher resistance of tef starch to Rst than the maize starch is an indication of inherent lower granule deformability and swelling, since these were positively correlated to Rst resistance in other native starches (Subramanian et al. 1994; Whistler and BeMiller, 1997; Han et al. 2002 a).

The cold paste viscosity (CPV) of all the tef starches (mean 292 RVU) was considerably lower than that of the maize starch paste (344 RVU). The highest CPV was recorded for DZ-01-196 (310 RVU). The lowest was recorded for DZ-01-99 (281 RVU) and DZ-Cr-37 (281 RVU) starches. Cold paste viscosity is related to the ability of the starch paste to form a gel after cooling (Whistler and BeMiller, 1997). Gelation occurs with junction zone formation (mostly through hydrogen bonding) re–associating the hydrated and dispersed starch molecules and can vary with the botanical sources of the starch, amylose and amylose–lipid complex, amount of water, other ingredients like proteins and temperature of cooling (Lii, Shao and Tseng, 1995; Whistler and BeMiller, 1997). High amylose (linear) containing starches re–associate more readily than amylopectin (branched). However, amylose–lipid complexing reduces re–association to some extent (Whistler and BeMiller, 1997). Tef starches showed a slight trend in their CPV, viz. higher amylose contents higher CPV (Tables 3.1 and 3.2). The exception in the case of the DZ-01-99 starch could be related to the variation in the other factors.

The setback viscosity (SBV) of all the tef starches (mean 101 RVU) was considerably lower than of the maize starch (161 RVU). The highest SBV was recorded for DZ-01-

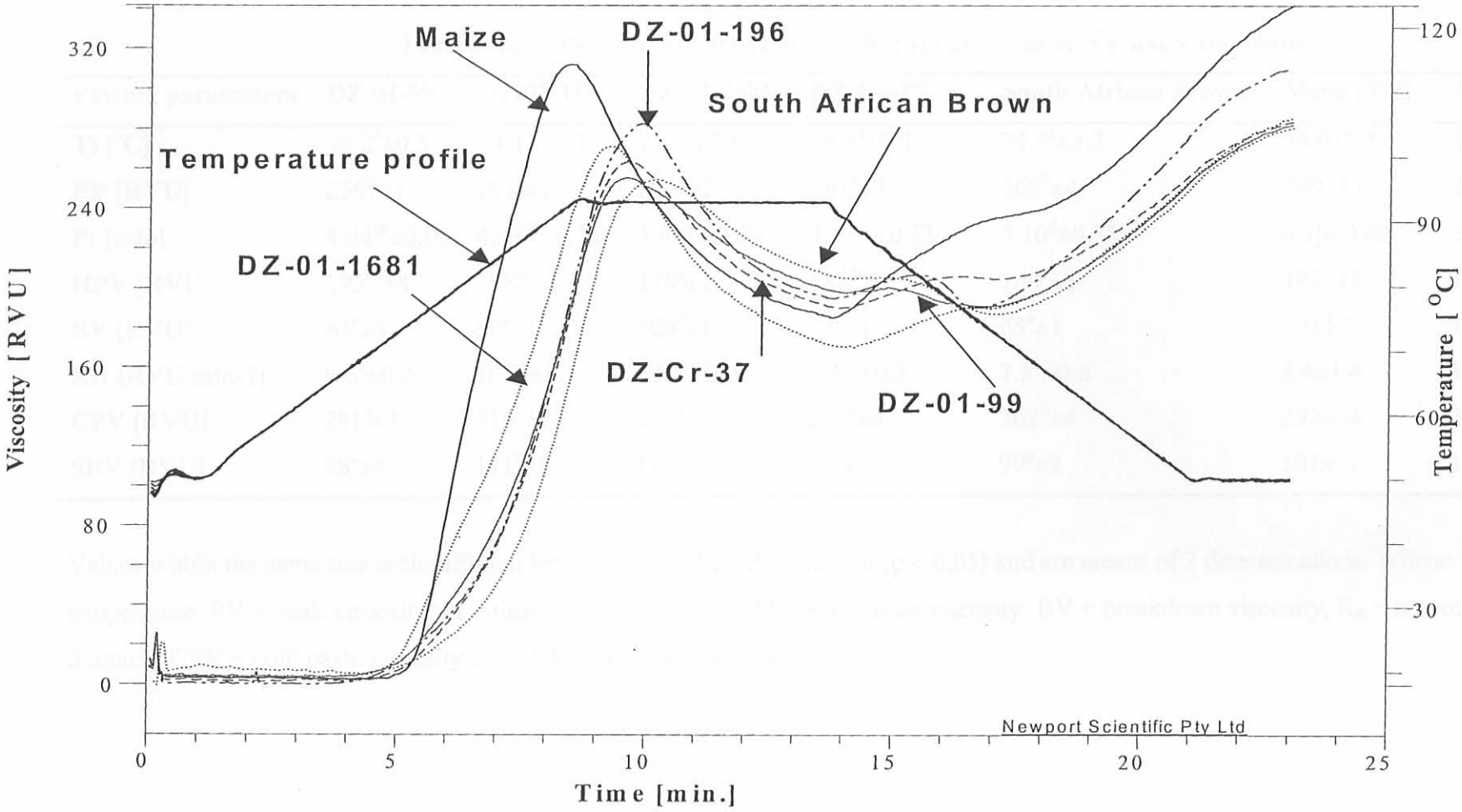


Figure 3.4. RVA pasting curves of starches from different tef varieties and maize

**Table 3. 2. Pasting properties of starch from different tef varieties and maize**

| Pasting parameters | DZ-01-99 | DZ-01-196 | DZ-01-1681 | DZ-Cr-37 | South African Brown | Mean (Tef) | Maize |
|---|---|---|---|---|---|---|---|
| **Ti [°C]** | $74.2^{a}\pm0.3$ | $74.4^{a}\pm1.0$ | $72.1^{a}\pm0.1$ | $74.8^{a}\pm0.1$ | $74.4^{a}\pm3.2$ | 74.0±1.1 | $74.1^{a}\pm0.1$ |
| **PV [RVU]** | $256^{a}\pm1$ | $291^{c}\pm2$ | $270^{b}\pm2$ | $261^{a}\pm4$ | $268^{b}\pm4$ | 269±13 | $313^{d}\pm2$ |
| **Pt [min]** | $4.04^{bc}\pm0.05$ | $4.43^{cd}\pm0.14$ | $3.94^{bc}\pm0.09$ | $3.43^{ab}\pm0.53$ | $5.10^{d}\pm0.71$ | 4.19±0.62 | $2.90^{a}\pm0.04$ |
| **HPV [RVU]** | $193^{cd}\pm4$ | $198^{de}\pm1$ | $170^{a}\pm1$ | $188^{bc}\pm2$ | $203^{e}\pm2$ | 190±13 | $184^{b}\pm2$ |
| **BV [RVU]** | $63^{a}\pm3$ | $92^{c}\pm1$ | $100^{d}\pm1$ | $73^{b}\pm1$ | $65^{a}\pm1$ | 79±17 | $129^{e}\pm3$ |
| **Rst [RVU min-1]** | $6.6^{a}\pm0.2$ | $10.2^{c}\pm0.1$ | $10.5^{c}\pm0.2$ | $7.1^{ab}\pm0.3$ | $7.8^{b}\pm0.8$ | 8.4±1.8 | $12.2^{c}\pm0.3$ |
| **CPV [RVU]** | $281^{a}\pm1$ | $310^{b}\pm9$ | $284^{a}\pm2$ | $281^{a}\pm4$ | $302^{b}\pm4$ | 292±14 | $344^{c}\pm4$ |
| **SBV [RVU]** | $88^{a}\pm4$ | $111^{b}\pm8$ | $114^{b}\pm1$ | $92^{a}\pm1$ | $99^{a}\pm2$ | 101±11 | $161^{c}\pm6$ |

Values within the same row with different letters are significantly different ($p < 0.05$) and are means of 2 determinations. Where: Ti = pasting temperature; PV = peak viscosity; Pt = time to peak viscosity; HPV = hot paste viscosity; BV = breakdown viscosity; $R_{st}$ = rate of shear thinning; CPV = cold paste viscosity and SBV = setback viscosity.

1681 (114 RVU), and the lowest was recorded for DZ-01-99 (88 RVU). The higher the SBV, the more syneresis is likely to take place (Newport Scientific, 1995). A preliminary observation on gel syneresis indicated that tef starch showed slower syneresis than that of the maize starch.

### 3.1.3.5. Water absorption index (WAI) and water solubility index (WSI)

The WAI of all the tef starches (mean 108 %) was considerably higher than that of the maize starch (86 %) (Table 3.1). The value of WAI for the maize starch is in agreement with that reported in the literature (80-95 %) (French, 1984). The highest WAI was recorded for DZ-01-1681 (114 %) and the lowest for DZ-01-99 (103 %). The minor compositional differences possibly contributed to the different tef starches in having slightly different WAI. The gel phase of the native starch granules is hydrophilic in nature (French, 1984) and is more penetrable by water and low molecular weight water–soluble solutes. Water absorption index is related to the amount and swelling degree of this gel phase (French, 1984). It reflects the extent of association of the molecules within the starch granule (French, 1984). The higher WAI of tef starch is probably also related to its smaller granule size. The smaller the size of starch granule, the more surface area and the higher the water absorption (French, 1984). The high WAI of tef starch possibly also contributes to the high volume of *injera* made from tef flour, since from the same weight of tef, maize, sorghum, wheat and barley flours, more *injera* is obtained from tef flour than the other flours (personal communication from tef improvement programme of the Debre Zeit Agricultural Research Centre, Ethiopia).

The WSI of all tef starches (mean 0.34 %) was considerably lower than that of the maize starch (mean 0.96 %) (Table 3.1). The highest WSI was recorded for DZ-Cr-37 (0.45 %). The lowest WSI was recorded for DZ-01-196 (0.23 %). Water solubility index reflects the strength of the micellar network within the starch granules (Qian, Rayas–Duarte and Grant, 1998). The leaching of small molecular weight polysaccharides will increase as the micellar network of the starch granules become weak (Qian et al. 1998).

### 3.1.4. Conclusions

Most properties of tef starch are different from maize starch. Tef starch shows considerably less swelling, lower breakdown and setback intrinsic viscosity. Tef starch has higher WAI and lower WSI. However, tef starch composition is similar to that of the normal native cereal starches. The gelatinisation temperature is similar to other tropical cereal starches. The gelatinisation temperature range is relatively narrow than maize because of relatively narrow uniform granule size distribution. The granule size difference could be one factor for considerable variation in the pasting, WAI and WSI. A small variation observed in the composition appeared to influence the tef starches to have slightly different pasting properties among the tef varieties studied. The low setback is associated to the slow syneresis. There could be potential to use tef starch where long duration of food storage is required.

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## 3.2. Chemical and Physical Characterisation of Grain Tef [*Eragrostis tef* (Zucc.) Trotter] Starch Granule Composition

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**Keywords:** *Eragrostis tef*; starch granule; X–ray diffraction; gelatinisation; DSC

## *Abstract*

Chemical and physical properties of starch granules isolated from five grain tef (*Eragrostis tef*) varieties were characterised and compared with normal maize starch. Endogenous starch lipids extracted with hot water saturated n-butanol and total starch lipids with n-hexane after HCl hydrolysis were 7.8 mg/g (mean) and 8.9 mg/g (mean), respectively, slightly lower than in the maize starch. The starch phosphorus content (0.65 mg/g) was higher than the maize starch but virtually the same as reported for rice starch. The starch granule-swelling factor was lower than the maize starch and extent of amylose leaching was higher. The starch granule X-ray diffraction pattern was characteristic of A type starch with a mean crystallinity of 37 %, lower than the maize starch and more similar to that reported for rice and sorghum starches. The starch DSC gelatinisation temperature was high, like other tropical cereals. To, Tp, Tc and ΔH were in the range 63.8-65.4, 70.2-71.3, 81.3-81.5 $^{o}$C and 2.28-7.22 J/g, respectively. The lower swelling, lower percentage crystallinity and lower DSC gelatinisation endotherms than maize starch suggest that the proportion of long amylopectin A chains in tef starch is lower than in maize starch.

### 3.2.1. Introduction

Tef [*Eragrostis tef* (Zucc.) Trotter] is a $C_4$ tropical cereal (Chapter 3.1.1). Grain tef is cultivated as a major cereal in Ethiopia and is the staple for the majority of Ethiopians (Chapter 3.1.1). Little information is available on the composition, physico-chemical properties and functionality of grain tef starch (Chapter 3.1). It is known that the granule is a compound type from which many simpler (2-6 μm in diameter) polygonal shaped granules are released on milling (Umeta and Parker, 1996; Chapter 3.1.3.1). The compound granule surface is smooth (Chapter 3.1.3.1). The amylose content and crude composition (ash, protein and ether extracted fat) of five-grain tef varieties was found to be similar to normal native cereal starches (Chapter 3.1.3.2). The *Kofler* hot stage gelatinisation temperature range (68-74-80 $^{o}$C) is typical of normal native tropical cereal starches (Chapter 3.1.3.3). The small granule size of tef starch when compared with maize starch was considered as one factor responsible for the considerable: lower paste viscosity (peak, breakdown and setback) (Chapter 3.1.3.4), higher water absorption index and lower water solubility index than the maize starch (Chapter 3.1.3.5). In the work reported here, the non–starch composition (lipids, phosphorus and other microelements: sodium, potassium, calcium and magnesium), swelling factor, extent of amylose leaching, X–ray diffraction pattern, and differential scanning calorimetry (DSC) of the tef starch granule is reported.

### 3.2.2. Materials and Methods

#### 3.2.2.1 Samples

Grain tef starch varieties (DZ-01-196, DZ-01-99, DZ-01-1681, DZ-Cr-37 and South African Brown) were as described in Chapter 3.1.2.1. Normal maize starch (Merck UniLAB, code: 587 14 00) was analysed for comparison.

#### 3.2.2.2. Starch granule extraction

Starch granules were extracted by dry/wet milling, sieving and centrifugation (Chapter 3.1.2.2).

#### 3.2.2.3. Starch lipids

Three replicate starch samples (2.0 g ± 0.1 mg) were used for each lipid extraction method. The hydrolysate total starch lipids (24 % HCl hydrolysis for 30 min followed extraction with n–hexane) were determined according to Food and Agricultural Organisation (FAO) (1986) methodology. Lipids on the surface of the starch granules were extracted with chloroform–methanol (CM, 2:1, v/v) at 28 $^{o}$C using a ratio of 16 mL solvent/2 g of starch (three x 2 h.) (Norja, Reinikainen, Olkku and Laakso, 1997). Internal starch granules lipids from the preceding surface lipid extracted starch granule samples were extracted with water–saturated n–butanol (WSB, 1:5, v/v) at 90 $^{o}$C using 16 mL solvent (three x 2 h.) (Morrison, Tan and Hargin, 1980). The extracted lipids were quantified by the gravimetric method.

#### 3.2.2.4. Microelements

*Phosphorus* was determined by the phosphomolybdate method after wet digestion of four replicate starch samples (Morrison, 1964).

*Potassium, Sodium, Calcium and Magnesium.* Three replicate starch samples (2 g ± 0.1 mg) were wet digested with 25 mL concentrated $HNO_3$ (69 %) at 350 $^{o}$C for one h. (Rains, 1991). After cooling, the sample was further digested at 350 $^{o}$C for over one h. with 15 mL concentrated $HClO_4$ (70 %) to colourless solution (ceasing of white fume $HClO_4$ evolution) (Rains, 1991). Then K, Na, Ca and Mg were analysed by atomic absorption (Model 210 VGP spectrophotometer, Buck Scientific, East

Norwalk, USA) by the air–acetylene flame atomisation technique using a characteristic radiation source generated for each element from their respective hollow cathode lamps (Buck Scientific, 1982). Accordingly, the absorbance (nm) of K, Na, Ca and Mg were read at 766.5 (slit 1.4), 589.0 (slit 0.4), 422.7 (slit 0.7) and 285.2 (slit 0.7), respectively. The amounts of K, Na, Ca and Mg were estimated from standard calibration curves prepared from KCl, NaCl, $CaCO_3$ and Mg ribbon, respectively (Buck Scientific, 1982).

### 3.2.2.5. Starch granule swelling

A starch swelling factor (SF) as a ratio of swollen starch granule volume to the dry starch granule volume was determined by Blue Dextran (Sigma D–5751, $M_r$ 2 x $10^6$) method using three replicate starch samples (*ca.* 100 mg ± 0.1 mg) in the temperature range of 35-90 $^{O}$C, at 5 $^{O}$C intervals (Tester and Morrison, 1990).

### 3.2.2.6. Extent of amylose leaching

Extent of amylose leaching was determined according to Hoover, Swamidas, Kok and Vasanthan (1996 b) in the temperature range of 50–95 $^{O}$C at 5 $^{O}$C intervals using three replicate starch samples (*ca.* 17.5 mg ± 0.1 mg) and 5 mL distilled water. After the sample was cooled and centrifuged (2000 x g, 10 min), the leached amylose was determined from the supernatant liquid (1.0 mL) by the iodine binding method of Chrastil (1987).

### 3.2.2.7 X–ray diffraction

A homogeneous loose starch sample powder was pressed with a glass slide into a Siemens diffractometer sample holder. The X–ray diffraction was obtained using an automated diffractometer (Siemens D–501, Karlsruhe, Germany) of: Cu Kα (1.5418 Å) with a radiation power setting of 40 kV (40 mA) in the range 3-70$^{O}$ of 2θ at 25 $^{O}$C, flat plate specimen rotating at 30 rpm, 1$^{O}$/1$^{O}$ divergence slit/scattering slit, receiving slits 0.05$^{O}$, step width 0.04$^{O}$ of 2θ, time per step 1.5 s. with secondary graphite monochromator and a detection by scintillation counting. The percentage crystallinity of the starch granule was determined from the ratio of crystalline area (Ac) to the total area drawn under the major diffraction peaks (Cheetham and Tao, 1998).

### 3.2.2.8. Differential scanning calorimetry (DSC)

*Method a. Samples from bulk sample*

A starch slurry in distilled water was prepared on the basis of 21 % starch (db) in bulk (50-100 mg) from which 15.0-20.0 mg was sampled into an aluminium hermetic DSC pan (40 µL capacity of maximum pressure rating 300 kPa) (Yu and Christie, 2001). The pan with its sample was hermetically sealed and allowed to equilibrate for more than one h. (Beta, Corke, Rooney and Taylor, 2000). The sample was analysed by DSC (DSC 2910, TA Instruments, Delaware, USA) at a heating rate of 10 $^{o}$C/min over the range of 20-180 $^{o}$C. From the DSC thermogram, To (onset), Tp (peak), Tc (conclusion) and gelatinisation enthalpy (ΔH) in J/g were calculated using TA instruments universal analysis software. Three replicates per sample were analysed.

*Method b. Samples by direct weighing on the pan*

A starch sample (3.0-5.0 mg) was weighed directly into an aluminium hermetic DSC pan (40 µL capacity of maximum pressure rating 300 kPa) to which 13-19 µL distilled water was added on the basis of 21 % starch (db) (Yu and Christie, 2001). The pan was covered with the lid and hermetically sealed. After equilibration for more than one h. (Beta et al. 2000), the sample was analysed as described in "a". Three replicates per sample were analysed.

### 3.2.2.9. Statistical analysis

At least three replicate experiments were analysed using Statistica for Windows (Statsoft, Tulsa, USA, 1995). One-way analysis of variance (ANOVA) was performed with means and compared at $p < 0.05$ using Fisher's least significant difference (LSD) test.

## 3.2. 3. Results and Discussion

### 3.2.3.1. Lipids

*Hydrolysate lipids:* the tef starches had slightly lower hydrolysate lipids (mean 8.9 mg/g) than the maize starch (9.9 mg/g) (Table 3.3). The hydrolysate lipids in DZ-01-99 and DZ-01-1681 were similar to that in the maize starch, whereas in DZ-01-196, DZ-Cr-37 and South African Brown the lipids were lower than in the maize starch (p

< 0.05). In tef varieties the highest lipids was for DZ-01-99 (9.6 mg/g) and the lowest was for DZ-Cr-37 (7.8 mg/g). Hydrochloric acid (24 %) is required to destroy the starch granule and protein matrices. Ethanol and formic acid forms ethyl formate *in situ* to aid the extraction of polar and non–polar lipids, which are then extracted by n–hexane (FAO, 1986). Since both bound and free lipids are extracted, hydrolysate lipids are regarded as total starch lipids (Vasanthan and Hoover, 1992 a). The maize total starch lipid in this work was slightly higher than reported in Vasanthan and Hoover (1992 a) (8.0 mg/g) and slightly higher than the maximum value in the range (5.0-9.0 mg/g) given in Galliard and Bowler (1987). The difference is in part probably due to differences in the nature of solvents used for lipid extraction, i.e. in this work n–hexane and *in situ* generated ethyl formate, whereas for example in Vasanthan and Hoover (1992 a) only n-hexane. The tef total starch lipid was higher than pearl millet (5.0 mg/g) (Hoover et al. 1996 b) and slightly higher than rice (7.6 mg/g) (Vasanthan and Hoover, 1992 a). The ether-extracted lipid (crude fat) (2.9 mg/g) (Chapter 3.1.3.2) in the same tef starch samples was much smaller than in this work because ether extraction was done from the whole starch granules.

*Chloroform-methanol (CM) extracted lipids*: starch granule surface lipid in tef starch (mean 4.3 mg/g) was slightly lower than in the maize starch (4.9 mg/g) (Table 3.3). In DZ-01-1681 and DZ-Cr-37 were similar as in the maize starch, whereas in DZ-01-196, DZ-01-99 and South African Brown lower ($p < 0.05$). In tef varieties DZ-01-196, DZ-01-99, DZ-01-1681 and South African Brown were similar ($p < 0.05$). By a similar extraction procedure to the present one, 3.7 mg/g in barley has been reported (Norja et al. 1997), and with a slightly different extraction procedure for only one h., 0.4 mg/g in maize, 0.5 mg/g in rice and 0.4 mg/g in wheat (Vasanthan and Hoover, 1992 a), and 0.6 mg/g in pearl millet (Hoover et al. 1996 b) were reported. In four non-waxy rice starch varieties, extraction with WSB at *ca.* 20 $^{o}$C for 10 min 1.4 mg/g (mean) was reported as non-starch lipids (Azudin and Morrison, 1986). At ambient temperature (*ca.* 28 $^{o}$C) mostly lipids on the surface of starch granules are extracted with CM due to limited penetration of the solvent into the granule interior to extract bound lipids (Vasanthan and Hoover, 1992 a; Hoover et al. 1996 b). The majority of such lipids (Vasanthan and Hoover, 1992 a) comprise triglycerides (TG), followed by free fatty acids (FFA), phospholipids (PL) and glycolipids (GL).

**Table 3.3.** **Lipid and mineral (phosphorus, potassium, sodium, calcium and magnesium) content (db) of the starch granules from different tef varieties and maize**

| Variety | DZ-01-196 | DZ-01-99 | DZ-01-1681 | DZ-Cr-37 | South African Brown | Tef (mean) | Maize |
|---|---|---|---|---|---|---|---|
| **Lipids: 24 % HCl [mg/g][2]** | $8.9^{bc}\pm0.8$[1] | $9.6^{d}\pm0.1$ | $9.4^{cd}\pm0.1$ | $7.8^{a}\pm0.1$ | $8.7^{b}\pm0.2$ | 8.9±0.7 | $9.9^{d}\pm0.1$ |
| **CM [mg/g][3]** | $4.2^{ab}\pm0.1$ | $4.1^{a}\pm0.6$ | $4.5^{abc}\pm0.5$ | $4.8^{bc}\pm0.1$ | $4.1^{a}\pm0.1$ | 4.3±0.3 | $4.9^{c}\pm0.6$ |
| **WSB [mg/g][4]** | $7.9^{bc}\pm0.4$ | $7.3^{a}\pm0.3$ | $8.4^{c}\pm0.2$ | $7.8^{ab}\pm0.3$ | $7.8^{ab}\pm0.4$ | 7.8±0.4 | $9.3^{d}\pm0.0$ |
| **Phosphorus (P) [mg/g]** | $0.69^{c}\pm0.02$ | $0.68^{c}\pm0.06$ | $0.69^{c}\pm0.01$ | $0.50^{b}\pm0.10$ | $0.69^{c}\pm0.01$ | 0.65±0.08 | $0.28^{a}\pm0.02$ |
| **Potassium (K) [µg/g]** | $21.7^{a}\pm2.9$ | $18.7^{a}\pm0.0$ | $23.5^{a}\pm5.0$ | $21.8^{a}\pm2.9$ | $22.0^{a}\pm2.9$ | 21.5±1.7 | $55.6^{b}\pm3.3$ |
| **Sodium (Na) [µg/g]** | $22.1^{a}\pm8.5$ | $18.4^{a}\pm5.4$ | $21.0^{a}\pm4.6$ | $22.0^{a}\pm7.4$ | $24.4^{a}\pm4.5$ | 21.6±2.2 | $163.4^{b}\pm3.8$ |
| **Calcium (Ca) [µg/g]** | $79.5^{b}\pm2.9$ | $95.5^{bc}\pm31.9$ | $109.5^{c}\pm1.0$ | $78.4^{b}\pm11.2$ | $102.7^{bc}\pm6.7$ | 93.1±13.9 | $28.2^{a}\pm6.4$ |
| **Magnesium (Mg) [µg/g]** | $40.7^{b}\pm1.6$ | $45.6^{c}\pm4.4$ | $47.6^{c}\pm2.6$ | $29.1^{a}\pm0.4$ | $59.5^{d}\pm0.8$ | 44.5±11.0 | $27.3^{a}\pm0.9$ |

[1]Values within the same row with different letters are significantly different ($p < 0.05$), [2]24 % HCl is a hydrolysate lipids extracted with n–hexane, [3]CM is chloroform–methanol (2:1, v/v) extracted lipids and [4]WSB is water saturated n–butanol extracted lipids (1:5, v/v).

Some of these lipids are those present *in situ* on the starch granule surface from partial degradation of amyloplast membrane lipids (Galliard and Bowler, 1987). Some are non–endogenous from spherosomes (lipid containing organelles in the aleurone and germ) associated to the starch granule on starch isolation (Galliard and Bowler, 1987). Extraction of starch granule surface lipids at ambient temperature can vary with starch isolation methods, lipid extraction procedures and solvent type, which makes direct comparison of different works with each other (Azudin and Morrison, 1986; Vasanthan and Hoover, 1992 a; Hoover et al. 1996 b; Norja et al. 1997) and with these data difficult.

*Hot water saturated n-butanol (WSB) extracted lipids:* the tef starches had lower endogenous (internal) lipids (mean 7.8 mg/g) than the maize starch (9.3 mg/g) (Table 3.3.). The endogenous lipids in DZ-01-196, DZ-Cr-37 and South African Brown were similar ($p > 0.05$). The highest in tef varieties was in DZ-01-1681 (8.4 mg/g) and the lowest was in DZ-01-99 (7.3 mg/g). The values obtained for tef starches were in the ranges reported for rice (6.3–11.1 mg/g) and barley (6.8–12.8 mg/g) by Morrison, Milligan and Azudin (1984). They are higher than pearl millet (4.3 mg/g) (Hoover et al. 1996 b), similar to maize (7.6 mg/g) and rice (7.1 mg/g) reported by Vasanthan and Hoover (1992 a), but lower than for the non–waxy rice starches (9.0–13.0 mg/g) reported by Azudin and Morrison (1986). In normal maize starch internal starch lipid was reported in the range 6.1-8.2 mg/g (Morrison et al.1984), which is slightly lower than this work. The difference is probably due to differences in the lipid extraction conditions (temperature, time and solvent type) and starch isolation methods. The hot (90 $^{o}$C) solvent extraction water enhances the swelling and partial disruption of the crystallinity of starch granules to extract internal lipids. The internal lipids can exist as inclusion complexes with amylose (LAM) (Morrison et al. 1984) or linked to the hydroxyl groups of the starch components via ionic or hydrogen bonding (Galliard and Bowler, 1987; Hoover et al. 1996 b) and mostly comprise lysophospholipids (LPL) and FFA (Galliard and Bowler, 1987; Vasanthan and Hoover, 1992 a).

### 3.2.3.2. Microelements

*Phosphorus* in tef starch (mean 0.65 mg/g) was higher than in maize starch (0.28 mg/g) (Table 3.3). Lowest phosphorus was recorded in DZ-Cr-37 (0.50 mg/g) and the

highest (0.69 mg/g) in DZ-01-196, DZ-01-1681 and South African Brown. The P content of native starches was reported as: 0.19 mg/g in maize, 0.57 mg/g in wheat and 0.90 mg/g in potato (Kasemsuwan and Jane, 1996), and 0.66 mg/g in rice and 0.59 mg/g in millet (Lim, Kasemsuwan and Jane, 1994). The phosphorus of native cereal starch (e.g. maize, wheat and the millets) is mostly in the form of phospholipids (Lim et al. 1994). In rice and sorghum starches, some amylopectin is phosphorylated to the extent of 0.031 mg/g (1.0 nmol glucose-6-phosphate/mg starch) and 0.028 mg/g (0.9 nmol glucose-6-phosphate/mg starch), respectively (Blennow, Engelsen, Munck and Møller, 2000). The amount of P in the tef starches was similar to rice, which also has compound starch granules. However, to establish the nature of the P more detailed investigation is required.

*Other microelements*. The mean K, Na, Ca and Mg contents of the tef starches were 21.5, 21.6, 93.1 and 44.5 μg/g, respectively (Table 3.3), compared to the maize starch 55.6, 163.4, 28.2 and 27.3 μg/g, respectively (Table 3.3). In the tef starches the lowest K was recorded in DZ-01-99 (18.7 μg/g) and the highest was in DZ-01-1681 (23.5 μg/g). The lowest Na was in DZ-01-99 (18.4 μg/g) and the highest was in South African Brown (24.4 μg/g). The lowest Ca was in DZ-Cr-37 (78.4 μg/g) and the highest was in DZ-01-1681 (109.5 μg/g). The Mg content of DZ-Cr-37 (29.1 μg/g) was the lowest and the highest was in South African Brown (59.5 μg/g).

Native starches contain mainly K, Na, Ca and Mg metal ions bound to the phosphate groups through ionic association (Swinkels, 1985; Whistler and BeMiller, 1997). The divalent metals (Ca and Mg) were more predominant in the tef starches than the monovalent metals (K and Na). In the maize starch, the reverse was found (i.e. monovalent metals were more predominant than the divalent ones). Phosphorylated starches possess two potential negative charges at the proximity of the phosphate moiety, whereas only one charge is found on the phosphate moiety of the lysophospholipid. For the phosphorylated starches it is likely that more divalent metals can be bound. This might hold true in the case of tef starch since its P content was higher than maize starch (Table 3.3).

### 3.2.3.3. Starch granule swelling factor

In all tef starches and maize starch, swelling increased gradually with temperature increase and a marked increase was observed at around 65 $^{O}$C until it reached maximum, tef starches at 80 $^{O}$C mean = 8.66 and maize starch at 85 $^{O}$C = 10.04 (Figure 3.5). Below 70 $^{O}$C no large difference was observed between the tef starches and the maize starch. At 70 $^{O}$C and above a large difference was observed between tef varieties and maize. At 75 $^{O}$C a significant difference ($P < 0.05$) among tef varieties was observed, and the highest SF was for DZ-Cr-37 (8.00) and the lowest was for DZ-01-1681 (6.91). The SF was reduced in the tef starches above 80 $^{O}$C, whereas in the maize starch this happened above 85 $^{O}$C.

In the tef and maize starches, the marked onset increase in swelling (around 65 $^{O}$C) was similar to their onset gelatinisation temperatures and the maximum swelling was slightly above their conclusion gelatinisation temperatures (around 80 $^{O}$C) (Chapter 3.1.3.3). This is in agreement with the literature on swelling and gelatinisation of normal native wheat, maize and barley, and waxy maize and waxy barley starch granules reported in Tester and Morrison (1990). On swelling, the possibility of amylose-lipid complex formation was reported (Tester and Morrison, 1990). The significant difference ($P< 0.05$) observed in SF among the tef starch varieties at 75 $^{O}$C could in part be related to differences in the lipid contents of the starches that could inhibit the swelling erratically. Swelling is apparently a property of amylopectin, which forms the crystalline components of the starch granule. The swelling and gelatinisation of a starch granule is largely influenced by the nature of amylopectin crystallites (Tester and Morrison, 1990). Swelling was reported (Tester and Morrison, 1990; Li and Yeh, 2001) to increase with heat of gelatinisation and with high proportion of long branch chain amylopectin molecules (degree of polymerisation, *dp* $\geq$ 35). It decreases with cross-linking and an increase in: amylose content, starch lipids and residual proteins (Tester and Morrison, 1990; Li and Yeh, 2001). The observed (in the gelatinisation range) smaller SF of tef starches compared to maize starch could suggest the amylopectin *dp* in the tef starch to be smaller due to the smaller crystallite proportion in tef starch (Table 3.4), whereas the amylose (Table 3.1) and lipid (Table 3.3) contents of both starches being virtually the same.

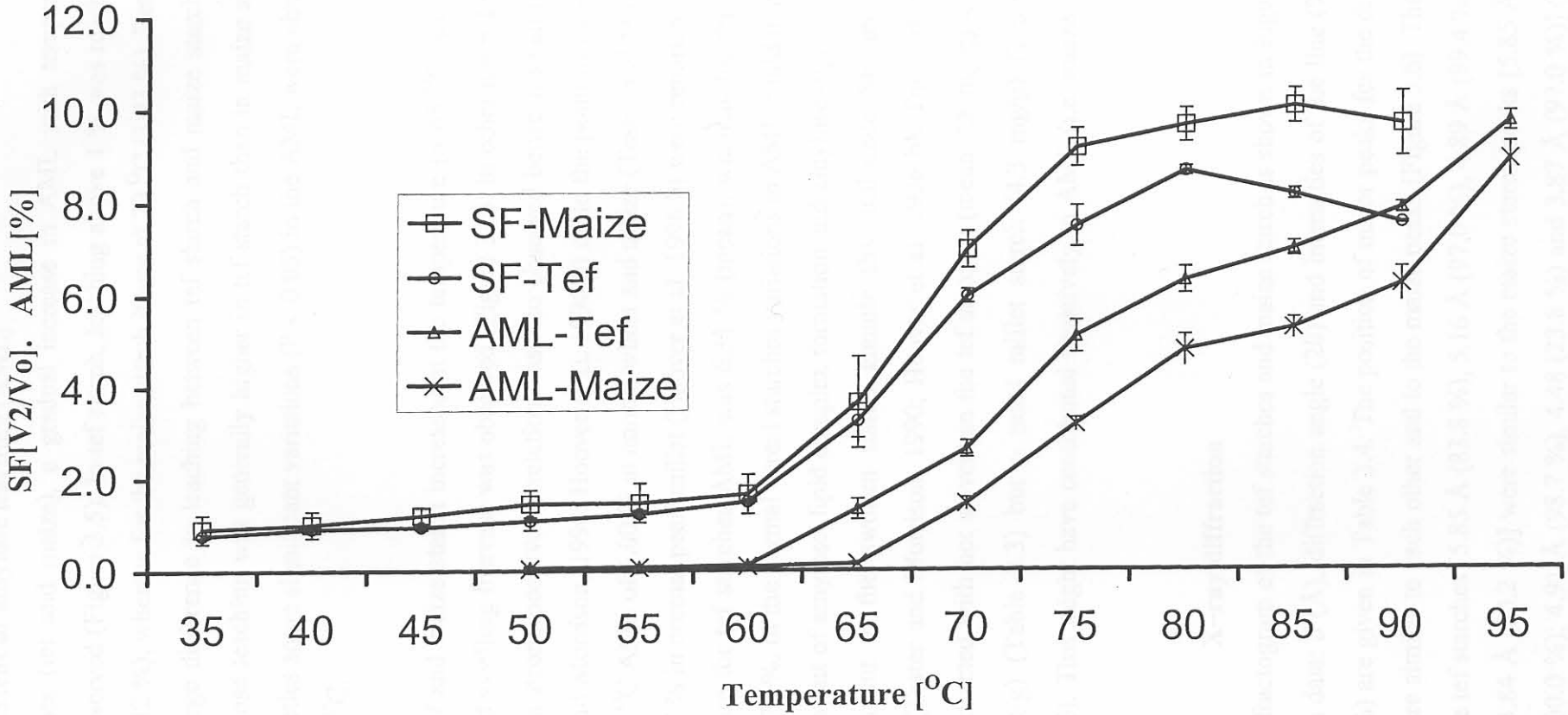


Figure 3.5. Swelling factor (SF) and amylose leaching (AML) of tef varieties (mean and range) and maize starches at different temperatures.

### 3.2.3.4. Extent of amylose leaching (AML)

For both starches (tef and maize), a gradual increase in AML with temperature increase was observed (Figure 3.5). In tef starch, leaching above 1 % was reached at 65 $^{O}$C (mean 1.32 %), whereas for the maize starch it was at 70 $^{O}$C (1.43 %). Between 65-90 $^{O}$C, a large difference in leaching between tef starch and maize starch was observed. Amylose leaching was generally higher in tef starch than in maize starch. Among tef varieties some significant variations ($P < 0.05$) in the AML were observed between 65–80 $^{O}$C.

The AML of tef and maize starch increased at the temperature (> 65 $^{O}$C) where, as stated, a marked swelling increase was observed (Figure 3.5). In other normal native cereal starches a strong positive correlation was also observed between swelling and AML (Tester and Morrison, 1990; Hoover et al. 1996 b) up to the point of maximum swelling. At 80 $^{O}$C AML of 6.00 % in normal wheat and maize (Tester and Morrison, 1990), and 7.67 % in normal pearl millet (Hoover et al. 1996 b) were reported. At the same temperature for tef starches AML was 6.31 % (mean), whereas for the maize starch it was 4.78 %. In the normal cereal starches variations in AML could in part be related to the extent of amylose–lipid complex formation and amylose–lipid complex steric entanglement in the swollen starch granule that influence SF and AML inconsistently (Tester and Morrison, 1990; Hoover et al. 1996 b). The endogenous starch lipids extracted with hot solvent in the tef starches (mean 7.8 mg/g) < maize starch (9.3 mg/g) (Table 3.3) but > pearl millet starch (4.3 mg/g) (Tester and Morrison, 1990). This might have contributed negatively to AML (i.e. maize < tef < pearl millet).

### 3.2.3.5. X–ray diffraction

The X-ray diffractogram of the tef starches and maize starch is shown in Figure 3.6. The diffraction data: *d* (Å), diffraction angle (2θ) and intensities of the line (%) and crystallinity (%) are given in Table 3.4. The position of major peaks for the different tef starches were similar to each other and to the maize peaks (Figure 3.6). The mean *d* values for the tef starches [5.85 Å (83.8 %), 5.16 Å (97.0 %), 4.89 Å (99.4 %), 4.41 (36.4 %) and 3.84 Å (80.2 %)] were similar to the maize starch peaks [5.85 Å (74.0 %); 5.17 Å (100.0 %); 4.91 Å (98.7 %), 4.48 (23.8 %) and 3.87 Å (97.0 %)] (Figure

3.6 and Table 3.4). For normal native maize starch, the major peak intensity were reported (Cheetham and Tao, 1998) at 5.91 Å, 5.22 Å, 4.98 Å, 4.50 Å and 3.89 Å, which is similar to these data. The three strong peaks at 5.80 Å, 5.20 Å and 3.80 Å are a characteristic of "A" type starches of monoclinic unit cell (a = 2.124 nm, b = 1.172 nm, c = 1.069 nm and $\gamma = 123.5^{O}$) (Zobel, 1988 a and b). The weak peak at 4.40 Å is a V pattern characteristic of lipid–amylose complex (Zobel, 1988 a and b). This was observed in the tef starches [mean 4.41 (36.4 %)] and in the maize starch [4.48 (23.8 %)] (Table 3.4). This indicates that some of the amylose exists as a complex with the LPL or FFA in the native tef starch granules.

The level of crystallinity of tef starches (mean 37 %) was lower than the maize starch (40 %). Literature values for normal maize, rice, sorghum, waxy rice and wheat starches are 40, 38, 37, 37 and 36 %, respectively (Zobel, 1988 b). The crystallinity of tef starch is thus similar to sorghum, waxy rice and rice starches. In the amylopectin molecule the A chain of a cluster is believed to form double helices with the adjacent A chain of another cluster and such array gives the crystalline order of starch granules (Cheetham and Tao, 1998; Noda, Takahata, Sato, Suda, Morishita, Ishiguro and Yamakawa, 1998). The crystalline level of starch granules is reported to be influenced by amylopectin A chain length, amount of double helices that are organised into a crystalline array, crystallite size, amylose content (Cheetham and Tao, 1998; Noda et al. 1998), and by degree of amylopectin phosphorylation (Blennow et al. 2000).

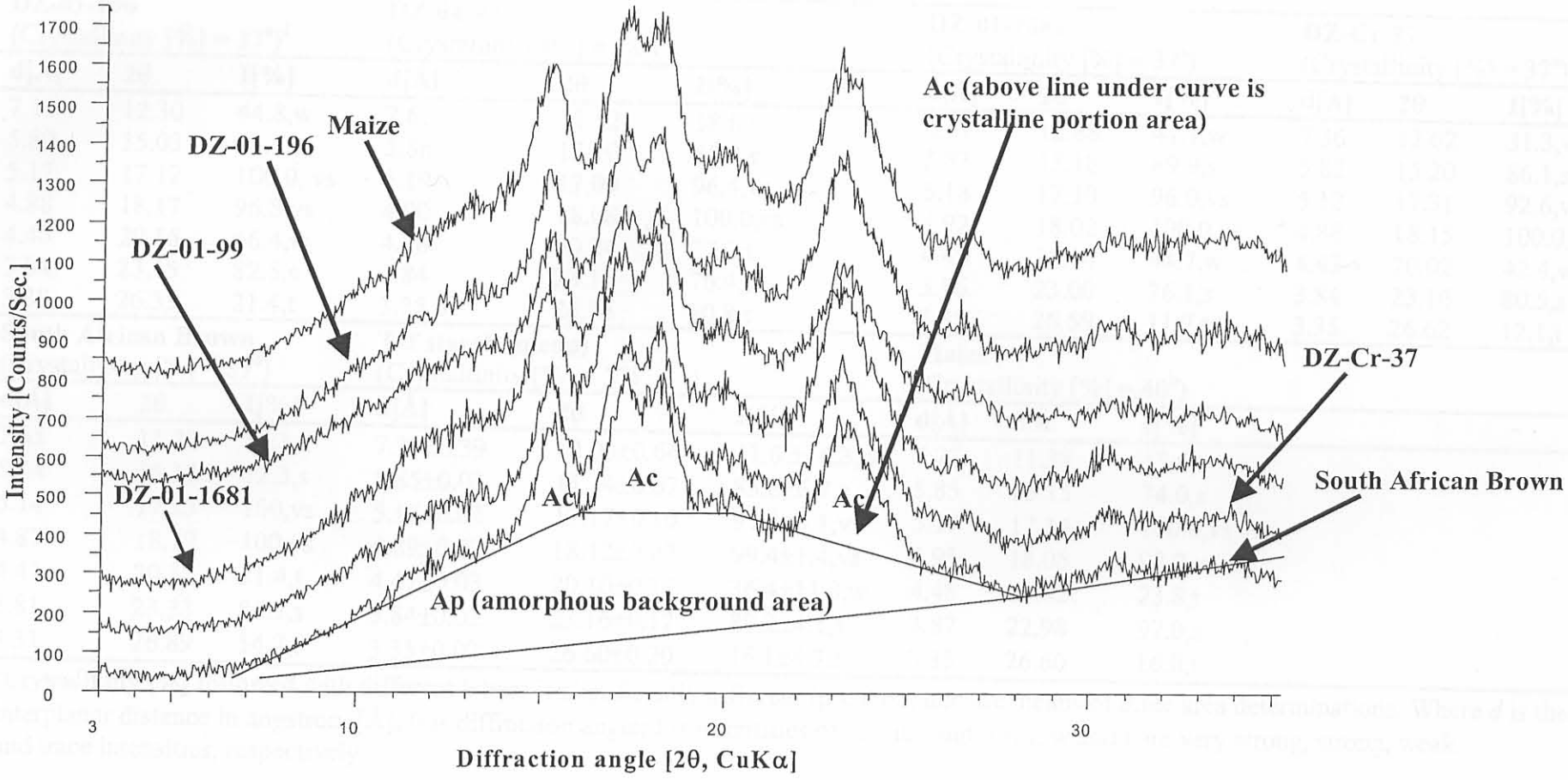


Figure 3.6. X–ray diffractogram of starches of different tef varieties and maize

**Table 3.4. X–ray diffraction pattern and crystallinity [%] of starches from different tef varieties and maize**

| DZ-01-196 (Crystallinity [%] = 37[a])[1] | | | DZ-01-99 (Crystallinity [%] = 38[a]) | | | DZ-01-1681 (Crystallinity [%] = 37[a]) | | | DZ-Cr-37 (Crystallinity [%] = 37[a]) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **d[Å]** | **2θ** | **I[%]** | **d[Å]** | **2θ** | **I[%]** | **d[Å]** | **2θ** | **I[%]** | **d[Å]** | **2θ** | **I[%]** |
| 7.19 | 12.30 | 44.8,w | 7.67 | 11.52 | 18.8,t | 6.87 | 12.88 | 47.1,w | 7.36 | 12.02 | 31.3,w |
| 5.89 | 15.03 | 85.4,s | 5.86 | 15.10 | 85.2,s | 5.83 | 15.18 | 89.9,s | 5.82 | 15.20 | 86.1,s |
| 5.17 | 17.12 | 100.0, vs | 5.19 | 17.09 | 96.4,vs | 5.18 | 17.10 | 96.0,vs | 5.12 | 17.31 | 92.6,vs |
| 4.88 | 18.17 | 96.8,vs | 4.90 | 18.08 | 100.0,vs | 4.92 | 18.02 | 100.0,vs | 4.88 | 18.15 | 100.0,vs |
| 4.40 | 20.16 | 46.4,w | 4.38 | 20.24 | 27.2,t | 4.45 | 19.94 | 44.7,w | 4.43 | 20.02 | 42.4,w |
| 3.84 | 23.15 | 82.5,s | 3.84 | 23.15 | 76.4,s | 3.86 | 23.00 | 76.1,s | 3.84 | 23.16 | 80.5,s |
| 3.38 | 26.33 | 21.4,t | 3.35 | 26.55 | 20.8,t | 3.35 | 26.59 | 11.7,t | 3.35 | 26.62 | 12.1,t |

| South African Brown (Crystallinity [%] = 37[a]) | | | Tef starch (mean) (Crystallinity [%] = 37) | | | Maize (Crystallinity [%] = 40[b]) | | |
|---|---|---|---|---|---|---|---|---|
| **d[Å]** | **2θ** | **I[%]** | **d[Å]** | **2θ** | **I[%]** | **d[Å]** | **2 Å** | **I[%]** |
| 7.85 | 11.27 | 16.1,t | 7.39±0.39 | 12.00±0.64 | 31.6 ±14.3,w | 7.77 | 11.38 | 12.6,t |
| 5.84 | 15.17 | 72.3,s | 5.85±0.03 | 15.14±0.07 | 83.8±6.7,s | 5.85 | 15.15 | 74.0,s |
| 5.14 | 17.25 | 100,vs | 5.16±0.03 | 17.17±0.10 | 97.0±3.1,vs | 5.17 | 17.14 | 100.0,vs |
| 4.87 | 18.19 | 100,vs | 4.89±0.02 | 18.12±0.07 | 99.4±1.4,vs | 4.91 | 18.05 | 98.7,vs |
| 4.41 | 20.12 | 21.4,t | 4.41±0.03 | 20.10±0.12 | 36.4±11.3,w | 4.48 | 19.82 | 23.8,t |
| 3.81 | 23.33 | 85.7,s | 3.84±0.02 | 23.16±0.12 | 80.2±4.1,s | 3.87 | 22.98 | 97.0,s |
| 3.31 | 26.89 | 14.7,t | 3.35±0.02 | 26.60±0.20 | 16.1±4.7,t | 3.35 | 26.60 | 16.0,t |

[1]Crystallinity [%] followed with different letters is significantly different ($p < 0.05$) and are means of three area determinations. Where *d* is the interplanar distance in angstrom [Å]; θ is diffraction angle; I is intensities of the line and vs, s, w and t are very strong, strong, weak and trace intensities, respectively

### 3.2.3.6. DSC

The DSC thermogram and the data evaluated for gelatinisation endotherms of starches from the different tef varieties and maize are shown in Figure 3.7 and Table 3.5, respectively. Gelatinisation endotherms of tef starches (mean) in method a: onset (To), peak (Tp) and conclusion (Tc) temperatures ($^{O}$C) and enthalpy (ΔH, J/g) were 63.8, 70.2, 81.5 and 2.28, respectively which are somewhat lower than in the maize starch (69.2, 73.5, 85.8 and 2.44, respectively). The ranges for To, Tp and Tc in the tef starches were 63.1 - 64.6, 69.3 - 70.9 and 80.1 - 84.3, respectively.

The Tp (70.2 $^{O}$C) of tef starch is in the range of other tropical cereal starches: rice (71.3 $^{O}$C) (Biliaderis, Page, Maurice and Juliano, 1986), sorghum (67.4 $^{O}$C) (Beta et al. 2000), pearl millet (68.5 $^{O}$C) (Hoover et al. 1996 b) and maize 72.9 $^{O}$C (Mistry and Eckhoff, 1992). The starch gelatinisation property measured by DSC is a manifestation of an irreversible dissociation of amylopectin molecular order involving melt of crystallite and double helical orders (Noda et al. 1998; Sahai and Jackson, 1999). A higher proportion of long chain length amylopectin fine structures forms longer double helices and contributes to a higher crystallite range which presumably requires high gelatinisation temperature and enthalpy to melt (Noda et al. 1998; Tester and Debon, 2000). The tef starch To, Tp and ΔH are relatively lower than the maize starch (Table 3.5). This suggests the proportion of long chain length amylopectin structures to be probably lower in tef starches than the maize starch, since a higher proportion of long chain length amylopectin structures is positively correlated with high To, Tp and ΔH (Noda et al. 1998). The lower % crystallinity in tef (mean 37 %) than maize starch (40 %) (Table 3.4), and the lower swelling factor of tef starches than the maize starch (Figure 3.5) also supports this hypothesis.

The DSC gelatinisation endotherm (To, Tp, Tc and ΔH) varies with experimental conditions like availability of water for hydrating the starch granules (Sahai and Jackson, 1999; Yu and Christie, 2001). Three tef varieties (DZ-01-196, DZ-01-99 and DZ-01-1681) along with the maize starch were studied by adding water directly to the sample in the DSC pan (method b) (Yu and Christie, 2001). For the tef starches (mean of three varieties) To, Tp, Tc temperatures [$^{O}$C] and ΔH [J/g] were 65.4, 71.3, 81.3

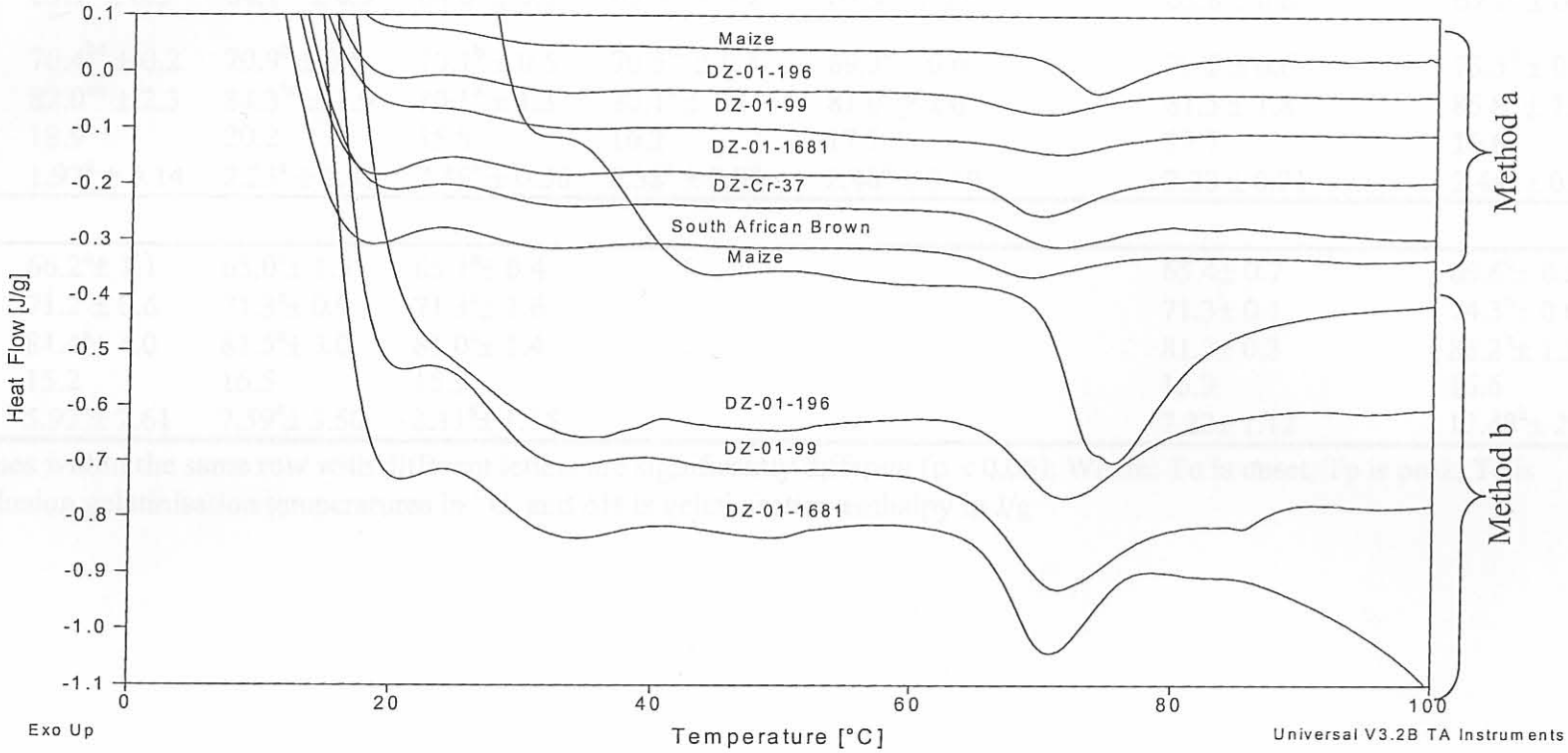


Figure 3.7. DSC thermograms of starches for different tef varieties and maize on the basis of 21 % starch (db) + 79 % water

**Table 3. 5. Differential scanning calorimetry data for starches from different tef varieties and maize starch**

| Variety | DZ-01-196 | DZ-01-99 | DZ-01-1681 | DZ-Cr-37 | South African Brown | Tef starch (mean) | Maize |
|---|---|---|---|---|---|---|---|
| **Method a** | | | | | | | |
| **To** | $63.1^{a} \pm 0.3^{1}$ | $64.1^{bc} \pm 0.9$ | $64.6^{c} \pm 0.5$ | $63.9^{bc} \pm 0.2$ | $63.3^{ab} \pm 0.6$ | $63.8 \pm 0.6$ | $69.2^{d} \pm 0.3$ |
| **Tp** | $70.4^{bc} \pm 0.2$ | $70.9^{c} \pm 0.5$ | $70.1^{b} \pm 0.5$ | $70.5^{bc} \pm 0.4$ | $69.3^{a} \pm 0.6$ | $70.2 \pm 0.6$ | $73.5^{d} \pm 0.4$ |
| **Tc** | $82.0^{ab} \pm 2.3$ | $84.3^{bc} \pm 1.9$ | $80.1^{a} \pm 1.3$ | $80.1^{a} \pm 1.4$ | $81.0^{a} \pm 2.6$ | $81.5 \pm 1.8$ | $85.8^{c} \pm 1.5$ |
| **Tc-To** | 18.9 | 20.2 | 15.5 | 16.2 | 17.7 | 17.7 | 16.6 |
| **ΔH** | $1.92^{a} \pm 0.14$ | $2.25^{a} \pm 0.81$ | $2.45^{a} \pm 0.56$ | $2.38^{a} \pm 0.09$ | $2.40^{a} \pm 0.59$ | $2.28 \pm 0.21$ | $2.44^{a} \pm 0.32$ |
| **Method b** | | | | | | | |
| **To** | $66.2^{a} \pm 1.1$ | $65.0^{a} \pm 1.5$ | $65.1^{a} \pm 0.4$ | | | $65.4 \pm 0.7$ | $69.6^{b} \pm 0.2$ |
| **Tp** | $71.2^{a} \pm 0.6$ | $71.3^{a} \pm 0.9$ | $71.3^{a} \pm 1.6$ | | | $71.3 \pm 0.1$ | $74.3^{b} \pm 0.6$ |
| **Tc** | $81.4^{a} \pm 4.0$ | $81.5^{a} \pm 3.0$ | $81.0^{a} \pm 1.4$ | | | $81.3 \pm 0.3$ | $86.2^{b} \pm 1.3$ |
| **Tc-To** | 15.2 | 16.5 | 15.9 | | | 15.9 | 16.6 |
| **ΔH** | $5.97^{a} \pm 2.61$ | $7.59^{a} \pm 3.50$ | $8.11^{a} \pm 1.15$ | | | $7.22 \pm 1.12$ | $12.48^{b} \pm 2.17$ |

[1]Values within the same row with different letters are significantly different ($p < 0.05$). Where: To is onset, Tp is peak, Tc is conclusion gelatinisation temperatures in $^{O}C$, and ΔH is gelatinisation enthalpy in J/g

and 7.22, respectively, which are lower than in the maize starch (69.6, 74.3, 86.2 and 12.48, respectively). The observed ΔH for maize starch is lower in method a, but is similar to data in method b when compared to the literature value (Mistry and Eckhoff, 1992; Sahai and Jackson, 1999). In the bulk sample preparation (method a), homogeneity of starch granule hydration is good but the possibility of moisture loss during transfer of sample from bulk preparation to DSC sample pan and precipitation of starch in the bulk were noted as shortcomings (Yu and Christie, 2001). By adding water directly to the sample in the pan (method b), moisture loss is minimised but homogeneity of hydration can be affected (Yu and Christie, 2001). With excess water (*ca.* 80 %) where moisture loss is minimal (method b) the effect is toward a slight increase in the onset gelatinisation temperature, narrowing of transition range and an increase in the gelatinisation enthalpy (Mistry and Eckhoff, 1992; Tester and Debon, 2000). In this study this trend was observed (To and ΔH in method a < in method b).

The DSC runs for two dry tef starches (DZ-Cr-37 and South African Brown) and dry maize starch of moisture content 5.4, 6.5 and 12.4 %, respectively showed high Tp endotherms, viz 155.0, 152.0 and 160.6 $^{O}$C, respectively (Figure 3.8). This is because at low moisture content (< 25 %), starches are known to show a high temperature endotherm in the range of 140-180 $^{O}$C due to the melt and uncoiling of double helices (Sahai and Jackson, 1999). The Tp of the three dry starches varied slightly, probably in part due to differences in their moisture content and nature of their helices (Sahai and Jackson, 1999).

Information about amylose lipid interactions can also be extracted from the DSC thermograms. Representative DSC thermograms where such interactions have occurred and data evaluated from the thermograms are shown in Figure 3.9 and Table 3.6, respectively. For the tef starches To, Tp and Tc in $^{O}$C and Δ H in J/g for the first endotherm after gelatinisation were in the ranges 90.0-92.2, 94.6-98.4 and 101.3-106.4, and 0.11-3.40, respectively, whereas for the maize starch these were 95.4, 99.2, 105.6 and 0.11-0.18 J/g, respectively. The second endotherm for the tef starches was in the ranges 102.5-111.7, 105.3-116.9, 110.0-123.5 and 0.02-3.78, respectively, whereas in the maize starch these were 116.6, 120.3, 127.3 and 0.09-1.62, respectively. The first endotherm after gelatinisation corresponds to the melt

region of form I (*ca.* 90-100 $^{O}$C) amylose–lipid complex, whereas the second higher temperature endotherm corresponds to the melt region (*ca.* 105-120 $^{O}$C) of the form II amylose–lipid complex (Biliaderis and Galloway, 1989). Form I is not crystalline and is a result of rapid nucleation with random distribution of helical segments (Biliaderis and Galloway, 1989). Form II is known as the V type crystalline structure of amylose–lipid complex (Biliaderis and Galloway, 1989). The X-ray diffraction pattern also in part supports this because in the tef starches and the maize starch a weak V–type crystalline diffraction pattern (Table 3.4) was observed. The DSC endotherms evaluated in the melt regions of form I and II of amylose–lipid complex showed slight variations among tef starch varieties and also with the maize starch (Table 3.6). This is because thermal transition of amylose–lipid complex is known to be affected by type of starch, chain length of the monoacyl/FFA and polar head of the lipids, and by the phase behaviour of the lipids (Eliasson, 1994). Among starches all these might not be the same.

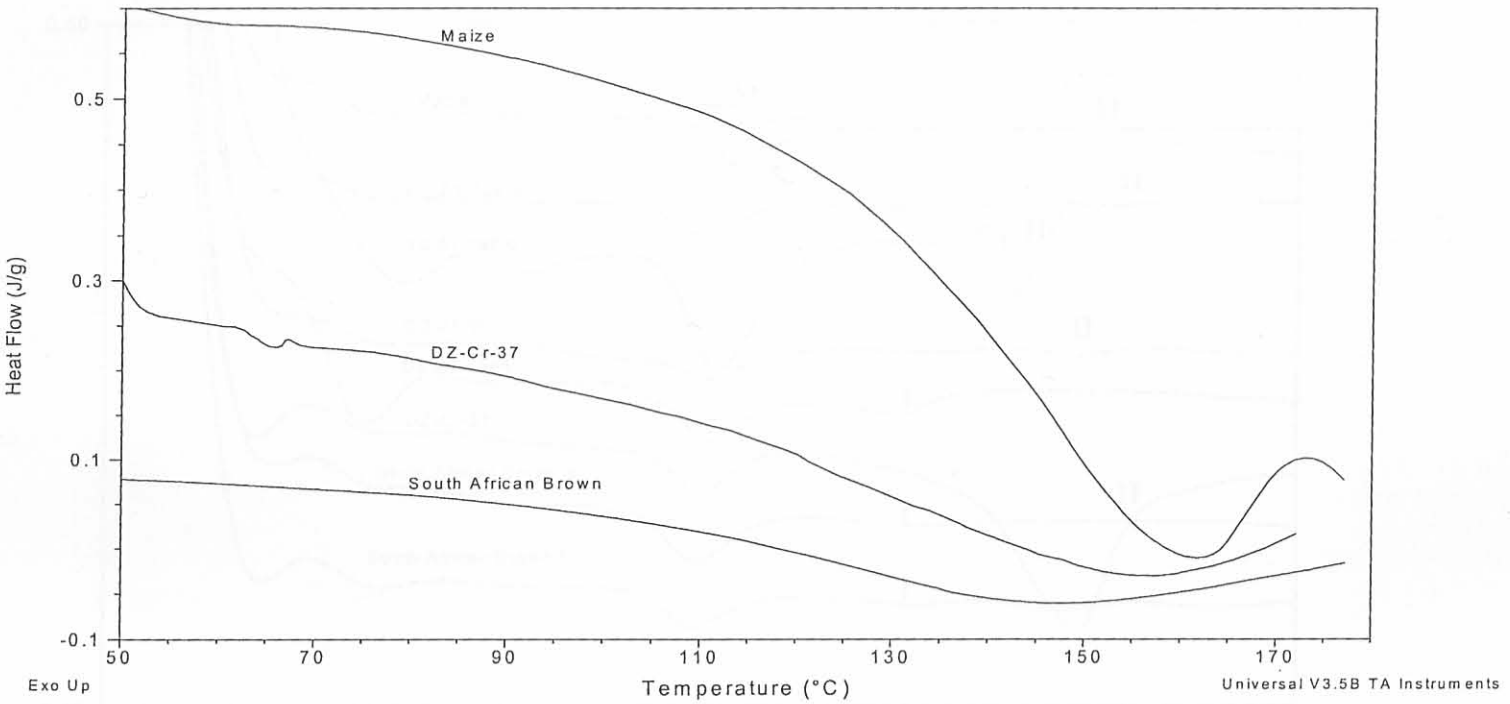


Figure 3.8. DSC thermograms of two dry tef starches and dry maize starch

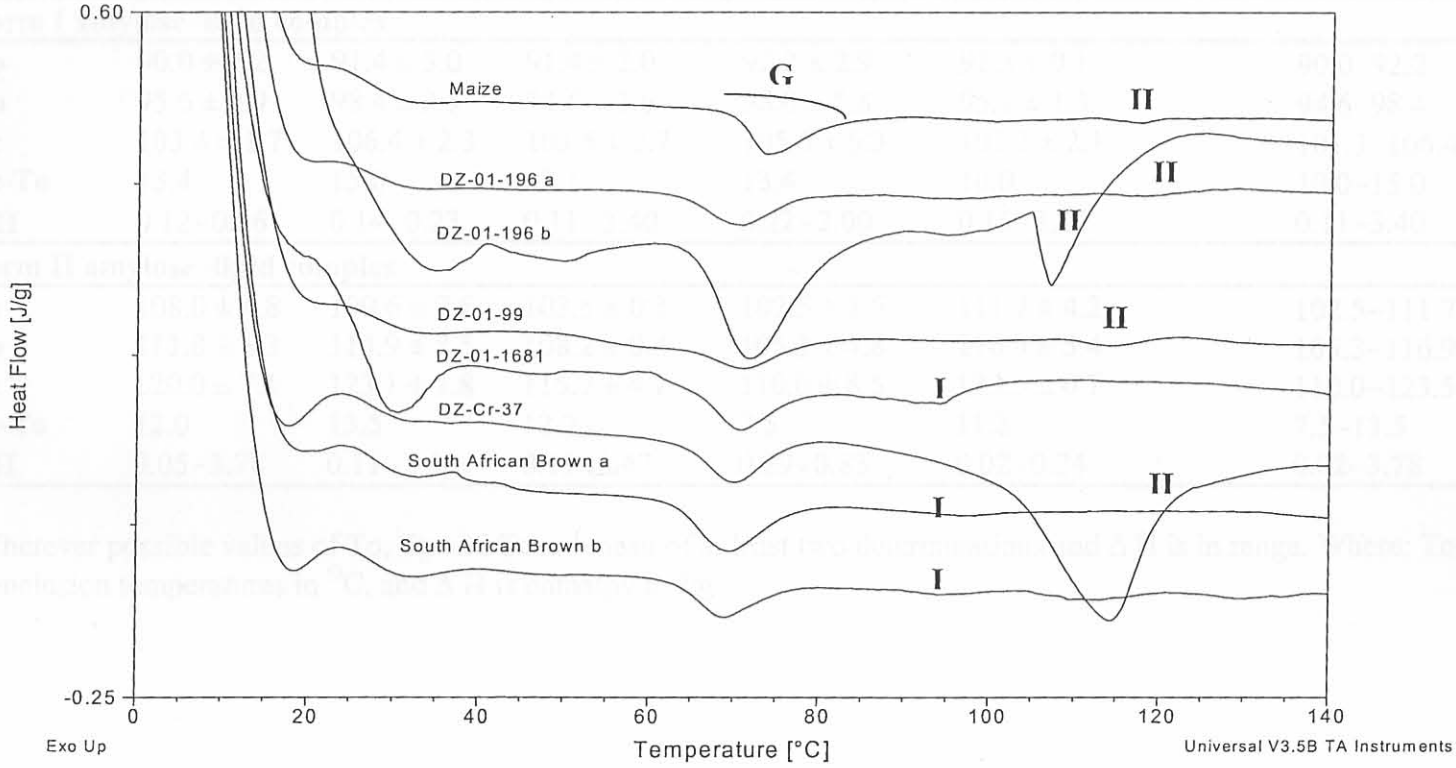


Figure 3.9. Samples from tef starch DSC thermograms where traces of amylose lipid complex melt transitions were observed. Where: G is gelatinisation transition region, I is for form I, and II is for form II amylose lipid complex melt transition regions.

**Table 3.6. Differential scanning calorimetry data of amylose–lipid complex endotherms for starches from different tef Varieties and maize**

| Variety | DZ-01-196 | DZ-01-99 | DZ-01-1681 | DZ-Cr-37 | South African Brown | Range (Tef) | Maize |
|---|---|---|---|---|---|---|---|
| **Form I amylose–lipid complex** | | | | | | | |
| **To** | 90.0 ± 2.2 | 91.4 ± 3.0 | 91.4 ± 2.0 | 92.2 ± 2.9 | 91.3 ± 0.1 | 90.0–92.2 | 95.4 ± 4.7 |
| **Tp** | 95.6 ± 0.9 | 98.4 ± 2.5 | 94.6 ± 1.6 | 98.0 ± 5.8 | 95.1 ± 1.3 | 94.6–98.4 | 99.2 ± 1.1 |
| **Tc** | 103.4 ± 1.7 | 106.4 ± 2.3 | 103.5 ± 2.7 | 105.6 ± 9.3 | 101.3 ± 2.1 | 101.3–106.4 | 105.6 ± 2.1 |
| **Tc-To** | 13.4 | 15.0 | 12.1 | 13.4 | 10.0 | 10.0–15.0 | 10.2 |
| **Δ H** | 0.12-0.26 | 0.14-0.23 | 0.11-3.40 | 0.22-2.00 | 0.15-2.49 | 0.11–3.40 | 0.11-0.18 |
| **Form II amylose–lipid complex** | | | | | | | |
| **To** | 108.0 ± 4.8 | 109.6 ± 7.6 | 103.5 ± 0.1 | 102.5 ± 1.5 | 111.7 ± 4.2 | 102.5–111.7 | 116.6 ± 3.3 |
| **Tp** | 111.8 ± 6.3 | 114.9 ± 8.5 | 108.2 ± 0.4 | 105.3 ± 4.8 | 116.9 ± 5.4 | 105.3–116.9 | 120.3 ± 2.7 |
| **Tc** | 120.0 ± 7.1 | 123.1 ± 7.8 | 115.7 ± 4.7 | 110.0 ± 8.5 | 123.5 ± 0.7 | 110.0–123.5 | 127.3 ± 3.3 |
| **Tc-To** | 12.0 | 13.5 | 12.2 | 7.5 | 11.8 | 7.5–13.5 | 8.6 |
| **Δ H** | 0.05-3.78 | 0.11-2.50 | 0.19-3.47 | 0.29-0.83 | 0.02-0.24 | 0.02–3.78 | 0.09-1.62 |

Wherever possible values of To, Tp and Tc are mean of at least two determinations and Δ H is in range. Where: To is onset, Tp is peak, Tc is conclusion temperatures in $^{O}$C, and Δ H is enthalpy in J/g

### 3.2.4. Conclusions

The level of endogenous starch lipids in tef starch granules is similar to other tropical cereals like maize and rice. Phosphorus content is similar to rice starch. The swelling factor is less than maize starch and amylose leaching is slightly higher than the maize starch. The X-ray diffraction pattern of tef starch granules is the A type pattern. The starch appears to be more amorphous than maize starch but similar to rice and sorghum starches in crystallinity level. The X-ray diffraction pattern of tef starch granules indicates that some of the amylose forms an inclusion complex with the endogenous lipids (LPL or FFA). The DSC gelatinisation temperature is similar to other tropical cereals. The lower swelling property, lower percentage crystallinity and lower DSC gelatinisation endotherms compared to maize starch suggest the proportion of crystallinity in the tef starch is less and probably the proportion of long amylopectin A chains is relatively lower.

**Acknowledgements**

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## 3.3: Functional Properties of Grain Tef [*Eragrostis tef* (Zucc.) Trotter] Starch

**Submitted to the journal *Starch/Stärke* (December 2002)**

**Keywords:** *Eragrostis tef*; Paste clarity; Gel texture; Retrogradation; α-Amylase digestion; Acid hydrolysis

*Abstract*

Functional properties of tef starch from five varieties were compared with commercial maize starch. In most tef varieties the paste clarity (measured as % T) was as opaque as the maize starch but less white. Tef starch gel texture was short and in most varieties was slightly firmer than the maize starch. Tef starch adhesiveness was less than maize starch. Retrogradation of tef starch evaluated as % gel syneresis under refrigeration (4 $^{O}$C) and freeze (-18 $^{O}$C) at 3, 7, 10 and 21 storage test days, was slower than maize starch. With three freeze-thaw cycle treatments a similar trend was observed. In tef starch initial digestion by α-amylase and hydrolysis by mild HCl was slightly higher than maize starch, probably in part because of smaller granule size and higher amorphous portion. Probably because of absence of surface pores in tef starch, α-amylase degradation was by surface erosion and the acid has gradually corroded the surface of the granules.

### 3.3.1. Introduction

Grain tef has compound type starch granules comprising many simple polygonal (2-6 μm in diameter) shaped individual granules (Umeta and Parker, 1996; Chapter 3.1). Studies of starch composition and physico-chemical properties of five grain tef varieties indicate that tef starch has low intrinsic viscosity (peak, breakdown and setback) compared to maize starch (Chapter 3.1). The research further showed that tef starch granule composition (amylose/amylopectin ratio, protein, ash and crude fat) is similar to normal native cereal starches. Starch gelatinisation temperature measured by *Kofler* hot stage microscopy (Table 3.1) and differential scanning calorimetry methods (Table 3.5) is high like other tropical cereal starches. Lipid and phosphorus contents are high (Table 3.3), similar to normal native rice starch. The swelling factor (Figure 3.5) and crystallinity level (Table 3.4) of tef starch is lower than the maize starch but amylose leaching is higher.

In Ethiopia, tef is traditionally used in various baked foods, mostly *injera* (fermented pancake-like spongy flat bread) (Parker, Umeta and Faulks, 1989). It is also used in traditional opaque beer, local spirits, porridge and *atmit* (thin porridge) (Umeta and Faulks, 1988). In all these food and beverage products tef starch granules are structurally transformed. During the baking of *injera*, starch is completely gelatinised to form a steam-leavened, spongy texture matrix, in which fragments of bran, embryo, micro-organisms and organelles are embedded (Parker et al. 1989). Gelatinised starches have a tendency to retrograde, which affects texture, shelf-life acceptability and nutritional value of foods (Whistler and BeMiller, 1997). The smaller setback and low cold paste viscosity of tef starch compared to maize starch is an indicator of slow retrogradation tendency (Chapter 3.1.3.4), which might have a positive role in respect of storage stability of food products made with tef starch.

Additionally, depending on the degree of enzyme (Whistler and BeMiller, 1997) or acid (Whistler and BeMiller, 1997; Jayakody and Hoover, 2002) treatment, starch can be depolymerised to different types of oligosaccharides (maltodextrins and glucose products). The mode of starch granule attack by enzymes can vary with starch and enzyme type (Whistler and BeMiller, 1997). For tef starch, this type of information is

limited to the fact that during fermentation for *injera* preparation, a few starch granules are eroded by amylase (Umeta and Faulks, 1988; Parker et al. 1989).

In this paper, some functional properties of tef starch are described: paste clarity, gel texture, retrogradation, and hydrolysis with porcine pancreatic α-amylase enzyme and hydrochloric acid.

## 3.3.2. Materials and Methods

### 3.3.2.1. Grain tef samples and starch granule extraction

Starch granules from DZ-01-196, DZ-01-99, DZ-01-1681, DZ-Cr-37 and South African Brown grain tef varieties were extracted by dry/wet milling, sieving and centrifugation (Chapter 3.1.2.2). Maize starch (Merck UniLAB, code: 587 14 00) was analysed for comparison.

### 3.3.2.2. Starch paste clarity

Starch paste clarity was studied by suspending three replicate starch samples (*ca.* 50 mg ± 0.1 mg, db) in 5 mL distilled water and heating the suspension to 95 $^{o}$C in a boiling water bath for 30 min with intermittent shaking at 5 min intervals (Craig, Maningat, Seib and Hoseney, 1989). While cooling to room temperature (*ca.* 5 min), 1 mL aliquots of the solutions were diluted to 10 mL (0.1 %) with distilled water. Percentage transmittance (% T) of the 0.1 % starch solution was determined against a water blank at 650 nm in glass cuvettes using a spectrophotometer (Lambda EZ 150, Perkin Elmer, Norwalk, USA). With the Lambda EZ 150 spectrophotometer, the 1 % starch solution gave % T < 2, because of this a 0.1 % starch solution was used.

### 3.3.2.3. Starch gel texture

Starch (10 %, db) was cooked in a Rapid Visco-Analyser (RVA model 3D, Newport Scientific, Sydney, Australia) using the RVA programme described in Chapter 3.1.2.7. The starch paste from the RVA canister was immediately hot filled into shallow circular plastic dishes (*ca.* 16 mm height x 37 mm diameter) and stored for 24 h. at *ca.* 23 $^{o}$C (Takahashi and Seib, 1988). Before hot filling the starch paste, the height of each dish had been increased approximately 5 mm (i.e. from 11 mm to *ca.* 16 mm height) with adhesive tape around the rim. After 24 h. of storage the adhesive tape was removed and a smooth surface was created by cutting the excess gel above the rim with a thread. Gel texture was analysed using TA-XT2 a texture analyser (SMS Stable Micro systems, Godalming, U. K.). The starch gel *ca.* 11 mm was compressed to a distance of 5 mm into the gel with a test speed of 0.5 mm/s using a cylindrical plunger P 20 (20 mm diameter) of pre test speed 2.0 mm/s and post test speed of 10.0 mm/s. The peak height force at 5 mm compression, termed firmness,

and the negative force area of the curve during retraction of the probe, termed adhesiveness, were recorded. After 24 h. storage, the texture of the gel was evaluated as short or long by cutting the gel with a smooth blade table knife.

### 3.3.2.4. Starch gel syneresis and freeze–thaw stability

A starch slurry (8 %, w/w, db) was cooked at 90 $^{o}$C in a water bath for 35 min with constant agitation using a glass rod. The paste was cooled to *ca.* 23 $^{o}$C over 3 h. Gel syneresis and freeze–thaw stability were evaluated according to Zheng and Sosulski (1998) using *ca.* 2.5-3.5 g starch paste. Syneresis (% gel weight) was determined gravimetrically by centrifuging the sample at 3030 x g for 10 min and then decanting off the water separated. Samples were stored at 4 $^{o}$C and -18 $^{o}$C and at the intervals of 3, 7, 10 and 21 days of storage syneresis was measured. For freeze–thaw stability evaluation a sample was treated three times with a freeze-thaw cycle (freeze storage at -18 $^{o}$C for 24 h., thawed at *ca.* 23 $^{o}$C for 6 h. and re-freezed at -18 $^{o}$C) and at the end of each thaw cycle syneresis was measured.

### 3.3.2.5. *In vitro* digestibility with porcine pancreatic α-amylase (PPA)

Starch (40 mg ± 0.1 mg) was suspended in 20 mL distilled water. A 5 mL (10 mg) starch suspension was treated with 2 µL PPA (A–6255) (Sigma, St. Louis, USA) in phosphate buffer pH 6.9 by incubating in a constant temperature (37 $^{o}$C) water bath for 5 h. (Knutson, Khoo, Cluskey and Inglett, 1982). The concentration of α-amylase was 33.3 mg/mL in 2.9 M NaCl containing 3 mM $CaCl_2$ .The PPA had an activity of 1020 units/mg. One unit is defined as the activity of α-amylase which liberates 1 mg maltose in 3 min at pH 6.9 and 20 $^{o}$C. At timed intervals of: 0.5, 1, 2, 3, 4 and 5 h, 1 mL was removed into 5 mL 99.8 v/v % ethanol, to which 1 mL 0.1 M hydrochloric acid was added to stop enzyme activity. The solution was centrifuged at 2500 x g for 5 min and 1 mL of the supernatant was analysed for soluble carbohydrate by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers and Smith, 1956). A blank was treated the same way and its absorbance was subtracted from the sample absorbance. The amount of solubilised carbohydrate was determined using a D–maltose standard (5–120 µg/mL) calibration curve.

For scanning electron microscopy (SEM), enzyme attacked starch after 2 h. hydrolytic reaction was washed with ethanol (2 x 3 mL) and dried in a forced air draught oven at 50 $^{O}$C for about 8 h. The samples were prepared for SEM as described in Chapter 3.1.2.3. Samples were viewed using a JEOL JSM 840 SEM (JEOL, Tokyo, Japan) operated at an accelerating voltage of 5 kV.

#### 3.3.2.6. Acid hydrolysis

Triplicate starch samples *ca.* 0.4 g ± 0.1 mg were suspended in 16 mL 2.2 M HCl (1 g/40 mL) and incubated at 35 $^{O}$C for up to 24 days (Jayakody and Hoover, 2002). The samples were re-suspended daily by shaking and at the intervals of 1, 2, 3, 4, 8, 12, 16, 20 and 24 days, 1 mL was withdrawn, neutralised (*ca.* 1 mL, 2.2 M NaOH) and centrifuged at 2500 x g for 5 min. From the supernatant 125 µL was analysed for soluble carbohydrate by the phenol-sulphuric acid method and the amount of solubilised carbohydrate was determined with a D–maltose calibration curve.

For SEM, samples at the intervals of 1, 4, 8, 16 and 24 days of reaction were filtered and sequentially washed with ethanol (2 x 10 mL) (70 %, v/v followed by 96 %, v/v) centrifuging at 2000 x g for 5 min. The washed samples were dried at 50 $^{O}$C in a vacuum oven for over 12 h. Samples for SEM were prepared and viewed as described in Chapter 3.1.2.3.

#### 3.3.2.7. Statistical analysis

At least three replicate experiments were analysed by one–way analysis of variance (ANOVA) and compared at $p < 0.05$ using Fisher's least significant difference (LSD) test, using Statistica for Windows (Statsoft, Tulsa, USA, 1995).

### 3.3.3. Results and discussion

#### 3.3.3.1. Paste clarity

The paste clarity (% T) of most of the tef starches (DZ-01-196, DZ-01-99 and DZ-01-1681) was similar to the maize starch (Table 3.7). However, in DZ-Cr-37 and South African Brown starches it was significantly ($p < 0.05$) lower than maize starch. The starch from the red tef varieties (DZ-01-99, DZ-01-1681 and South African Brown) appeared less white than the starch from the white tef varieties (DZ-01-196 and DZ-

Cr-37) and maize starch (visual observation). Whiteness is related to the association of starch chains after pasting that reflect or scatter light (Craig et al. 1989). Whiteness is also influenced in part by non–starch components like residual proteins, lipids and trace polyphenols (Craig et al. 1989). The presence of polyphenols, which are found in the red tef varieties (Urga, Fite and Biratu, 1997 a) might have influenced the starch colour, as occurs with sorghum starch (Beta, Corke, Rooney and Taylor, 2000).

The paste clarity of starch is reported to be influenced by the interplay of: degree of swelling, granular remnants in the paste, junction zone formation of starch molecules in the paste, existence of amylose–lipid complex, cross–linking, trace non–starch components (protein, lipids, phosphorus and polyphenols) and pH (Craig et al. 1989). The % T of a paste measured at the same wavelength can also vary with type of spectrophotometer used (Craig et al. 1989). However, under uniform experimental condition the results are reproducible. Using a Varian DMS 80 spectrophotometer, paste clarity was ordered: maize < waxy maize < wheat < cassava < potato (Craig et al. 1989). On the basis of this, the paste clarity of tef starches can be categorised as being the same or slightly less clear than maize starch.

### 3.3.3.2. Gel texture

The gel texture of starches from different tef varieties and maize after 24 h. storage at ambient temperature (*ca.* 23 $^{o}$C) is shown in Figure 3.10. The starch gel of DZ-01-196 was softer than maize starch, whereas DZ-01-99 and South African Brown were firmer than maize starch ($p < 0.05$) (Table 3.7). The highest gel firmness was observed for South African Brown and the lowest was for DZ-01-196. The gel firmness of DZ-01-1681 and DZ-Cr-37 tef starches were similar to the maize starch ($p < 0.05$).

The gel of normal cereal starches of sufficient concentration (*ca.* 6–20 %) is a composite of leached continuous amylose matrix with swollen granular remnants (deformable particles) acting as fillers in the continuous phase (Hermansson and Svegmark, 1996). The gel firmness of normal cereal starch has been positively correlated with leached amylose (Tsai, Li and Lii, 1997) and starch granule associated

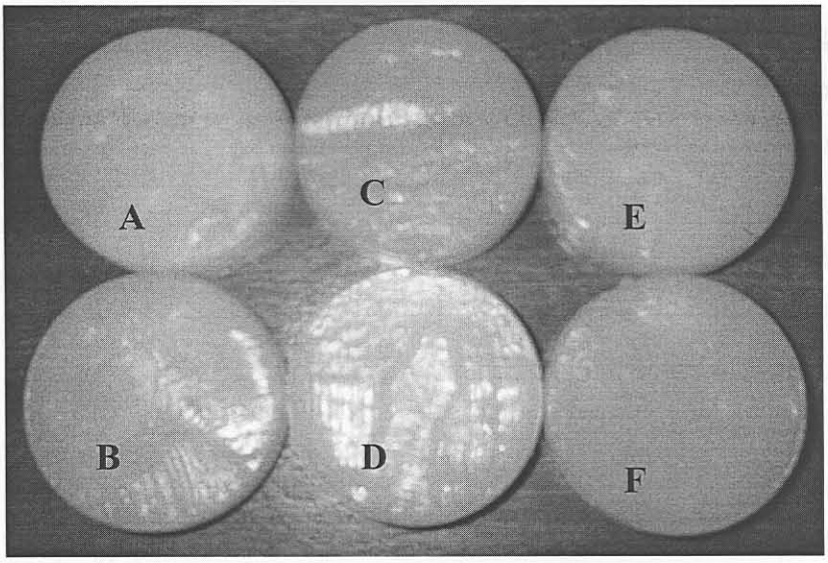


Figure 3.10. Starch gels from different tef varieties and maize (A = Maize, B = DZ-01-196, C = DZ-01-99, D = DZ-01-1681, E = DZ-Cr-37 and F = South African Brown) after 24 h. storage at ambient temperature (*ca.* 23 $^{O}$C)

proteins (Han, Campanella, Guan, Keeling and Hamaker, 2002 b), and negatively with starch lipids (Takahashi and Seib, 1988) and phosphorus content (Lin and Czuchajowska, 1998). In normal rice and maize starches, the interaction of leached amylose with remnants of swollen granular structures was considered a major factor for the reinforcement of the starch gel matrix rather than re-association of leached amylose alone (Tsai et al. 1997). The higher amylose leaching (Figure 3.5) and proteins (Table 3.1) in tef starches than in the maize starch presumably contribute positively to greater gel firmness of some of the tef starches, whereas the higher phosphorus content (Table 3.3) would negate this. Comparison of starch gel texture parameters determined by different workers is difficult due to differences in experimental conditions (sample size and shape, ratio of compressing probe size versus sample, extent of deformation, cross-head speed, number of bites and replicates per mean value) (Pons and Fiszman, 1996). However, under uniform experimental conditions, gel firmness (7–25 % starch) of normal native maize starch < normal native wheat starch (Takahashi and Seib, 1988) but > normal native oats (Zhou, Robards, Holmes and Helliwell, 1998) and rice starches (Tsai et al. 1997). On the basis of this, the gel firmness of tef starches is similar to that of maize and wheat starches.

The adhesiveness of tef starch gel (mean 16.9 g.s) was in all cases significantly ($p < 0.05$) less than maize starch (31.8 g.s) (Table 3.7). Adhesiveness is the ability of starch gel to stick to other objects (Pons and Fiszman, 1996). When the attractive force in the starch gel matrix is strong, the sticking of the probe to the gel surface is reduced (Pons and Fiszman, 1996). The data suggest that the intermolecular forces in tef starches gel are stronger than in maize. Most probably also the interaction of leached amylose to remnants of swollen granular structures is also stronger, since this interaction is more positively correlated to reinforcement of the starch gel matrix than re–association of leached amylose alone (Tsai et al. 1997).

**Table 3.7. Paste clarity and gel texture of starch from different tef varieties and maize**

| Variety | Transmittance [%] | Firmness [g] | Adhesiveness [g.s] | Gel texture (visual) |
|---|---|---|---|---|
| **DZ-01-196** | $28.7^{b} \pm 0.8$ | $586.0^{a} \pm 3.6$ | $13.7^{b} \pm 1.8$ | Short |
| **DZ-01-99** | $28.2^{b} \pm 0.5$ | $781.7^{c} \pm 21.7$ | $27.4^{d} \pm 3.0$ | Short |
| **DZ-01-1681** | $30.6^{c} \pm 1.2$ | $717.8^{b} \pm 27.8$ | $19.5^{c} \pm 2.0$ | Short |
| **DZ-Cr-37** | $25.3^{a} \pm 0.8$ | $702.3^{b} \pm 31.4$ | $19.2^{c} \pm 2.5$ | Short |
| **South African Brown** | $24.8^{a} \pm 0.6$ | $833.4^{d} \pm 19.8$ | $4.8^{a} \pm 1.8$ | Short |
| **Tef (mean)** | $27.5 \pm 2.4$ | $724.2 \pm 93.3$ | $16.9 \pm 8.4$ | Short |
| **Maize** | $29.3^{bc} \pm 0.8$ | $689.5^{b} \pm 33.4$ | $31.8^{e} \pm 0.7$ | Short |

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

### 3.3.3.3. Retrogradation

All tef starch varieties showed similar syneresis to each other in all treatments (Table 3.8). Water separated (mean of 4 and -18 $^{O}$C, and freeze–thaw treatment) from fresh tef starches paste and maize starch paste was similar ($p < 0.05$). By a similar method (Zheng and Sosulski, 1998) water separated from fresh normal native cereal starch pastes was ordered: maize > barley > oat > rice. After 3 days, syneresis was substantially lower in tef starches than maize starch in all cases. A similar trend was observed at 7, 10 and 21 storage days, and with the freeze–thaw cycle treatments. Starch retrogradation for various botanical sources was ordered: wheat, maize > rice, cassava, potato>> waxy maize (Jacobson, Obanni and BeMiller, 1997). On the basis of this the retrogradation tendency of tef starches is less than maize and wheat starches.

In tef and maize starches stored at 4 $^{O}$C and -18 $^{O}$C, syneresis increase was higher between 3 to 10 days than 10 to 21 days. For all tef starches and maize starch, syneresis was greater at 4 $^{O}$C than –18 $^{O}$C. The data suggest that retrogradation in both tef starches and maize starch was enhanced more by freeze–thaw treatments than freeze storage. This is because on freezing ice crystals are formed leaving a non–frozen starch–rich phase and on thawing water can be easily expressed leading to more syneresis than freeze storage alone (Yuan and Thompson, 1998).

Starch gel retrogradation is reported to be affected by the interplay of storage temperature, starch granule composition, amount of amylose solubilised, degree of polymerization (*dp*) of amylopectin A chains and *dp* amylose (Gudmundsson, 1994). Water influences the glass transition temperature ($T_g$) of starch gels (Fredriksson, Silverio, Andersson, Eliasson and Aman, 1998). For starch gel of 45-50 % water, the $T_g$ is *ca* -5 $^{O}$C, retrogradation is high between 23–-5 $^{O}$C and very low at freeze storage temperatures $T_g$ < -5 $^{O}$C (Gudmundsson, 1994). In this work similar results were observed (i.e. retrogradation was higher at 4 $^{O}$C storage than –18 $^{O}$C). This is because nucleation for re–crystallisation is known (Gudmundsson, 1994) to increase exponentially with decreasing temperature down to $T_g$ (-5 $^{O}$C) and crystal growth is to be favoured at temperature just below melting temperature ($T_m$) (*ca.* 23 $^{O}$C).

In starch retrogradation, gelation of amylose is a short term effect and the highest retrogradation tendency is observed for *dp* 80–100 amylose (Lu, Jane and Keeling, 1997). However, in the long term retrogradation is due to re-crystallisation of amylopectin (Gudmundsson, 1994). In waxy maize starches retrogradation was positively correlated to *dp* 14–24 amylopectin unit chains and negatively to *dp* 6–9 unit chains (Yuan and Thompson, 1998). The slower retrogradation tendency of tef starches than the maize starch in part suggest that is probably due to a higher proportion of 6–9 *dp* amylopectin A chains in tef starch than the maize starch, whereas the proportion of 14–24 *dp* amylopectin A chains may be less than the maize starch.

Lipids are also known to retard starch retrogradation by directly forming complexes with amylose, slightly interacting with amylopectin outer chains, interfering with the possible slight co–recrystallisation of amylose and amylopectin, and changing water distribution in the gel (Gudmundsson, 1994). In the lipid fractions, monoacyl lipids and free fatty acids are known to form complexes with amylose (Whistler and BeMiller, 1997). Tef starches had total lipids slightly lower than maize starch (Table 3.3) but the extent of amylose lipid complex formation may probably not follow the same trend. In fact, in the X–ray diffraction of native tef and maize starch granules, the intensity (I) for an indicator of V–type diffraction of amylose lipid complex ($d$ = 4.4 Å) was stronger in tef starches (I = 36 %) than maize starch (I = 24 %) (Table 3.4), which suggests the extent of amylose–lipid complex formation is slightly higher in tef starch than maize starch. Thus, the slower retrogradation of tef starch in part could probably be related to the extent of amylose–lipid complex formation.

Table 3.8. Syneresis of starch [%] from different tef varieties and maize after storage at 4 $^{O}$C and –18 $^{O}$C, and freeze–thaw treatments

| Storage at 4 $^{O}$C | | | | | |
|---|---|---|---|---|---|
| **Variety** | **Fresh paste** | **3 Days** | **7 Days** | **10 Days** | **21 Days** |
| **DZ-01-196** | $2.6^{a} \pm 0.3$ | $6.7^{a} \pm 0.3$ | $13.1^{a} \pm 0.3$ | $21.2^{a} \pm 0.6$ | $31.5^{a} \pm 1.1$ |
| **DZ-01-99** | $2.7^{a} \pm 0.3$ | $6.9^{a} \pm 0.3$ | $13.5^{a} \pm 0.3$ | $21.8^{a} \pm 0.7$ | $32.5^{a} \pm 0.0$ |
| **DZ-01-1681** | $2.4^{a} \pm 0.4$ | $6.6^{a} \pm 0.8$ | $12.8^{a} \pm 0.7$ | $21.0^{a} \pm 1.1$ | $31.6^{a} \pm 1.2$ |
| **DZ-Cr-37** | $2.5^{a} \pm 0.2$ | $6.4^{a} \pm 0.2$ | $12.5^{a} \pm 0.8$ | $20.3^{a} \pm 1.0$ | $30.5^{a} \pm 1.1$ |
| **South African Brown** | $2.5^{a} \pm 0.5$ | $6.6^{a} \pm 1.0$ | $12.8^{a} \pm 2.0$ | $20.8^{a} \pm 3.3$ | $31.5^{a} \pm 4.1$ |
| **Tef (mean)** | $2.5 \pm 0.1$ | $6.6 \pm 0.2$ | $12.9 \pm 0.4$ | $21.0 \pm 0.5$ | $31.5 \pm 0.7$ |
| **Maize** | $2.9^{a} \pm 0.2$ | $8.9^{b} \pm 0.4$ | $16.8^{b} \pm 0.8$ | $27.1^{b} \pm 1.0$ | $40.4^{b} \pm 1.9$ |
| **Storage at -18 $^{O}$C** | | | | | |
| **Variety** | **Fresh paste** | **3 Days** | **7 Days** | **10 Days** | **21 Days** |
| **DZ-01-196** | $2.6^{a} \pm 0.6$ | $4.0^{a} \pm 0.4$ | $6.1^{a} \pm 0.5$ | $9.2^{a} \pm 0.2$ | $14.2^{a} \pm 0.3$ |
| **DZ-01-99** | $2.7^{a} \pm 0.2$ | $4.2^{a} \pm 0.4$ | $6.3^{a} \pm 0.5$ | $9.9^{a} \pm 0.7$ | $14.7^{a} \pm 0.8$ |
| **DZ-01-1681** | $2.6^{a} \pm 0.5$ | $4.1^{a} \pm 0.5$ | $6.0^{a} \pm 0.7$ | $9.4^{a} \pm 1.1$ | $14.2^{a} \pm 1.1$ |
| **DZ-Cr-37** | $2.4^{a} \pm 0.2$ | $3.5^{a} \pm 0.2$ | $5.5^{a} \pm 0.2$ | $8.8^{a} \pm 0.6$ | $13.7^{a} \pm 0.9$ |
| **South African Brown** | $2.4^{a} \pm 0.4$ | $3.9^{a} \pm 0.5$ | $5.7^{a} \pm 0.2$ | $9.1^{a} \pm 0.2$ | $14.0^{a} \pm 0.3$ |
| **Tef (mean)** | $2.5 \pm 0.1$ | $3.9 \pm 0.3$ | $5.9 \pm 0.3$ | $9.3 \pm 0.4$ | $14.2 \pm 0.4$ |
| **Maize** | $3.0^{a} \pm 0.1$ | $5.8^{b} \pm 0.4$ | $9.5^{b} \pm 0.5$ | $15.0^{b} \pm 0.9$ | $22.2^{b} \pm 1.7$ |

**Table 3.8. Continued**

| Variety | Freeze–thaw cycle* treatment | | | |
|---|---|---|---|---|
| | Fresh paste | 1st cycle | 2nd cycle | 3rd cycle |
| **DZ-01-196** | $2.6^{a} \pm 0.7$ | $4.1^{a} \pm 0.6$ | $7.1^{a} \pm 0.7$ | $12.3^{a} \pm 0.9$ |
| **DZ-01-99** | $2.4^{a} \pm 0.3$ | $3.7^{a} \pm 0.1$ | $6.5^{a} \pm 0.1$ | $11.5^{a} \pm 0.1$ |
| **DZ-01-1681** | $2.7^{a} \pm 1.1$ | $4.0^{a} \pm 0.9$ | $7.1^{a} \pm 1.2$ | $12.4^{a} \pm 1.2$ |
| **DZ-Cr-37** | $2.6^{a} \pm 0.3$ | $3.9^{a} \pm 0.3$ | $7.1^{a} \pm 0.1$ | $11.9^{a} \pm 0.5$ |
| **South African Brown** | $2.4^{a} \pm 0.4$ | $3.6^{a} \pm 0.4$ | $6.7^{a} \pm 0.4$ | $11.4^{a} \pm 0.7$ |
| **Tef (mean)** | $2.5 \pm 0.1$ | $3.9 \pm 0.2$ | $6.9 \pm 0.3$ | $11.9 \pm 0.5$ |
| **Maize** | $3.0^{a} \pm 0.2$ | $5.6^{b} \pm 0.1$ | $10.6^{b} \pm 0.1$ | $17.4^{b} \pm 0.6$ |

Values within the same column with different letters are significantly different ($p < 0.05$). Where * is one freeze–thaw cycle = freeze storage at –18 $^{O}$C for 24 h., thaw to *ca.* 25 $^{O}$C for 6 h. and refreeze at –18 $^{O}$C.

### 3.3.3.4. Starch *in vitro* digestibility with PPA

For the tef starches at 1, 3 and 5 h. hydrolysis, the digestibilities were in the range: 8.7-11.9, 20.4-24.0 and 29.5-35.3 %, respectively, and for maize starch 3.8, 20.8 and 31.0 %, respectively (Table 3.9). Digestibility up to two h. was relatively higher in the tef starches than maize starch and thereafter the difference was minimal. This is probably because during the early period, the amorphous portion is readily degraded (Sreenath, 1992), which is higher in tef starches (63 %) than in maize starch (60 %) (Chapter 3.2.3.5).

The native tef starch granule is smooth with no surface pores (Figure 3.11 A), whereas in maize starch surface pores are evident (Figure 3.11 B). The mode of PPA attack on all tef starch varieties appeared to be the same. Because of this SEM (Figure 3.11) is only shown for DZ-01-196 (C), DZ-01-1681 (D) and South African Brown (E) starches. The mode was surface erosion that enlarged at each shell level in to the granule (Figure 3.11, C, D and E). In some granules, surface eroded large holes were observed (Figure 3.11, C, E). In maize starch, the mode of α-amylase attack is known as random pitting (holes) (Sreenath, 1992) and this was observed (Figure 3.11, F). Since at the early stage of digestion, the amorphous portion is preferentially attacked (Oates, 1997), the difference in the mode of attack between tef starch and maize starch indicates that the tef and maize starch granules are different in their amorphous and crystalline lamellae distribution. Enzymic starch degradation within the same starch preparation is not uniform and will differ granule by granule, with an already attacked granule being hydrolysed more rapidly (Oates, 1997). In this work, the same was found, since some granules appeared less attacked (Figure 3.11, C, D, E). Wheat, barley and rye starch granules (A type) as well as cassava and sweet potato starch granules have susceptibility zones and their mode of attack is endocorrosion (channels from selected points on the surface towards the centre of the granule) creating pits that sink into the interior (Oates, 1997). In normal maize and rice starches the mode of attack is also endocorrosion with pitted channels enlarged at each shell level through the granules (Oates, 1997). In tef hydrolysis is also endocorrosion in nature (Figure 3.11, C, D and E). However, potato and some B type starches are slightly eroded by exocorrosion on the entire granule surface or portion of it (Oates, 1997).

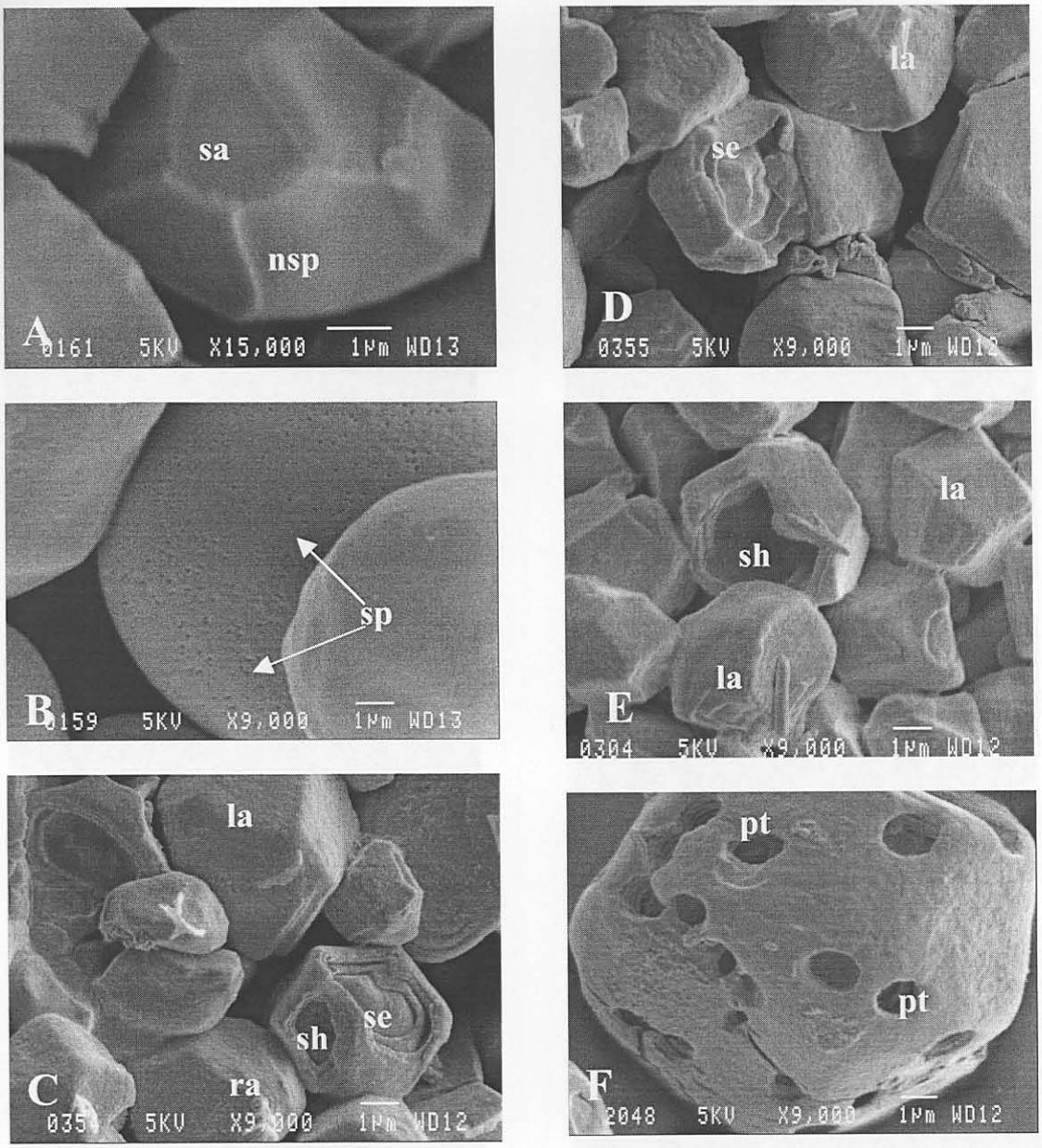


Figure 3.11. SEM of porcine pancreatic α-amylase treated starches from different tef varieties and maize. A and B are untreated and C–F are treated. A = DZ-01-196, B = Maize, C = DZ-01-196, D = DZ-01-1681, E = South African Brown and F = Maize, sa = smooth surface and sharp edged, nsp = no surface pores, sp = surface pores, se = surface erosion, sh = surface eroded hole, la = less attacked and pt = pit

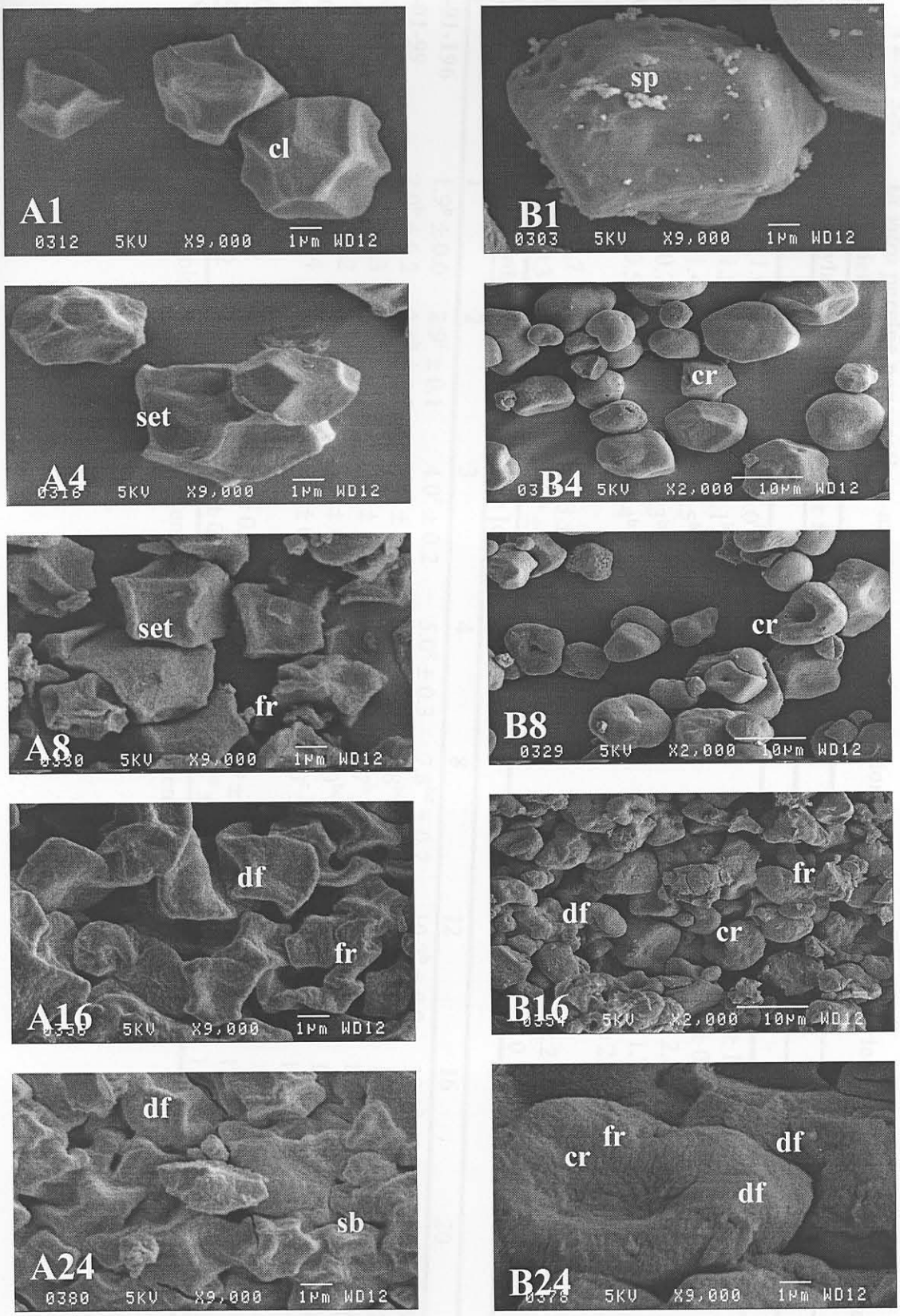


Figure.3.12. SEM of mild HCl treated starch granules from tef variety DZ-01-196 and maize after 1, 4, 8, 16 and 24 days of treatment. A = Tef, B = Maize, cl = clean, sp = spots, set = surface etching, fr = fragmented, df = deformed, sb = solubilised and cr = crater

**Table 3.9.** ***In vitro*** **porcine pancreatic α-amylase enzyme digestion and mild hydrochloric acid hydrolysis of starches from different tef varieties and maize**

| Variety | α-amylase digestion [%] over time [h.] | | | | | |
|---|---|---|---|---|---|---|
| Time [h.] | 0.5 | 1 | 2 | 3 | 4 | 5 |
| DZ-01-196 | $5.1^{b} \pm 1.6$ | $9.1^{b} \pm 1.9$ | $16.0^{b} \pm 1.4$ | $23.5^{ab} \pm 1.9$ | $29.0^{a} \pm 1.1$ | $31.5^{ab} \pm 1.1$ |
| DZ-01-99 | $5.3^{b} \pm 1.5$ | $11.9^{c} \pm 1.6$ | $18.1^{b} \pm 1.9$ | $23.5^{ab} \pm 2.4$ | $30.7^{a} \pm 1.7$ | $32.6^{bc} \pm 0.6$ |
| DZ-01-1681 | $3.5^{b} \pm 1.4$ | $9.6^{bc} \pm 0.2$ | $15.5^{b} \pm 0.7$ | $21.7^{ab} \pm 0.1$ | $28.4^{a} \pm 2.2$ | $29.5^{a} \pm 2.8$ |
| DZ-Cr-37 | $4.8^{b} \pm 0.7$ | $8.7^{b} \pm 1.9$ | $16.3^{b} \pm 1.2$ | $24.0^{b} \pm 2.6$ | $30.0^{a} \pm 3.1$ | $35.3^{c} \pm 1.7$ |
| South African Brown | $4.7^{b} \pm 0.5$ | $11.0^{bc} \pm 2.2$ | $15.4^{b} \pm 2.4$ | $20.4^{a} \pm 2.4$ | $29.5^{a} \pm 4.2$ | $33.9^{bc} \pm 2.0$ |
| Tef (mean) | $4.7 \pm 0.7$ | $10.1 \pm 1.3$ | $16.3 \pm 1.1$ | $22.6 \pm 1.5$ | $29.5 \pm 0.9$ | $32.6 \pm 2.2$ |
| Maize | $1.4^{a} \pm 0.3$ | $3.8^{a} \pm 0.3$ | $9.4^{a} \pm 3.6$ | $20.8^{ab} \pm 1.4$ | $28.1^{a} \pm 1.0$ | $31.0^{ab} \pm 0.7$ |

| Variety | Acid hydrolysis [%] over time [days] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 3 | 4 | 8 | 12 | 16 | 20 | 24 |
| DZ-01-196 | $1.9^{b} \pm 0.0$ | $2.9^{c} \pm 0.1$ | $4.0^{c} \pm 0.2$ | $5.0^{c} \pm 0.3$ | $7.8^{bc} \pm 0.2$ | $10.7^{b} \pm 0.9$ | $13.1^{b} \pm 1.0$ | $17.6^{b} \pm 0.6$ | $20.4^{b} \pm 2.3$ |
| DZ-01-99 | $2.0^{b} \pm 0.2$ | $2.6^{b} \pm 0.1$ | $3.7^{b} \pm 0.3$ | $4.7^{bc} \pm 0.1$ | $7.8^{bc} \pm 0.1$ | $11.3^{b} \pm 0.5$ | $14.1^{b} \pm 0.3$ | $18.6^{cd} \pm 0.2$ | $22.7^{bc} \pm 0.3$ |
| DZ-01-1681 | $2.0^{b} \pm 0.3$ | $2.5^{b} \pm 0.1$ | $3.4^{b} \pm 0.2$ | $4.7^{bc} \pm 0.2$ | $7.7^{b} \pm 0.4$ | $11.1^{b} \pm 0.5$ | $13.6^{b} \pm 0.9$ | $17.9^{bc} \pm 0.5$ | $21.5^{bc} \pm 1.3$ |
| DZ-Cr-37 | $2.0^{b} \pm 0.2$ | $2.5^{b} \pm 0.1$ | $3.4^{b} \pm 0.2$ | $4.6^{b} \pm 0.1$ | $8.0^{bc} \pm 0.2$ | $11.5^{b} \pm 0.5$ | $14.0^{b} \pm 0.3$ | $18.7^{cd} \pm 0.2$ | $23.0^{c} \pm 1.4$ |
| South African Brown | $2.0^{b} \pm 0.4$ | $2.5^{b} \pm 0.2$ | $3.6^{b} \pm 0.1$ | $4.8^{bc} \pm 0.3$ | $8.0^{c} \pm 0.1$ | $11.8^{b} \pm 0.9$ | $14.1^{b} \pm 0.1$ | $18.9^{d} \pm 0.8$ | $22.4^{bc} \pm 0.4$ |
| Tef (mean) | $2.0 \pm 0.0$ | $2.6 \pm 0.2$ | $3.6 \pm 0.2$ | $4.8 \pm 0.2$ | $7.9 \pm 0.1$ | $11.3 \pm 0.4$ | $13.8 \pm 0.4$ | $18.3 \pm 0.6$ | $22.0 \pm 1.1$ |
| Maize | $1.3^{a} \pm 0.2$ | $1.8^{a} \pm 0.1$ | $2.9^{a} \pm 0.1$ | $4.2^{a} \pm 0.1$ | $6.8^{a} \pm 0.1$ | $9.1^{a} \pm 0.4$ | $11.4^{a} \pm 0.8$ | $14.2^{a} \pm 0.5$ | $16.7^{a} \pm 1.0$ |

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

Enzymic starch degradation can be affected by the interplay of: kinetics involved (enzyme diffusion to starch surface, adsorption and catalysis), starch granule morphology, crystalline type, crystalline and amorphous distribution, starch granule composition, starch state (native or gelatinised) and enzyme type (Oates, 1997). In maize and cassava starches, digestibility with α-amylase was greater in smaller starch granules than larger (Franco, Preto and Ciacco, 1992). Amylolysis for different crystalline types is in the order of: A ≥ V > C > B (Gérard, Colonna, Buléon and Planchot, 2001). Digestion of different starches by α-amylase was found to be in the order of: rice > maize > wheat > sweet potato > potato (Sarikaya, Higasa, Adachi and Mikami, 2000). The granule size (Chapter 3.1.3.1) and amorphous proportion (Chapter 3.2.3.5) of tef and rice starches are similar and during the earlier stages, digestibility of tef starch was greater than in maize starch. All this suggests that extent of native tef starch digestibility is similar to rice starch. Amylolysis by PPA was found to be high for amylopectin with a relatively high proportion of 2-7 *dp* and fewer 8-50 *dp*, because of possible lower crystallites proportion in the former than latter (Gérard et al. 2001). The slightly higher PPA digestion of tef starch than the maize starch suggests that in tef starch amylopectin chains with 2–7 *dp* are slightly more abundant than in maize starch.

### 3.3.3.5. Starch acid hydrolysis

At all incubation times tef starch was slightly more etched by mild HCl than maize starch (Table 3.9). In both tef starch and maize starch, the increase in hydrolysis was slightly faster before 8 days than thereafter. This is because in part during the earlier stages, acid rapidly attacks amorphous regions, thereafter the crystalline regions are attacked slowly (Jenkins and Donald, 1997).

Acid treated tef starch varieties observed by SEM appeared to be the same. Because of this only SEM of DZ-01-196 at intervals of 1, 4, 8, 16 and 24 days are shown (Figure 3.12 A). At 1 day, the surface of tef starch granule appeared clean with slight etching (Figure 3.12 A1), whereas small spots were seen on the surface of maize starch granules (Figure 3.12 B1). At 4 days, the tef starch granules were slightly surface etched and denuded (Figure 3.12 A4), whereas in maize starch granules, the surface appeared clean but on one side, in some granules, a crater started to appear

(Figure 3.12 B4). At 8 days, tef starch granules were deeply surface etched, denuded and some fragments also appeared (Figure 3.12, A8), whereas in the maize starch granules more craters appeared (Figure 3.12, B8). After 24 days most granule features of tef starch granules were either deformed or lost and the granules appeared more like solubilised starch (Figure 3.12 A24), whereas in the maize starch granules more deformation and craters appeared (Figure 3.12 B24). In tef, due to absence of surface pores (Figure 3.11 A), the acid reaction seemed to be at the surface (Figure 3.12 A1–A24). In maize, due to presence of surface pores (Figure 3.11 B), the acid is known to corrode inside the granule (Jayakody and Hoover, 2002), as observed (Figure 3.12 B1–B24).

In normal maize, rice, oats, waxy maize, amylomaize V and VII starches, the extent of hydrolysis before 8 days was reported to be influenced by the interplay of amount of amylose complexed with the lipids, amylose content, starch granule size and pores on the granule surface, whereas beyond 8 days it was influenced by mode of $\alpha$ (1→6) bond distribution between amorphous and crystallite regions, amylopectin content and packing nature of double helices within the crystallites (Jayakody and Hoover, 2002). The smaller tef starch granule size (Chapter 3.1.3.1) and higher amorphous percentage than maize starch (Chapter 3.2.3.5) presumably contributed positively to the earlier greater extent of hydrolysis than maize starch. However, the absence of surface pores (Chapter 3.1.3.1) in the tef starch negated this. Comparing the data of Jayakody and Hoover (2002) and Singh and Ali (2000), hydrolysis with mild HCl can be ordered: waxy maize > rice > maize ≈ oats > wheat > finger millet > cassava > potato. On the basis of this, the hydrolysis of tef starch is similar to normal cereal starches that are hydrolysed slightly faster than normal maize starch (e.g. rice).

### 3.3.4. Conclusions

The paste clarity of tef starch is similar to maize starch. The gel texture of tef starch is short and is generally firmer than maize starch. The retrogradation tendency is slower than maize starch, which is beneficial for applications where starch staling is preferred to be reduced. Alpha–amylase degradation of tef starch is by surface erosion, endocorrosion in nature, with channels that enlarge through granules at each shell

level. Mild acid gradually denudes the surface, deforms and fragments the granules. The alpha–amylase and mild HCl degradation of tef starch seems similar to rice starch degradation. Since the extent and type of tef starch modification by alpha–amylase and mild HCl are slightly different from maize starch, the amorphous and crystalline lamellae distribution in the tef starch granule is also most probably different. The nature of enzyme modified and acid modified products could also possibly be different from maize starch degradation products, in terms of their *dp*.

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