2. INTRODUCTION

2.1. Statement of Problem

Grain tef is cultivated in Ethiopia as a major cereal food crop and represents about 20 % of total cereal production in the country (Central Statistical Authority, CSA, 1997). The USA and South Africa also produce grain tef for human consumption (National Research Council of the USA, NRC, 1996). In some countries (Australia, Kenya, India and South Africa) it is cultivated as a forage crop (Ebba, 1969; NRC, 1996). In Ethiopia, foods prepared from grain tef are staples for the majority of Ethiopians and tef *injera* (a fermented, flat, spongy and pancake–like sour bread) is regarded as the national staple food (Ebba, 1969). Tef is also used to prepare porridges, *atmit* (fermented thin porridge), opaque beer and local spirits (Ebba, 1969). In the USA, tef usage in some products like breakfast cereals, banana bread, waffles, great chocolate cake, icing and filling has been promoted (Ketema, 1993). Tef contains some 73 % (g) carbohydrate (NRC, 1996), of which the largest proportion is starch. In all these food products the starch is structurally manipulated; in most cases gelatinised, pasted and/or gelled.

Starch is the major source of carbohydrates in the human diet (Lillford and Morrison, 1997). In addition to being a nutrient, starches and modified starches are used for their functionalities, such as in thickening, gelling, stabilising, sweetening, bulking, texturising, fat replacement, film–formation, foam strengthening, antistaling, dusting, moisture and flavour binding applications (Lillford and Morrison, 1997). Starches are also used in other industries like alcohol (Kennedy, Cabral, Sá-Correia and White, 1987), paper (Mentzer, 1984), adhesives (Kennedy and Fischer, 1984), plastics (Otey and Doane, 1984), pharmaceuticals and cosmetics (Jane, 1997). In the food industry, granular starch has been commonly used as a dusting agent for candy, a carrying agent for baking powder, and as a mould for gum drop manufacture (Jane, 1997). Starch pastes are used in food products for thickening, fillings, fat mimetics, and providing body texture to beer and soft drinks (Thomas and Atwell, 1999). Starch gel also gives desirable textures to foods, such as crispy coatings to fried chicken and fish (Thomas and Atwell, 1999). The extent of starch gelatinisation in baked foods

strongly affects product properties, including its storage behaviour and rate of digestion (Whistler and BeMiller, 1997). Some quality defects in food products, such as bread staling and loss of viscosity and precipitation in soups and sauces are due to retrogradation of starch (Whistler and BeMiller, 1997). Thus, information on the physico-chemical properties of tef starch such as gelatinisation, pasting, solubility, gellation and retrogradation are technically important (i.e. for processing, distribution and storage) in the utilisation of tef starch based foods.

The physico-chemical property and functionality of a starch are influenced by its composition (amylose/amylopectin ratio, lipids, proteins, phosphorus and other trace microelements: potassium, sodium, calcium and magnesium) and molecular size of amylose and amylopectin (Swinkels, 1985). Starches from different botanical origins have different compositions and ultrastructures (Swinkels, 1985). They can have different merits and shortcomings with respect to processing requirements (resistance to shear, high temperature and low pH processing conditions). Suitable starch functionality is an important factor for the success of new product development in food or non–food application of starches. Research on different botanical sources of starches is always important because it will add new information and can lead to alternative opportunities for the exploitation of the starch if more desirable and more suitable functionalities are offered by the novel starch.

Information available on the properties of grain tef starch is very limited and is only related to the preparation of *injera* (Umeta and Faulks, 1988; Parker, Umeta and Faulks, 1989; Umeta and Parker, 1996). Ethiopia has different landraces and released varieties of tef which are used as food ingredients, ranging from local beverages to various baked products. Thus, in view of the fact that tef is used as a major food ingredient by most Ethiopians, and its potential to be used in different other food applications (worldwide), there is a need to generate basic scientific information. In this thesis, therefore, the composition, physico-chemical and functional properties of five grain tef starch varieties are reported. The information will be helpful in food processing and other related industries where starch is used. It will also help breeders to know their materials better and to define breeding strategies in a more refined way.

2.2. Literature Review

2.2.1. Description of tef crop, origin, cultivation and production

2.2.1.1. Description of the tef plant

Tef is a C_4 self–pollinated tetraploid cereal plant with a chromosome number of 2n = 4X = 40 (Berhe, 1975; Ketema, 1993). The tef field plant and panicles for some varieties are shown in Figure 2.1. The root system is fibrous and most stems are erect, some are bending or elbowing types (Ketema, 1993). It has a panicle type of inflorescence showing different forms (from loose to compact) (Ketema, 1993). Its spikelets have 2–12 florets (Ketema, 1997). Each floret has a lemma, palea, three stamens, mostly two ovaries (in some exceptional cases three) and feathery stigmas (Ketema, 1997). In most varieties the plant height ranges from 50–120 cm (Tefera, Ayele and Assefa, 1995). A single tef plant can produce up to 50 000 grains (Tefera et al. 1995). The grain comes in a range of colours from milky white (preferred among rich people) to almost dark brown (consumed mostly by the poor) (Ketema, 1993). The most common colours are very white, white, light brown and dark brown. The tef grain size is 0.9 to 1.7 mm in length and 0.7 to 1.0 mm in diameter (Ebba, 1975). Umeta and Parker (1996) described the grain as very small, oval, uniform in size (1.0–1.2 mm in length) with an individual mass (mean) 0.62 mg ± 0.05 for white tef and 0.83 mg ± 0.02 for red tef. For 1000 grains, a mass of 50 g was recorded for wheat, whereas for tef it was 0.6–0.8 % of the wheat mass (Almgard, 1963). Lester and Bekele (1981) described the grain weight as being generally ≤ 2 mg.

2.2.1.2. Name and origin of grain tef

Tef [*Eragrostis tef* (Zucc.) Trotter] belongs to the family of Poaceae, subfamily Eragrostoidae, tribe Eragrosteae and genus *Eragrostis* (Costanza, DeWet and Harlan, 1979). About 300 species are known in the genus *Eragrostis* (Bekele and Lester, 1981). Chloridoideae is synonymously used for Eragrostoidae of tef. Vernacular

names in different parts of the world are: Afrikaans: *tef, gewone bruin tef* (*ou bruin*); Arabic: *tahf*; English: *tef, teff*, Williams lovegrass; Ethiopian: *tafi* (*taafi*) (Oromo/Afar/Sodo), *tafe-e* (Had); *t'ef, teff, taf* (Amarinya, Tigrinya languages); French: *mil èthiopien;* Malawian: *chimanganga, ndzungula (Ch), chidzanjala (Lo)* (NRC, 1996).

The tef crop is indigenous to the central highlands of Ethiopia (Costanza et al. 1979). Ethiopia is also considered as the major world centre for the genetic diversity of tef (Ebba, 1975; Ketema, 1993). The Ethiopian Biodiversity Institute has conserved some 3842 accessions of tef for varietial improvement study and to reduce genetic erosions (Ketema, 1997). However, the exact details of the domestication of tef are unclear (Costanza et al. 1979). It is believed first to have been domesticated by Cushites (Costanza et al. 1979), and perhaps also by Semites (Vavilov, 1951). There is also a report that tef seed and straw have been recognised in Neolithic sites in Ethiopia dated at about 2600 BC (Tatham, Fido, Moore, Kasarda, Kuzmicky, Keen and Shewry, 1996).

2.2.1.3. Grain tef cultivation and production

Tef can adapt to a wide range of environments (moisture stress, high rain fall, different soil types and wide range altitudes) (NRC, 1996). It can grow from near sea level to altitudes of over 3000 m. However, the best conditions for tef cultivation are 1800–2100 m above sea level, a temperature range of between 10–27 ^{O}C over the cultivation period, an annual rainfall of 750–850 mm and a growing season rainfall of 450–550 mm (Ketema, 1997).

Tef is known to have fewer disease and pest problems as compared to other common cereals (maize, sorghum, wheat and barley). The grain is less attacked by weevils and other storage pests (Stewart and Yirgou, 1967; Ketema, 1997). It is stored relatively safely under traditional storage conditions (Ketema, 1997). However, the productivity of tef is low and the average national yield recorded is less than 1 tonne per hectare (9.4 q/ha) (CSA, 1997). Lack of high yielding cultivars, lodging, weeds, water–logging, low moisture and low fertility conditions have been noted as major

factors that contribute to the low grain yield of tef (Ketema, 1993). Threshing of its tiny seeds is also not simple. However, breeders and agronomists particularly in Ethiopia, are trying to solve such technical production problems to improve yield and nutritional composition (Berhe, 1975; Tefera et al. 1995; Hundera, Bechere and Tefera, 1999; Hundera, Tefera, Assefa, Tefera, Kefyalew and Girma, 2000).

From 1970 to 1995 ten improved tef varieties were released for large–scale production in some parts of Ethiopia (Tefera et al. 1995). The yield of these improved varieties under improved technologies (fertilizer, weed control, appropriate harvesting and threshing of the grain) were in the range of 17–22 q/ha. Recent literature even indicates that some improved varieties can yield as high as 27 q/ha under improved technologies in a suitable location (Hundera et al. 2000).

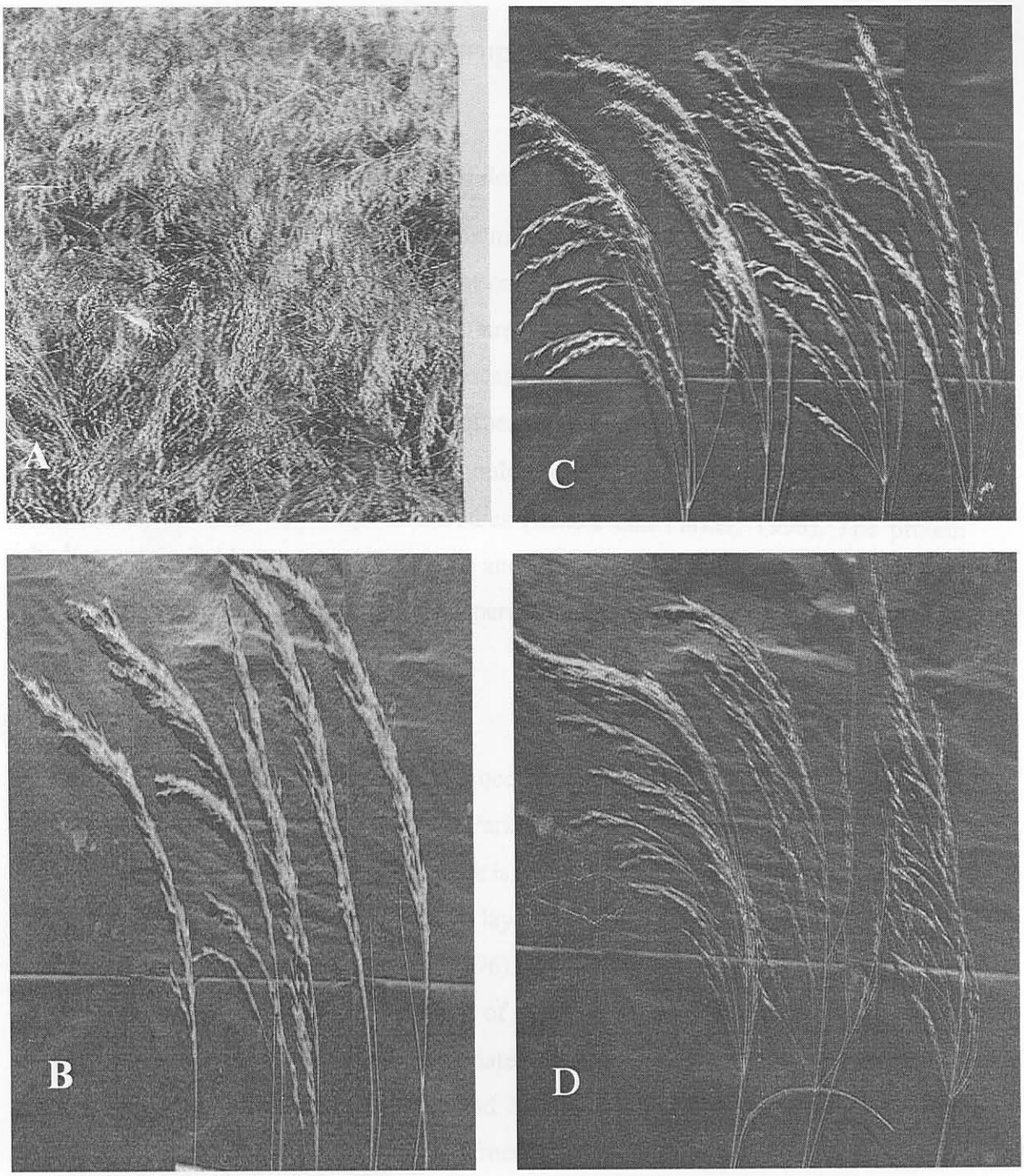

Figure 2. 1. The tef plant: (A) tef field of DZ-01-196; (B) panicles of DZ-01-196 very white seeded; (C) panicles of DZ-Cr-37 white seeded and (D) panicles of DZ-01-99 brown seeded (Tefera et al. 1995)

2.2.2. Physico–chemical property and usage of grain tef

2.2.2.1. Grain tef an anatomical description

The central endosperm of the tef grain is mealy (floury or opaque) and the outer cells of the endosperm are horny (translucent or vitreous) (Parker et al. 1989; Umeta and Parker, 1996). The starch granules in tef are compound type representing the contents of one amyloplast. Most granules are located in the central endosperm, while a few are located in the outer cells of the endosperm (Umeta and Parker, 1996). On milling, polygonal shaped individual starch granules of size between 2–6 μm are released along with small groups of protein bodies (Umeta and Parker, 1996). The protein bodies are individual entities in nature and spherical in shape, most of which are located in the outer cells of the endosperm with a few in the central part of the endosperm (Umeta and Parker, 1996).

Like other small cereal grains, the germ occupies a relatively large proportion of the kernel and is rich in protein and lipids (Parker et al. 1989; Umeta and Parker, 1996). The aleurone layer, which is one cell thick is rich in protein and lipid bodies (Parker et al. 1989). Outside the aleurone layer, one layer of fused mesocarp and endocarp of the pericarp is found (Umeta and Parker, 1996). Within the pericarp there is a testa, the thickness of which varies with the colour of the grain (Umeta and Parker, 1996). The testa of red tef is filled with pigmented material, tannins or polyphenolic compounds (Umeta and Faulks, 1988; Urga, Fite and Biratu, 1997 a). Like sorghum, tef also contains starch granules in the pericarp (Umeta and Parker, 1996).

2.2.2.2. Grain tef biochemical composition

Tef grain biochemical composition from various sources is given in Table 2.1. Comprehensive data including vitamins, essential amino acids and micronutrients are cited in NRC (1996). The protein content of the grain is in the range of 9.38–13.33 % (Jansen, Dimaio and Hause, 1962; Rouk and Mengesha, 1963; Assefa, 1978; Areda, 1995; NRC, 1996; Hundera, 1998). The protein content was noted as slightly higher

than in the normal sorghum, maize or oats (NRC, 1996). The fractional protein composition of the grain was reported (Assefa, 1978) as: glutelins 45 %; albumins 37 %; prolamins 12 % and globulins 7 %, whereas according to Bekele (1995) it was reported as 28–42 %; 24–39 %; 3–15 % and 14–34 %, respectively. Tef protein was cited as being essentially free of gluten of the type found in wheat (NRC, 1996). The main protein fractions, viz. albumins, glutelins and globulins are the most digestible types of cereal proteins and it is presumed that their digestibilities are also high in tef (NRC, 1996). Major prolamins of tef were characterised and found similar to the α–prolamins of maize, sorghum and *coix* (Tatham et al. 1996). The amino acid composition of tef protein is reported to be similar to that of egg, except for its lower lysine content (Jansen et al. 1962). Through analysis of the data of Jansen et al. (1962) and Areda (1990), the amino acid profile of tef was reported as excellent, except that its lysine content was lower as compared to rice and oats (Ketema, 1993).

Fibre, fat and ash contents were reported in the ranges of 1.98–3.50 %, 2.00–3.09 % and 2.66–3.00 %, respectively (Rouk and Mengesha, 1963; NRC, 1996). The calcium, iron, copper, zinc, aluminum and barium contents of tef are reported high, as compared to barley, wheat and sorghum (Mengesha, 1966). The relatively high iron content reported by some workers (Rouk and Mengesha, 1963; Mengesha, 1966) was ascribed as in part contamination of the grain with soil during harvest (Almgard, 1963; Besrat, Admasu and Ogbai, 1980; Ramachandran and Bolodia, 1984; Mamo and Parsons, 1987; Areda, Ketema, Ingram and Davis, 1993). Contamination of tef with soil under Ethiopian harvesting conditions is impossible to prevent and contributes to the high iron content in the tef diet (Besrat et al. 1980). In Ethiopia, an absence of iron deficiency anaemia among tef consumers was reported to be due to high iron content in the diet (Besrat et al. 1980). Even though there is a contribution of soil contamination to high iron in the diet, Ramachandran and Bolodia (1984), however, concluded that the absence of iron deficiency anaemia is as largely due to fermentation, since most food products of tef like *injera* are fermented. Recent studies also indicate that due to the destruction of phytic acid during fermentation the bio–availability of micro–nutrients is higher in fermented tef food products than in unfermented products (Urga et al. 1997 a; Urga, Keshava and Narasimha, 1997 b; Urga and Narasimha, 1998; Urga and Keshava, 1998; Urga, Narasimha, Sasikala and

Vishwantha, 1998).

The carbohydrate content of tef was recorded as 73 % (g) of which the largest proportion is starch (NRC, 1996). The limited information available on tef starch is related to the fermentation step in the making of *injera*, which indicates that a few starch granules are eroded by amylase (Umeta and Faulks, 1988; Parker et al. 1989; Umeta and Parker, 1996). On fermentation (72 h.) before baking of *injera* about 9 % of the starch was utilized by fermenting organisms (Umeta and Faulks, 1988). Free sugars were estimated at *ca* 2.7g/100 g (db) before fermentation, of which the predominant sugar was sucrose and while on fermentation and in the baked *injera,* fructose was found to be predominant. Free sugar was observed to increase until 24 h. hereafter it decreased (Umeta and Faulks, 1988). Lactic and acetic acid were reported to be the major organic acids produced (90 %) with other small quantity volatile fatty acids (VFAs) (propionic, isobutyric, n–butyric, isovaleric, n–valeric, isocaproic and n–caproic acids) on fermentation (Umeta and Faulks, 1989). In the baked *injera* the concentration of lactic acid remained constant, acetic acid content was reduced due to evaporative loss while for the VFAs an increase in concentration for propionic, n–valeric and n–caproic acids was observed. Other acids remained nearly unchanged (Umeta and Faulks, 1989). The sour taste of *injera* was reported to be mainly due to lactic and acetic acids, whereas the other VFAs were the main contributors to the flavour of *injera* (Umeta and Faulks, 1989).

Table 2. 1. Tef grain biochemical composition (db)

Biochemical class	Compound or Micro–element	Values, where available in a range	Summary	Remark
Moisture (%)		11.0[14]	11.0	
Protein (%) (N x 6.25)		9.69-12.56[1], 10.83-11.67[2], 11.70[5], 10.81-13.33[12], 11.0[13], 9.6[14], 9.38-12.75[16]	9.38-13.33	
Carbohydrate (%)		73.0[13, 14]	73.0	
Starch (%)		78.5-78.7[9]	78.5-78.7	At 0:00 h. tef flour dough fermentation
Crude fibre (%)		1.98-2.95[2], 3.50[13], 3.00 [14]	1.98–3.50	
Fat (%)		2.69-3.09[2], 2.60[13], 2.00 [14]	2.00–3.09	
Ash (%)		2.66-2.70[2], 3.00[13], 2.90 [14]	2.66–3.00	
Food energy (kJ/100 g)		1405.80 [14]	1405.80	
Microelements (mg/100 g)	Calcium (mg)	216-223[2], 140-170[4], 104-111[8], 159 [14]	104–223	
	Chloride (mg)	13 [14]	13	
	Chromium (μg)	250 [14]	250	
	Copper (mg)	2.30-5.30[4], 0.91-1.04[8], 0.70 [14]	0.7–5.3	
	Iron (mg)	5.20-5.90[3],10.60-19.60[4], 8.44–12.14[6] 5.30-6.00* [6], 4.90-5.10[8], 10.78-14.88[11], 4.73– 6.02+ [11], 5.80 [14]	4.73–19.60	*Acid and +Water washed grain samples
	Magnesium (mg)	155-190[4], 138-141[8], 170 [14]	138–190	
	Manganese (mg)	1.6-3.0[4], 3.4-4.1[8], 6.4[14]	1.6– 6.4	
	Phosphorus (mg)	402-417[2], 415-480[4], 453-466[8], 378 [14]	378–480	
	Potassium (mg)	330-570[4], 556-563[8], 401 [14]	330–570	
	Sodium (mg)	21.2-25.2[4], 11.8-13.1[8], 47 [14]	11.8–47.0	
	Zinc (mg)	5.6–6.7[4], 3.7– 3.8[8], 2[14]	2.0– 6.7	
Vitamins	Vitamin A (RE)	8[14]	8	

Table 2. 1. Continued

	Thiamin (mg)	0.30 [14]	0.30
	Riboflavin (mg)	0.18 [14]	0.18
	Niacin (mg)	2.50 [14]	2.50
	Vitamin C (mg)	88 [14]	88
Amino acids (% of protein recovered)			
	Aspartic acid	6.17-7.15[7], 5.8–6.3[17]	5.80–7.15
	Threonine	3.34[1], 4.25-4.38[7], 4.32 [10], 4.32[13], 2.80 [14], 2.4–3.3[17]	2.40– 4.38
	Serine	5.06-5.56[7], 2.8–3.2[17]	2.80–5.56
	Glutamic acid	23.05-24.89[7], 18.7–21.2[17]	18.70–24.89
	Proline	5.07-6.28[7],10.5–11.4[17]	5.07–11.40
	Glycine	3.77-4.09[7],1.7–2.5[17]	1.70–4.09
	Alanine	5.49-6.03[7],14.0–14.7[17]	5.49–14.70
	Cysteine	2.50[1], 0.73-1.09[7], 2.50[13], 1.90[14], 0.5–0.8[17]	0.50–2.50
	Valine	5.25[1], 5.10-5.94[7], 5.46 [10], 5.46[13], 4.10 [14], 9.0–9.9[17]	4.10–9.90
	Methionine	2.79[1], 3.14-4.58[7],4.06 [10], 4.06[13], 2.10 [14], 2.0–2.6[17]	2.00–4.58
	Isoleucine	4.07[1], 3.81-4.17[7], 4.00[10], 4.00[13], 3.20 [14], 3.9–5.4[17]	3.20–5.40
	Leucine	7.73[1], 8.31-8.80[7], 8.53[10], 8.53[13], 6.00 [14], 9.1–9.7[17]	6.00–9.70
	Tyrosine	2.20[1], 3.63-4.04[7],3.84[10], 3.84[13], 1.70 [14], 2.5–3.1[17]	1.70–4.04
	Phenylalanine	4.87[1], 5.38-5.87[7], 5.69 [10],5.69[13], 4.00 [14], 2.7–4.8[17]	2.70–5.87
	Histidine	2.14[1], 2.76-3.70[7], 3.21 [10], 3.21[13], 2.2–2.4[17]	2.14–3.70
	Lysine	3.11[1], 2.94-3.97[7], 3.68[10],3.68[13], 2.30[14],1.4–1.7[17]	1.40–3.97
	Arginine	3.54[1], 4.48-6.24[7],5.15[10], 5.15[13], 2.9–3.6[17]	2.90–6.24
	Tryptophan	1.30[1], 1.30 [10], 1.30[13], 1.20 [14]	1.20–1.30
Fatty acids (%)	Palmitic (C16:0)	13.95-16.40[16]	13.95–16.40
	Palmitoleic (C16:1)	0.10-0.60 [16]	0.10–0.60
	Stearic (C18:0)	3.00-3.70 [16]	3.00– 3.70
	Oleic (C18:1)	23.30-23.45 [16]	23.30–23.45

Table 2. 1. Continued

	Linoleic (C18:2)	41.25-46.45 [16]	41.25–46.45	
	Linolenic (C18:3)	6.85-9.90 [16]	6.85– 9.90	
	Arachidic (C20:0)	0.60-0.90 [16]	0.60– 0.90	
	Arachidonic (C20:1)	0.50-1.15 [16]	0.50–1.15	
	Behenic (C22:0)	0.25-1.05 [16]	0.25–1.05	
	Erucic (C22:1)	0.00-0.85 [16]	0.00 – 0.85	
Antinutrients	Tannin (mg/100 g)	881 [15]	881	At 0:00 h. tef flour dough fermentation
	Phytate (mg/100g)	707 [15]	707	At 0:00 h. tef flour dough fermentation
	Trypsin inhibitor activity (TIU/g)	5584 [15]	5584	At 0:00 h. tef flour dough fermentation

Sources: [1]Jansen et al. (1962), [2]Rouk and Mengesha (1963), [3]Almgard (1963), [4]Mengesha (1966), [5]Assefa (1978), [6]Besrat et al. (1980), [7]Lester and Bekele (1981), [8]Mamo and Parsons (1987), [9]Umeta and Faulks (1988), [10]Areda (1990), [11]Areda et al. (1993), [12]Areda (1995), [13]Ketema (1993), [14]NRC (1996), [15]Urga et al. (1997 a), [16]Hundera (1998), [17]Bekele (1995)

Where: RE is retinol equivalent.

2.2.2.3. Grain tef usage

Food products processed from tef are staples for the majority of Ethiopians (Ebba, 1969). Tef was cited as having as good or even better nutritional value than major grains like wheat, barley and maize because it is used in the form of whole grain, viz. the germ and bran are consumed along with the endosperm (Ketema, 1993; NRC, 1996).

Tef flour is widely used for making *injera* (Ketema, 1993). The fermentation step for *injera* making involves two phases lasting for a total of 24 to 72 h. (Gashe, 1985). Fermentation is initiated by mixing the flour with water (1:1.6 ratio) to a homogeneous slurry. The initial 18 to 24 h. is notable for vigorous evolution of gas and maximum dough expansion. At about 30 to 33 h. an acidic yellowish liquid appears on the surface of the dough. This phase is characterised by a decrease in the gas evolution up to 31 h., an increase in the liquid volume up to 48 h. and a pH decrease to below 5.8. The first stage of fermentation results in a liquid/solid separation after about 24 h. The liquid layer is then poured off. About 10 % of the fermenting dough is mixed with water (1:3 ratio) and boiled for 2 to 5 min. to a thick paste called *absit* (a dough binder). This is cooled and added to the fermentation vat signalling the second stage of fermentation. The second phase is characterised by a short duration of dough expansion and gas formation that spans from 30 min. to 2 h., signalling the termination of the second stage of fermentation.

A complex group of microorganisms are reported to be involved in the fermentation of tef (Gashe, Girma and Besrat, 1982; Gashe, 1985). Bacteria belonging to the *Enterobacteriaceae* family were reported to initiate fermentation (Gashe et al. 1982; Gashe, 1985). During the first 18 h. of fermentation the activities of this group of bacteria reduce the pH of the dough to about 5.8. A group of lactic acid bacteria (*Leuconostoc mesenteroides, Streptococcus faecalis, Pediococcus cerevisiae, Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus fermentum*) were reported to be involved at the later stage of fermentation (18 to 72 h.) in reducing the dough pH from 5.8 to 3.8 (Gashe, 1985). During the later stage of fermentation (22 to 24 h.), yeasts of two genera *Saccharomyces (S. exiguus, S. dairensis*) and *Torulopsis*

holmii were reported to be involved (Gifawesen and Besrat, 1982). In the later stage (*ca* 48 h.), yeast genera of *Candida (C. krusei, C. lambica and C. sorbosa*) and *Pichia kudriavzevii* were predominantly isolated from the yellow liquid separated from dough (Gifawesen and Besrat, 1982). The group of bacteria species involved in the fermentation were described as a contributor of amylase enzymes to partially degrade the native starch granules, in addition to the amylase present in the grain (Gashe et al. 1982). A bacterial species (*Bacillus sp.* A–001), was also isolated and characterised as an amylase producing bacteria (Lealem and Gashe, 1994).

Injera can be baked any time after 24 h. of fermentation (Ebba, 1969; Gashe et al. 1982). Three types of *injera* are usually prepared from tef, depending on the duration and nature of fermentation involved (Stewart and Getachew, 1962; Ebba, 1969). These are: *injera* made from dough lacking *absit,* which is characterised by a soft thin, fine appearance, sour taste without eyes of *injera* (air cells). The second type of *injera* is called *aflegna,* which is made from relatively unfermented paste of 12 to 24 h. fermentation and is characterised by a sweet flavour, inviting odour and a rusty red underside. The third type is called *komtata injera,* which is made from over fermented paste and is very sour tasting.

Tef is also used for the preparation of traditional alcoholic drinks called *tella* (opaque beer) and *katikalla* (local sprit) (Ebba, 1969; Ketema, 1993). The flour is used to make sweet dry unleavened *kitta* (bread), *muk* (gruel) and *genefo* (porridge) (Ebba, 1969; Ketema, 1993). In the USA, tef was recommended as a good thickener for soups, stews and gravies (Ketema, 1993). Its mild, slightly molasses like sweetness makes tef easy to include in porridges, pancakes, biscuits, cookies, cakes, stir–fry dishes, casseroles, soups, stews and puddings (Ketema, 1993; NRC, 1996).

In all these food and beverage products, the physico–chemical properties of tef starch play a vital role in affecting the processing, storage stabilities, food value, wholesomeness and to the overall product acceptability attributes. The physico–chemical properties of a given starch are influenced by the starch granule size, shape, amylose/amylopectin (ratio, their ultrastructures and degree of their

association and separation in the granule) and minor component (water, lipids, proteins, phosphorus and other trace contaminants incorporated to the starch granule on its isolation) (Galliard and Bowler, 1987; Gidley, 2001). This review of the literature shows that there is very limited information on the nature of tef starch (only few papers related to preparation of *injera* and grain anatomy) and virtually no information on the fundamental physico–chemical properties, functionality and on the granule composition. In the following section, relevant information on what is known about other starch granule structures, starch composition, fundamental properties and functionality will be reviewed.

2.2.3. Nature, chemistry and structure of starch granules

2.2.3.1. Nature of a starch granule

Starch occurs in various plant organs: cereal grains (maize, rice, wheat, barley, oat, sorghum, rye, the millets, tef, etc), legume seeds (peas and beans), roots (sweet potatoes, cassava, arrowroot and yam), tuber plants (potato and canna) and plant stems pith (sago and palm) as tiny (0.5 to 175.0 μm in diameter) white granules (Jane, Kasemsuwan, Leas, Zobel and Robyt, 1994; Whistler and BeMiller, 1997). The shape and size of starch granules can vary between different botanical sources and also slightly within the same botanical sources (Table 2.2) (Hoseney, 1994; Jane et al. 1994).

The major components of a starch granule are two polysaccharides of α-D (+)-glucose (an hexose sugar) (Figure 2.2) the polymers, amylopectin and amylose (Figure 2.3) (Whistler and BeMiller, 1997). In normal starches, amylose comprises *ca.* 18-33 %, whereas amylopectin is *ca.* 72-82 % of the granule (Buléon, Colonna, Planchot and Ball, 1998). In amylo starches (some mutant genotypes of maize, rice and barley), amylose is *ca.* 55-70 % and amylopectin is *ca.* 30-45 %, whereas in waxy genotype starches (maize, rice, sorghum and barley) amylose is < 1 % and amylopectin is at least *ca.* 96 % (Buléon et al. 1998). Maize, potato, wheat, cassava, rice, waxy maize and amylomaize are common sources of commercial starches (Swinkels, 1985; Whistler and BeMiller, 1997).

The monomer glucose is biosynthesised in the green plant leaf by condensing carbon dioxide and water with sunlight energy trapped by chlorophyll (Taylor, 1998). Starch is biosynthesised in the plastid (chloroplast in the photosynthetic cells and amyloplast in the non-photosynthetic cells) organelles of plant cells (Keeling, 1997; Smith, Denyer and Martin, 1997). The transient starch deposited in the leaf chloroplast during the day is partially broken in the night and transported to the storage site (Taylor, 1998). At the storage site, the granule starts to grow from the hilum (growth centre) by apposition (addition of starch polysaccharides to the outside of the granule)

radially to the various levels of growth rings (French, 1984). In the granule, the two polymers (amylopectin and amylose) align outward from the central hilum perpendicular to the growth rings and to the granule surface (Figure 2.4) (Davis, 1994).

The synthesis of starch granules in the plastid is effected by the coordinated action of various enzymes, viz ADP glucose pyrophosphorylase (EC 2.7.7.27), bound starch synthases (EC 2.4.1.21), branching enzymes (EC 2.4.1.18) and debranching enzymes (EC 3.2.1.41 and EC 3.2.1.68) (Keeling, 1997; Smith et al. 1997). Enzyme ADP glucose pyrophosphorylase synthesises ADP glucose, a substrate for the starch polymer (Smith et al. 1997; Morell, Li and Rahman, 2001). In the orderly packing of amylopectin molecules, starch branching enzyme, starch synthase and debranching enzymes are involved (Smith et al. 1997). Granule bound starch synthase I (GBSSI) is responsible for the synthesis of amylose (Denyer, Waite, Motawia, Møller and Smith, 1999).

Even though there are advances in the understanding of the nature of the enzymes involved in starch granule biosynthesis, there is still incomplete information on how these enzymes contribute and control the bio-assembly of different starch granules (Keeling, 1997; Myers, Morell, James and Ball, 2000). There is also a lack of in-depth knowledge on the ultrastructure of the starch granule to design a rational biosynthesis of the starch granule with predictive functionality through biotechnology (Keeling, 1997; Ridout, Gunning, Parker, Wilson and Morris, 2002).

2.2.3.2. Structure of amylopectin (core structural former of the starch granule)

The molecular weight of amylopectin is in the range of *ca.* 1.0×10^7 to 5.0×10^8 (Whistler and BeMiller, 1997). Amylopectin is regarded as a core structural former in the various starch granule organisations (Gallant, Bouchet and Baldwin, 1997; Myers et al. 2000). At a molecular bonding level, amylopectin is a polymer of D (+)-glucose via α-(1→4) glucan backbone to which a number of α-(1→6)-glucan branch linkages

are attached (Figure 2.3 b). About 4–5 % of amylopectin structure is branched (Hizukuri, Takeda, Maruta and Juliano, 1989).

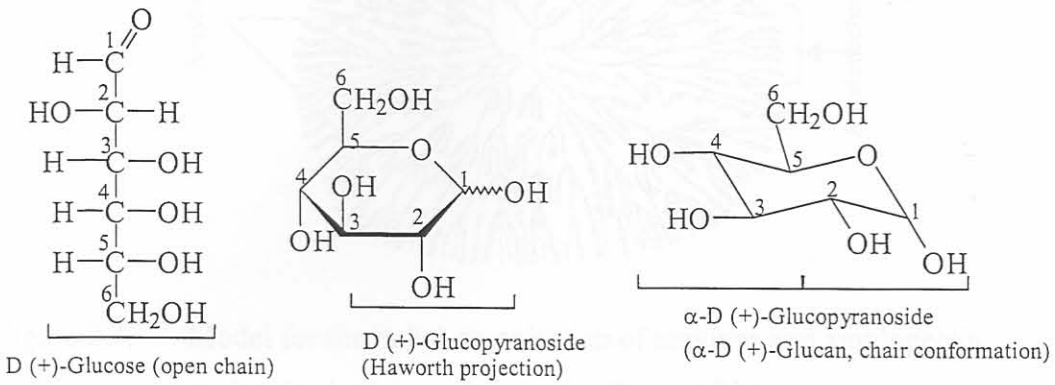

Figure 2.2. Open and cyclic structures of D (+) glucose

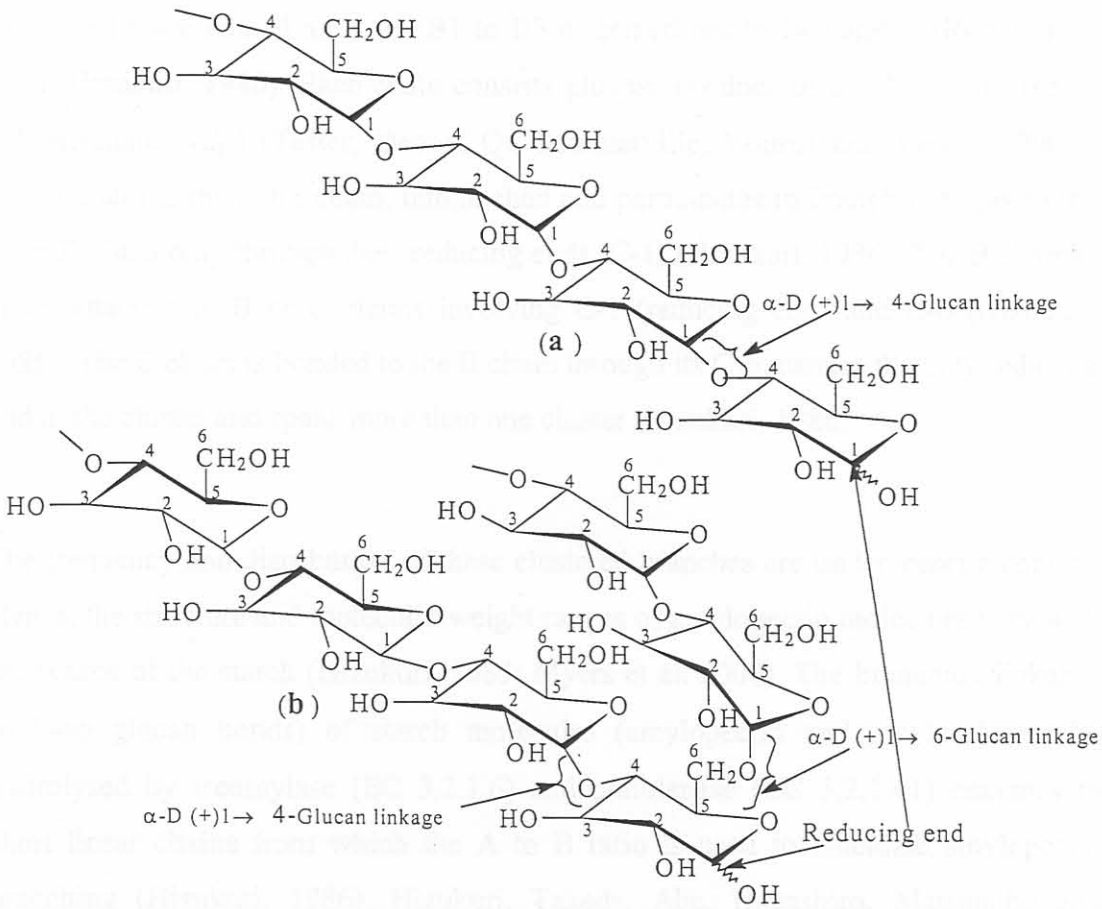

Figure 2.3. Segment of the amylose (a) and amylopectin (b) polymers

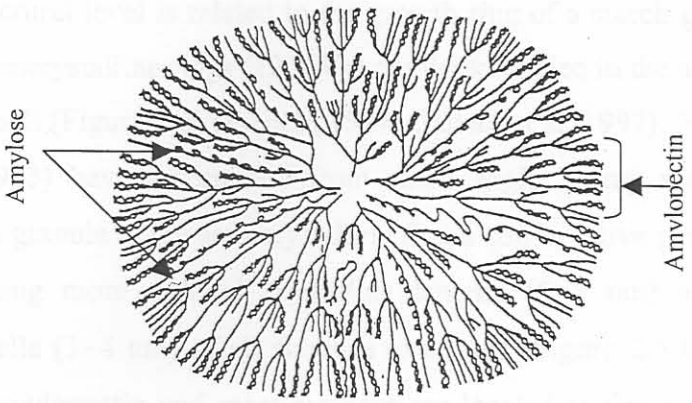

Figure 2.4. Model for the radial organisation of amylose and amylopectin molecules in the starch granule (Davis, 1994)

A model for the structure of amylopectin is the cluster (racemose) model (Figure 2.5 a) (Robin, Mercier, Charbonniere and Guilbot, 1974). The cluster chains of amylopectin are named as A, B (B1 to B3 or sometimes to B4) and C (Robin et al. 1974; Hizukuri, 1986). Each chain consists glucose residues of *ca.* 20-25 degree of polymerisation (*dp*) (Tester, Debon, Qi, Sommerville, Yousuf and Yusuph, 2001). The A chain is the outer chain, unbranched and participates in branch linkages to the inner B chain only through their reducing ends (C-1) (Hizukuri, 1986). The B chain is either attached to B or C chains involving C-1 (reducing end) and C-6 (Hizukuri, 1986). The C chain is bonded to the B chain through its C-6, carries the only reducing end in the cluster and spans more than one cluster (Hizukuri, 1986).

The frequency and distribution of these clustered branches are under genetic control. Hence, the structure and molecular weight ranges of amylopectin molecules vary with the source of the starch (Hizukuri, 1985; Myers et al. 2000). The branching linkages (α-1$\rightarrow$6 glucan bonds) of starch molecules (amylopectin and amylose) can be hydrolysed by isoamylase (EC 3.2.1.6) and pullulanase (EC 3.2.1.41) enzymes to short linear chains from which the A to B ratio is used to elucidate amylopectin branching (Hizukuri, 1986). Hizukuri, Takeda, Abe, Hanashiro, Matsunobu and Kiyota (1997) reported A to B ratio that varies from 1.0: 1.5 to 1.5: 1.0 by reviewing the results of different workers. The variation in the ratio was attributed to the accumulation of slight experimental errors and to the use of different methods.

The second structural level is related to the growth ring of a starch granule, which is composed of semicrystalline rings (120–400 nm thick) buried in the amorphous (120–400 nm thick) shell (Figure 2.6) (French, 1984; Gallant et al. 1997). Jenkins, Cameron and Donald (1993) have determined from small angle X-ray scattering (SAXS) studies of starch granule in the semicrystalline ring a conservative periodicity of 9 nm for the alternating more ordered crystalline lamella (5-6 nm) and less ordered amorphous lamella (3-4 nm) of all starches examined (Figure 2.5 b). The branched cluster site of amylopectin and most amylose are located in the amorphous lamella (Donald, Perry and Waigh, 2001). Amylopectin forms long-range double helices in the crystalline lamella and short-range double helices due to more steric interaction with amylose in the amorphous lamella (Donald et al. 2001).

The third structural level was proposed as the blocklet model (Figure 2.7) with evidence from atomic force microscopy (AFM) (Gallant et al. 1997; Baldwin, Adler, Davies and Melia, 1998; Ohtani, Yoshino, Hagiwara and Maekawa, 2000; Baker, Miles and Helbert, 2001; Ridout et al. 2002) and transmission electron microscopy (TEM) of a starch granule (Gallant et al. 1997). The AFM image of a starch granule revealed the presence of spherical protrusions (nodules) described as a blocklets of size 20 to 500 nm depending on the botanical sources of a starch and their location within the granule (Gallant et al. 1997; Baldwin et al. 1998). Blocklets can be thought as an assembly of amylopectin side chain clusters (Gallant et al. 1997; Baldwin et al. 1998). A single cluster forms a blocklet of size 10 nm, 2 to 5 clusters forms 20–50 nm size blocklets and 5 to 50 clusters forms 50–500 nm size blocklets (Baldwin et al. 1998). The alternating semicrystalline and amorphous growth rings were proposed as formed from the assembly of two or three layers of blocklets (Gallant et al. 1997).

The blocklets size was viewed as being larger in the crystalline region than in the amorphous due to less steric hindrance by amylose in the crystalline region (Gallant et al. 1997). The starch granule surface pores (0.10–0.30 μm in diameter) and radial channels (0.07–0.10 μm in diameter) observed on the surface and inside starch granules, respectively (Fannon, Hauber and BeMiller, 1992; Fannon, Shull and BeMiller, 1993; Huber and BeMiller, 2000) were proposed to be a result of junction zones between the blocklets (Gallant et al. 1997).

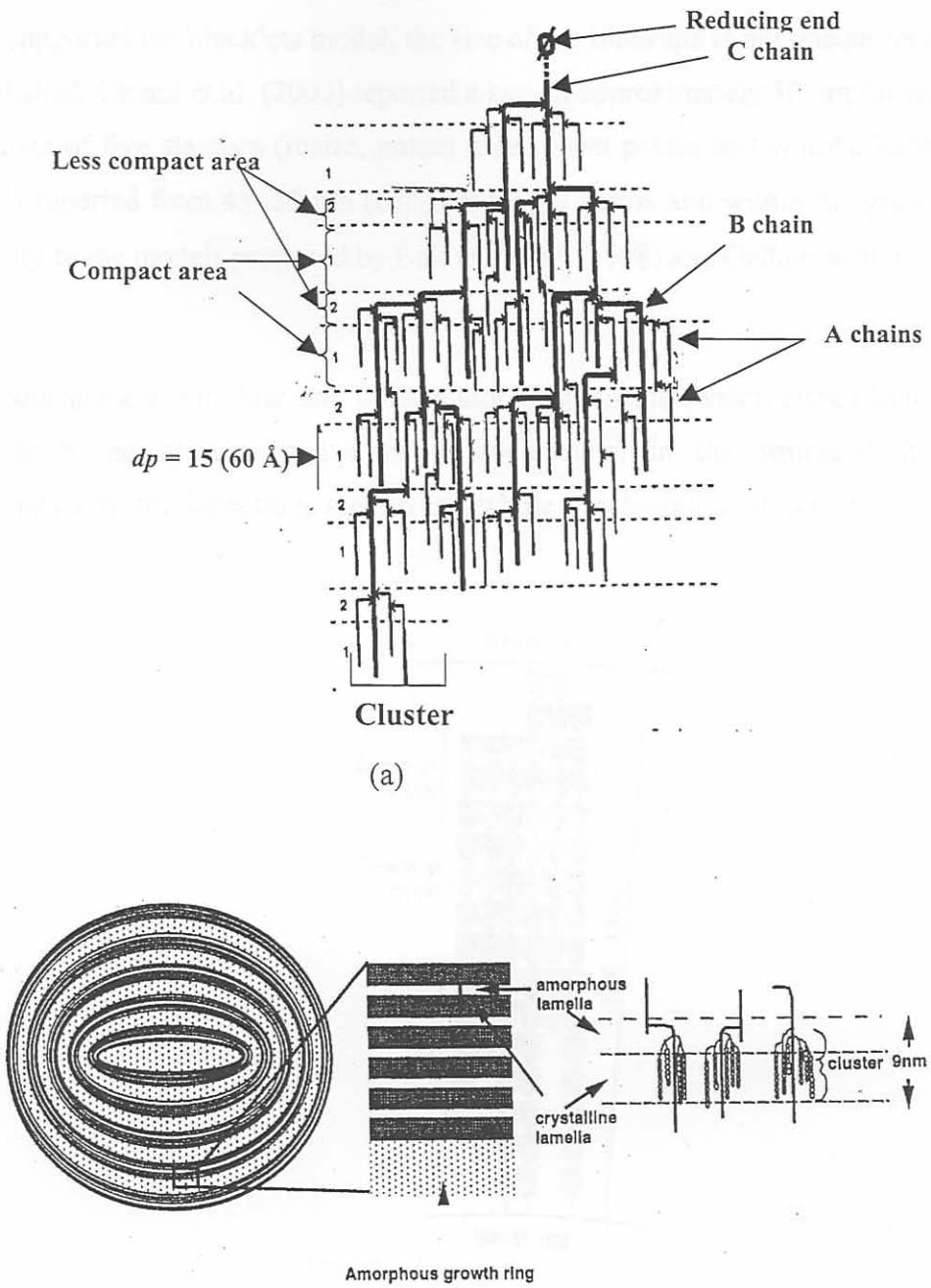

Figure 2.5. A model for amylopectin. (a) Cluster model (short chain branches are clustered on long chain) of amylopectin with branches A and B (Robin et al. 1974). (b) Crystalline and amorphous lamellae of amylopectin (Donald et al. 2001)

Even though most recent AFM of starch granules (Ohtani et al. 2000; Krok, Szymonska, Tomasik and Szymonski, 2000; Baker et al. 2001 and Ridout et al. 2002) have supported the blocklets model, the size of the blocklets is not unequivocally well established. Ohtani et al. (2000) reported a size of approximately 30 nm for individual blocklets of five starches (maize, potato, rice, sweet potato and wheat). Ridout et al. (2002) reported from 45-85 nm (constant range) across and within the growth rings, contrary to the models proposed by Baldwin et al. (1998) and Gallant et al. (1997).

The packaging of amylose and other minor components (water, starch lipids, starch granule bound proteins and trace microelements) in the structural framework established by amylopectin is viewed as a whole starch granule structure.

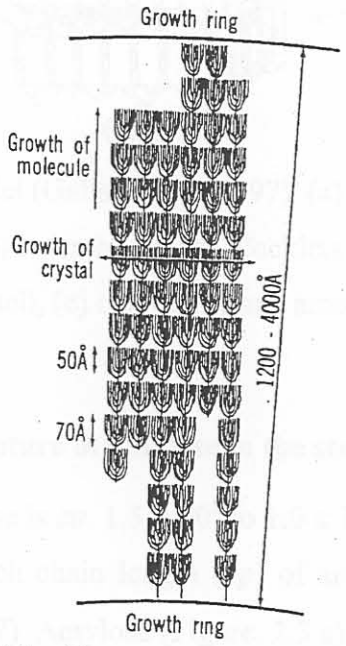

Figure 2.6. Schematic representation of amylopectin within the growth ring of a starch granule (French, 1984)

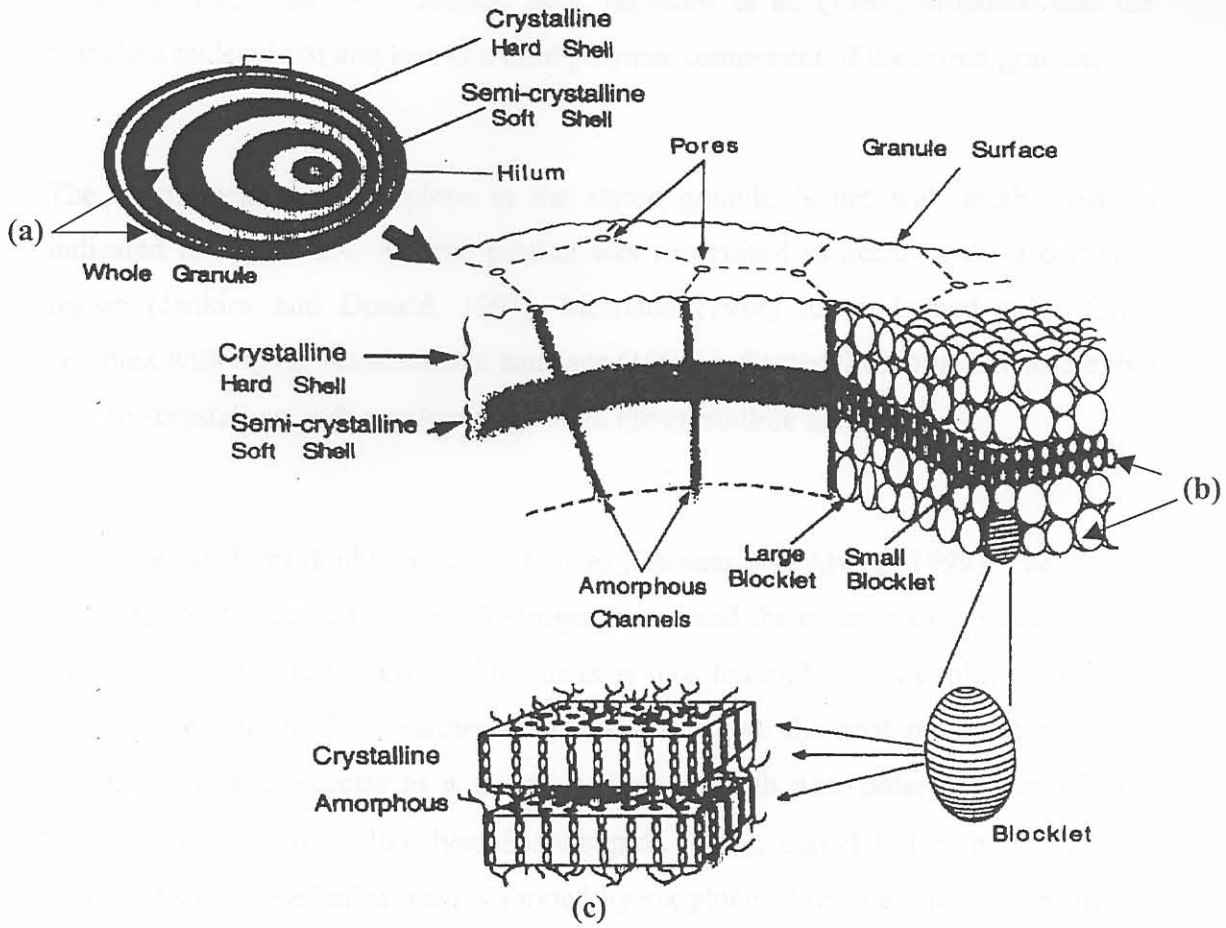

Figure 2.7. The blocklet model (Gallant et al. 1997). (a) The growth rings of the starch granule, (b) assembly of the blocklets in the crystalline and semicrystalline shell, (c) crystalline and amorphous lamellae

2.2.2.3. Structure and nature of amylose in the starch granule

The molecular weight of amylose is *ca.* 1.5 x 10^5 to 1.0 x 10^6 (Davis, 1994; Whistler and BeMiller, 1997). The branch chain length (*dp*) of amylose ranges from 500 to 5000 (Galliard and Bowler, 1987). Amylose (Figure. 2.3 a) is essentially a linear α-D-(1→4) glucan polymer of D-glucose with a few molecules branched via α-D-(1→6) to the extent of 0.27-0.68 % in the whole molecule (Hizukuri et al. 1997). The branched molecule can contain 9-20 branch points per chain of *ca.* 200-700 *dp* (Hizukuri et al. 1997; Tester et al. 2001). Curá, Jansson and Krisman (1995) reported 2.2 % of molecules branched, for whole molecules. However, they pointed out the limitations of analytical methods that can successfully separate linear amylose

molecules from the few branched ones. Hizukuri et al. (1989) proposed that the branched molecule of amylose is a third polymer component of the starch granule.

The exact location of amylose in the starch granule is not well established, as indicated in Figure 2.4. A large portion was recognised to occur in the amorphous region (Jenkins and Donald, 1995). Morrison (1988) has indicated some forms complex with lipids. Kasemsuwan and Jane (1994) indicated that some amylose exists as a co-crystalline with amylopectin within the crystalline lamella.

Amylose can form double or single helices (Thomas and Atwell, 1999). The interior of the helix is occupied by most hydrogen atoms and the exterior side is occupied by hydroxyl groups. The interior of the helix is thus hydrophobic (lipophilic) in nature and is amenable to form *clathrates* (inclusions where the host molecule entraps a second molecular species as a guest), complexes with non-polar portions of fatty acids, monoacyl lipids like lysophospholipids (LPL), diacyl lipids, poly (I_3^-) and some alcohols. The helical turn is formed by six glucosyl residues and six methylene groups of the complexing agent (monoacyllipids, fatty acids and linear alcohols) (Figure 2.8) (Biliaderis and Galloway, 1989; Tufvesson and Eliasson, 2000). However, with branched chain alkyl compounds like 1,1–dimethyl ethanol and bulkier molecules like 1-naphthol the helical turn is formed by 7 and 8 D-glucosyl residues, respectively. There are two thermally distinct amylose-lipid complexes, i.e. forms I and II (Biliaderis and Galloway, 1989). The differential scanning calorimetry (DSC) endothermic transition temperature of form I complex is *ca.* 94-100 ^{o}C and form II is *ca.* 100-125 ^{o}C (Eliasson, 1994). Form II amylose–lipid complex is more ordered with a distinct crystalline region (Eliasson, 1994). The stability and variation of endothermic transition temperature of amylose-lipid complexes are dependent on the nature of the ligands, type of starch, water and temperature (Eliasson, 1994).

After starch granule dispersion with dilute dimethyl sulphoxide (DMSO) or NaOH or KOH, amylose forms a selective precipitate with 1–butanol (Cornell, McGrane and Rix, 1999). This selective precipitation is used to separate amylose from amylopectin

during ultrastructure characterisation of amylose and amylopectin (Cheetham and Tao, 1997; Chen, Fringant and Rinaudo, 1997).

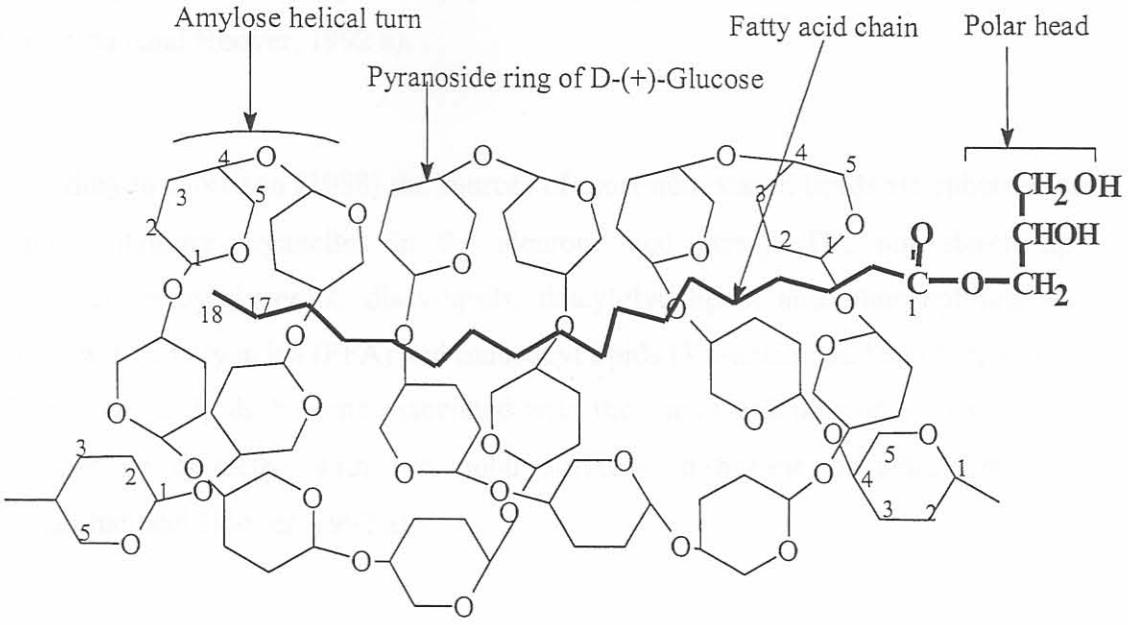

Figure 2.8. Schematic diagram of the amylose-lipid complex

Complexes of polyiodide with long helical segments of amylose give the characteristic blue colour, which is used in the identification of starch and quantitative determination of amylose in starches and flours (Morrison and Laignelet, 1983). Amylose binds iodine to the extent of *ca.* 19 % (w/w) of its weight (Whistler and BeMiller, 1997). The branch chain lengths (*dp*) of amylopectin are too short to form a long-range complex with polyiodide, hence instead of a blue colour a reddish purple complex is formed with amylopectin (Whistler and BeMiller, 1997).

2.2.2.4 Minor components of the starch granule

2.2.2.4.1. Lipids

The lipids associated with native starch granules can be divided into three groups: Non-starch, Surface bound and Internal (true or endogenous or bound) starch lipids (Morrison, 1988). The nature and content of lipids associated with the starch granule varies with the botanical source of the starch (Morrison, 1988 and 1995). The total lipids in nonwaxy cereal starches are in the range 0.60-1.20 %, whereas in waxy

maize and potato starches they are *ca.* 0.20 % and *ca.* 0.06 %, respectively (Whistler and BeMiller, 1997). Only cereal starches are recognised to contain lipids inside the starch granules (Morrison, 1988 and 1995). Legume, tuber and root starches have no internal lipid and contain generally much less lipid than the normal cereal starches (Vasanthan and Hoover, 1992 a).

According to Morrison (1988) the sources of most non-starch lipids are spherosomes (lipid containing organelles in the aleurone and germ). The non-starch lipid comprises triacylglycerols, diacyllipids, diacylglycolipids and phospholipids with very few free fatty acids (FFA) and monoacyl lipids (Vasanthan and Hoover, 1992 a). Most of these lipids become associated with the starch granules on starch isolation and can be extracted with non-polar solvents (n-hexane or petroleum ether) (Vasanthan and Hoover, 1992 a).

The endosperm fat containing proteinaceous matrix is also implicated, in part, as a source of starch surface bound lipids (Morrison, 1988). These lipids penetrate the granule periphery during steeping and wet milling. However, some of the surface bound starch lipids are those present on the starch granule surface *in situ* arising from partial degradation of the amyloplast membrane lipids surrounding the developing starch granules (Galliard and Bowler, 1987). The starch surface bound lipids comprise monoacyl lipids like lysophospholipids (LPL) and FFA. These lipids can form inclusions with amylose located at the periphery of the starch granule surface (Morrison, 1988 and 1995). They can be extracted with chloroform-methanol (2:1) (v/v) at *ca.* 25–28 ^{o}C (Vasanthan and Hoover, 1992 a).

Internal lipids are endogenous to native cereal starch granules and are usually composed of LPL and FFA (Morrison, 1995). These lipids form V-type amylose lipid complexes in the native starch granules. On starch gelatinisation more of these complex are formed (Morrison, 1988 and 1995). Thus, inside the native starch granules some of these lipids exist as lipid complexed amylose (LAM) and some are as lipid free amylose (FAM) (Tester and Morrison, 1990; Morrison, 1995). The biochemical role of the internal starch lipids of cereal starch granule is yet obscure

(Morrison, 1988 and 1995). However, their quantity is known to vary with long linear α-1,4-glucan (amylose) content and in response to the environmental temperature in the field (Morrison and Gadan, 1987; South, Morrison and Nelson, 1991). The extraction of internal lipid requires hot (90-100 ^{O}C) solvent: n-propanol-water, 3: 1 (v/v) or water–saturated 1-butanol, 1:5 (v/v) (Morrison and Coventry, 1985). In the extraction, water is required to enhance the swelling and gelatinisation of the starch granules and n-propanol or 1-butanol is used to dissolve the lipids.

The LAM are believed to inhibit starch gelatinisation and pasting, whereas the FAM enhances it to occur at lower temperatures (Tester and Morrison, 1990; Morrison, 1995). The LAM also reduce α-amylase starch digestion and retrogradation of starch paste via re-crystallization of the solubilised starch molecules (Whistler and BeMiller, 1997). Lipids in the starch can impart undesirable flavours (via oxidation of unsaturated fatty acid chains) (Swinkels, 1985). They also make the starch paste more opaque or cloudy (Swinkels, 1985). In starch pastes and films, the complexes also reduce the thickening power and binding force of the gelatinised starches (Swinkels, 1985).

2.2.2.4.2. Proteins

Proteins that adhere to the surface and inside of starch granules are collectively called starch granule associated proteins (SGAPs) (Baldwin, 2001). Depending on botanical origin and methods used to isolate the starch granules, the SGAPs level can vary from 0.06-0.50 % (Swinkels, 1985). Cereal starches (maize, wheat and waxy maize) contain the highest levels of proteins (0.25-0.50 %), whereas potato and cassava contain approximately 0.06 % and 0.10 %, respectively (Swinkels, 1985).

By sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) the apparent molecular weight of the most common ten SGAPs were determined as *ca.* 5, 8, 15, 19, 30, 60, 77, 86, 95 and 149 x 10^3 M_r (relative molecular mass) which are characterised as different from the common cereal prolamin storage proteins (Baldwin, 2001). The *ca.* 5, 8, 15, 19 and 30 x 10^3 M_r proteins are called surface proteins because they can be extracted without much influencing the starch granule

structure by 0.1M NaCl, 1 or 2 % SDS, 10 mM dithiothreitol (DTT), 50 % (v/v) propan-2-ol or with toluene at room temperature (Baldwin, 2001). Some of these surface proteins were adsorbed to starch granule surface from the surrounding storage proteins (Baldwin, 2001). The 15 x 10^3 M_r polypeptides were characterised as *friabilin* proteins and the 30 x 10^3 M_r as a glycoprotein (Baldwin, 2001). The *ca.* 60, 77, 86, 95 and 149 x 10^3 M_r proteins are called internal proteins and are residual starch synthase enzymes. The extraction of internal protein requires starch granule swelling/gelatinisation and strong detergents (at least 1–2 % SDS at 50 ^{O}C or above) because they are embedded inside the starch granules by entanglement or covalent bonded with amylose and amylopectin molecules (Duarte, Robinson and Freeman, 1995; Baldwin, 2001). The 60 x 10^3 M_r proteins were described as the starch granule bound starch synthase I (SGBSS I), and the reminder were its isoforms (Baldwin, 2001).

The SGAPs can affect starch granule swelling, gelatinisation and pasting properties (Zeng, Morris, Batey and Wrigley, 1997; Han, Campanella, Guan, Keeling and Hamaker, 2002 a). The interaction of *friabilin* (*puroindoline*–a and –b) with lipids on the surface of the starch granules has been implicated in grain softness in wheat (Baldwin, 2001). Baking properties of flours and unwanted flavour, colour and odour in starch products of glucose syrups are influenced by the SGAPs (Baldwin, 2001). They can also act as a barrier to enzyme attack and to starch reaction with modifying agents (Baldwin, 2001).

2.2.2.4.3. Ash and Phosphorus

Ash: starch consists trace amount (0.1–0.5 %) of inorganic mineral elements, collectively called ash (Swinkels, 1985; Whistler and BeMiller, 1997). The ash content of native starches can vary depending on the degree of phosphorylation (genetically controlled), agronomic practices and starch isolation methods used (Nielsen, Wischman, Enevoldsen and Møller, 1994). Potato starch contains the highest ash (0.4 %) due to its high degree of phosphorylation. Maize, wheat, cassava and waxy maize starches contain 0.1, 0.2, 0.2 and 0.1 % ash, respectively (Swinkels, 1985). Next to phosphorus, the main components of native starch ash are calcium, potassium, magnesium and sodium metal ions (Swinkels, 1985). These metal ions

exist in the native starches as bound to phosphate groups through ionic association and they can modify the charge of starch granules and its dispersion in water (Galliard and Bowler, 1987).

Phosphorus: in the native starch granule, phosphorus occur as starch phosphate monoesters, phospholipids and inorganic phosphate (Kasemsuwan and Jane, 1996). The phosphorus content and its form can vary with botanical source, maturity and growing conditions of the plant (Kasemsuwan and Jane, 1996). Phosphorus of normal cereal starch (0.016-0.065 %) is mainly in the form of phospholipid, whereas in potato (0.089 %) and tuber starches (0.004-0.021 %) it is mainly in the form of starch phosphate monoester (Lim, Kasemsuwan and Jane, 1994). For normal cereal starches, phosphorus is highest in rice starch (0.065 %) and is lowest in maize starch (0.016 %) (Lim et al. 1994). Starch phosphorylation is more confined to the amorphous region of amylopectin, with long chain amylopectin being more phosphorylated than the short chain (Blennow, Bay–Smidt, Wischmann, Olsen and Møller, 1998; Blennow, Engelsen, Munck and Møller, 2000). One or more phosphate is bonded to a unit chain length of 30-100 glucose (Blennow et al. 2000). The probability of phosphorylation to carbon in glucose is 60-70 % at C-6, 30-40 % at C-3 or trace at C-2 (Figure 2.9) (Blennow et al. 2000).

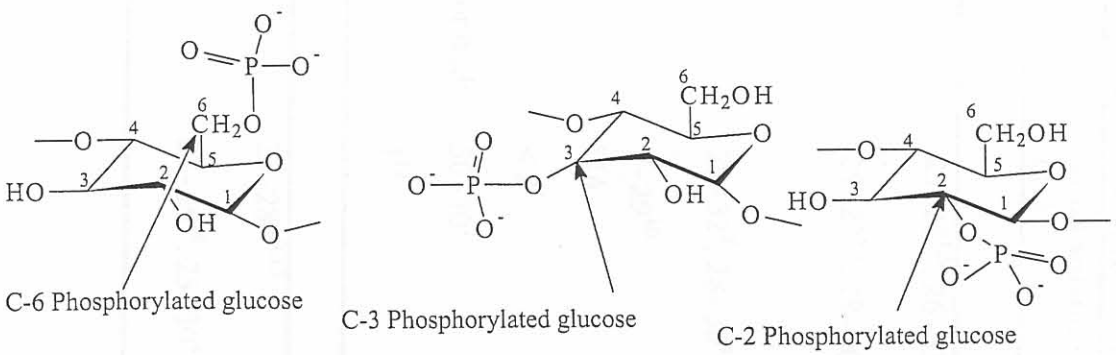

Figure 2.9. Positions of phosphorylation on the glucose residues of starch

Starch functionality can be affected by the content and nature of phosphorus (Swinkels, 1985; Blennow, Bay–Smidt and Bauer, 2001). The phosphate monoester of potato starch imparts low gelatinisation temperature, high paste viscosity and clarity, and contributes to slow retrogradation to potato starch (Swinkels, 1985). The phospholipid in wheat is known to reduce the paste clarity and pasting viscosity (Kasemsuwan and Jane, 1996).

Table 2. 2. Some properties of starch granules from certain cereals, pseudocereals, tubers, roots, stem pith and legumes

Starch source	Diameter (μm)	Shape	Total amylose (%)	Crystallinity (%)	Remarks
Tropical cereals					
Maize *(Zea mays)*	2-30[1]	Polygonal or spherical[1,2]	25-28[1,3], 26-33[4]	A, 40[5]	
Sorghum *(Sorghum bicolor)*	10-30[6]	Polygonal or round[5]	25-27[6,7], 28-30[4]	A, 37[5]	
	5[6]	Polygonal or round[5]			
Pearl millet *(Pennisetum glaucum)*	2-21[6]	Round or polygonal, deep surface indentations[6]	29-32[8], 28-30[4]	A[8], NA	
Rice *(Oryza sativa)*	3-8[6,9]	Polygonal[6,9]	7-29[10]	A, 38[5]	Compound type
Tef *(Eragrostis tef)*	2-6[11]	Polygonal[11]	NA	NA	Compound type
Waxy maize	2-30[1]	Irregular polygonal[6]	< 2[1]	A, 40[5]	
Amylomaize	2-24[1]	Irregular polygonal, some with rod or buds[1]	50-70[1]	B, 15-22[5]	
Waxy rice	3-8[6]	Irregular polygonal[6]	1[12]	A, 37[5]	Compound type
Temperate cereals					
Wheat *(Triticum aestivum)*	20-35[9]	Lenticular[2]	25-28[1,9,13]	A, 36[5]	Trimodal in hard red winter wheat[1]
	2-10[3]	Round[2]			
Barley *(Hordeum vulgare)*	15-32[6]	Disk shaped or elliptical[6]	22-26[14], 25-30[4]	A, 22-27[14]	Trimodal in some work[14]
	2-3[6]	Round[6]			

Table 2. 2. Continued

Oats (*Avena sativa*)	2-15[6]	Irregular polygonal and pockmarked on several granules[6]	28-30[15], 23[16], 25-29[4]	A, 33[5]	Compound type starch
Rye (*Secale cereale*)	22-36[6] 2-3[6]	Lenticular[6] Round[6]	28[17], 26[5]	A, 34[5]	
Pseudocereals					
Amaranthus (*Amaranthus cruentus*)	1-2[18]	Polygonal[18]	8[18]	A, 46[18]	Compound popcorn ball[18]
Quinoa (*Chenopodium quinoa*)	1-2[18]	Polygonal[18]	12[18]	A, 35[18]	Aggregated[18] (compound)
Buckwheat (*Fagopyrum esculentum*)	3–9[27], 2–14[28]	Round, polygonal[27]	47*[27], 21[28]	A[28], NA	Often aggregated[28]
Tuber plants					
Potato (*Solanum tuberosum*)	15-75[6]	Oval, smooth[3,6]	20[3], 21[1], 22[5], 23[17]	B, 28[5]	
Canna (*Canna edulis*)	30-100[6]	Smooth, ellipsoidal and spherical shaped[6]	28[5]	B, 26[5]	
Roots					
Cassava (*Manihot esculenta*)	4-35[1]	Oval, truncated–kettle drum[1]	17[1], 18[5]	C, 38[5]	
Sweet potatoes (*Ipomea batatas*)	5-30[1]	Polygonal round[1]	20[5], 23[19], 19[20]	A or C, 38[20]	
Yam (*Dioscorea abyssinica*)	4-20[19]	Rounded shaped[21]	30[21]	B[21], NA	
Arrowroot (*Maranta arundinacea*)	15-70[22]	Smooth and oval shape[6]	19[23]	NA	
Plant stem pith					
Sago (*Metroxylon sagu*)	20-60[22]	Deformed ellipsoid with pitting indentations toward one end side of ellipsoid[24]	24-31[25]	C, 28-33[25]	

Table 2. 2. Continued

Legumes				
Black bean *(Phaseolus vulgaris)*	10–45[6], 7–30[26]	Oval, irregular, round[6,26]	27–30[26]	C, 17–22[26]
Pinto bean *(Phaseolus vulgaris)*	10–27[6], 6–32[26]	Oval, irregular, elliptical[6,26]	35–36[26]	C, 25–26[26]
Navy bean *(Phaseolus vulgaris)*	14–28[26]	Irregular, oval, elliptical[6,26]	28–29[26]	C, 19–21[26]
Chick pea *(Cicer arietinum)*	10–27[6], 9–30[26]	Irregular round with indented cut at one end[6]	23[26]	C, 18[26]
Smooth pea *(Pisum sativum)*	14–32[26]	Irregular round[6]	24[26]	C, 20[26]
Lentil bean *(Lens culinaris)*	10–20[6], 7–28[26]	Smooth ellipsoidal with indentations[26]	24–25[26]	C, 19[26]

Sources: [1]Whistler and BeMiller (1997), [2]Snyder (1984), [3]Thomas and Atwell (1999), [4]Morrison, Milligan and Azudin (1984), [5]Zobel (1988 b), [6]Jane et al. (1994), [7] Beta, Corke, Rooney and Taylor (2000), [8]Hoover, Swamidas, Kok and Vasanthan (1996 b), [9]Hoseney (1994), [10]Morrison and Azudin (1987), [11]Umeta and Parker (1996), [12]Lii, Shao and Tseng (1995), [13]Vansteelandt and Delcour (1999), [14]Tang, Ando, Watanabe, Takeda and Mitsunaga (2001), [15]Tester and Karkalas (1996), [16]Hoover and Senanayake (1996), [17]Kennedy et al. (1987), [18]Qian and Kuhn (1999), [19]McPherson and Jane (1999), [20]Hoover (2001), [21]Gebre–Mariam and Schmidt (1998), [22]Corbishley and Miller (1984), [23] Erdman (1986), [24]Sim, Oates and Wong (1991), [25]Ahmad, Williams, Doublier, Durand and Buléon (1999), [26] Hoover and Ratnayake (2002), [27]Qian, Rayas–Duarte and Grant (1998), [28]Zheng, Sosulski and Tyler (1998).

Where: NA is information not available, and *apparent amylose

2.2.4. Some physico-chemical and functional properties of starch granules

2.2.4.1. Starch granule crystallinity

Starch granules are composed of crystalline and amorphous regions (Zobel, 1988 a and b). Based on their X-ray diffraction pattern, starch granules can be grouped into four types: A, B, C and V (Zobel, 1988 a). The crystalline percentage of native starch granules ranges from 15 to 45 % (Zobel, 1988 b). The long-range left handed, double-helical, parallel-stranded amylopectin arrangement is believed to be responsible for the crystalline domain in the granule (Imberty, Buléon, Tran and Párez, 1991). The average chain length (CL) of amylopectin is one basic factor that determines whether a starch granule is A or B type (Hizukuri et al. 1997). The A type is formed when the glucosyl average CL is shorter than 20 and is more densely packed than B type starches (Hizukuri et al. 1997). The B type is formed when it is longer than 22. The C type (mixture of A and B) is formed by between 20 to 22 glucosyl unit CL (Hizukuri et al. 1997).

The unit cell of A type starch is monoclinic [a = 2.124 nm, b = 1.172 nm, c = 1.069 nm and γ = 123.5 ^{o}] formed by 12 glucose residues (6 double fold) and *ca.* 8 molecules of water (Parker and Ring, 2001) (Figure 2.10 a). In the B type starches it is hexagonal (a = b = 1.850 nm and c = 1.040 nm) formed by 12 glucose residues (6 double fold) and *ca.* 30 to 40 water molecules (Parker and Ring, 2001) (Figure 2.10 b). The genetics of the plant determines the chain length of amylopectin and thereby influence its crystallinity and branching patterns (Hizukuri et al. 1997). The A type starch granule is favoured by warm and dry environmental conditions, whereas B is favoured by wet and cold environmental conditions (Hizukuri et al. 1997). Heat moisture treatment can induce changes from B to A, whereas only sulphate ions are known to induce changes from A to B (Hizukuri et al. 1997). The A type diffraction is a characteristic of most cereal starches (Zobel, 1988 a). The A type is distinguished by strong intensity peaks (*d* value) at 5.8, 5.2, 4.9 and 3.8 Å (Zobel, 1988 a).

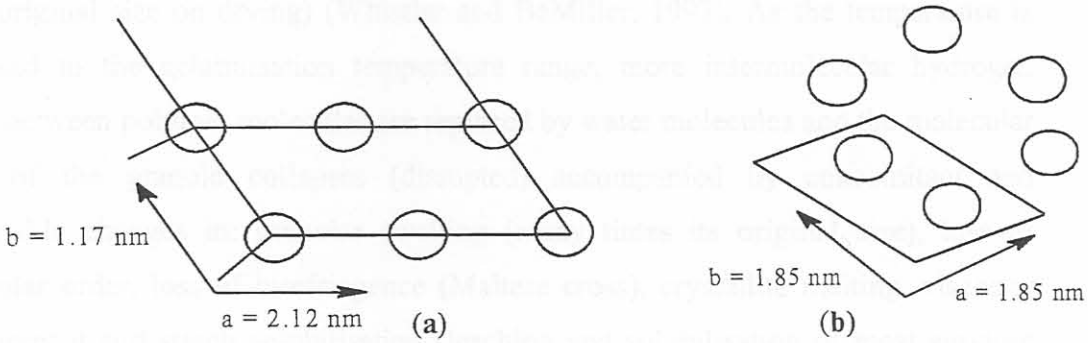

Figure 2.10. A model for arrangement of double helices in a unit cell for A and B crystalline forms of starch (Parker and Ring, 2001). (a) Monoclinic unit cell packing for A type, (b) hexagonal unit cell packing for B type

The peak at 4.4 Å is an indication of amylose–complexed with lipids. Tuber (potato), root (yam) and high amylose starches give B type diffraction (Zobel, 1988 a). The B starches show peaks (*d* in Å) at 15.8–16.0, a broad medium intensity at 5.9, a strong intensity line at 5.2 and a medium intensity doublet at 4.0 and 3.7. Legume, for example pea, starches give a C diffraction pattern, which is a combination of A and B type diffraction. The C diffraction pattern is the same as A, except for the addition of a medium to strong peak at about 16.0 Å (Zobel, 1988 a). The V form diffraction pattern is a characteristic of amylose–complexed with FFA and monoacyl lipids, which often becomes more distinct on starch granule gelatinisation (Zobel, 1988 a). The hydrated V form peaks (*d* value) at 12.0, 6.8 and 4.4 Å, whereas the dehydrated V form shows reduced peaks at 11.3, 6.5 and 4.3 Å (Zobel, 1988 a).

2.2.4.2. Starch granule gelatinisation, pasting and retrogradation

2.2.4.2.1. Starch granule gelatinisation

Starch gelatinisation is a broad concept and mechanistically can be viewed as a disruption of hydrogen bonds between starch polymer chains (disruption of molecular order) by heating in the presence of plasticiser (mostly water) or by applying simultaneous shear force and temperature (mechanically) (Colonna, Buléon and Mercier, 1987). At low temperature *ca.* < 45 ^{o}C (below gelatinisation temperature), in the presence of a sufficient amount of water, the starch granule is hydrated, mostly through the amorphous region and imbibes reversibly (swells slightly and then returns

to its original size on drying) (Whistler and BeMiller, 1997). As the temperature is increased to the gelatinisation temperature range, more intermolecular hydrogen bonds between polymer molecules are replaced by water molecules and the molecular order of the granule collapses (disrupted) accompanied by concomitant and irreversible changes in: granular swelling (many times its original size), loss of molecular order, loss of birefringence (Maltese cross), crystallite melting, viscosity development and starch solubilisation (leaching and solubilisation of most amylose molecules) (Parker and Ring, 2001). The point of initial gelatinisation and the range over which it occurs is governed by starch concentration, method of observation, granule type, and heterogeneities of the granule population under observation (Atwell, Hood, Lineback, Varriano–Marston and Zobel, 1988). For an individual starch granule, the loss of birefringence starts from the granule centre and gelatinisation is accomplished over a 1 - 2 ^{o}C temperature range, whereas for a whole population of the granules, the range is over 10 ^{o}C (Liu, Lelievre and Chee, 1991).

2.2.4.2.2. Starch pasting

Starch pasting is the phenomenon following gelatinisation in the dissolution of starch and it involves granular swelling, exudation of molecular components from the granule, and eventually, total disruption of the granules (Atwell et al. 1988). Pasting can be described as a continuation of gelatinisation but not exactly separated from gelatinisation and is usually related to the development of viscosity (Thomas and Atwell, 1999). At its peak viscosity, starch is regarded as fully pasted (i.e., usually when the largest percentage of swollen intact granules are present). On pasting, a viscous starch paste of continuous (dispersion of solubilised starch molecules) and discontinuous (the outer remainder portion of the granule and fragments) phases can be formed (Whistler and BeMiller, 1997). After starch is fully pasted, the viscosity of maximum swollen starch granules can be reduced with shearing (shear thinning) because of the disintegration of the swollen starch granules and alignment of solubilised starch molecules in the direction of the shear (Hoseney, 1994). On cooling of the pasted starch, depending on the amylose content, a gel or viscoelastic paste can be formed (Thomas and Atwell, 1999). The higher the amylose content of the starches, the more the paste will set to a firm and cuttable gel. Low amylose starches like waxy starches set to cohesive and viscoelastic pastes.

2.2.4.2.3. Starch retrogradation

Starch retrogradation is a process, which occurs when the molecules comprising gelatinised starches begin to reassociate in an ordered structure (Atwell et al. 1988). In its initial phases, two or more starch chains may form a simple juncture point which then may develop into more extensively ordered regions. Ultimately, under favourable conditions, a crystalline order appears. The pasted starch will retrograde because of the solubilised starch polymers have a tendency to reassociate (Gudmundsson, 1994). The junction zone can be formed via hydrogen bonding, weak van der Waals attractions and/or entanglements, which eventually forms double helices between starch chains and crystalline structural orders (Tako and Hizukuri, 2002). On the process of retrogradtion, a gradual exudation of water (starch syneresis) from the gel also takes place (Gudmundsson, 1994). The retrogradation rate of a starch paste varies with botanical source of the starch (Table 2.3) and is influenced by the amount of water in the paste, storage temperature, presence of minor components (lipids, phosphorus and protein), amylose/amylopectin ratio and starch molecule chain length (*dp*) (Gudmundsson, 1994).

Amylose molecules because they are linear, reassociate more readily to form a gel and are responsible for the short-term rheological changes in starch-based foods (Gudmundsson, 1994). Amylopectin recrystallises very slowly and is responsible for the long-term rheological changes in starch-based foods. Amylose reaches its recrystallisation limit in about 2 days, whereas for amylopectin to approach a limiting value, 30 to 40 days are required (Gudmundsson, 1994). The crystallinity of an amylopectin gel is fully reversible on heating, whereas in amylose it is not (Zobel 1988 b). On retrogradation of starch, the undesirable changes that can occur to foods are: (a) increase in opacity of the product, (b) development of coarse grainy structures, (c) excessive increase in consistency and even congelation, and (d) loss of water holding capacity and proliferation of microbial spoilage (Schoch, 1968). Due to starch retrogradation, for example transparent fruit–pie fillings may turn dull and opaque, starch thickened sauces may lose their smooth creamy consistency and become curdled. On seepage of water from the gel, the structure of starch–based foods can

become microsponge like. Staling of bread and other baked products, which is mostly related to the retrogradation of amylopectin is one other example of retrogradation impact (Gudmundsson, 1994). However, sometimes retrogradation is beneficial, such as in the production of type III resistant starch (retrograded starch which is less absorbed in the small intestine of healthy individuals) (Alonso, Escrig, Carrón, Bravo and Calixto, 1999). In certain breakfast cereals, parboiled rice and Japanese *harusame* noodles, starch retrogradation is promoted to modify the structural, mechanical or organoleptic properties (Karim, Norziah and Seow, 2000).

2.2.4.3. Some functional features of starches from different botanical sources

A summary of functional properties (gelatinisation temperature, pasting temperature, relative viscosity, paste texture, paste clarity, paste flavour, shear resistance and retrogradation tendency) of starches from common cereals (tropical and temperate), pseudocereals, tuber plants, roots, plant stem pith and legumes is given in Table 2.3. Based on this, a brief discussion for each group follows.

2.2.4.3.1. Cereal starches

The gelatinisation temperature of tropical cereal starches (maize, sorghum, rice and the millets) is relatively higher than the temperate cereal starches (wheat, barley, oats and rye) (Whistler and BeMiller, 1997). For most tropical cereals the range is about 65-85 ^{o}C, whereas for temperate cereals it is about 50-65 ^{o}C. This is because tropical cereal starches are synthesised under high environmental temperatures as opposed to relatively cold environmental temperatures for temperate cereals. At high environmental temperatures, crystallite formations were implicated to be enhanced and thereby cause higher temperature of gelatinisation (Tester, 1997). The pastes of normal native cereal starch (maize, sorghum and rice) are characterised as: opaque, medium viscous with reasonable shear resistance that gel quickly (Swinkels, 1985; Whistler and BeMiller, 1997). The paste texture of most cereal starches is short with the exception of low amylose (7-22 %) rice starch (Hamaker and Griffin, 1990) and oats (Zhou, Robards, Holmes and Helliwell, 1998). In low amylose rice the paste is described as fairly long or sticky and in oats as less firm or more elastic. Wheat and

rye starch paste are characterised by late onset of firmness gain (two-three fold) on cooling and the gel is as firm as maize starch (Schierbaum and Kettlitz, 1994). Due to amylose-lipid complex formation, the paste clarity of most normal cereal starches is regarded as opaque (Swinkels, 1985). In oats the paste is fairly clear (Zhou et al. 1998) and in some varieties of red or white pericarp sorghum of high polyphenol level the paste is slightly pink in colour (Beta et al. 2000). The flavour of most normal cereal starch pastes is mealy-like, which is relatively more distinct in wheat due to high level of SGAPs (Swinkels, 1985). In oats, the paste is slightly rancid in flavour due to high level of starch lipids (Zhou et al. 1998). Rice starch paste flavour is bland, smooth, and creamy and provides texture to food similar to fat (fat substitute) (Champagne, 1996). The shear resistance of most normal cereal starches is reasonably high when compared to potato starch, in part because of high lipids and SGAPs in cereal starches (Swinkels, 1985). In pearl millet the shear resistance of the paste is high like in the legume starches because of this the bonding force in the interior of the granules are implicated as being strong (Hoover et al. 1996 b). The retrogradation tendency of most normal cereal starches is high as compared to potato and cassava starches, in part because of smaller molecular size (*dp*) of amylose, since retrogradation is high for low *dp* (200–1200) amylose (Swinkels, 1985). Retrogradation in oats is low in part because on gelatinisation and pasting amylose and amylopectin leaching takes place together and this possibly retards amylose preferential gelation (Zhou et al. 1998). Retrogradation in low amylose rice is medium because of low amylose content (Lii et al. 1995).

The thickening power of all waxy (amylopectin) cereal starches is high because of large molecular size (*dp*) of amylopectin, the almost absence of amylose and negligible lipids (Swinkels, 1985). Their shear resistance is poor as compared to non-waxy cereal starches in part because of almost absence of amylose, negligible lipids and also the maximum swollen starch granules have a tendency to easily disintegrate on shearing. Waxy cereal starch paste is fairly clear due to negligible amylose-lipid complex formation. The paste will not set to a stiff gel, rather it is soft and at low concentration is free flowing because of negligible amylose (Zobel, 1988 b). Retrogradation of waxy cereal starches is very low because of absence of amylose (Swinkels, 1985). The high amylose (amylo) (*ca.* 50-80 %) starches do not develop a

peak viscosity but set very quickly to firm gel on cooling, because of high amylose molecular interaction (Zobel, 1988 b).

2.2.4.3.2. Pseudocereal (amaranth, quinoa and buckwheat) starches

The gelatinisation temperature of amaranth and quinoa starches is as high as tropical cereal starches because their cultivation is most prevalent in tropical regions (Qian and Kuhn, 1999). The gelatinisation temperature of buckwheat starch is also high even though its cultivation is most prevalent in temperate regions. The possible high level of amylose–lipid complexation was implicated to change the water distribution and thereby to raise the gelatinisation temperature (Qian et al. 1998). The thickening power of amaranth (medium) and quinoa (low) are relatively less than normal cereal starches (maize, sorghum and wheat) in part because of their very small granule size. However, the thickening power of buckwheat is higher than wheat starches and weaker bonding force in the granule was implicated to cause (Biacs, Aubrecht, Léder and Lajos, 2002). The paste texture of amaranth is long and that of quinoa and buckwheat are short in part because of low amylose in amaranth (Qian and Kuhn, 1999) and high in quinoa (Qian and Kuhn, 1999) and buckwheat (Qian et al. 1998). The shear resistance of quinoa and amaranth is medium because small swollen granules are resistant to shear breakdown (Qian and Kuhn, 1999). Buckwheat starch is very resistant to shear breakdown and higher granule rigidity and/or higher amylose leaching was implicated to cause (Zheng et al. 1998). The retrogradation tendency is higher in quinoa than in amaranth (Qian and Kuhn, 1999). The retrogradation tendency of buckwheat starch is medium to high, in part because of high amylose (Qian et al. 1998). Due to the very small granule size of *ca.* 1-2 μm in diameter (Table 2.2), amaranth and quinoa starches are recommended for use as a fat mimetic (Koziol, 1993).

2.2.4.3.3. Tuber plant (potato and canna) starches

The gelatinisation temperature of potato starch is lower than tropical cereal starches, whereas for canna it is similar to tropical cereal starches. The granule size of potato and canna are very large and their swelling power is also very large (Hoover, 2001). Potato starch has no phospholipids but its amylopectin is highly phosphorylated and

carries negative charges (Swinkels, 1985). This favours low pasting temperature, very high paste viscosity and clarity. The paste of potatoes is long or stringy (Swinkels, 1985). Potato starch is used in dry mix soups, cakes and in some extruded breakfast cereals (Thomas and Atwell, 1999). The thickening power of canna starch is also very high with clear paste and short texture (Piyachmkwan, Chotineeranat, Kijkhunasatian, Tonwitowat, Prammanee, Oates and Sriroth, 2002). The shear resistance is low-medium in potatoes (Swinkels, 1985) and high in canna (Piyachmkwan et al. 2002). The retrogradation tendency is very high in canna and is low to medium in potato.

2.2.4.3.4. Root (cassava, sweet potato, yam and arrowroot) starches

The gelatinisation temperature of cassava starch is relatively low as compared to tropical cereal starches, in part, because the root starches are synthesised under the influence of soil moisture, which possibly favours cool temperatures as opposed to cereal starches which are synthesised in open air (Sriroth, Santisopasri, Petchalanuwat, Kurotjanawong, Piyachomkwan and Oates, 1999). The average *dp* of cassava amylopectin is very high as compared to any other normal starches but is less than waxy maize starch (Swinkels, 1985). Cassava starch develops reasonably high viscosity compared to cereal starches because of relatively larger average *dp* of amylopectin, low lipid and relatively low amylose content. The paste is clear, bland-testing, has low resistance to shear and has low retrogradation tendency (Swinkels, 1985). Cassava starch is used in confections (nougats, caramels and toffees) and flavoured dairy products (Thomas and Atwell, 1999). The gelatinisation temperatures of sweet potato, yam and arrowroot are similar to cassava starch. The paste viscosities of sweet potato and yam are similar to cassava starch but arrowroot starch has lower viscosity (Hoover, 2001). The shear resistances of sweet potato and yam starch are very high, possibly being caused by the presence of strong bonding forces within the granule interior (Hoover, 2001). Shear resistance of arrowroot starch is medium (Hoover, 2001). The retrogradation tendency of sweet potato is very high, whereas yam and arrowroot are high (Hoover, 2001). The paste of arrowroot starch is slightly yellowish with a bitter flavour (Erdman, 1986).

2.2.4.3.5. Plant stem pith (sago) starch

The gelatinisation temperature of sago starch is high, like tropical cereal starches, with medium-high viscosity and a moderately clear paste (in some coloured slightly brownish) of long texture (Sim et al. 1991). The shear resistance and retrogradation tendency of sago starch is medium-high. The metal (aluminum, iron, magnesium, calcium) and sulphur ions in the sago starch are considered as a factor for the lower firmness of sago starch as compared to wheat, cassava and potato starches (Sim et al. 1991). Interaction of iron with the polyphenols present in the pith is considered as a factor for the slight brownish colour of the starch (Sim et al. 1991).

2.2.4.3.6. Legume (beans and peas) starches

The gelatinisation temperature of legume (black bean, pinto bean, navy bean, chickpea, smooth pea and lentil bean) starches is as high as the tropical cereal starches (Hoover and Ratnayake, 2002) probably because legume starches occur as dense granules with low water uptake and restricted swelling property (Sosulski, Yook and Arganosa, 1997). The viscosity of black bean starch is medium-high, pinto bean low and the rest are medium. The shear resistance of all legume starches is very high and indeed with shearing the viscosity increases because of their high amylose content (Hoover and Ratnayake, 2002). The retrogradation tendency of all legume starch is medium-high.

Among the starch types described above, the most important commercial sources of starches are maize, potato, wheat, cassava, rice and waxy maize. It seems that other sources of starch are rarely utilized or their functional potentiality and economic significance are little known. In some food processing applications and under some storage conditions, the functionality of the widely used native starches are in some aspects limited. The native starch gels can be degraded due to high shear, high sterilisation temperature and low pH processing conditions. During storage and distribution, the performance of native starches can be negatively affected due to starch retrogradation. Chemically modified starches are often used to overcome such limitations at the expense of cost. An opportunity is to research and characterise

potential native starches of diverse and unique functionality. This review indicates that different sources of starch from different botanical sources have different granule size, compositions [(amylose/amylopectin ratio, lipids, proteins, phosphorus and microelement (potassium, sodium, calcium and magnesium)], structures (ultrastructure of amylose and amylopectin) and functionality (Tables 2.2 and 2.3). At present tef usage is mostly confined to Ethiopia for traditional food applications, ranging from local beverages to different baked products. Research work that generates scientific information on grain tef starch composition, structure and functionality might help to diversify its usage worldwide in food, food related and in other industries where starch is used.

2. 3. Objectives

The overall objectives of this research were to:

1. Characterise composition, physico-chemical and functional properties of tef starch from five different grain tef varieties.
2. Compare the properties of tef starch with commercial maize starch.
3. Determine whether tef starch has any unique and useful functional properties in food and non–food applications.

Specific objectives of the work were to determine:

1. tef starch granule composition.
2. tef starch granule structure and water interaction properties.
3. tef starch granule degradation properties.
4. gelatinisation properties of tef starch.
5. pasting properties of tef starch.
6. retrogradation properties of tef starch

2. 4. Hypotheses

- Tef starch composition, physico-chemical and functional properties can vary slightly between tef varieties because such variations have been found in other starch containing plant species.
- The physico–chemical and functional properties of tef starch may be influenced by the fact that tef starch granules are compound.
- Tef starch physico-chemical properties like X-ray diffraction pattern, gelatinisation, pasting, retrogradation, water absorption and water solubility indexes are in general similar to tropical C4 cereals (sorghum, maize and millets) because tef is a tropical C4 cereal.

Table 2. 3. Some functional properties of starch from certain cereals, pseudocereals, tubers, roots, stem pith and legumes

Starch source	Gelatinisation temperature (^{O}C)	Pasting temperature (^{O}C)	Relative viscosity	Paste texture	Paste clarity	Paste flavour	Shear resistance	Retrogradation tendency
Tropical cereals								
Maize ***(Zea mays)***	62-80A[1], 62-72A[2],64-76B[4]	75-80[3]	Medium[3]	Short[3]	Opaque[3]	Cereal flour[3]	Medium[3]	High[3]
Sorghum ***(Sorghum bicolor)***	60-76A[5], 68-78B[2]	68-70[5]	Medium-high[5]	Short[5]	Opaque in some slight pinked[5]	Cereal flour[3]	Low-medium[5]	High[5]
Pearl millet ***(Pennisetum glaucum)***	62-76B[6]	89-90[6]	Low-medium[6]	Short[6]	Cloudy[6]	Cereal flour[3]	High[6]	Medium-high[6]
Rice ***(Oryza sativa) (Normal amylose 20-33%)***	68-78A[2], 72-89B[7]	85-92[8], 93[9]	Medium-high[7]	Short flaky and dry[8]	Opaque[10]	Bland, smooth, creamy, fat substitute[11]	Low-medium[7]	Medium-high[7]

Table 2. 3. Continued

Rice *(Oryza sativa) (Low amylose, 5-20 %)*	68-78A[2], 64-82 B[7]	85-92[8]	Medium-high[7]	Long, sticky[8]	Opaque[10]	Bland, smooth, creamy, fat substitute[11]	Low medium[7]	Medium[7]
Tef (*Eragrostis tef*)	NA	NA	NA	NA	NA	NA	NA	NA
Waxy maize	63-72A[1]	65-70[3]	Medium-high[3]	Long reversible, free flowing at low con.[3,12]	Fairly clear[3]	Clean[3]	Low[3]	Very low[3]
Amylomaize	66-170[1]*	66-170[1]*	No peak very low[1]	Stiff, irreversible[12]	Opaque[1]	NA	Very low[3]	Very high strong gel[1]
Waxy rice	65A[12], 64-85B[7]	85-92[8]	Medium-high[7]	Soft, reversible and free flowing at low con.[12]	Fairly clear[3]	Bland, smooth, creamy, fat substitute[11]	Low[3]	Very low[3]

Table 2. 3. Continued

Temperate cereals								
Wheat ***(Triticum aestivum)***	52-85[1], 58-64A[2], 53-64B[13]	80-85[3]	Low-medium[3]	Short[3]	Cloudy[3]	Flour like[3]	Medium[3]	High[3]
Barley ***(Hordeum vulgare)***	52-60A[2], 52-70B[14]	85–86[15]	Low-medium[15]	Short[15]	Opaque[15]	NA	Low-medium[15]	Medium-high[15]
Oats (***Avena sativa)***	45-74B[16], 52-74B[17]	81-93[18]	Medium[18]	Long[18]	Fairly clear[18]	Slight rancid[19]	Low-medium[18]	Low[18]
Rye ***(Secale cereale)***	48-54A[20], 52-54B[20], 57-70B[2]	79-87[20]	Low-medium[20]	Short[20]	Opaque[20]	Flour like[20]	Low-medium[20]	High[20]
Pseudocereals								
Amaranth (***Amaranthus cruentus***)	66-87B[21]	72[21]	Low[21]	Long[21]	NA	Fat substitute[21]	Medium[21]	Low-medium[21]
Quinoa ***(Chenopodium quinoa)***	60-71B[21]	67[21]	Medium[21]	Short[21]	NA	Fat substitute[21]	Medium[21]	Medium-high[21]
Buckwheat (***Fagopyrum esculentum***)	61–80B[30], 63–81B[31]	75[31]	Medium–high[30]	Short[31]	NA	NA	High[31]	High[31]

Table 2.3. Continued

Tuber plants								
Potato (*Solanum tuberosum*)	58-65A[1], 50-68A[2], 63-71B[22]	60-65[3]	Very high[3]	Long, stringy[3]	Very clear[3]	Slight earthy[3]	Low-medium[3]	Low-medium[3]
Canna (*Canna edulis*)	65-70A[22], 69[23]	NA	Very high[23]	Short[23]	Very clear[23]	NA	High[23]	Very high[23]
Roots								
Cassava (*Manihot esculenta*)	59-70A[1], 57-76B[22]	65-70[3]	High[3]	Long[3]	Quite clear[3]	Bland[3]	Low[3]	Low[3]
Sweet potato (*Ipomea batatas*)	58-72B[24]	68–77[22]	Medium-high[22,24]	NA	NA	NA	Very high[22,24]	Very high[22,24]
Yam (*Dioscorea abyssinica*)	64–75B[22]	73-76[22]	High[25]	NA	NA	NA	Very high[25]	High[25]
Arrowroot (*Maranta arundinacea*)	61-86B[22]	72-76[26]	Low[26]	NA	A slight yellowish[27]	Slight bitter flavour[27]	Medium[26]	High[26]
Plant stem pith								
Sago *(Metroxylon sagu)*	71[+]B[28]	74-76[28]	Medium-high[28]	Long[28]	Moderately clear, some brownish[28]	NA	Medium-high[28]	Medium-high[28]
Legumes								
Black bean *Phaseolus vulgaris)*	62-84B[29]	70-75[29]	Medium-high[29]	NA	NA	NA	Very high[29]	High[29]
Pinto bean *(Phaseolus vulgaris)*	72-81B[29]	80-82[29]	Low[29]	NA	NA	NA	Very high[29]	Medium-high[29]

Table 2.3. Continued

Navy bean *(Phaseolus vulgaris)*	66-85B[29]	70-72[29]	Medium[29]	NA	NA	NA	Very high[29]	Medium[29]
Chick pea *(Cicer arietinum)*	59-78B[29]	75[29]	Medium[29]	NA	NA	NA	Very high[29]	Medium-high[29]
Smooth pea *(Pisum sativum)*	61-75B[29]	74–75[29]	Low-medium[29]	NA	NA	NA	Very high[29]	High[29]
Lentil bean *(Lens culinaris)*	61-79B[29]	72[29]	Medium[29]	NA	NA	NA	Very high[29]	Medium-high[29]

Sources: [1]Whistler and BeMiller (1997), [2]Snyder (1984), [3]Swinkels (1985), [4]White, Abbas, Pollak and Johnson (1990), [5]Beta et al. (2000), [6]Hoover et al. (1996 b), [7]Lii et al. (1995), [8]Hamaker and Griffin (1990), [9]Hoover, Sailaja and Sosulski (1996 a), [10]Morrison and Azudin (1987), [11]Champagne (1996), [12]Zobel (1988 b), [13] Sasaki and Matsuki (1998), [14]Tang et al. (2001), [15]Czuchajowska, Klamczynski, Paszczynska and Baik (1998), [16]Tester and Karkalas (1996), [17]Hoover and Senanayake (1996), [18]Zhou et al. (1998), [19]Liukkonen and Laakso (1992), [20]Schierbaum and Kettlitz (1994), [21]Qian and Kuhn (1999), [22]Hoover (2001), [23]Piyachmkwan et al. (2002), [24]McPherson and Jane (1999), [25]Gebre–Mariam and Schmidt (1998), [26]Erdman (1986), [27]Corbishley and Miller (1984), [28]Sim et al. (1991), [29]Hoover and Ratnayake (2002), [30]Qian et al. (1998), [31]Zheng et al. (1998).

Where: NA is information not available; A is gelatinisation transition temperature measured by birefringence loss on a *Kofler* hot stage; B is gelatinisation transition temperature measured by DSC and (+) is peak gelatinisation temperature by DSC. *(Amylomaize starch starts to loss birefringence at about 66 ^{O}C, produces essentially no viscosity under ordinary cooking at 95–100 ^{O}C of starch slurry and cookout does not occur until the temperature reaches 160–170 ^{O}C)[1]. A "short" paste is one that breaks abruptly when allowed to flow from a stiring rod, as opposed to a "long"(cohesive) paste, which forms long strings under the same conditions.