

**EFFECTS OF PROCESSING PROCEDURES AND CULTIVAR ON
THE PROPERTIES OF CASSAVA FLOUR AND STARCH**

BY

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Dedicated

**To my wife, Gloria, my children, Monica, Alejandro and Juan Manuel,
my parents, Alfonso and Leyda, brothers, Adolfo and Francisco Javier, and of
course to my grandmother, Elisa, for their love and encouragement**

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ABSTRACT

The aim of this research was to widen the knowledge of the physicochemical properties of cassava starch and flour and to understand the factors which influence their functional characteristics, including both genetic and environmental effects as well as processing procedures.

A range of chemical and physical techniques which included the Brabender viscoamylograph, Bohlin CS rheometer, Brabender farinograph, WAXS, GPC, SE-HPLC and Coulter counter have been used to examine the structural and behavioural characteristics of both cassava starch and flour.

The results suggested that the functional behaviour of native cassava starches of different origins varies widely and appeared to be associated with molecular structure and the architecture of the starch granule. The viscosity and the mechanical properties of the pastes produced by gelatinization were determined by the degree of swelling and the amount and proportion of amylose and amylopectin in the solvent phase of the pastes. The amylopectin was present in the solvent phase in substantial quantities which varied between 37 and 57% of the total starch solubilized. In native cassava starches, the amylose appeared to have a high molecular weight (M_w 19×10^5 - 11×10^5). The constituent chains of the amylopectin molecule did not vary in length with cassava starches of different origins, but their relative population did which was reflected in minor differences in the chromatographic profiles. Starch granules

containing long chain amyloses and amylopectin with a high degree of branching were found to release reduced amounts of molecules into the liquid phase of the pastes, and *vice versa*. Where the pastes contained a high proportion of amylopectin and long amylose molecules the resultant gel was surprisingly weak

Cassava starch processed to produce "sour" starch, or fermented, and sun dried starch, was found to have suffered degradation to an extent where 77-86 % of the starch was solubilized during aqueous heating. The extent of the degradation was influenced by the cassava cultivar. The "sour" starch proved superior to unfermented and fermented, oven dried starches in the production of baked products with an expanded texture..

Starch extracted from cassava roots which had been stored for a short period, during which physiological deteriorative processes could have occurred, was found to show a slight reduction in the pasting viscosity which was not related to granular or molecular size or organization.

Rural, factory-extracted starch was found to have a reduced paste viscosity as a consequence of fermentation and contamination with peel residues from the roots.

Cassava flour properties were influenced by the conditions of preparation. Drying temperature, milling procedure and particle size could be selected and controlled to give cassava flours of the desired functional properties.

CHAPTER 1

INTRODUCTION

1.1 CASSAVA - A TROPICAL CROP

Cassava (*Manihot esculenta* Crantz) is a starchy root crop and has been cultivated in tropical America for over 5000 years. However, it is now grown throughout the tropical world. It is a high energy, staple food crop (520 kJ/100 g in contrast to the potato which only provides 319 per 100 g) and is now estimated to feed about 500 million people in 92 countries in the tropics and subtropics (CIAT, 1993).

To satisfy such requirements, it is not surprising that the global production of cassava amounted to 154 million tons of fresh roots in 1991 (FAO, 1992). The five major cassava producing countries are Brazil (25 million tons), Nigeria (20), Thailand (20), Zaire (18), and Indonesia (16). The total area harvested is about 16 million hectares, with 57% in Africa, 25% in Asian and 18% in Latin America (CIAT, 1993).

The growing of cassava, either by the individual or commercially, is limited to the hotter areas of the world; however, within this zone, it is cultivated under enormously different climatic and soil conditions. In Colombia, a country with diverse ecological conditions, cassava grows in the high rainfall areas of the Andes at 2000 meters, in the semi- arid areas of the Guajira, in the rich soil of the Cauca and Tolima

Valleys, and the tropical rain forest of Putumayo (Cook, 1985).

Typically, the crop is grown between 30° north and 30° south of the equator, in areas where the annual mean temperature is greater than 20°C. However, in highland areas near the equator (the Andean zones of Colombia, Ecuador and Peru), cassava grows in areas where the mean annual temperature is as low as 17°C, but fluctuations about this mean are slight (Cook 1985). These well adapted, cold cultivars are found only in the Andean zone and they do not yield well in areas that have higher temperatures.

The roots are the main portion of the plant to be used with a typical composition range of: moisture 62 to 66%, starch 28 to 33%, sugars 0.4 to 1.2%, protein 0.4 to 1.5%, dietary fibre 1.4 to 1.6%, fat 0.1 to 0.3% and ash 0.5 to 1% (Bradbury and Holloway, 1988). Annual consumption is greatest in Africa, averaging 96 kg per capita but average world consumption is 18 kg per capita. About 85 % of the world cassava crop is used domestically for food (58%), animal feed (28%), industrial uses (3%), and wastage (11%). The remainder 15% is exported, as either chips, pellets, or starch, mainly by Thailand and Indonesia, to the European Union, Eastern Europe, the Russian Federation and Japan (CIAT, 1993)

1.2 CASSAVA STARCH

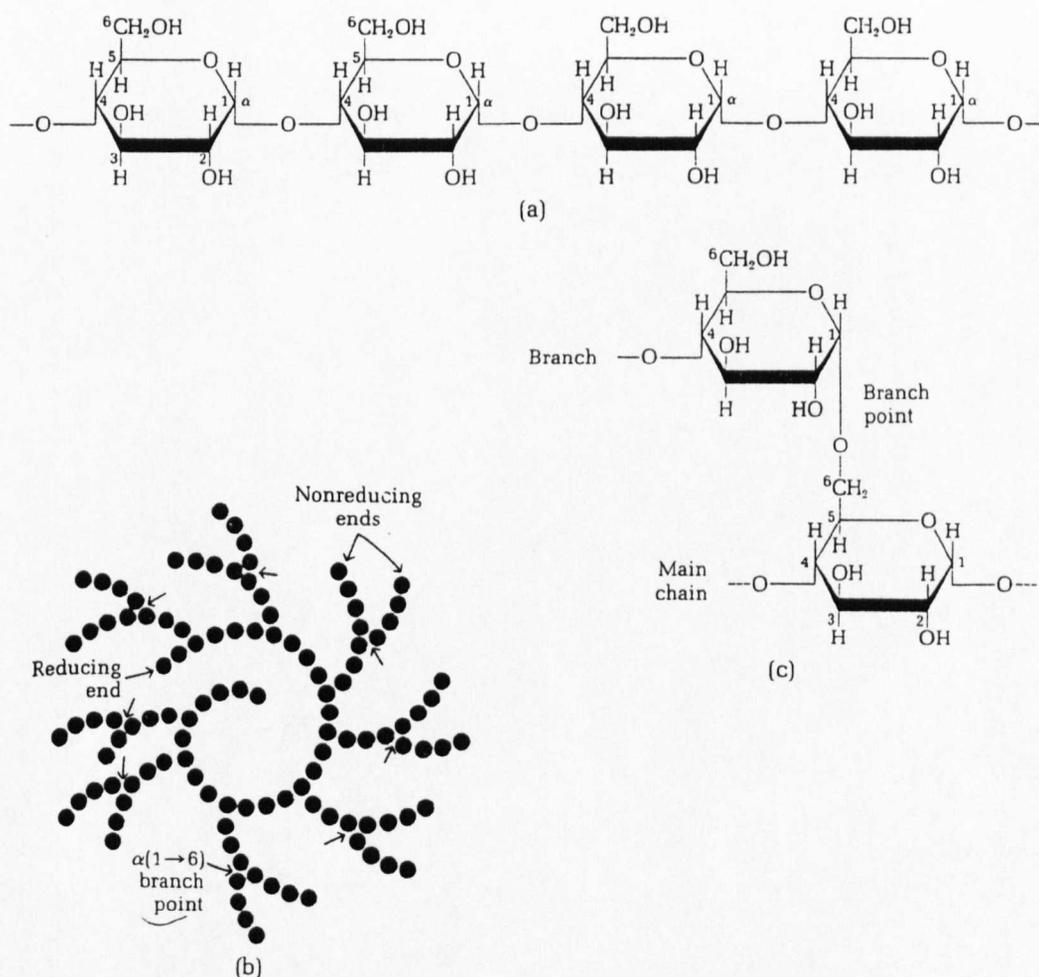
Starch is the major carbohydrate in the cassava roots; values ranging from 73.7 to 84.9 % dry root weight have been reported for a number of cassava cultivars

(Rickard *et al.*, 1991). Cassava starch, as a raw material in the food industry, has the reputation of possessing a high viscosity, a high water binding capacity, a quite clear to translucent fluid paste, a bland taste, low tendency to retrograde, and a long cohesive texture (Glicksman, 1969). Rickard *et al.* (1991) reviewed the literature on the physico-chemical properties of cassava starch available and observed some variability in the properties. It was not possible to infer from the information available whether the variations reported were genetically or environmentally induced, or if they were caused by differences in the techniques of detection and assessment employed. The extraction procedure used could also have an effect on the properties of the starch depending on the presence of impurities and the extent of mechanical damage in the starch granules. A review of recent research reports on cassava starch shows that there are some variations in the distinctive properties which were related to cultivar, age, and growth season (Asaoka *et al.*, 1991, 1992; Wheatley *et al.*, 1992; Chuzel, 1992; Moorthy, 1994). However, it was difficult to relate many of the physico-chemical properties of the extracted starch to variations in texture of fresh roots and the quality of products derived from the cassava.

Starch polysaccharides. It is known that starch consists chemically of two main polysaccharides, amylopectin and amylose (Figure 1.1 a and b), and the proportion of these components varies from one cultivar to another. Most of the functional properties of native starches are due to the relative proportion of these macromolecules, their molecular structure and to the organization of amylose and amylopectin within the starch granule. The amylose content of cassava starch has been reported to range from 13.6 to 23.8 % (Rickard *et al.*, 1991). Wheatley *et al.* (1992) evaluated 560 cassava

clones from the core collection of 630 accessions established at CIAT (Cali, Colombia) and found a range of amylose of 15-28% using an iodo-colorimetric method.

Figure 1.1 (a) Amylose linear chains of D-glucose units in $\alpha(1 \rightarrow 4)$ linkage. (b) Amylopectin. Each circle represent one glucose residue. The $\alpha(1 \rightarrow 6)$ linkages are indicated by the small arrows. (c) Structure of a branch point. (From : Lehninger, 1992).



Structure of amylose. Amylose is essentially a linear molecule, with few branches, consisting of up to several thousands D-glucopyranose residues linked together mainly by α -D (1 \rightarrow 4) bonds (Figure 1.1a). The incomplete conversion of amylose to maltose by β -amylase suggests the presence of some branches linked through α (1 \rightarrow 6) bonds. This enzyme cannot hydrolyze α (1 \rightarrow 6) linkages and, therefore, the β -amylolysis (expressed in maltose) is only 100% for a linear chain (Guilbot and Mercier, 1985). The β -amylolysis range for the amylose of cassava has been reported to lie between 64 and 75% (Hizukuri *et al.*, 1981 and Takeda *et al.*, 1987).

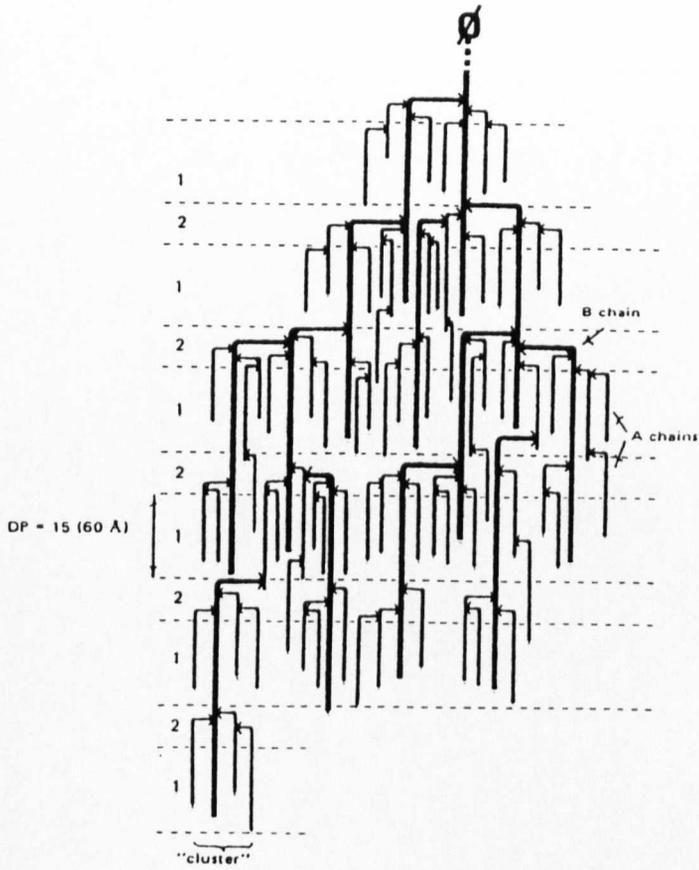
One of the important characteristics of amylose molecules is their ability to complex with iodine. The amylose chain forms a helix around the polyiodide ion to give a characteristic blue colour (maximum absorption wavelength, λ_{\max} , 620-680 nm) which is distinct from the amylopectin-iodine complex that forms a reddish-purple colour (λ_{\max} 550 nm). The interaction of amylose with iodine is strongly dependent on its chain length, complexing being more pronounced with longer amylose molecules (Bailey and Whelan, 1961). In solution, amylose can adopt several conformations including a random coil (in water or neutral solutions), an interrupted helix (in alkali and dimethyl sulphoxide) or a helix with 6 glucopyranose residues per turn (in the presence of complexing agents) (Banks *et al.*, 1969).

The range of average chain lengths of the whole molecule of amylose in cassava is reported to be DP_n 2600-3642 number-average degree of polymerization,

and DP_w 6680-7710 weight-average degree of polymerization (Hizukuri *et al.*, 1981; Takeda *et al.*, 1984; Hizukuri and Takagi, 1984; Suzuki *et al.*, 1985 and Ong *et al.*, 1994).

Structure of amylopectin. Amylopectin is one of the largest natural polymers with an average DP_n between 3×10^5 and 3×10^6 (Zobel, 1988). The weight-average molecular weight of cassava amylopectin has been reported to be 450×10^6 (Banks *et al.*, 1972). Amylopectin is a highly branched polymer, with a tree-like configuration, and β -amylolysis is limited to a range of 49-58% due to the high number of α (1 \rightarrow 6) branch points (Figure 1.1b and c). The average length of the constituent chains of cassava amylopectin has been reported to be about DP_n 26 (Hizukuri, 1986). The amylopectin chains can be classified into three categories (Manners, 1985): A chains, which are linked to B chains by their reducing end groups through the α (1 \rightarrow 6) linkage, the B chains in turn are linked to other B chains or to the C chains (only one per molecule) which carries the sole reducing end group of the macromolecule. The short A chains are organised as double helices and form clusters at regular intervals throughout the molecule linked together by the longer B chains. This cluster model (Robin *et al.*, 1974) is generally accepted as representative of the structure of amylopectin (Figure 1.2). Hizukuri (1986) proposed a more refined cluster model with four types of B chains (*i.e.* B₁, B₂, B₃, and B₄) of increasing chain length. The model of Hizukuri (Figure 1.3a) was derived from the polymodal, instead of bimodal and trimodal (Manners, 1995) size exclusion chromatograph chain profiles from cassava amylopectin as shown in Figure 1.3b. Hizukuri model assumes that, the A and B₁

Figure 1.2 Cluster model of amylopectin proposed by Robin (1974). 1, Compact area; 2, less compact area rich in branching points; Ø, reducing unit.

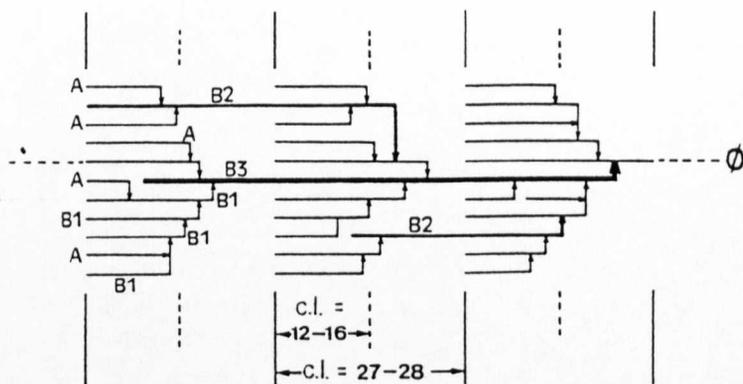


chains (which represent 82-91% of the total chains) are the constituents of a single cluster, whilst the B₂ and B₃ chains participate in two or three clusters in tandem respectively and the remaining (1%), the B₄ chains, could participate in four clusters (in tandem). The average chain lengths of the fractions A, B₁, to B₄ in a sample of cassava amylopectin have been reported by Hizukuri (1986) to be 12, 21, 42, 69 and 115 respectively. Hood and Mercier (1978) have proposed 15 as the length of the A chain in cassava amylopectin. Ong *et al.* (1994) have confirmed a polymodal chain length distribution in amylopectin of a sample of cassava starch from Malaysia.

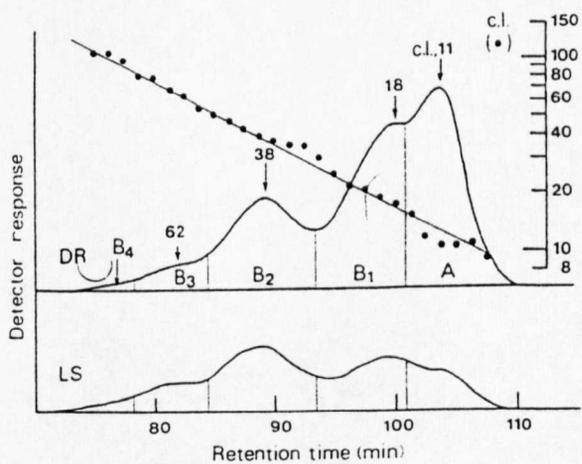
Organization of the starch granule. Native starch granules can be identified by their shape and size. Cassava starches have been identified as round, spherical or round to angular. The granules have been reported to have range of sizes from 5 to 20 μm (Rickard *et al.*, 1991). The structure of the starch granules depends on the way in which amylose and amylopectin are associated by intermolecular hydrogen bonds. These macromolecules are associated throughout the granules and the degree of mutual binding is responsible for the structural heterogeneity. The growth of the starch granules is by deposition on the outside of successive layers of increasing organization. Starch granules have a layered ultrastructure, and terms such as growth rings, layers, shells and lamellae are used to name the macro-structural units of the granule. Thin sections of starch granules have revealed a regular arrangement of concentric layers or rings, with alternating amorphous and semicrystalline regions (French, 1984; Guilbot and Mercier, 1985). At higher levels of organization, the semicrystalline rings are composed of stacks of alternating crystalline and amorphous

Figure 1.3 (a) Cluster model of amylopectin suggested by Hizukuri (1986). \emptyset , Reducing unit; —, (1 \rightarrow 4) α -D-glucan chain; \rightarrow , α -(1 \rightarrow 6) linkage. (b) Gel-permeation h.p.l.c of cassava amylopectin.

(a)

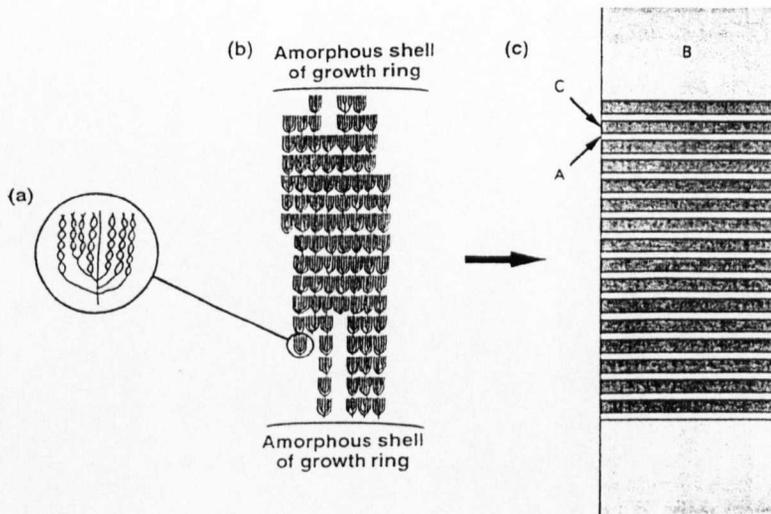


(b)



lamellae (Jenkins *et al.*, 1993). The crystalline lamellae are associated with the amylopectin (French, 1984; Guilbot and Mercier, 1985; and Jenkins *et al.*, 1993). The currently accepted crystalline structure consists of a radial arrangement of clusters of amylopectin. Each cluster contains a region high in branching points (the amorphous lamella) and a region where short chain segments of amylopectins have formed double helices (the crystalline lamella) (Figures 1.4a and b). Cameron and Donald (1992), have modelled the starch granule structure as a finite number of lamellae of alternating crystalline regions C and amorphous regions A, embedded in a background region B, assumed to correspond to the amorphous growth ring (Figure 1.4c). French (1985) has suggested that with cereal grains, growth rings represent periodic growth of the starch granule and daily fluctuations in carbohydrate available for starch deposition. The amorphous region, between the crystalline dense rings, is relatively more susceptible to acid and enzymic degradation than dense crystalline zones which are relatively incapable of radial swelling owing to the constraints of the molecular chains which run perpendicular to the growth rings. However, the surrounding (intercrystalline) amorphous region is readily penetrated by water and undergoes limited reversible swelling that involves swelling of the entire granule. It seems that the changes in starch granules during drying are primarily due to the loss of water from the amorphous phase (also called gel phase) leading to the formation of interchain and intrachain hydrogen bonds (Guilbot and Mercier, 1985).

Figure 1.4 (a) A single amylopectin cluster with double helix formation. (b) Schematic representation of the arrangement of amylopectin molecules within a semi-crystalline growth ring. (c) A model of the structure in terms of a stack of lamellae alternating in crystalline regions C and amorphous regions A, embedded in an amorphous region.
From: Jenkins *et al.* (1993)



The semi-crystalline structure of the starch granules diffract the X-rays. The X-ray diffraction technique (Zobel, 1964) detects the double helices packed into regular arrays, resulting in an X-ray diffraction pattern of an amorphous halo onto which are superimposed a series of broad diffraction peaks. The positioning of the peaks and their intensity depend on the starch granule source. Three main patterns, A-, B- and C- types, have been identified to describe native starch granules. Generally the A-type starch granules include the cereal starches, the B-type the starches from tubers and fruits and C-type from cassava starches (Zobel, 1964). Patterns of C-type are intermediate between A- and B-type patterns and are probably due to mixtures of A- and B-type crystallites, either within individual granules, or as mixtures of A- and B- type granules. In general, amylopectin molecules of A-type starches have a larger proportion of shorter chains (chain length average of DP_n 26) than those of the B-type starches (DP_n 36), while the chain length of amylopectins of the C-type (DP_n 28) are intermediate (Hizukuri, 1985). It was suggested by Hizukuri (1985) that the C-type starches could give a varying type of crystalline structure depending on the environmental temperature, whereas A- and B-type starches are insensitive to temperature. In the review of Rickard *et al.* (1991) cassava starch was reported with X-ray diffraction patterns of A-, C- and a C- close to A-type.

Aqueous heating characteristics of starch. Starch heated in water changes little until an energy level high enough to dissociate the relatively weak bonding is reached. The granules then swell tangentially and fully hydrated starch molecules separate from the intricate micellar network and diffuse into the surrounding medium (Leach *et al.*,

1959). Swelling is irreversible up to the point at which the molecular structure within the granules is disrupted and molecular order is lost. Over a relatively narrow temperature range, all the granules swell irreversibly and are said to have undergone gelatinization. The range of temperatures over which cassava starch has been reported to undergo gelatinization has been reviewed by Rickard *et al.* (1991); an average range is between 56 and 67 °C. It was reported that small variations in the gelatinization temperature range occurred between cassava cultivars from the same location but larger variations were obtained in cassava starches from different countries.

At temperatures above the gelatinization range the starch granules continue to swell (hydrogen bonds continue to break), and there is diffusion of starch macromolecules into the water. There are therefore two parallel events, granule swelling and starch solubilisation. Cassava starch has been shown to exhibit a two-stage swelling, and a swelling power lower than potato starch but higher than starches from yam, sweet potato, plantain, and colocacia. Solubility has also been reported to be higher than that of yam but less than that of potato starches (Rickard *et al.*, 1991). A significant variation in the swelling characteristics of cassava starches from plants of different ages and genetic constitution was found by Moorthy and Ramanujan (1986). During their study it was observed that, during the growth period the size of the granules and the molecular size of the starch did not change appreciably, suggesting that the changes in the swelling abilities of the granules were the result of changes in the associative binding forces of starch molecules.

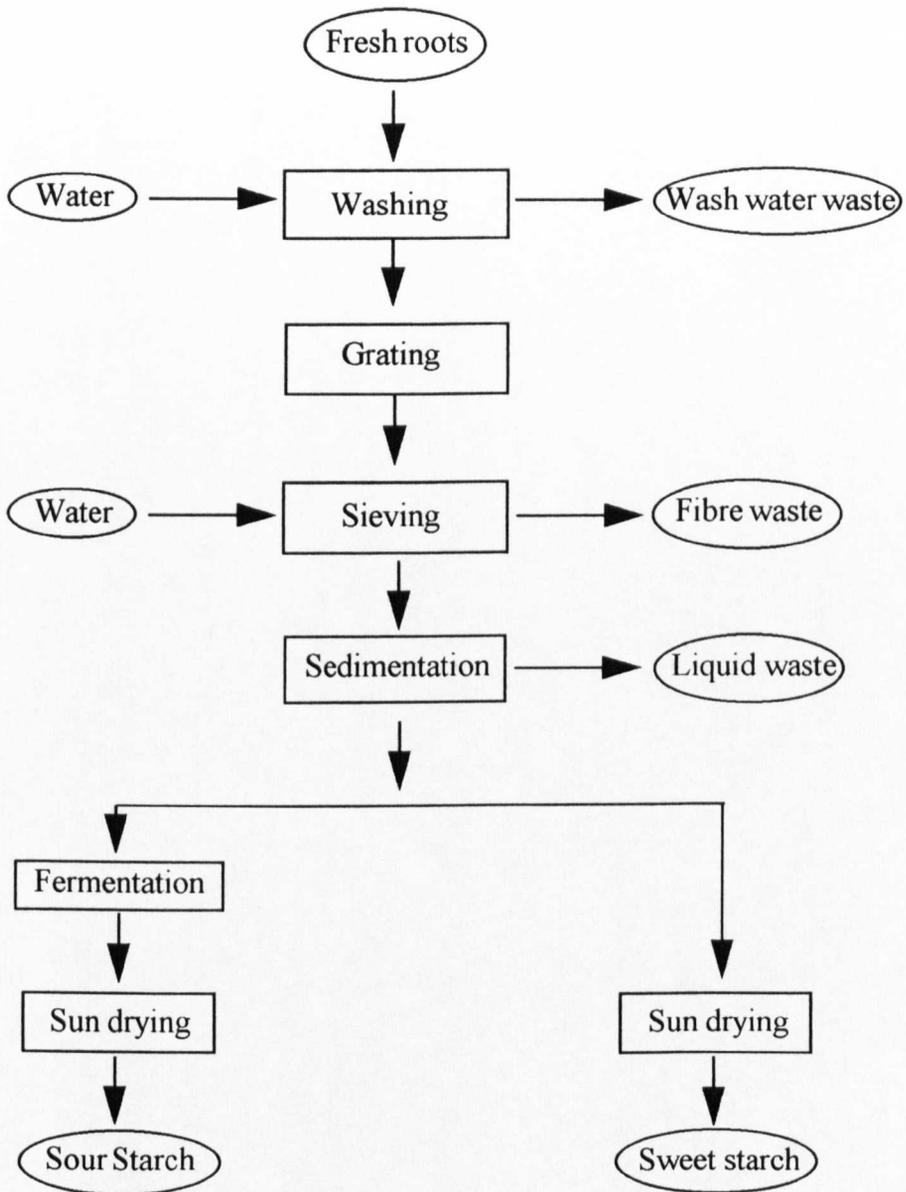
After the swelling of the granules and starch solubilisation, the starch aqueous suspension is transformed into a viscous paste. Upon cooling, the paste can develop a gel-like viscoelastic quality depending on the retrogradation tendency of the solubilised starch in the paste, which is largely determined by the affinity of the hydroxyl groups in one molecule for another. This process occurs mainly between the amylose molecules (in the early stages) present in the solvent phase. Suzuki *et al.* (1985) suggested that the lower retrogradation tendency exhibited by cassava starch paste might be caused by the higher weight-average molecular weight of the amylose fraction. The viscosity profiles of suspensions of cassava starch during pasting have been characterised by the Brabender visco-amylograph but comparison of the viscosity data has been difficult due to the different test conditions used (Rickard *et al.*, 1991). Initial pasting temperatures ranging from 52 to 66°C in aqueous starch suspensions (6%) of seven Indian cultivars of cassava were reported in the review by Rickard *et al.* (1991). A significant variation in the viscograph data has been found between starches of cassava cultivars grown in CIAT (Colombia) (Wheatley *et al.*, 1992, Chuzel, 1992; Fernández *et al.*, 1994). Wheatley *et al.* (1992) observed significant differences in the Brabender viscosity data between cassava cultivars with the highest and lowest total cyanogen contents although the initial pasting temperatures were similar. The high cyanogen cultivars had lower pasting viscosity values than the low cyanogen cultivars. In their study, it was also found that amylose content did not correlate with the starch functional properties evaluated using the Brabender visco-amylograph (*i.e.* ease of cooking, gel instability and set back viscosity).

1.3 SOUR CASSAVA STARCH

Sour cassava starch is a product with a distinctive flavour and distinct functional properties. When a sour starch-water dough is oven-baked it expands. Sour starch is irreplaceable in the manufacture of traditional cheese breads in Latin America: *pandebono and pandeyuca* in Colombia and *biscoicho* in Brazil. Sour starch is made from sweet (native) cassava starch which is naturally fermented and then sun dried. Sun drying is essential to give the fermented starch the required baking expansion property. The general process of production of sour starch is presented in Figure 1.5. In Colombia, sour starch production is a small-scale rural industry (1-5 ton of fresh roots/day). In Brazil production is also rural but with an average process capacity of up to 120 tons of roots/day. The factories in Colombia produce 5000-6000 ton of sour starch/year (Brabet, 1994). In Colombia, the fermentation lasts from 20 to 30 days but in Brazil for about 60 days. The sun drying is completed in 1-2 days with a load of 2-3 kg of wet starch/m². One hundred kg of fresh roots [moisture content of 63.7 (±2.6) %] produce 20.8 (±7.6) kg of sour starch [(moisture content of 12.8 (±1.2) %] (Chuzel, 1992). The yield of sour starch is a function of the cassava cultivar, the growth conditions, age of the plants and the climatic conditions at the time of harvesting the roots. The yield of sour starch has been reported to decrease with the time of storage of the roots post-harvest (Brabet, 1992).

In several samples of sour starch from different cultivars, the amylose content of sour starches appears to remain unmodified (Camargo, et al., 1985). The shape and the size of the granules of sweet and fermented starches have not shown significant

Figure 1.5 Flow chart of the process of extraction of sweet starch and production of sour starch.

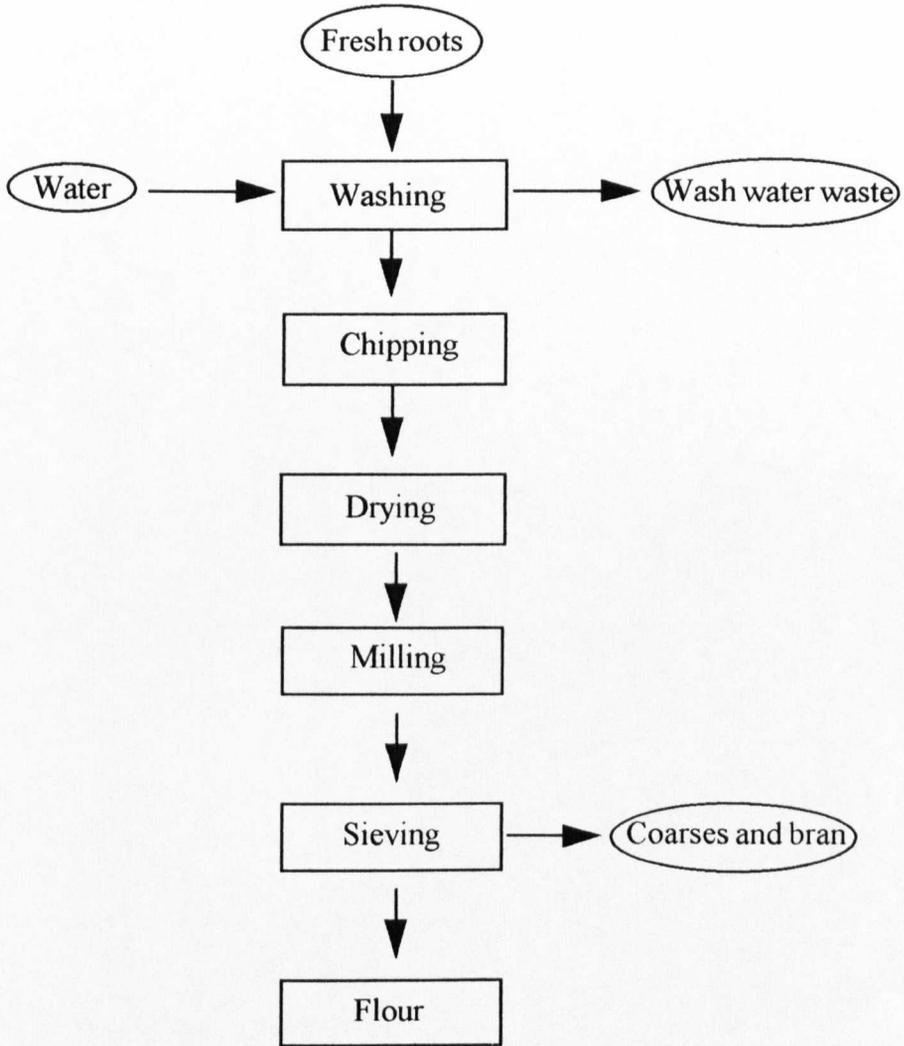


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1.4 CASSAVA FLOUR

Cassava flour is made by slicing or chipping peeled roots, then drying and milling them (Figure 1.6). The chemical compositions of laboratory prepared cassava flours from several cassava cultivars grown at CIAT (Palmira, Colombia) have fallen in the ranges: starch 85.7-86.1%, crude fibre 4.3-4.4%, protein (N x 6.25) 2.9-3.6%, total sugars 3.6-4.1% and ash 2.2-2.9% (Rodriguez, 1992). The proportion of the components can vary according to the method and conditions of the milling and sifting process (Alonso, 1992). Some studies have shown that it is feasible to make bread without wheat using cassava flour (Eggleston *et al.*, 1993b; Defloor, *et al.*, 1994 and 1995), which resembles a product intermediate between cake and bread. It has also been demonstrated that cassava flour can readily be incorporated into wheat bread at levels as high as 10-20% to produce a bread loaf of acceptable quality of the bread loaf (Ciacco, *et al.*, 1978; CIAT *et al.*, 1988; Almazan, 1990; Eggleston *et al.*, 1993a). Although mixing cassava flour with wheat flour will lower the protein content of the resulting loaf, this disadvantage could easily be overcome by fortifying the cassava flour with soybean flour (CIAT *et al.*, 1988 ; Eggleston, *et al.*, 1992). Cassava flour is used in Indonesia in many other products, such as biscuits, pies, pastry, crispy chips and meat balls (INDOKASAVA) and is sometimes used to extend wheat flour for noodles (Cock, 1985). In India it is used in making spaghetti and noodles. A survey, as well as experimental trials, in the food companies in Colombia have shown that cassava flour can beneficially be incorporated into food products such as processed meats (sausages), traditional pastries, instant soups, pastas, batter mixes, biscuits, condiment mixes and bread crumbs (Ostertag, 1992).

Figure 1.6 Flow chart of production of cassava flour in a pilot plant in CIAT (Palmira, Colombia)



The bread making quality of composite cassava-wheat flour has been shown to be affected by the cassava cultivar and the age of the plant at harvest. In bread baking trials with flours from cassava cultivars grown in CIAT (Palmira, Colombia) and harvested between 8 and 14 months, an increase in the quality of cassava-wheat bread was demonstrated from 8 to 12 months. In this study, it was suggested that changes in the diastatic activity in the flours could have affected the specific volume of the bread (CIAT *et al.*, 1988). The diastatic activity was measured in cassava flour from several cultivars, when used with wheat flour, in baking tests carried out in IITA (Ibadan, Nigeria). Flours with high diastatic activity (and indirectly with low paste viscosity) had a deleterious effect on the baking properties (Eggleston *et al.*, 1993a). Wheatless cassava breads from flours with high diastatic activity (*i.e.* above ~ 145 mg of maltose) produced dense, pudding-like breads which were therefore unsuitable; bread specific volumes were reliably predicted from the diastatic activity and the Brabender peak viscosity. The diastatic activity was found to be dependent on the moisture content of the freshly harvested roots (Eggleston *et al.*, 1993b).

The rheological and baking properties of doughs made from wheat-cassava flour blends have been investigated (Ciacco *et al.*, 1978 and Eggleston *et al.*, 1993). As the cassava flour level was increased, the Brabender farinograph parameters, *e.g.* developing time and stability of the dough, decreased. Although acceptable bread could be produced with 10% cassava flour, difficulties were encountered with the dough handling properties (Ciacco, *et al.*, 1978). Changes in the functional properties of cassava flours have been related to the presence of fibrous materials which can

restrict swelling in hot water (Moorthy *et al.*, 1993).

1.5 POST-HARVEST DETERIORATION OF CASSAVA

Cassava roots are highly perishable and will deteriorate within a few days of harvest (Wenham, 1996). Two distinct deterioration processes have been identified: an initial deterioration caused by endogenous physiological processes, and a secondary deteriorative process dominated by high microbial activity (Booth, 1976). The physiological deterioration is seen first in damaged areas of the roots as a blue-black discoloration of the vascular tissue which rapidly spreads to a general discoloration of the root storage parenchyma (Wheatley, 1989). The rapid physiological deterioration of cassava roots often begins within 24 h of harvest. It is considered to be a humidity sensitive wound response and involves an increase in enzyme activity and the production of phenolic compounds (Rickard, 1985).

The storage of cassava roots is therefore limited to a few days. Post-harvest deterioration causes a reduction in root quality, which leads to economic losses as the roots remain unsold or sell at a discount price. The main reason for processing the roots into a variety of traditional products in the tropics is to avoid the rapid losses caused by cassava deterioration.

Cassava roots with visible signs of physiological deterioration are considered to have poor eating and processing qualities. In Latin America, the cassava starch industry is reported to experience severe limitations, including poor availability of

fresh roots, and low starch extraction efficiency (Chuzel, 1991). The sedimentation of starch from deteriorating roots is considered by processors in Colombia to be less efficient than from fresh roots. Recent results from CIAT (unpublished) have shown that starch extraction yields were significantly reduced by post-harvest deterioration. Storage of the roots for more than 36 hours could also alter the colour and flavour of the sour starch produced (Ruiz, 1991). Starch losses due to deterioration of roots are of significant economic importance in a countries such as Indonesia, where in 1978 about one-third of all cassava utilized went into starch production (Wenham, 1995).

Observations made on the implications of a physiological deterioration on the root usage were compiled by Wenham (1995):

- * Roots suffering deterioration took longer to cook, had an unpleasant bitter flavour and an unattractive off colour;
- * *Fufu* (a fermented flour from soaked roots) made from deteriorating roots had a lower and less desirable elasticity than *fufu* prepared from fresh roots;
- * Cooked roots were difficult to pound;
- * *Gari* (a roasted, fermented product from grated roots), when prepared from deteriorating roots, had lower and less desirable swelling properties than *gari* from fresh roots.

1.6 AIMS OF THIS RESEARCH

Cassava starch and flour have been reported to have intrinsic functional properties which depend on the cultivar, age of the plant at harvest, growth and harvest season. Environmental factors (especially the temperature) could have a marked effect in the synthesis of starch in cassava plants. The processing conditions of the roots could also affect the properties of cassava starch and flour.

The origin of the variability in the functional properties of cassava starch requires investigation to determine whether they are a consequence of the two major polysaccharides present in the starch, their structure, relative composition and the granular organization. Understanding the physicochemical and functional properties of native cassava starches could be of great importance to cassava plant breeders and geneticists for the production of plants with particular attributes in their starches.

The majority of cassava starch factories in Latin America have technical problems, related to the poor efficiency of the extraction process and the quality of the final product. The determination of the functional and other properties of starch from these factories will show the extent of the change in quality due to the process conditions.

Cassava flour has potential as an ingredient in the food industry in Latin America. Obviously, high quality flour is required for use in existing and potentially new, high grade products. Cassava flour is often produced by associations of farmers

in rural enterprises with a production scale of 2000-3000 kg of roots/day. The prevailing rural processing technology needs some refinement to produce cassava flour of the quality required for the food industry.

The aim of this thesis is to widen the knowledge of the physico-chemical properties of cassava starch and flour. This research investigates,

(a) the relation between the physico-chemical and functional properties of cassava starch by examining the effect of cultivar and plant growth environment, the effect of fermentation and sun drying in naturally modified starches, and the effect of using stored roots that could exhibit some physiological deterioration;

(b) the extent of modification of the properties of cassava flour in relation to drying temperatures and milling methods.

CHAPTER 2 GENERAL MATERIALS AND METHODS

2.1 CASSAVA CULTIVARS

Of the world core collection of cassava germplasm held at the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia 550 cultivars have been evaluated for their cyanide and dry matter content of the roots, and the apparent amylose content of their starches (Wheatley *et al.*, 1992). The results showed considerable variability and from these measurements the cultivars were grouped in ten clusters as shown in Figure 2.1.

The cultivars of cassava studied in this research were selected in CIAT from the cluster groups in Figure 2.1. A total of thirty one cultivars were chosen (Table 2.1) to include popular varieties from Latin America, Asia and Africa which possessed desirable organoleptic qualities both of the cooked roots and their products.

Figure 2.1: Representation of clusters of cassava cultivars, using three factors as axes (Factor 1: total cyanogen content, Factor 2: dry matter content, Factor 3: amylose content.)

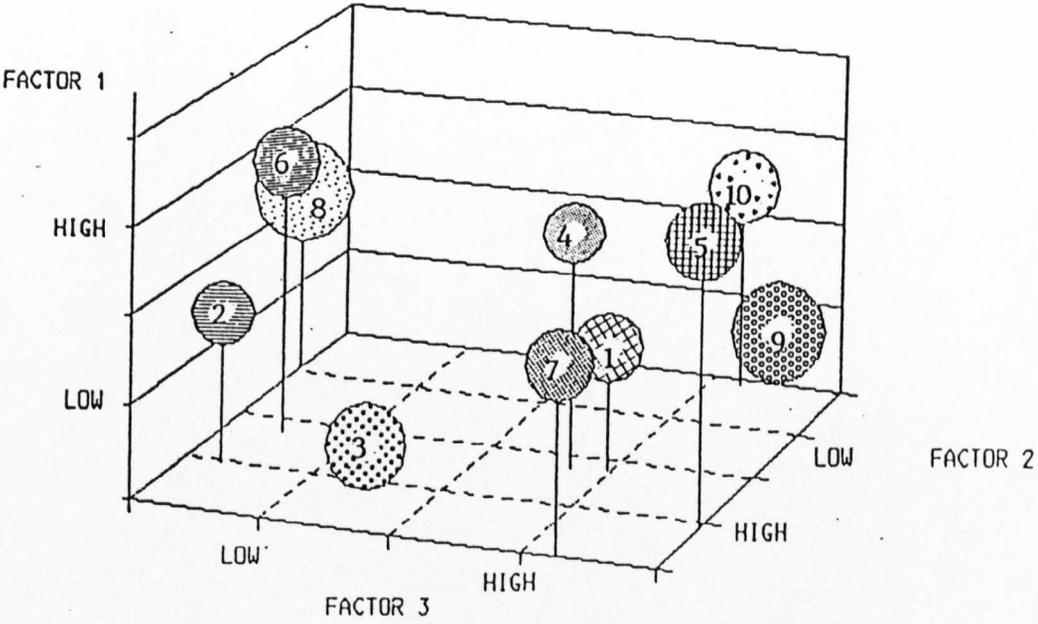


Table 2.1 : Cassava cultivar, including hybrid parents and some common names

Cluster	Cultivar	Hybrid parents	Name	Main use
	MCol 22			Cooking
1	MCol 2066		Chiroza	Cooking
	MBra 897			
	CG915-1	MBra 12 x MCol1643		
2	MMex 59			
	MPar 105		Caballero-i	Cooking
	Mcol 1468		CMC 40	
3	MCR 35			
	MCol 1522		Algodona	Starch
	CG 1-37	MBra 12 x MCol 22		
4	MVen 77			
	MPer 196			
	CG 1141-1	MMex 11 x MCol 65		
5	MCol 72			
	MMal 1		M4 (India)	Cooking
	MCol 1684			
	MVen 25			Casabe
6	MThai 1		Rayong 1	Chips
	MCol 2485		Unee Rucu	Casabe*
	MBra 1162			

Table 2.1 (Continued)

Cluster	Cultivar	Hybrid parents	Name	Main use
7	MCol 2215 CM3306-4	CMC 40 x P11	Venezolana	Cooking
	CM 2772-3	CM727-14 x MPan 12B		
8	CM 489-1 MBra 881	MCol 882 x MVen 270	Branca st. C.	Starch
	CG 402-11	MCol 1522x MCol 647		
9	CG1118-121 MCol 1132	MCol 2017x MCol2060		
10	CG 165-7 MMal 2 MNga 2	MCol 1495x MPan 90	Black Twig TMS 30001	Starch Gari**

* Casabe: a baked, flat cake or tortilla-like bread made from squeezed cassava mash.

**Gari: a roasted, fermented product from grated cassava roots.

2.2 PREPARATION OF THE STARCH SAMPLES

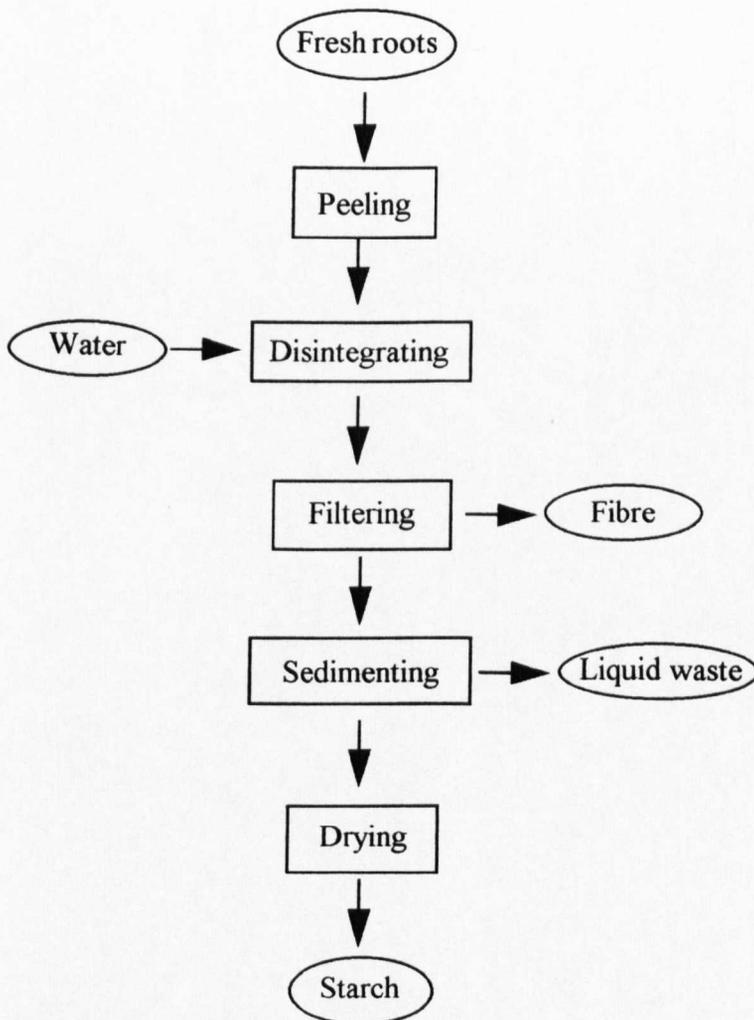
2.2.1 Starches for the study of cultivar and environmental variation

Twenty nine cassava cultivars were produced by CIAT in an experimental field at Palmira, Colombia (Zone A). During the growth period (May 1993 - January 1994) the average daily temperature was 23.4°C and there was a total rainfall of 644 cm. In addition, seven cultivars were produced by CIAT in another location at Pivijay on northern coast of Colombia (Zone B). In this zone, during the growth (October 1993 - June 1994), period the average temperature was 31°C and there was a total rainfall of 807 cm.

When the plants were 9 months of age, the roots from a random selection of plants were harvested. The fresh roots were laboratory processed by CIAT within 20 hr of being harvested and the starch granules isolated following the process outlined in Figure 2.2. Approximately 1-2 kg. of roots per cultivar were processed by manual peeling, washing and cutting of parenchyma into small pieces, liquidizing the tissue with distilled water in a Waring blender (10 minutes), filtering the resulting starch suspension through two laboratory sieves (aperture size of 150 μm and 106 μm), settling (6 hours) the starch from the suspension into plastic pans, decanting several times with water to clear the supernatant from the non-starch components, and leaving the wet starch in the pan for sun drying for 8 hours. The resultant granular white product had a moisture content of 10-11 % (wb).

The dry starch samples (200 - 300 g. per cultivar) were packed in plastic containers and shipped to the United Kingdom. Before analysis, the samples were gently crushed in a mortar and sieved so as to pass through a 51 μm laboratory test sieve.

Figure 2.2: Process steps for cassava starch isolation in the laboratory

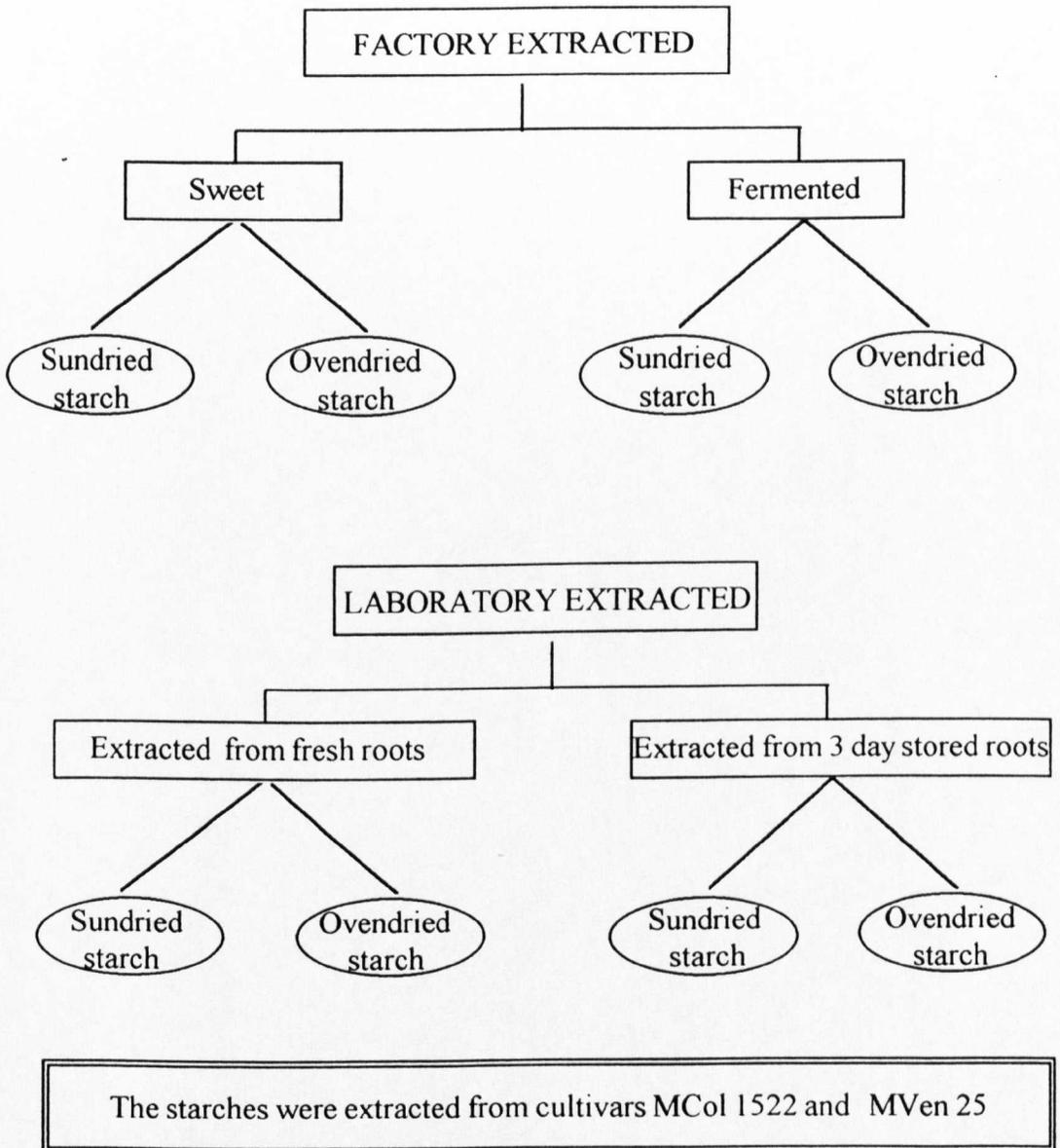


2.2.2 Starches for the study of the influence of processing variables

Two cassava cultivars MCol 1522 and Mven 25 were planted in CIAT, Palmira Colombia in April 1994, and the roots harvested when the plants were 14 months of age.

Starch granules were isolated and processed to produce the samples designated in Figure 2.3. The factory extracted samples were processed in a small rural factory in Colombia, in which sweet and fermented (or sour starch) were produced as shown in Figure 1.5 (Section 1.3). A total of 1000 kg was processed per cultivar. The soil residues on the roots were removed in a rotary drum washer, the washed and unpeeled roots were grated on the sharp surface of a rotary solid cylinder, the disintegrated mass was filtered in excess of water by sieving it through cloths on the internal surface of a hollow cylinder, the fibre free starch slurry was poured into tanks and left to sediment for 6 hr. After decanting the supernatant, two samples of about 4 kg each of sedimented starch (sweet starch) were dried immediately; one was sun dried for 8 hours and the other oven dried at 40°C for 24 hr. The rest of the sedimented starch was left to ferment for 30 days and finally to sun dry for 8 hours. A sample of about 4 kg of the fermented starch was also oven dried with the same conditions as the sweet sample.

Figure 2.3: Cassava starch samples prepared to investigate the effect of processing on their properties.



The laboratory extracted samples were prepared by the procedure described in Section 2.2.1 designed for the isolation of highly refined, small amounts of native starch. The starches were extracted from both fresh roots within 10 hr of being harvested and from roots which had been stored at room temperature (25-30°C) for about 3 days. As with the factory extracted starches the samples were dried using the same conditions (sun and oven)..

A total of 8 experimental samples of starch per cultivar were obtained. The samples (600 g) were packed in plastic containers and shipped to the United Kingdom. The dried granulated starch samples were gently crushed to powder in a mortar so as to pass through a 106 µm laboratory test sieve.

2.3 PREPARATION OF THE FLOUR SAMPLES

2.3.1 Flours for the study of the influence of processing variables

Two cultivars of cassava, CM3306-4, MVEN 25, were produced by CIAT in Palmira Colombia, and the roots harvested when the plants were 10 months of age.

The flour samples were processed in Palmira, Colombia in an experimental unit of the Cassava Utilisation Section of CIAT. The general process of cassava flour production is outlined in Figure 1.6 (Section 1.4). The process conditions of the experiments are specified in Figure 2.4.

For each drying experiment, 500 kg of roots were harvested and processed daily. The roots were thoroughly washed in a mechanical drum washer, and the washed, unpeeled roots were cut on a rotary chipping disk machine. The fresh chips were loaded (*circa* 475 kg) into a bin dryer with a 3 m² floor area producing a layer of about 0.15 m thick. The material was dried using an upward flow of air. Different batches were dried at 40, 60 and 80 °C.

The dried chips from each drying experiment were broken to particules of around 4 to 5 mm in size using an experimental roller mill made in CIAT with rollers set with a gap of 600 µm and rotated at 300 rpm. The material produced from each drying experiment was split into four portions for milling. The specifications and operating conditions of the mills were as follows.

Hammer mill. This was an experimental 24 hammer - mill (made locally) operated at 5800 rpm (a peripheral speed of 91 m/s at the hammers) with a 3.17 mm (1/8 in.) screen,

Roller mill. A mill designed for small processing operations (125-250 kg/hr) (China Machinery and Equipment Import and Export Company, Harbin, China) was operated with rollers at a speed ratio of 1:2.45 (184 rpm:450 rpm) and with a separation of 300 μm for a first pass of material and 30 μm for the second pass. The surface of the rollers had 18 teeth/in,

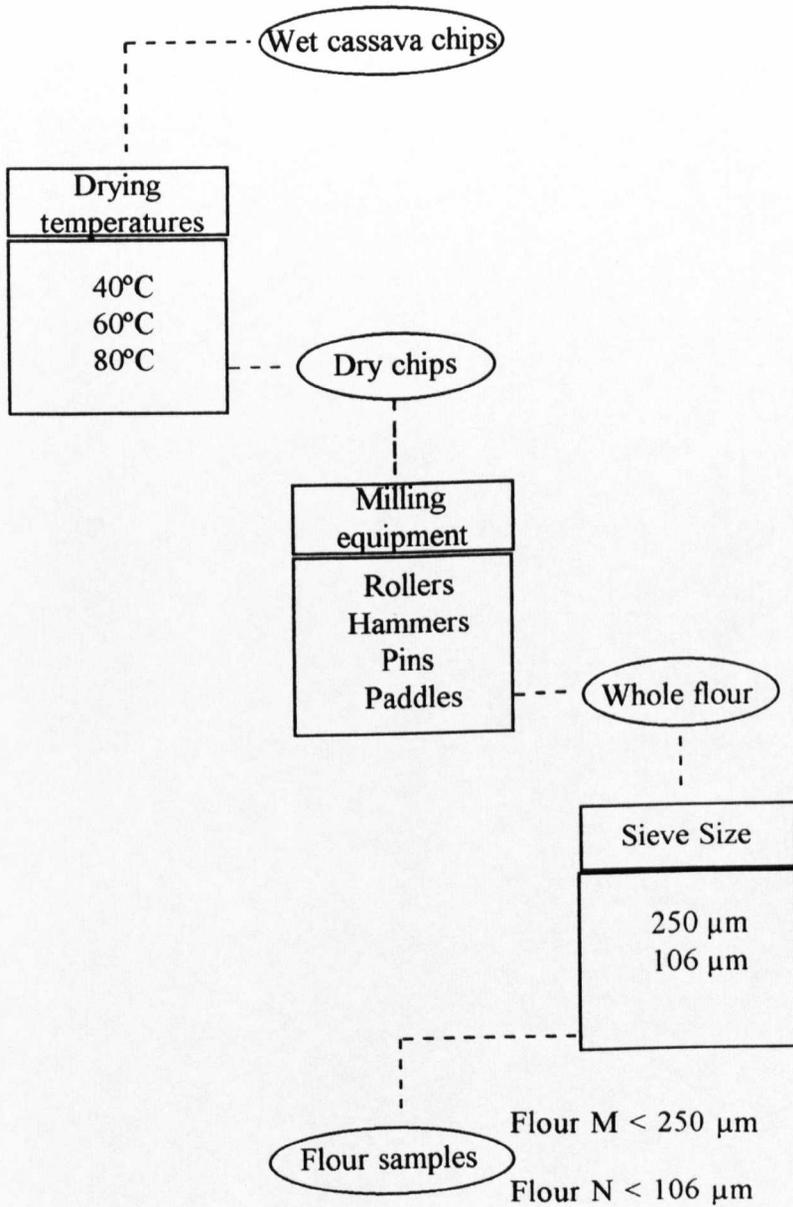
Paddle mill. This was an experimental equipment manufactured at CIAT which consisted of a paddle auger operated at 1800 rpm which fed into two consecutive cylindrical sifters of screen sizes 5 mm and 250 μm respectively,

Pin mill. This was a commercial mill (model BAC200, A.B.N.O., Lindgrens Maskinfabrikset, Malmo, Sweden) operated at 2800 rpm with a 0.5 mm screen.

The whole flour sample from each mill was sieved to two size grades of flour, one to pass through a 250 μm and the other through a 106 μm laboratory test sieve.

A total of 24 experimental samples of flour per cultivar were obtained; samples of 300 g were sent to the United Kingdom for analysis.

Figure 2.4: Experimental protocol to investigate the effects of processing conditions on the properties of cassava flour.



2.4 TESTS METHODS IN STARCHES

2.4.1. Aqueous swelling and solubility of starch pastes

Swelling volume and solubility of 1% (w/v) starch pastes were measured using the procedure described by Mat Hashim *et al.* (1992), which is based in concepts from Schoch (1964). Starch samples (0.15 g dry matter) were suspended in distilled water to a final volume of 15 ml in a hermetically sealed 40 ml universal sample bottle and shaken gently in a water bath at 95 °C until the suspension became translucent i.e. until gelatinization of the starch was complete. The samples were held in the water for another 60 minutes, cooled and then transferred into a 15 ml conical centrifuge tube and centrifuged in a swing out CENTAUR 2 at 2200 rpm (approx. 1000xg) for 20 minutes.

The volume of the sediment was read directly from the tube and the result determined as swelling volume in ml per 100 ml of suspension.

The soluble solids in the test solutions were determined gravimetrically by drying 3 ml aliquot of the supernatant at 105°C for 24 h. The solubility was given as the total weight of solids in the supernatant expressed as a percentage of the total dry weight of the original starch in the suspension. The tests were carried out in triplicate.

2.4.2 Pasting characteristics of starch suspensions

Starch samples (25 g dry matter) were suspended in distilled water to a final volume of 500 ml and analysed in a Brabender Viscoamylograph type PT-100. The starch suspensions (5%) were heated to 95 °C, held for 20 minutes at 95 °C, cooled to 50 °C and held for 20 minutes at 50 °C. The heating and cooling rates were both set at 1.5 °C/min.

From the pasting profiles the following values were obtained: initial pasting temperature, peak viscosity, viscosity at the end of 20 minutes at 95°C, viscosity at 50 °C (at the end of the cooling period) and viscosity after 20 minutes at 50 °C.

2.4.3 Mechanical properties of starch pastes.

The viscoelastic behaviour of cassava starch pastes were studied using an oscillatory small strain rheometry. The measurements were performed on a Bohlin CS Rheometer run in the oscillatory mode, with a 40 mm plate-and-plate measurement system set to a separation of 1 mm.

Starch samples (0.6 g) were suspended in distilled water to a final volume of 10 ml in screw top 40 ml Universal sample bottles. The starch suspensions were heated in a 90 °C water bath, with gentle shaking to ensure that granules remained in suspension until gelatinization had occurred i.e. when the suspensions became translucent. The bottles were left in the water bath for an additional period of 15 minutes before being immediately transferred to a water bath at 65 °C. Samples were held for a further period of 2 hr for stabilization of the paste.

Approximately 1 ml of the starch pastes at 65 °C were loaded and pressed without delay between the plates previously heated to 65 °C; paraffin oil was added at the sample boundary to prevent evaporation of water during the tests. The samples were then sheared at an oscillation frequency of 1 Hz and at a constant strain of 0.01 mm (at this strain, all samples were in the linear strain region).

Two consecutive tests were performed while the samples were sheared. Firstly, the pastes were cooled from 65 to 25 °C at a rate of 1.5 °C/min and held at 25 °C for a further period of 60 minutes and, secondly, a frequency sweep was performed from 0.01 to 5 Hz. at 25 °C. The basic rheological parameters from a dynamic oscillatory test were measured: storage modulus G' , loss modulus G'' , phase angle δ ($\tan \delta = G''/G'$) and dynamic viscosity, η . Test conditions and the data collection process were under computer control by the Bohlin Rheology AP computer software, version 4.70.

2.4.4 Size distribution of starch granules

The Coulter Counter Model TAPII (Coulter Electronic Ltd., Luton, UK) was used to determine the granule size distribution. The equipment was fitted with a 100 μm orifice tube and calibrated with a latex suspension of 20 μm particles. Starch samples (40 mg) were suspended in 1 ml of electrolyte (Coulter Isoton II) and about 60 μl of this suspension was added to 150 μl of the electrolyte in which the starch granules were tested. The size distribution was monitored and collected in a range from 3.57 to 45.24 μm . Each sample was tested in duplicate. From the size

distribution data, values of the geometric mean particle size d_{gw} and geometric standard deviation S_{gw} were calculated:

$$\ln \overline{d_{gw}} = \frac{\sum (W_i \ln d_i)}{\sum W_i}$$
$$\ln \overline{S_{gw}} = \frac{\sum (W_i (\ln d_i - \ln \overline{d_{gw}})^2)}{\sum W_i}$$

W_i = Coulter Counter counts in channel i

d_i = Expected mean size of particles counted per channel i

The calculations of d_{gw} and S_{gw} were based on the assumption that the granule sizes were distributed as a log normal distribution; low fibre biological materials, either ground or whole are for all the practical purposes log normally distributed (Headley and Pfof, 1968). Rasper (1971) has already applied this approach to the measurement and analysis of starch granule sizes.

2.4.5 X-ray diffraction patterns and crystallinity of starch powders

A wide angle X-ray diffractometer system was used (Philips, PW1730, ADP 15, Eindhoven, the Netherlands) equipped with a PW1050/25 goniometer with a Cu target (monochromatic $K\alpha$ radiation $\lambda=0.154$ nm). Powdered, dried starch samples (9-11%,wb) were packed tightly into the sample holder of the diffractometer and diffraction data collected over an angular range from 4 to 32° 2θ at step intervals of

0.05 2θ . This angular range has been shown to encompass all the significant diffraction peaks for starch crystallites. The data were collected under computer control by a BBC/Torch computer. The X-ray patterns were identified visually by comparison with the diffractogram types as defined by Zobel (1964). The crystallinity (%) was calculated by comparing the area under the crystalline peaks with the total area of the diffractogram using a computer program written by S. Wynne-Jones, based on the method of Nara and Komiya (1983).

2.4.6 Iodo-colorimetric measurement of amylose content.

The procedure was based on the International Standard ISO 6647 (ISO,1987) method for amylose determination of rice flour (approx. 90 % starch). The method of preparation of the standard curve was modified to take account of the higher starch content of the cassava starch samples.

Starch samples (100 mg dry matter) were weighed, wetted with 1 ml ethanol and suspended in 9 ml of 1N NaOH solution and left overnight at room temperature. After 18-20 hr, the gelatinized-solubilized starch was transferred into a 100 ml volumetric flask, diluted to 100 ml with deionised water and shaken vigorously to disperse the gel. Aliquots (5 ml) of this solution were transferred into a 100 ml volumetric flask containing 50 ml of deionised water, neutralised with 1 ml 1M acetic acid, mixed with 2 ml of iodine solution (0.2 % I_2 in 2% KI) and made up to volume with deionised water. The colour was left to develop for about 20-25 minutes and the absorbance read at 620 nm in a spectrophotometer (LKB Biochrom, Ultraspec, 4050,

Croydon, Surrey, UK). A sample blank was prepared simultaneously with the samples.

Standards were prepared in a similar manner by mixing alkaline solubilized solutions of potato amylose (ICN Biomedicals, Inc. 1263 South Chilicothe Road, Aurora, Ohio 44202, USA) and waxy rice amylopectin (prepared in CIRAD-CA, Montpellier, France). The solutions of potato amylose and waxy rice amylopectin were mixed in proportions of amylose (by volume) ranging from 0 to 30 %. Aliquots (5 ml) of the standard mixtures were neutralised, reacted with the iodine solution, diluted to 100 ml and the colour absorbance read along with the samples.

2.4.7 Identification of starch components and amylopectin chain lengths by conventional gel permeation chromatography (GPC).

The enzymic debranching of whole starch accompanied by size exclusion chromatography has been demonstrated in this and many other laboratories to be as a valuable method for investigating the structure of starch (Asaoka *et al.*, 1991, 1993; Ong , 1994).

Native cassava starch was debranched with isoamylase and the resulting constituents separated by size exclusion chromatography GPC. The technique was based on the procedures developed originally by Ikawa *et al.* (1981) and Inouchi *et al.* (1983), which were modified and tested with cassava starch by Asaoka *et al.*, 1991. The system used in this research had been set up and used extensively by Ong *et al.*(1994).

Debranching of starch samples

Starch samples (40 mg) were dispersed and dissolved in NaOH (0.25 ml, 2M) and deionised water (0.25 ml) for 3 hr at 40 °C. The starch pastes were diluted and neutralised with water (3.5 ml) and HCl (approx. 0.9 ml to obtain a pH 6.2-6.4). The starch solutions were buffered with acetate buffer (5 ml, 60 mM at pH3.5) and incubated with isoamylase (30 µl) (*ex Pseudomonas amyloclavata*, EC 3.2.1.68, 59000 units/mg protein, 1mg protein/ml, purchased from Hayashibara Biochemical Laboratories, inc., Okayama, Japan) for 24 hr at 40 °C. If samples were not used immediately, the debranched starch solution was diluted with 2X10 ml ethanol and concentrated in a rotary evaporator at 40 °C to increase their storage life at 5°C . The dried samples were redissolved with NaOH (0.53 ml) and deionised water (1.57 ml) followed by centrifugation at 2300g and 1 ml of the supernatant per sample was injected into the GPC system.

GPC system and calibration

The GPC system consisted of two packed 2.2 cm x 100cm glass columns (Amicon Ltd., Glos., UK.) serially connected and arranged for descending flow. The columns were packed with Fractogel TSK HW55(S) and with HW50(S)(from Merck-BDH Co., UK). The column eluent consisted of an aqueous solution (pH=11) composed of NaOH (0.2%),NaCl(0.2%) and sodium azide (0.02%). The eluent was pumped at a rate of about 30 ml/hr and a programmable Gilson fraction collector, model 203 (Anachem Co., Luton, UK) was set to collect 80 tubes at 100 drops per tube.

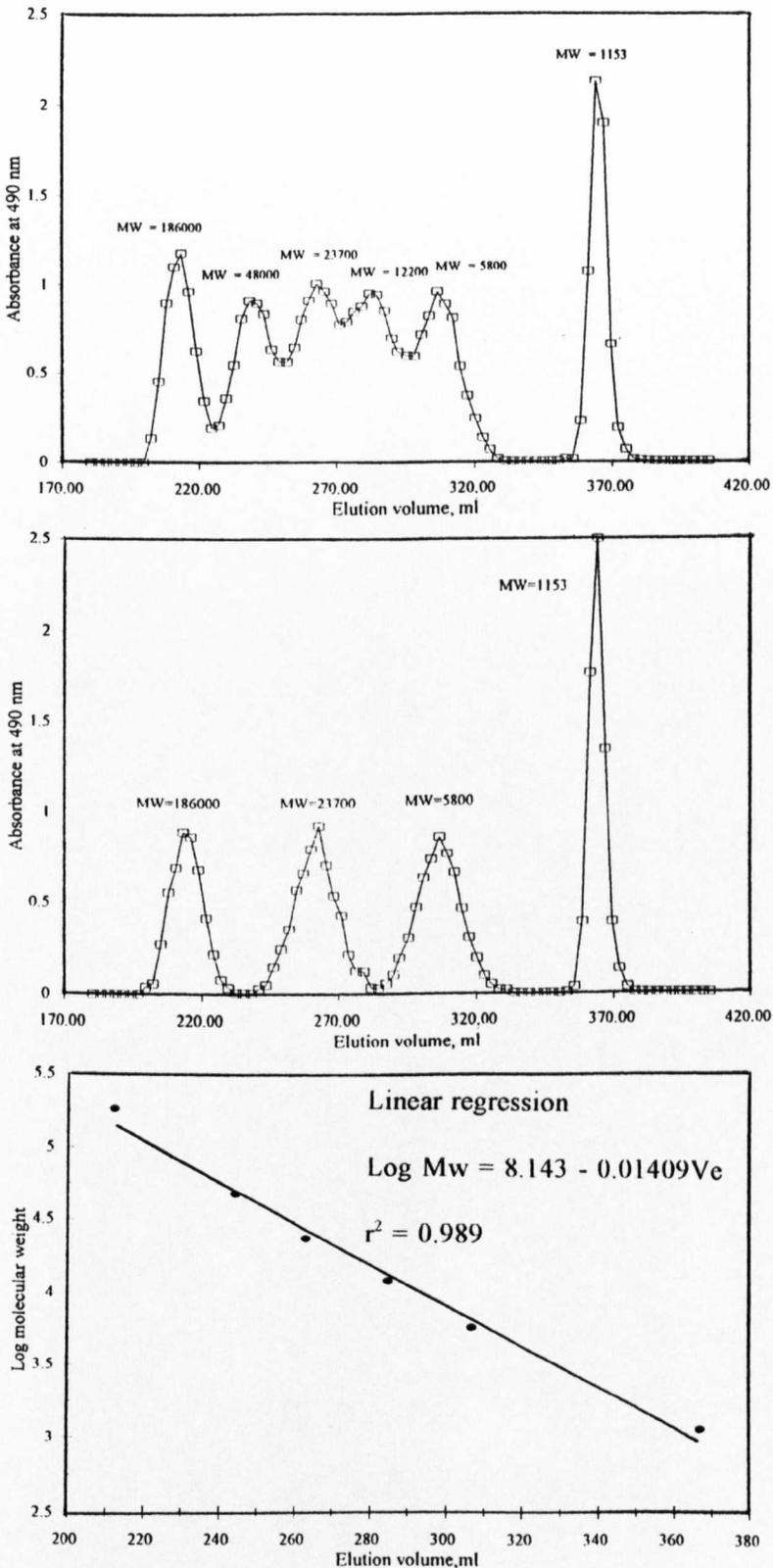
The size exclusion columns were calibrated by operating the system under the same conditions as those ultimately required for the starch samples. The mono-dispersed pullulan or polymaltotriose standards (4mg/ml) (Polymer Laboratories, Church Stretton, UK) were injected into the columns. The standard molecular weights (M_w) were as follows: P-200 (186000), P-100 (100000), P-50 (48000), P-20 (23700), P-10 (12200), and P-5 (5800). Maltoheptaose (1153), a sugar standard from Sigma Chemical Co., was also used. Examples of chromatograms and the derived relationship of molecular weight and elution volume are displayed in Figure 2.5.

Analysis of the fractionated starch components

Aliquots of the fractions collected in the tubes were chemically analysed for total sugar content determination by the phenol sulphuric acid method (Dubois *et al.*, 1956). A volume of 200 μ l per fraction was mixed vigorously with 200 μ l of phenol (5% w/v) and 1000 μ l of concentrated sulphuric acid. After 30-40 minutes the absorbances of all 80 fractions collected were read at 480 nm and analysed in a computer controlled microplate reader spectrophotometer (Dynatech MR 5000, Dynatech Laboratories Ltd, Billingshurst, West Sussex, UK). D-glucose standard solutions (Diagnostic Glucose Standard Solution, Sigma) were tested over a range of 0-300 μ g/ml. The column eluent was used as a blank for the samples and standards.

The results from the sugar tests were used to calibrate the chromatograms. The chromatograms were divided into three major fractions, namely, amylose, and long and short chains of amylopectin. The boundaries between fractions were derived

Figure 2.5 : (a) GPC chromatograms of molecular weight markers, (b) The relationship between molecular weights and elution volumes derived from the chromatograms.



from the wavelenghts of maximum absorbance of the starch-I₂ complex tests identified in research on cassava starch by Asaoka *et al.* (1991, 1993). The relative weight of each chromatogram fraction was calculated from its total carbohydrate content derived from the sugar test results. The degrees of polymerization (DP) of the amylopectin fractions were measured at peak positions using the derived linear relationship between molecular weight standards and elution volume. The degree of polymerization was calculated by dividing the molecular weight by 162, which is the molecular weight of a glucose unit minus the water molecule.

2.4.8 Structural analysis of starches by a size exclusion high performance liquid chromatograph system (SE-HPLC) assisted by a multi-angle laser-light-scattering system (MALLS) and a differential refractometer (RI) .

In the last few years, the SE-HPLC technique has been used widely for the structural study of starch from samples of either pre-isolated amylose and amylopectin, debranched starch or whole starch (Hizukuri and Takagi, 1984; Hizukuri, 1985, 1986; Hizukuri and Maehara, 1990; Yuan *et al.*, 1993; Bradbury and Bello, 1993; Ong *et al.*, 1994; Wang and White, 1994).

SE-HPLC-MALLS-RI system

The same SE-HPLC system was used as that developed by Ong *et al.* (1994) for the structural analysis of starch in the Food Sciences laboratory, University of Nottingham. The SE-HPLC system and detectors consisted of an on-line degasser

(Degassers, DG-1200, Uniflow, HPLC Technology Co. Macclesfield, UK); an HPLC pump (Waters 590, Waters Associates Ltd., Northwich, Cheshire, UK); a rheodyne injector (Rheodyne 7125, HPLC Technology, Macclesfield, UK); a multi-angle laser light photometer (Dawn F, Wyatt Technology Inc., Santa Barbara, USA) with a He-Ne laser operating at 632 nm consisting of 15 detectors ranging from 22° to 160°; a differential refractometer (Wyatt Optilab 903, Wyatt Technology Inc., Santa Barbara, USA); a guard column (TSK GEL PWXL, 6mm x 40mm, Anachem, Luton, UK) and 5 size exclusion columns connected as follows: TSK GEL G3000 PWXL, 2 Asahipack GS-320H, TSK G2500 PWXL and TSK GEL G-OLIGO PWXL. The TSK columns (7.8 mm x 300 mm) were from Anachem Co., UK, and the Asahipak columns (7.8 mm x 250mm) were from Rhone-Poulenc, Manchester, UK. The columns were arranged in an HPLC oven (Anachem Co., UK). The mobile phase or eluent was phosphate buffer (0.1 M Na₂HPO₄ and 0.02 % sodium azide adjusted to pH 8.6 with 0.05 M NaH₂PO₄) previously filtered under vacuum through a 0.2 µm nylon membrane filter in a glass Millipore solvent filtration system.

The eluates were first examined by the Dawn F light scattering photometer and subsequently by the Optilab refractive index concentration detector. The response signal of the detectors was automatically recorded by an IBM PC computer. The digitized signals were processed with the Wyatt Technology ASTRA 2.04 and EASI 6 software. The refractive index increment (dn/dc) which is the change of the refractive index of the solution as a function of the solute concentration was set to 0.15 ml/mg for the analysis (Banks *et al.*, 1969). From the ASTRA software the

following set of data were recorded for each chromatogram: elution time (or elution volume), RI detector response, and the signals of 15 light-scattering detectors. The RI response is proportional to the concentration or the mass of the eluted materials and the light-scattering intensities to their molecular weights. The chromatogram ASTRA files were converted to ASCII text files for downstream processing.

The size exclusion column set was calibrated using solutions of mono-disperse pullulan and sugar standards (1mg/ml) (purchased from Polymer Laboratories, UK). The pullulan standard set included those specified in section 2.3.7 plus P-800 (M_w : 853000) and P-400 (M_w : 380000). The set of sugar standards included: stachyose tetrahydrate (M_w : 738), D-glucose (M_w : 180); and ethanediol (M_w : 62) (ethanediol was obtained from Fisons Scientific Equipment, Loughborough, UK). The elution volume, V_e , for each standard was determined from the position of peak maximum elution position. Figure 2.6 shows the elution profiles of the standards which eluted in a permeation volume between 23.5 (near the void volume) and 48 ml. The relationship between $\log(M_w)$ and elution volume is also displayed.

Analysis of debranched starch

Amylose and amylopectin were separated in the size exclusion columns following enzymatic hydrolysis. The procedure of enzymic debranching of starch was based on that of Hizukuri (1985) but without the pre-isolation treatment with butanol as described by Ong *et al.* (1994).

Figure 2.6: (a) SE-HPLC-RI chromatograms of standards of known molecular weights.

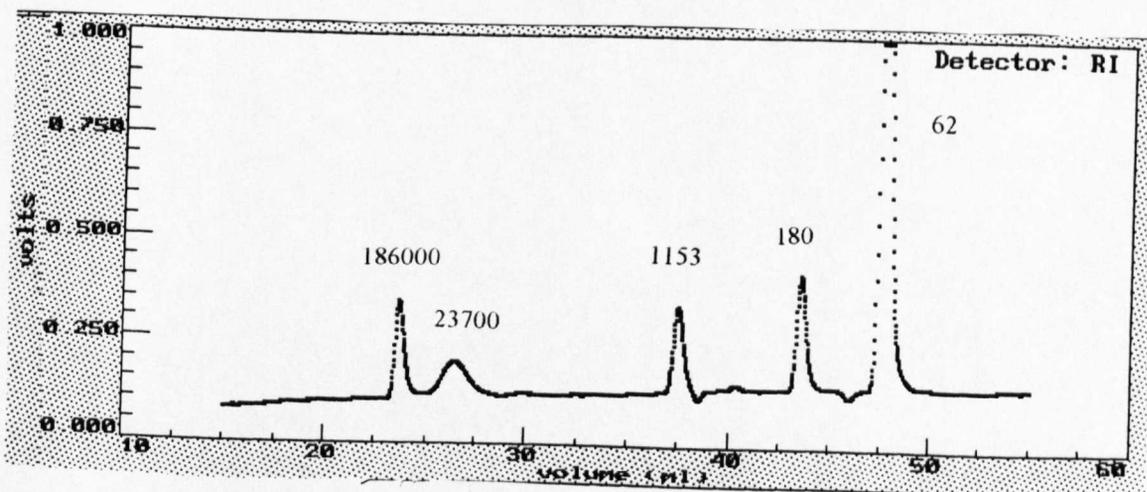
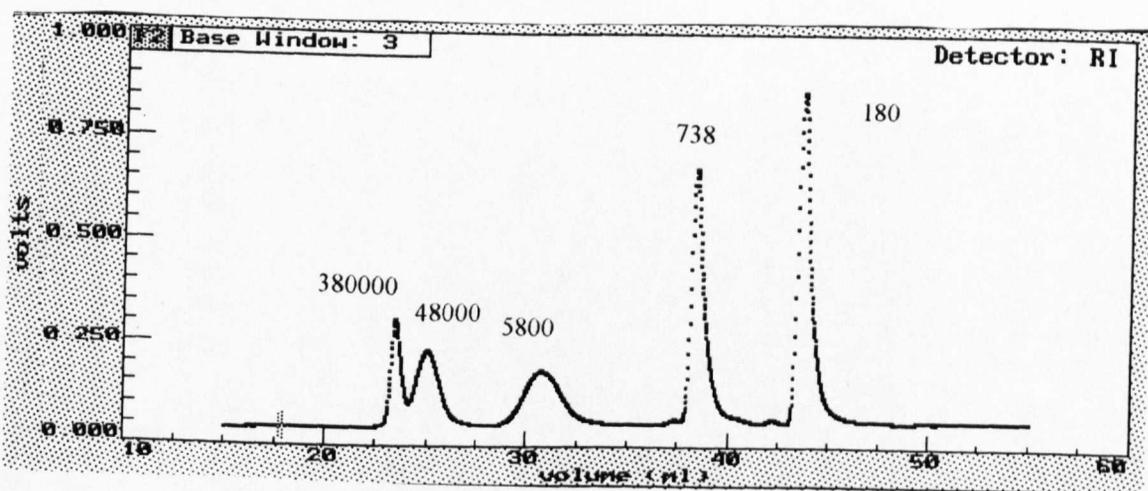
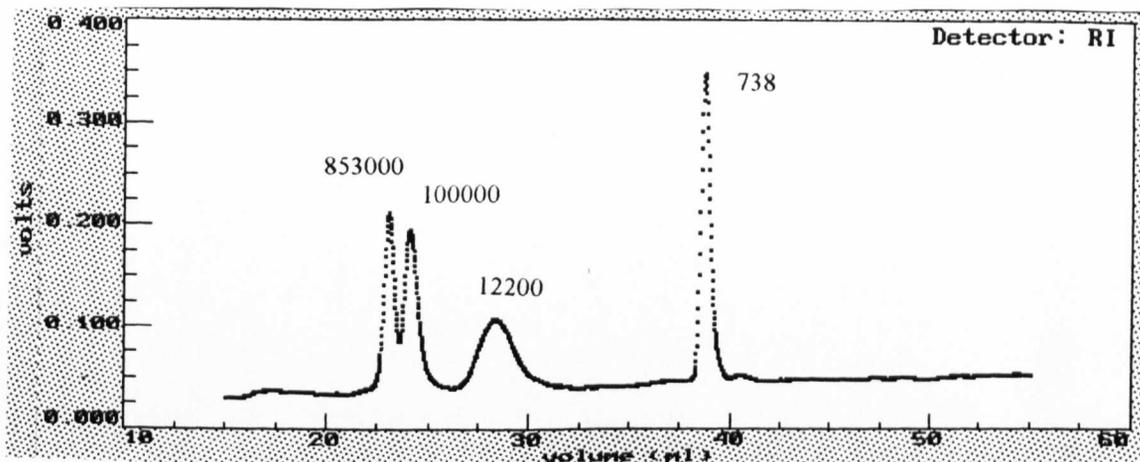
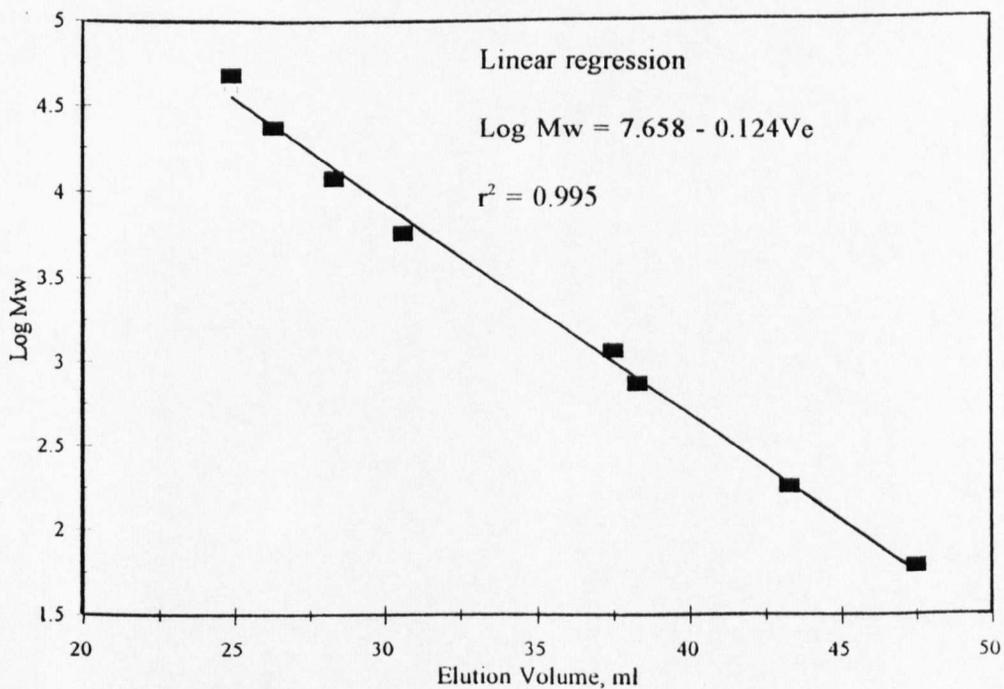
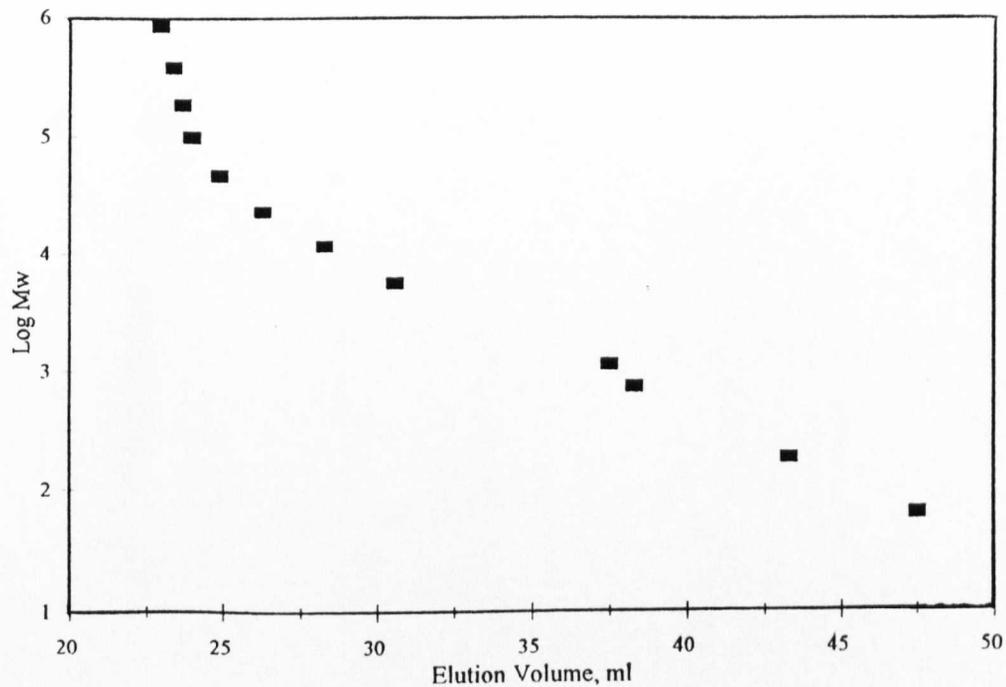


Figure 2.6: (b) Relationship between molecular weights and elution volumes derived from the chromatograms (a), showing the linear range used in the analysis.



Starch samples (75 mg) were dispersed in 3.71 ml deionised water by heating in a boiling water bath at 96°C for 6 min. After a fast cooling to 45° C, the dispersions were buffered with 0.25 ml 1M acetate solution pH 3.5, tempered at 45°C and incubated with 55 µl of isoamylase *ex Pseudomonas amyloclavata* for 2.5 hr at 45°C. To inactivate the enzyme, the digests were then mixed with 1 ml 0.5 M sodium phosphate and heated in a boiling water bath for 3 min. The completely clear, hydrolysed cassava starch solutions were centrifuged at 1400xg for 10 min. Aliquots (500 µl) of the supernatants were filtered through a 0.45 µm syringe filter (Whatman, 13 mm, PVDF) and 100µl injected into column system via a sample loop rheodyne injection valve attached directly onto the oven containing the SE-HPLC system. Possible aggregation and retrogradation of amylose were minimized by maintaining the debranched solutions at 45-50°C before centrifugation, filtration and injection into the SE-HPLC system. Prior to the tests the column system and eluent were equilibrated at 40°C overnight. The eluent was degassed with the on-line degasser in the SE-HPLC system. The flow rate was set at 0.5 ml/min and the total elution volume was 55 ml.

The *absolute* molecular weight distribution of the amylose was calculated from the light-scattering computer data files with the ASTRA 2.04 and EASI 6 computer software. As the light-scattering signals were too weak from the debranched, lower molecular weight amylopectin elutates, it was assumed that the amylopectin fractions would coelute on a size exclusion column with the pullulan and sugars of identical molecular weight. The molecular weight of the amylopectin elutates were calculated

as *relative* molecular weight distributions derived from the relationship of log molecular weight and elution volume of the standards. A linear relationship was found to exist between the elution volumes of the standards and their log molecular weights between 48000 and 63 (Figure 2.6) *i.e.* $\log m = \beta - \alpha V_e$. The fitting parameters of this straight line were the slope $\alpha = 0.124$ and the intercept $\beta = 7.658$.

The unresolved amylopectin peaks in the chromatograms were preliminarily analysed by dividing the chromatograms into three fractions, drawing vertical lines at selected minima and inflection points on the elution profile. The resulting three sections were considered to match fractions of high molecular weight (HMW), intermediate molecular weight (IMW), and low molecular weight (LMW) (Yuan *et al.*, 1993). A Pascal computer program, written by Dr. P. Tokarczuk of the University of Nottingham, was used to read the chromatogram file and to divide the chromatogram into sections between user-chosen specific limits, to numerically integrate the sectional areas, to calculate weight and number average molecular weights (M_n and M_w) and degree of polymerization (DP). The values of M_n and M_w were averaged over each amylopectin section according to the following equations (Yau *et al.*, 1979):

$$M_n = \frac{\sum C_i}{\sum (C_i/M_{wi})}$$

$$M_w = \frac{\sum C_i M_{wi}}{\sum C_i}$$

where C_i is the eluate mass concentration (i.e. the RI detector response) at elution volume i (V_{ei}) and M_{wi} is its corresponding molecular weight derived from the calibration curve.

Further analyses were carried out using a more sophisticated mathematical approach aimed at deconvoluting the chromatogram to characterize the contributing amylopectin components. The components were distinguished by fitting a series of Gaussian peaks to the concentration response curve (i.e., the RI detector response). The fitting procedure was accomplished with the aid of the computer program PeakFit™ non-linear curve-fitting software v3.0 (Jandel Scientific, Germany). PeakFit is a user-interactive, command-driven program which uses the Marquardt-Levenberg algorithm for non-linear curve fitting. The area Gaussian function included in the software was selected for the curve fitting. After reading the data file of the chromatogram, a number of Gaussian-shaped curves were placed under the experimental RI response displayed on the screen, and fitting estimates were set graphically by adjusting Gaussian component forms so that the overall sum curve closely matched the data. The numerical iterative fitting process was then started which proceeded until the fit converged. PeakFit does not consider a fit to have converged until the χ^2 , the sum of the squares for the fit, is unchanged within the eighth significant figure for five full interactions. Each fitted Gaussian component is characterized by its peak elution position (P), peak height (H) and width (W). These were the input variables along with the variables of the molecular weight calibration equation for the calculation of the M_n and M_w values of the amylopectin components.

The values of M_n and M_w were averaged over each fitted Gaussian component using the functional form of a Gaussian curve, denoted here as $I(V_e)$ (i.e. RI fitted response) and the molecular weight line function $m(V_e)$ (i.e. the column calibration equation). The equations of M_n and M_w were derived from an integration procedure, which resulted in expressions involving the Gaussian fitted parameters P and H , and the calibration line parameters α and β . These equations were also used in an analogous program but based on a somewhat different computational strategy (Ong *et al.*, 1994). The technical concepts and mathematical foundations of the above approaches are well described in the following references (Rektorys, 1969; Fishman *et al.*, 1991; Hoagland *et al.*, 1993; Rundel, 1991):

$$I(V_e) = H \exp - \left(\frac{V_e - P}{W} \right)^2$$

$$m(V_e) = 10^{\beta - \alpha V_e} = \exp(\delta - \gamma V_e); \text{ where: } \delta/\beta = \gamma/\alpha = \ln 10$$

$$\overline{Mn} = \frac{\int I(V_e) dV_e}{\int \frac{I(V_e)}{m(V_e)} dV_e} = \exp(\delta - \gamma P) \exp - ((1/2) \gamma W)^2$$

$$\overline{M} = \frac{\int I(V_e) m(V_e) dV_e}{\int I(V_e) dV_e} = \exp(\delta - \gamma P) \exp((1/2) \gamma W)^2$$

The degree of polymerization values (DP_n and DP_w) were calculated by dividing the molecular weights by 162, which is the molecular weight of a glucose unit minus the water molecule. The following parameters were reported per amylopectin component: number average degree of polymerization (DP_n), average weight degree of polymerization (DP_w), relative weight, and relative mole content (i.e., the relative weight divided by the corresponding DP_n). This calculation process was accomplished on Quattro Pro™ Notebook software.

Analysis of aqueous exudates from the starch granules

Soluble starch leachates were analysed by the SE-HPLC-MALLS-RI system. Starch pastes (1%) were prepared (95°C for 1 hr and centrifuged) as described in Section 2.3.1. Aliquots (3.71 ml) of the supernatant were subjected to isoamylase debranching and chromatographed using the conditions previously described.

Suspensions of starch cultivar MCol 1522 were also pasted at 95 °C but held for different time periods : the time until the suspension became translucent *i.e.* when the starch gelatinized *i.e.* about 30-40 s, and additional periods of 0.5 hr, 1 hr, and 2 hr. Following centrifugation, the supernatants (3.71 ml) were also assayed as above..

Using the chromatograms, the relative weights of the amylose eluates were measured from the peak areas. The amylose molecular weight distribution was calculated from the light-scattering data using the ASTRA software.

Analysis of whole starch

Further studies on the molecular structure of cassava starch were carried out in the SE-HPLC-MALLS-RI system but with a different column arrangement with a larger exclusion volume. The gel permeation system was designed to try to isolate amylopectin and amylose without the previous debranching treatment. The five columns were connected in the following order: TSK G6000 PWXL, TSK G5000 PWXL; TSK G4000 PWXL, TSK 3000 PWXL and and TSK G2500 PWXL. A calibration of this column system with the pullulan and sugar standards (Section 2.3.8) resulted in a permeation volume between 26 and 50 ml (Figure 2.7). Starch samples (10-20 mg) were dispersed and solubilised by adding 0.25 ml deionised water and 0.25 ml 1M NaOH solution, flushing with N₂ and leaving overnight at 40 °C. The starch solutions were neutralised with 0.25 ml 1M acetic acid, diluted with 3.25 ml of deionised water and 1ml 0.5 M sodium phosphate and heated for 2 minutes in boiling water followed by centrifugation at 1400xg for 10 min. Aliquots (100 µl) from the centrifuged supernatants were filtered through a 0.45 µm syringe filter and injected into the SEC-HPLC system which was run with the same conditions as previously described.

The elution profiles of the starch eluates were generated by the ASTRA software. The molecular weights of the eluates were averaged over the full elution profile using the light-scattering data and the ASTRA software.

2.5 TEST METHODS FOR CASSAVA FLOUR

2.5.1 Particle size distribution

Whole flour samples (100 g) were shaken through 838, 300, 250, 212, 150, 106 μm sieves on a Ro-Tap Sieve Shaker for 45 min.

2.5.2 Pasting characteristics of flour suspensions

Flour (27 g dry matter) was suspended in distilled water to a volume of 450 ml and poured into the bowl of a Brabender Viscoamylograph type PT-100. The slurry (6%) was heated from 25 to 95 °C, held for 20 minutes at 95 °C and cooled to 50 °C. The heating and cooling rates were set at 1.5 °C/min. From the viscoamylograph the following empirical parameters were obtained: the initial temperature of gelatinization, peak viscosity, ease of cooking, gel instability, and gelation index.

2.5.3 Fibre content

Total Dietary Fibre

Total dietary fibre (TDF) includes hemicelluloses, celluloses, lignins, nondigestible oligosaccharides, pectins, gums and waxes (Sigma, 1993). By using a combined enzymatic and gravimetric method, total dietary fibre was determined in the flour samples. The determination is based on the method published by the AACC (1995a), and has been applied to tuber and root-based products at the Natural

Resources Institute (UK). The samples were not defatted since the fat content of the roots ranged from 1.2 to 1.7% (reported by CIAT) and the procedure was considered to be unnecessary with samples containing less than 5% fat. As the protein in the samples (reported by CIAT as 2.2-2.4 %) was considered to be completely digested by the enzymatic treatment, the step of determining the remnants of protein in the digested residues was not deemed necessary for these samples. Dried samples were gelatinized and partially hydrolysed with heat-stable α -amylase and then enzymatically digested with protease and amyloglucosidase to remove the protein and starch present in the sample. Ethanol was added to precipitate the soluble dietary fibre. The residues were filtered and washed with ethanol and acetone. After drying, the residues were weighed and finally ashed. Total dietary fibre is the weight of the residues less the weight of the ash. Duplicated 1g samples were weighed into 400 ml incubation flasks, buffered with 50 ml 0.05M, pH 6.0, phosphate solution, and incubated with 0.2 ml of heat-stable α -amylase solution (Sigma Chemical Co.) at 95°C for 30 minutes with gently shaking every 5 minutes. After cooling to room temperature, the solutions were adjusted to pH 7.5 with 0.17N NaOH solution and incubated with 0.1 ml of protease solution [50 mg/ml solution of protease (Sigma Chemical Co.) in phosphate buffer] at 60°C for 30 minutes in a shaking water bath. After cooling to room temperature, the solutions were adjusted to pH 4.5 with 0.21M phosphoric acid solution and incubated with 0.3 ml of amyloglucosidase (Sigma Chemical Co.) at 60°C for 30 minutes with continuous agitation. Ethanol 96% (280 ml), preheated to 60°C, was added to the digested solutions and left to precipitate for 1 hr. The precipitates were filtered into preweighed crucibles installed in a vacuum line, and the residues washed successively

with three 20-ml portions of 78% ethanol, two 10-ml portions of 96% ethanol, and two 10-ml portions of acetone. The crucibles containing the residues were dried overnight at 105 °C. After cooling in a desiccator and weighing to the nearest 0.1 mg, the residues were incinerated at 525 °C for 5 hr. The TDF is the weight of the residue less the weight of the ash, expressed as a percentage of the original sample weight.

Crude Fibre

Crude fibre was also determined; the standard method of the AOAC (1995) was used without any modification.

2.5.4 Physical testing of dough

Since a dough cannot be developed with cassava flour alone, tests were conducted by preparing wheat/cassava flour blends with a 20 % level of cassava flour. The quality of the wheat flour used was graded as a *strong flour*. A Brabender Farinograph (Brabender OHG, Duisburg, Germany) with a large mixing bowl (300 g flour) was used for testing the rheological properties of the dough. The farinograph method used was derived from the method published by the AACC (1995b). Flour mixtures of 300 ± 0.1 g (13 % moisture basis) were placed in the farinograph bowl and blended for one minute until the zero-minute line on the recording paper was reached. At that instant, a volume of water was added from a large burette approaching that of the expected absorption of the flour blend (from prior experiments this volume was around 62-63 ml). If the mixing curve appeared to level off at a value larger than 500 BU, more water was carefully added until the curve

peak was approximately centered on the 500-BU line. The machine was permitted to run until the curve reached the 400-BU line. The following data were obtained from the curves: water absorption (ml/ 100 g of flour blend), development time (min), dough stability (min) and degree of softening (BU). Control samples of 100 % wheat flour were also tested along with the blends.

2.6 STATISTICAL ANALYSIS

Where appropriate, analyses of correlation were made in this study by calculating the Pearson r product moment correlation from the UNISTAT statistical package (Version 4.7) (UNISTAT Ltd., London). The levels of significance were established at 95% ($P < 0.05^*$), 99% ($P < 0.01^{**}$) or 99.9 ($P < 0.001^{***}$) confidences.

CHAPTER 3
PHYSICOCHEMICAL PROPERTIES OF NATIVE
CASSAVA STARCH:
CULTIVAR AND ENVIROMENTAL VARIABILITY

3.1 RESULTS AND DISCUSSION

3.1.1. Pasting characteristics of starch suspensions

Starch granules, when heated as an aqueous suspension, imbibe water and swell after they reach a certain critical temperature as part of a phenomenon termed gelatinization (Atwell *et al.*,1988). Upon continuation of the heating, swelling proceeds and simultaneously starch molecules diffuse out of the granules and dissolve. In due course, some swollen granules rupture as well as more starch diffuses from those granules retaining their integrity. As a consequence, the viscosity of the system changes in the course of these physical events, which are frequently referred to as starch pasting (Pomeranz, 1991). In the current investigations cassava starch suspensions (5%,w/v) were pasted, with continuous stirring and their viscosities recorded using the Brabender Viscoamylograph (Section 2.4.2)

The pasting profiles (*i.e.* the viscoamylograms) varied widely between samples. Figure 3.1 shows an example of the viscoamylograms in which is illustrated the range of the pasting profiles obtained from all the samples. Outstanding features of two types of viscosity profiles were observed: (a) single stage gelatinization with

high to medium peak viscosity and high to medium viscosity breakdown on continuous heating; and (b) single stage gelatinization with low peak viscosity and low breakdown. All starch samples from Zone B plants were in the latter group. The important viscoamylogram parameters of the samples are summarised in Table 3.1. The results show, from the Zone A starches, that there was an effect of cultivar on the pasting trends but, on comparing the pasting data from Zones A and B starches, it is clear that the two different environments where the plants were cultivated exerted pronounced effects upon the properties of the starches. A similar, significant variation in the pasting viscosity response of starches of other cassava cultivars grown in different experimental situations has been reported in previous studies (Rosenthal *et al.*, 1974; Olorunda *et al.*, 1981; Asaoka *et al.*, 1991; Moorthy; 1994; Wheatley *et al.*, 1992; Chuzel, 1992). We conclude that both endogenous and environmental factors may modify the pasting behaviour of cassava starches under study. This behaviour and allied properties will be a matter of investigation in the following sections.

The starches from the plants grown in Zone B were different from those of Zone A plants in that they had higher initial pasting temperatures (71.5 - 74.8° C) and exhibited lower viscosities on pasting. The low pasting viscosities exhibited by the Zone B starches have not hitherto been reported for native cassava starch. Rather, it has been suggested that the pasting characteristics of cassava starch all exhibit a relatively high degree of swelling resulting in a high peak viscosity, followed by a rapid paste breakdown (Rickard *et al.*, 1991). As indicated in Section 2.2.1, since Zone B had an average environmental temperature higher than Zone A, the

different pasting responses of the starches might be related to the two different ambient temperatures during the growth period. The effect of the environmental temperature on plants and specifically on the pasting properties of their starches has been reported by Hizukuri (1969), who suggested that the higher environmental temperatures generated more intermolecular forces in the starch granules which resisted swelling and resulted in higher pasting temperatures and lower maximum viscosity. Further results on cassava starch properties in the next sections will be related to this environmental effect.

Figure 3.1 Brabender viscoamylograph curves of 5% native cassava starch aqueous pastes.

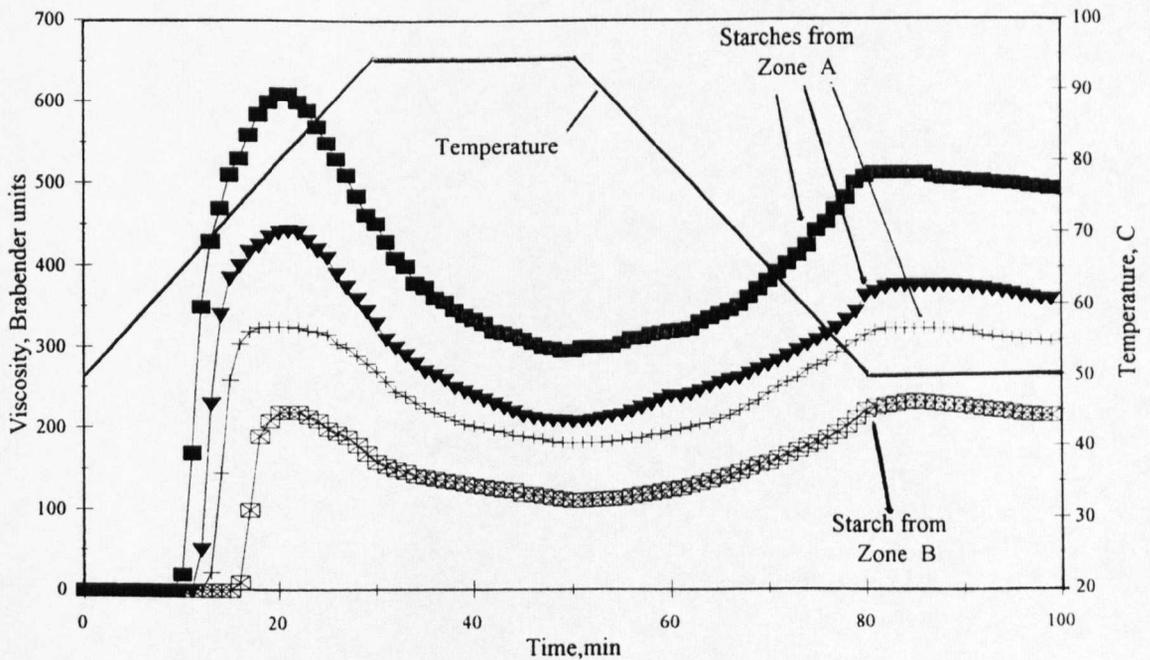


Table 3.1: Brabender pasting parameters of native cassava starches.

Cluster	Cultivar	PT (°C)	PkV (B.U.)	V_{95C} (B.U.)	V_{95 C,20 min} (B.U.)	V_{50C} (B.U.)	V_{50 C,20min} (B.U.)
Starches from Zone A							
1	MCol 22	67.0	410	300	180	330	320
	MCol 2066	64.7	390	260	160	300	290
	MBra 897	64.3	330	270	170	300	280
2	CG915-1	63.9	430	320	180	320	300
	MMex 59	62.0	440	290	180	300	290
	MPar105	65.4	360	250	160	300	290
3	MCol 1468	65.4	390	300	180	300	280
	MCR 35	63.9	600	420	260	400	390
	MCol 1522	61.5	540	360	240	400	380
4	CG1-37	64.8	450	330	210	380	360
	MVen 77	63.6	430	310	190	340	320
	MPer 196	62.2	555	340	230	380	360
5	CG1141-1	65.3	360	280	180	320	310
	MCol 72	66.1	450	360	230	400	370
	MMal 1	67.6	320	260	170	310	300
6	MCol 1684	63.4	500	280	180	340	320
	MVen 25	63.0	470	220	140	250	230
	MThai 1	64.5	400	280	180	330	320
	MCol 2485	62.1	550	330	230	400	370
	MBra 1162	62.1	530	310	210	390	370

Table 3.1: (Continued)

Cluster	Cultivar	PT (°C)	PkV (B.U.)	V _{95C} (B.U.)	V _{95 C,20 min} (B.U.)	V _{50C} (B.U.)	V _{50 C,20min} (B.U.)
7	MCol 2215	66.3	390	310	200	350	340
	CM3306-4	64.6	560	420	270	440	420
	CM2772-3	64.1	450	320	200	360	340
8	CM 489-1	64.3	510	360	220	390	370
	MBra 881	64.0	500	300	200	360	340
9	CG 402-11	63.2	520	330	220	380	360
	CG 1118-121	63.5	610	440	290	500	480
	MCol 1132	63.6	520	370	250	450	430
10	CG 165-7	64.2	470	320	210	370	350
Starches from Zone B							
4	CG1-37	73.9	220	180	120	195	200
	MPer 196	72.1	220	160	110	230	210
6	MVen 25	71.5	230	190	140	230	220
	MBra 1162	73.4	250	180	120	240	220
8	MBra 881	72.4	290	230	170	280	270
10	MMal 2	74.8	200	180	140	310	280
	MNga 2	72.5	250	210	160	310	290

PT: Initial pasting temperature; PkV: Peak viscosity; V_{95C}: Viscosity at 95°C
V_{95C,20 min}: Viscosity after 20 min. at 95°C; V_{50C}: Viscosity on cooling to 50°C
V_{50C,20min}: Viscosity on cooling and holding for 20 min. at 50°C .

3.1.2. Swelling and solubility of the starch pastes

The product from aqueous pasting of the starch is a cooked starch paste, which is composed of a mixture of undissolved swollen granules and starch in solution (Pomeranz, 1991).

The swelling volumes and the solubilities of native cassava starches in 1% aqueous suspensions pasted at 95°C (Section 2.4.1) were determined (Table 3.2). These properties varied significantly between cassava cultivars. Zone B starches exhibited the highest solubilities and the lowest swelling volumes. It was noticed, perhaps not surprisingly, that wherever the samples had a low swelling volume they had a high solubility (Figure 3.2), *i.e.* it appears that the swelling volume was restricted by the amount of starch which leached from the granules. Moorthy (1986) also observed this tendency in cassava starch, and pointed out that changes in those properties could be due to an alteration of the inherent physical binding or associative forces between molecules in the starch granules, which may be weakened considerably in certain cultivars leading to low swelling volume and high solubility. However, in this research other factors will also be considered, such as the starch granule size and its molecular composition and structure.

Figure 3.2 Solubility and swelling volume relationship in native cassava starch pastes.

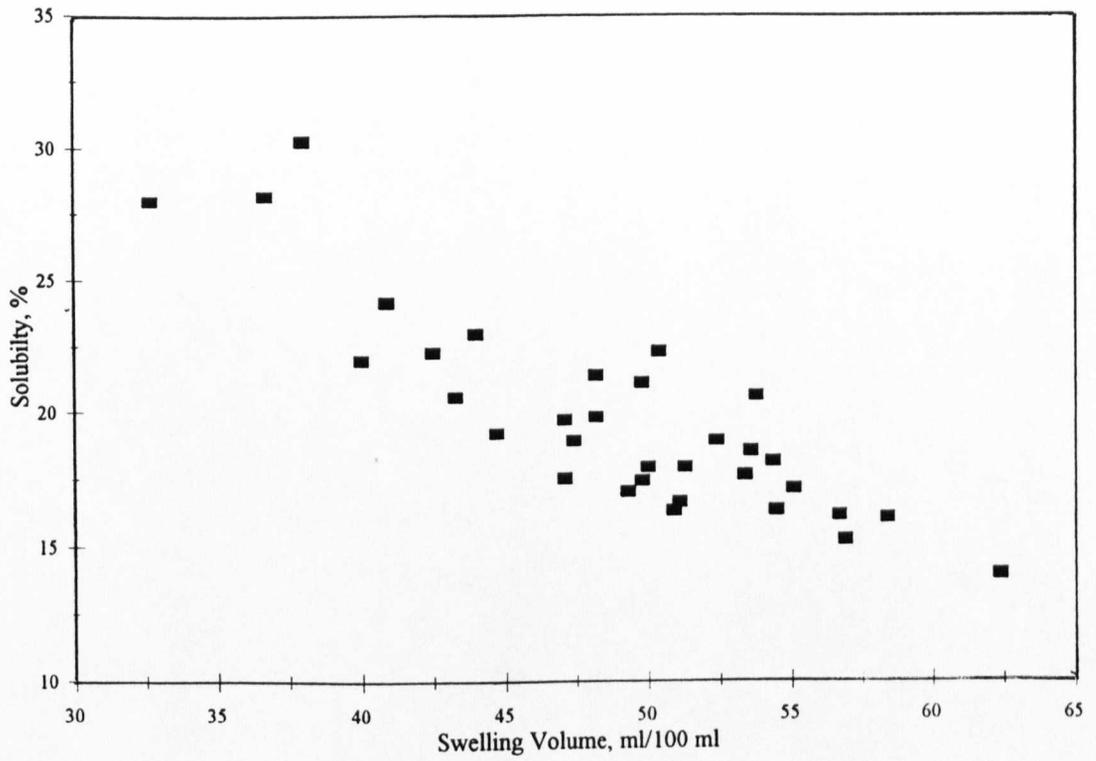


Table 3.2 Solubility and swelling volume of cassava starch in 1% aqueous pastes.

Samples from cassava cultivars planted at Zone A			
Cluster	Cultivar	Solubility * (%d.b)	Swelling volume * (ml\100 ml)
1	MCol 22	21.1±1.1	49.8±0.4
	MCol 2066	22.3±0.9	50.4±0.8
	MBra 897	22.3±0.9	42.5±0.4
2	CG915-1	20.6±0.9	53.8±0.8
	MMex 59	18.9±1.0	52.4±1.0
	MPar 105	23.0±0.7	44.0±0.0
3	Mcol 1468	19.8±0.9)	48.2±1.7
	MCR 35	16.3±0.5	50.9±0.8
	MCol 1522	17.5±1.1	47.1±0.3
4	CG 1-37	18.9±0.6	47.4±1.2
	MVen 77	19.7±1.3	47.1±0.8
	MPer 196	17.4±1.0	49.8±0.4
5	CG 1141-1	21.4±0.2	48.2±1.4
	MCol 72	16.3±1.2	54.5±2.4
	MMal 1	24.2±0.5	40.9±0.4
6	MCol 1684	18.1±0.6	54.4±1.9
	MVen 25	16.0±0.8	58.4±1.0
	MThai 1	20.6±1.5	43.3±0.0
	MCol 2485	17.9±0.9	50.0±0.0
	MBra 1162	16.6±1.6	51.1±0.8
7	MCol 2215	17.9±1.0	51.3±1.7
	CM 3306-4	15.2±1.1	56.9±0.4
8	CM 2772-3	17.1±1.6	55.1±1.5
	CM 489-1	18.5±1.1	53.6±1.0
	MBra 881	13.9±1.0	62.4±0.8
9	CG 402-11	17.6±1.3	53.4±2.3
	CG1118-121	17.0±1.2	49.3±1.2
	MCol 1132	19.2±1.3	44.7±1.4
10	CG 165-7	16.1±1.1	56.7±0.0

Table 3.2 (continued)

Samples from cultivars planted at Zone B

Cluster	Cultivar	Solubility (Hot-water-soluble starch, %db)	Swelling Volume (ml/100ml)
4	CG1-37	28.2±0.6	36.7±0.0
	MPer 196	28.0±0.4	32.7±0.0
6	MVen 25	30.3±0.4	38.0±0.0
8	MBra 881	22.0±0.3	40.0±0.0

* Mean of triplicate determinations

It was evident that the different swelling and solubilization trends of the starches were reflected in the viscosity profiles on pasting. Lower viscosities on pasting meant higher starch solubilities and, consequently, lower swelling volumes, as seen from the relationship between starch solubility and Brabender peak viscosity observed in Figure 3.3. Additionally, the higher Brabender initial pasting temperatures resulted from the restricted granule swelling caused by the higher starch solubilization [pasting temperature correlated positively with starch solubility ($r = 0.77$, $P < 0.001^{***}$)]. Moorthy (1994) did not observe any correlation between viscosity and swelling volume when comparing eight cassava starch varieties grown in India. Interestingly, it was noted that the MMal 1 starch (known as M4 in India) had the lowest swelling volume among the Zone A starches, paralleling the results observed with the M4 starch when compared to other starches from the cassava cultivar grown in India (Moorthy, 1994).

3.1.3 Mechanical properties of starch pastes and gels

Upon cooling a paste, the dispersed starch molecules reassociate at junction zones into an ordered structure, and ultimately under favourable conditions crystallize out of solution as part of the phenomenon of retrogradation and gelation (Atwell *et al.*, 1988). The final product is a gel in which thickening and rigidity develop. Starch gel pastes are considered to be a composite in which swollen starch granules reinforce a continuous matrix of entangled amylose molecules (Ring, 1985). Starch gels exhibit a viscoelastic rheological response, which can vary from a weak, viscous to an elastic, strong gel. The dynamic viscoelastic properties of cassava starch pastes were measured

and then subjected to dynamic viscoelastic tests during further cooling from 65 to 25°C, and during an additional short period at 25 °C (Section 2.4.3).

Figure 3.3 Brabender peak viscosity relationship with native cassava starch solubility in aqueous pastes.

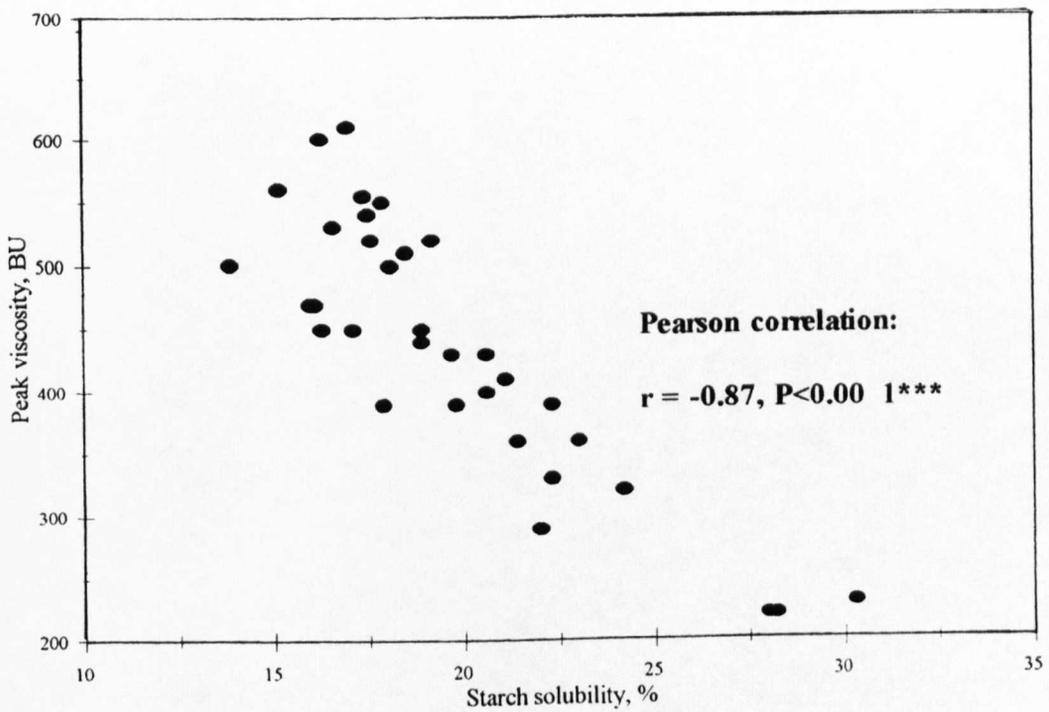


Figure 3.4a-b shows the typical profiles obtained of storage modulus (G'), loss modulus (G''), and phase angle (δ) for the MCol 1522 starch paste. As the paste was cooled from 65 to 25 °C (Figure 3.4a), the G' and G'' values increased, and δ decreased as a direct response of the aggregation and molecular rearrangements of the dispersed and solubilized starch components. The starch paste behaved like a gel with G' higher than G'' and with the values of the phase angle, δ , between 28 and 20 degrees. The frequency sweep test carried out within 30 minutes after the pastes reached 25°C (Figure 3.4b) showed that the fresh paste at 25°C exhibited a dependence of the shear moduli with frequency. Both moduli increased with the frequency increment.

Development of storage modulus (G') and phase angle (δ) upon cooling of the cassava starch pastes.

The viscoelastic response shown by the pastes on cooling varied widely. In Figure 3.5a and b are shown the results from a selected group of starch pastes which illustrate the profiles obtained for G' and δ upon cooling. The different responses of G' observed in the starch pastes on cooling were, maybe, a consequence of differences in the fine structure of the amylopectin (Section 3.1.7.2) and the molecular composition of the soluble phase in the pastes (Section 3.1.8).

The plateau values of G' in Figure 3.5a (during the holding period at 25 °C) were selected and expressed as $\log(G')$ (a gel strength index). The $\log(G')$ values ranged from 0.9 to 1.6 (Table 3.3). The starch gel strength was correlated with the amylose proportion in the solubilized starch in the pastes ($r = 0.73$, $P < 0.01^{**}$) (Section

3.1.8) and to some extent to the molecular structure of amylopectin (*i.e.* A/B ratio, Section 3.1.7) in the native starches, ($r = 0.48$, $P < 0.05^*$).

Frequency dependence of G' modulus in fresh pastes

The frequency dependence of the shear moduli (G', G'') may give valuable information about the nature of the network structure of a starch paste or gel. A material which is frequency independent over a large time scale range is solid-like and, as such can be used as a characteristic of a true gel system (Biliaderis, 1992; Doublier, 1990). Figure 3.6 shows the change in G' with frequency, a response which suggests that the cassava starches had only formed weak gels, not structures with permanent cross-links which are typical of true gels. The behaviour of cassava starch pastes as weak gels was probably related to the lack of a continuous phase rich in amylose. Instead it contained about equal amounts of solubilized amylose and amylopectin (Section 3.1.8).

The shear modulus, G' , is related to the frequency, f , by the linear function $\log(G') = k + n \log(f)$, where the value of the slope n is a measure of the frequency dependence of the gel strength. Low n values means less frequency dependence and hence a more solid-like sample. The values of the starch samples were calculated, and ranged from 0.19 to 0.32 (Table 3.4). This indicator of the rigidity of the gel structure was also related to the composition of the starch leached out from the granules (Section 3.1.8).

Figure 3.4 (a) Changes in G' , G'' and delta in 6% native cassava starch paste (MCol 1522) upon cooling (65 to 25 °C) at 1.5 °C/min. (b) Frequency dependence of G' , G'' and delta of the paste at 25 °C.

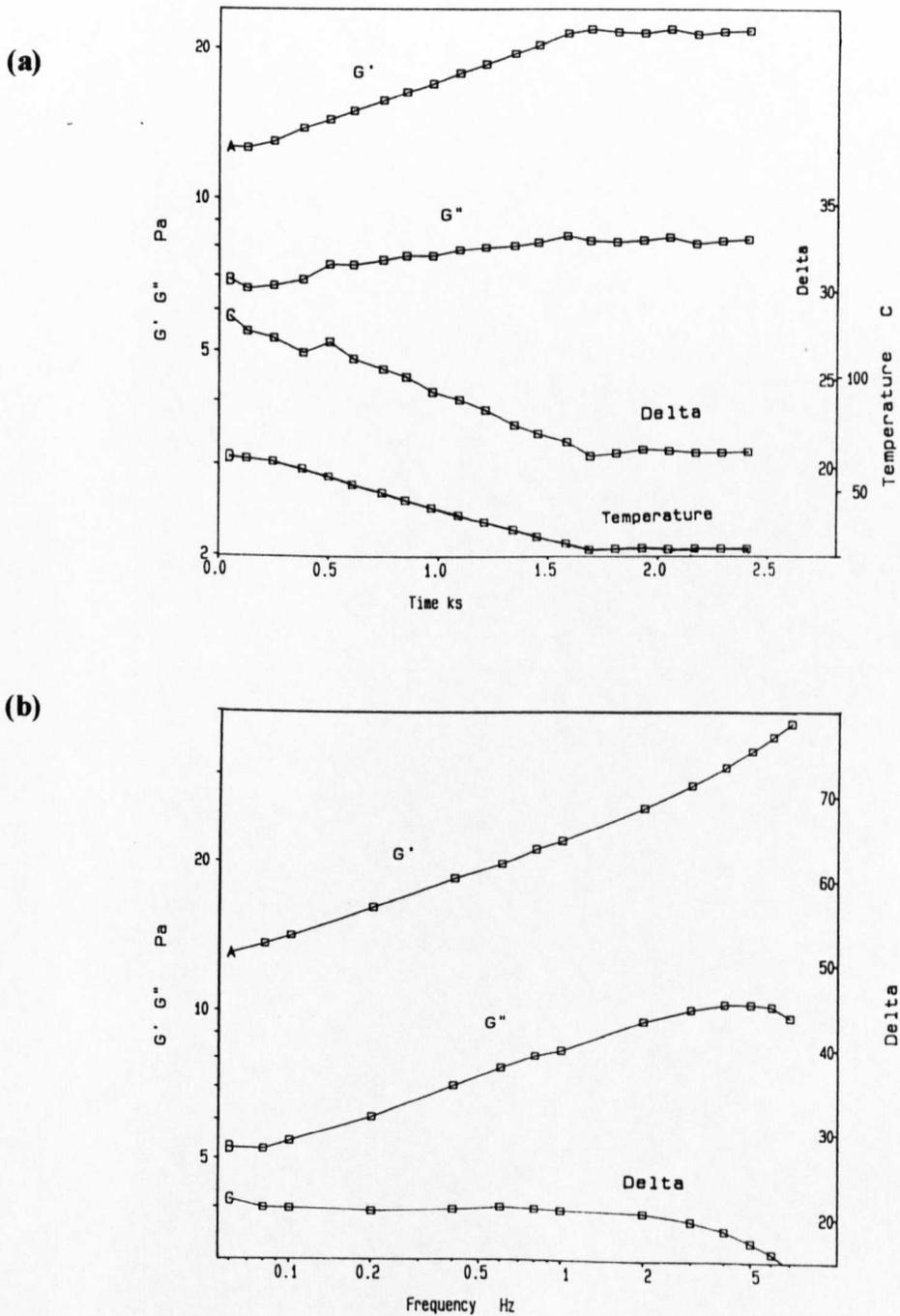


Figure 3.5 Changes in G' and delta in 6% native cassava starch pastes upon cooling (65 to 25 °C) and holding at 25 °C for 15 minutes.

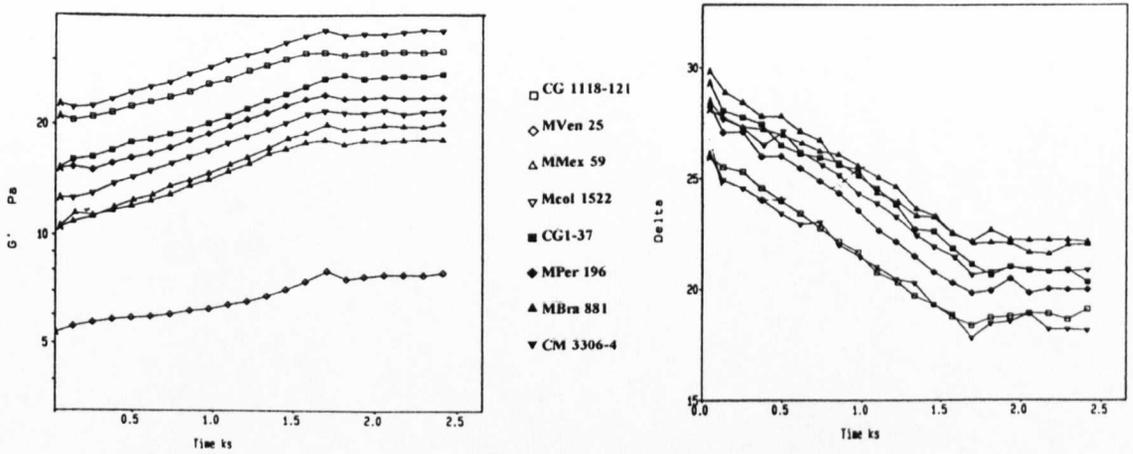


Figure 3.6 Frequency dependence of G' in 6% cassava starch pastes at 25 °C.

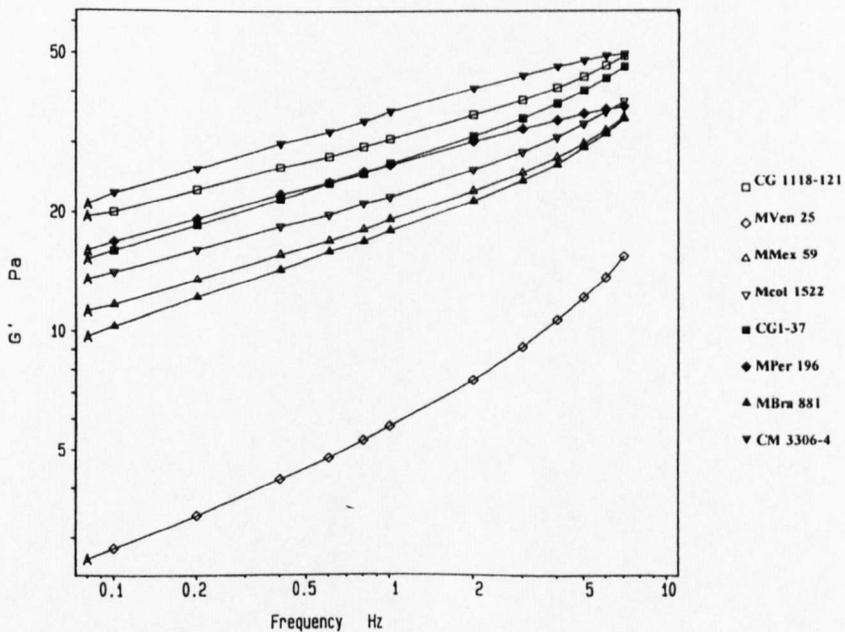


Table 3.3 Strength of cassava starch pastes at 25°C as measured by the logarithm of storage modulus G' at frequency 1 Hz.

Cluster	Cultivar	Log(G)	Cluster	Cultivar	Log(G)
1	MCol22	1.34	6	MCol 1684	1.24
	MCol 2066	1.35		MVen 25	0.9
	MBra 897	1.39		MThai 1	1.28
		MCol 2485		1.39	
2	CG915-1	1.25	7	MBra 1162	1.32
	MMex 59	1.26		MCol 2215	1.36
	MPar 105	1.28	CM3306-4	1.6	
3	Mcol 1468	1.34	8	CM 2772-3	1.35
	MCR 35	1.40		CM 489-1	1.39
	MCol 1522	1.34		MBra 881	1.0
4	CG 1-37	1.44	9	CG 402-11	1.4
	MVen 77	1.35		CG1118-121	1.5
	MPer 196	1.38		MCol 1132	1.5
5	CG 1141-1	1.44	10	CG 165-7	1.2
	MCol 72	1.44			
	MMal 1	1.48			

Table 3.4 Frequency dependence, as measured by the slope n of the curve $\log(G')$ vs frequency (f), of cassava starch pastes at 25°C.

Cluster	Cultivar	n	Cluster	Cultivar	n
1	MCol22	0.23	6	MCol 1684	0.25
	MCol 2066	0.26		MVen 25	0.32
	MBra 897	0.22		MThai 1	0.27
		MCol 2485		0.23	
2	CG915-1	0.23		MBra 1162	0.22
	MMex 59	0.24	7	MCol 2215	0.22
	MPar 105	0.23		CM3306-4	0.21
3	Mcol 1468	0.22	8	CM 2772-3	0.22
	MCR 35	0.21		CM 489-1	0.22
	MCol 1522	0.21		MBra 881	0.26
4	CG 1-37	0.23	9	CG 402-11	0.24
	MVen 77	0.24		CG1118-121	0.19
	MPer 196	0.21		MCol 1132	0.23
5	CG 1141-1	0.23		CG 165-7	0.26
	MCol 72	0.24			
	MMal 1	0.24			

Viscoelastic character of the starch gels after 24 hr at 4°C.

Starch pastes from four cultivars grown in both Zones A and B were also compared by testing them in a separate experiment using the methodology previously described. Thereafter, the fresh pastes were stored for 24 hr at 4°C, equilibrated to 25°C, and subjected to retesting for viscoelasticity measurements.

There was an interesting reversal after storage in the viscoelastic behaviour of the fresh starch pastes from Zones A and B. Figure 3.7a-c shows, with MPer 196 starch, that the fresh paste from Zone A starch had higher G' compared with that from Zone B starch (Figure 3.7a and b), but the reverse was observed with the aged pastes (Figure 3.7c). Figure 3.8a and b shows the above effect by comparing the storage modulus G' (at 25°C and 0.6 Hz) of the starch pastes from the four cultivars. The higher gel rigidity of Zone B aged pastes is also evident from the values of the frequency dependence indices (n) in Table 3.5.

Starches from Zone B compared to those from Zone A exhibited a higher starch solubilization (Section 3.1.3) of both amylose and amylopectin, with a higher relative proportion of amylopectin in the leached starch (Section 3.1.8). These features might be responsible for the gel strength which development in the aged pastes prepared with starches from Zone B. The high concentration of amylopectin in solution might additionally have delayed the gelation of soluble amylose in the fresh pastes and could have contributed to the developing of the gel structure after storage at 4°C as deduced from research by Ring *et al.* (1987) and Svegmak *et al.* (1993).

Figure 3.7 (a) Development of G' upon cooling (65 to 25 °C) and holding at 25 °C for 15 minutes in 6% pastes of MPer 196 native cassava starch extracted from Zone A and Zone B plants, and (b) the frequency dependence of G' in the fresh and (c) in the aged pastes .

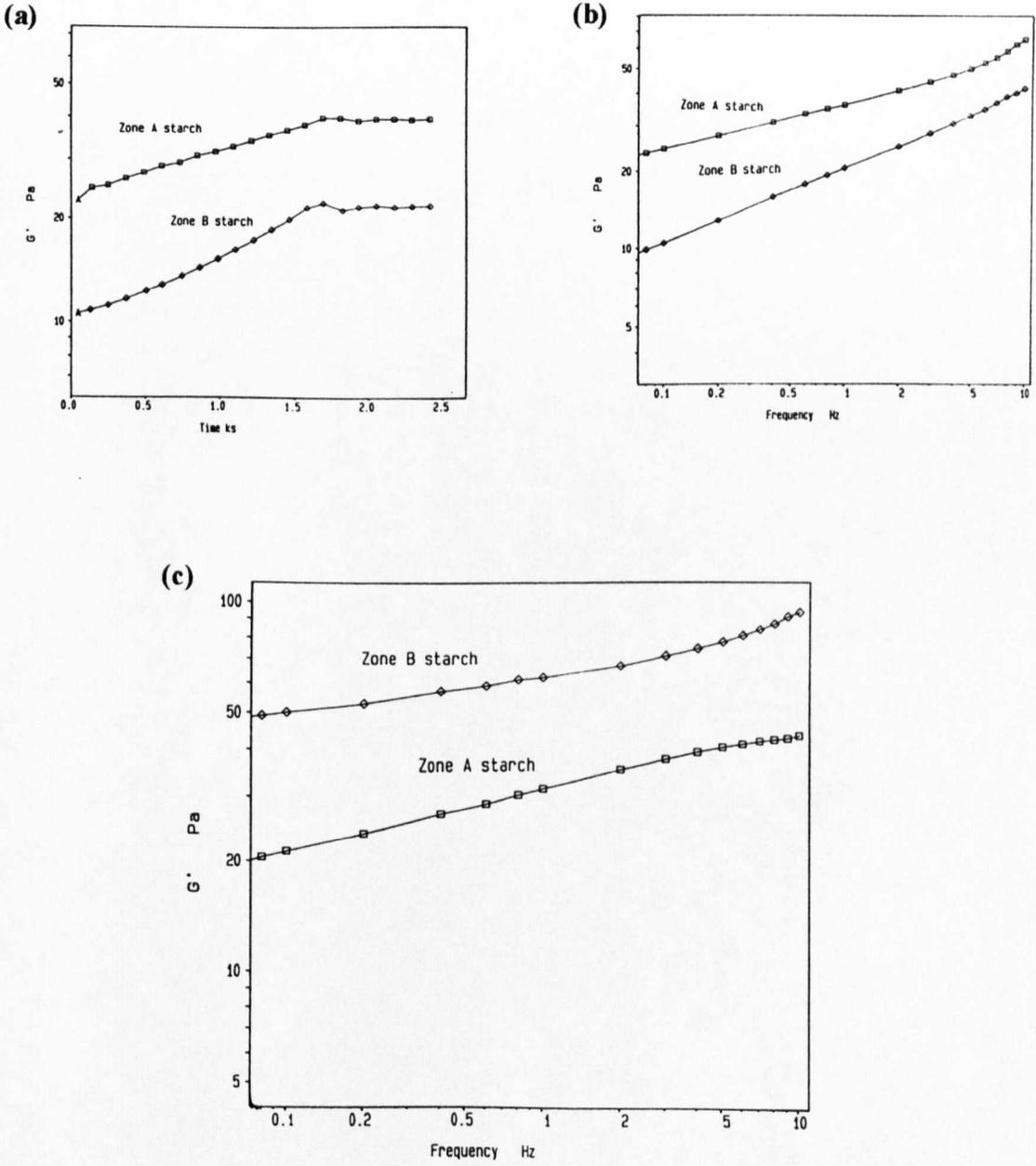


Figure 3.8 (a) Storage modulus (at 0.6 Hz and 25°C) in fresh and (b) in stored pastes of native cassava starches extracted from plants of Zones A and B.

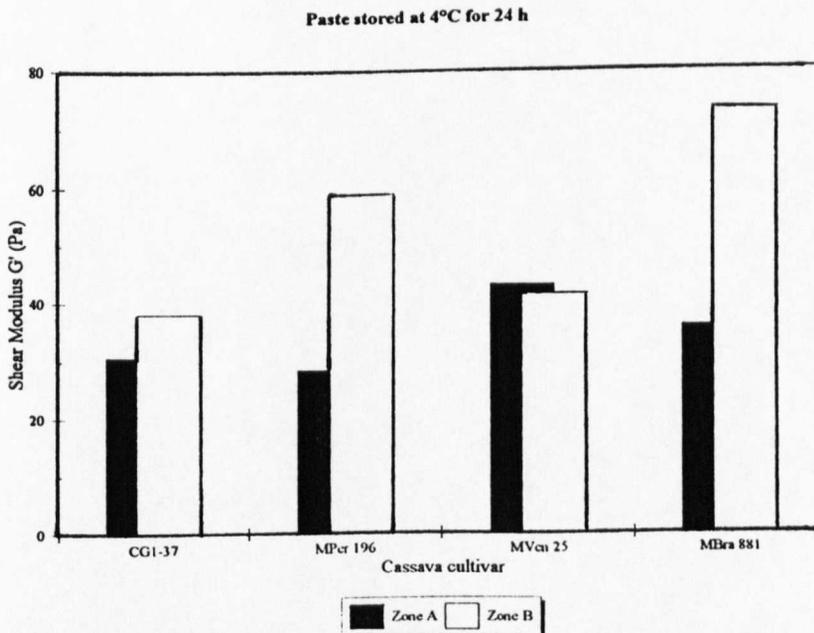
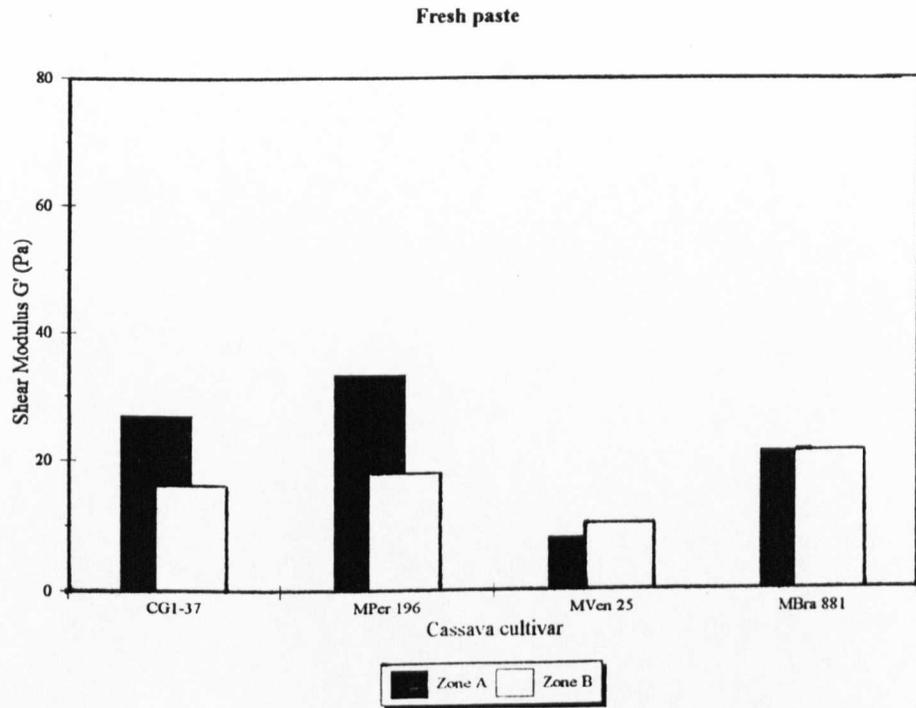


Table 3.5 Small strain rheology- frequency dependence (n) as a mean of gel structure rigidity of fresh and stored pastes of cassava starches extracted from plants grown in Zones A and B.

Cultivar	Zone A	Zone B
Fresh pastes tested at 25 °C		
CG1-37	0.21	0.25
MPer 196	0.19	0.29
MVen 25	0.30	0.34
MBra 881	0.23	0.25
Pastes stored at 4 °C for 24 hr and tested at 25 °C		
CG1-37	0.18	0.11
MPer 196	0.16	0.12
MVen 25	0.12	0.13
MBra 881	0.13	0.09

3.1.4. Granule size distribution of the starch granules

The size and particle size distribution of the starch granules are physical factors which might contribute to the pasting behaviour and the rheological response of the starches as concluded by Rasper (1971), Wong and Lelievre (1981) and Eliasson (1986). The cassava starch granules powders were subjected to particle size measurements (Section 2.4.4). The particle size distribution varied between starch samples and the range is observed from the distributions shown in Figure 3.9. Zone B starches had a higher proportion of small granules than Zone A starches. The mean particle sizes and their standard deviations are in Table 3.6, where it is observed that the starches had a range of d_{gw} from 8.8 to 12.4 μm , with standard deviations ranging from 1.47 to 1.65. Starches from Zone B had the lowest d_{gw} in the range from 8.8 to 10.8 μm . and their size distributions were narrower than the vast majority (72%) of the starches from Zone A as is evident from the standard deviations.

The differences in the granule size between the samples could partially explain their different behaviour in water in relationship to starch solubility ($r = -0.55$, $P < 0.001^{***}$) and the Brabender peak viscosity on pasting ($r = 0.52$, $P < 0.001^{***}$). On the one hand, increased solubilization might be expected from a mass of small granules since more starch could leach out more readily through the greater surface area per unit mass than would be the case with large granules. On the other hand, reduced solubilities in masses of large granules resulted in increased swelling volumes (Figure 3.10) and consequently enhanced maximum pasting viscosities.

Figure 3.9 Typical granule size distributions obtained in native cassava starch powders.

* The numbers described as the granules sizes are shown as follows:

1: 3.57 μm , 2: 4.47 μm , 3: 5.65 μm , 4: 7.15 μm , 5: 8.94 μm , 6: 11.4 μm , 7: 14.2 μm , 8:

17.88 μm , 9: 22.36 μm , 10: 28.28 μm , 11: 35.9, 12: 45.24 μm .

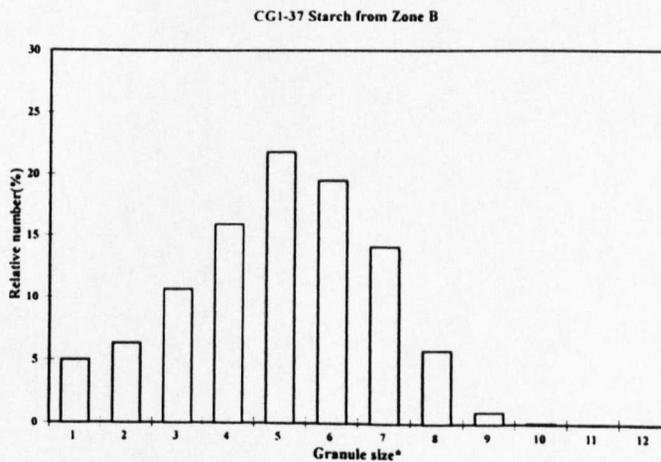
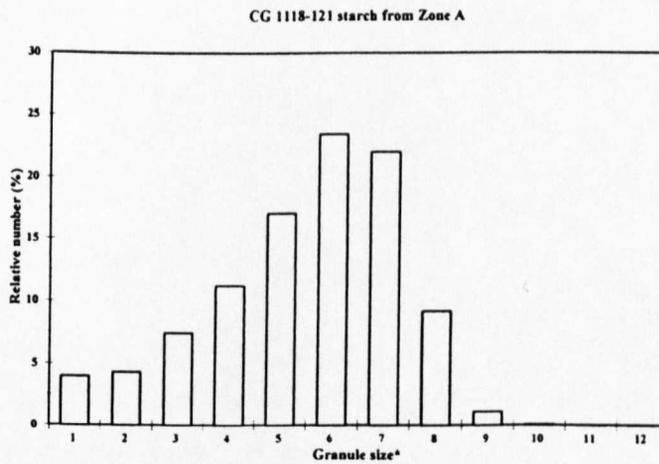
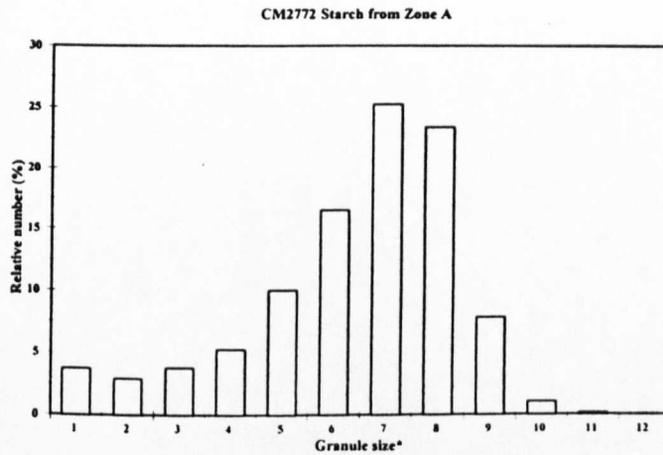


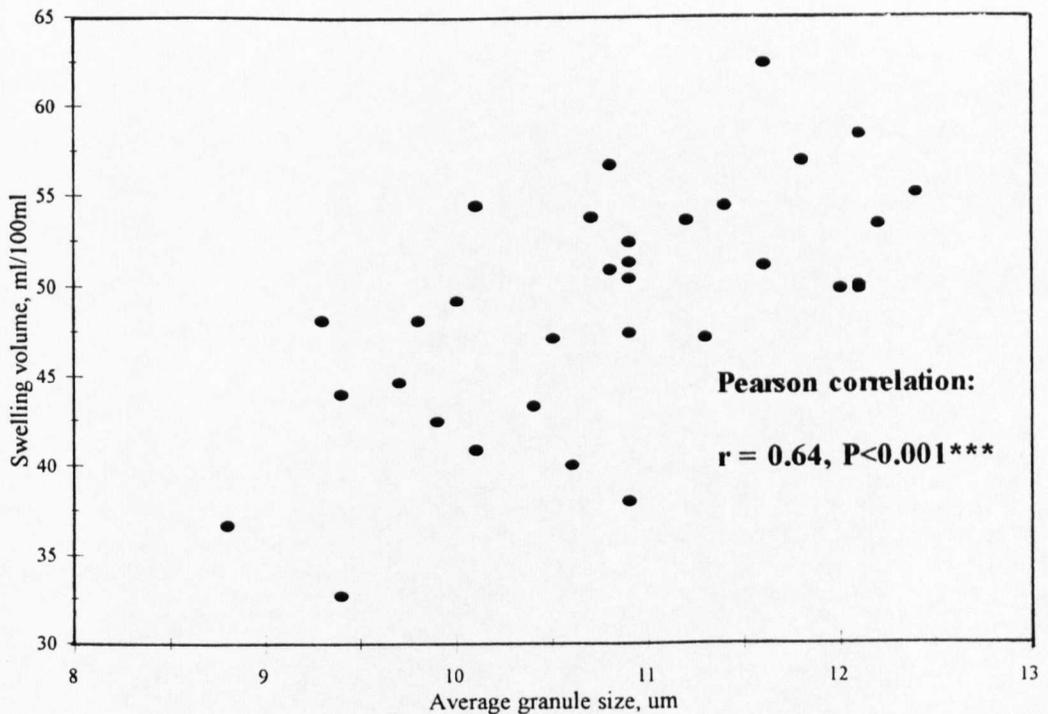
Table 3.6 Geometric mean diameter (d_{gw} , μm) and geometric standard deviation (s_{gw}) of the distribution of the size of starch granules.

Starches from Zone A							
Cluster	Cultivar	d_{gw}	s_{gw}	Cluster	Cultivar	d_{gw}	s_{gw}
1	MCol22	12.1	1.51	6	MCol 1684	11.4	1.56
	MCol 2066	10.9	1.58		MVen 25	12.1	1.60
	MBra 897	9.9	1.55		MThai 1	10.4	1.57
2	CG915-1	10.7	1.57		MCol 2485	12.1	1.56
	MMex 59	10.9	1.54		MBra 1162	11.6	1.55
	MPar 105	9.4	1.52	7	MCol 2215	10.9	1.53
3	Mcol 1468	9.8	1.54		CM3306-4	11.8	1.53
	MCR 35	10.8	1.54	8	CM 2772-3	12.4	1.58
	MCol 1522	10.5	1.54		CM 489-1	11.2	1.61
4	CG 1-37	10.9	1.56		MBra 881	11.6	1.65
	MVen 77	11.3	1.54	9	CG 402-11	12.2	1.59
	MPer 196	12.0	1.59		CG1118-121	10.0	1.53
5	CG 1141-1	9.3	1.57		MCol 1132	9.7	1.54
	MCol 72	10.1	1.53	10	CG 165-7	10.8	1.53
	MMal 1	10.1	1.51				

Starches from Zone B							
Cluster	Cultivar	d_{gw}	s_{gw}	Cluster	Cultivar	d_{gw}	s_{gw}
4	CG1-37	8.8	1.53	8	MBra 881	10.6	1.51
	MPer 196	9.4	1.53				
6	MVen 25	10.9	1.53	11	MMal 2	9.5	1.50
					MNga 2	9.2	1.47

The results of the studies of the molecular structure of the starches (3.1.7.2) suggested that the granule size is correlated to some degree to the extent of short branches in the amylopectin and to the chain length of the amylose. For example, more highly branched amylopectin and longer amylose chains are expected to be present in the larger cassava granules.

Figure 3.10 Relationship of swelling volume in native cassava starch pastes and the granule size in their starch powders.

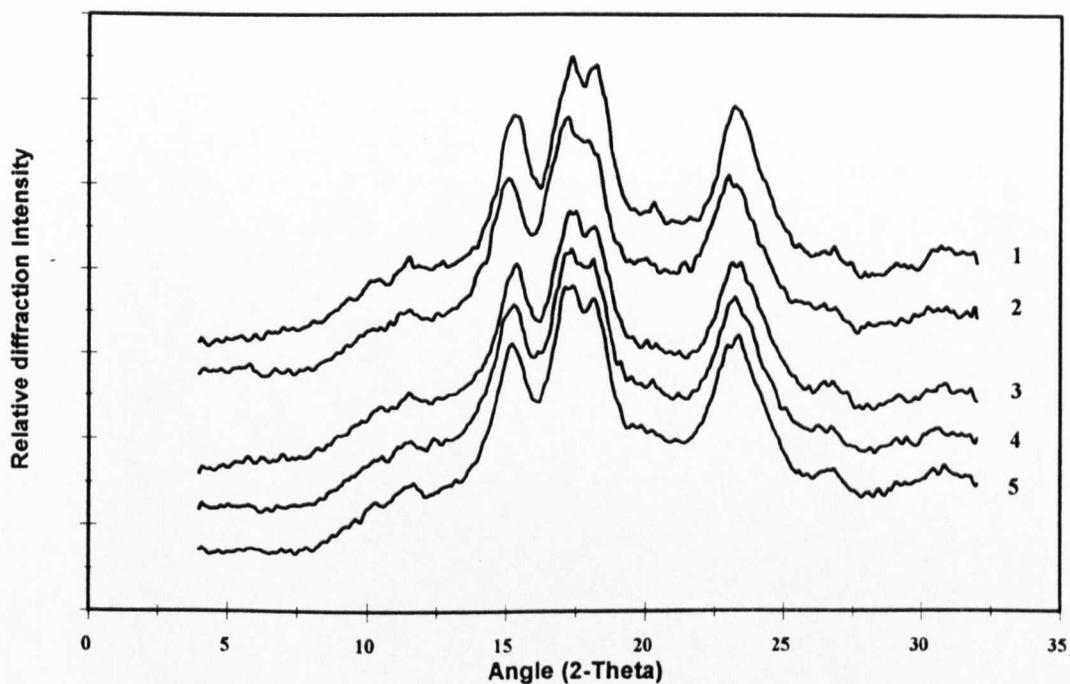


3.1.5 X- ray diffraction patterns and crystallinities of starch powders

Wide-angle X-ray diffraction studies (Section 2.4.5) of nineteen native cassava starches yielded A-type and C-type patterns in Zone A starches but exclusively A-type patterns in starches from Zone B. If the C-type patterns were closer to an A pattern, they were designated as Ca-type starch, following Hizukuri (1969). Figure 3.11 presents a selection of the X-ray pattern types. Cassava starches have been reported to have an A-type pattern by Rosenthal *et al.* (1974), Hizukuri (1985), Franco *et al.* (1988), Moorty (1994), Linnecar (1995); whilst a C-type pattern has been reported by Dreher and Berry (1983) and Zobel (1988), and a C-type, close to A-type pattern, by Asaoka *et al.* (1991, 1993). The presence of an A-type pattern in all the starches from cassava plants grown in the environmental conditions of Zone B (Section 2.2.1) coincides with the conclusions of Hizukuri(1969) who found that high environmental temperatures of 28°C or above favoured the formation of A-type crystal patterns in starches from sweet potato and soybean seedlings. The levels of crystallinity in the starch granules were calculated by the separation and integration of the relative areas of the crystalline peaks under the crystalline and amorphous X-ray diffraction peaks. The crystallinities of the starches varied from 40.0 to 47.7%, with the highest values in Zone B starches (Table 3.7). The crystallinity levels are in agreement with the expected values for A and C-type crystal structures observed by Zobel (1988), who reported a value of 38% for cassava starch. The crystallinities showed some correlation with the initial pasting temperature ($r = 0.58, P < 0.01^{**}$) and the Brabender peak viscosities ($r = -0.55, P < 0.01^{**}$). The above relationships are in line with the view that a starch with greater crystallinity would probably result in

both the hydration and swelling of the amorphous regions being delayed to a higher temperature of gelatinization (TG) and the subsequent destabilization of the crystallites (Blanshard, 1987). Data by Zobel (1988) show increased TG's (determined by loss of optical birefringence) in the more crystalline samples of starch of different botanical origin with A-type crystals. In the present research, the gelatinization temperature the (TG) was not determined, but the initial pasting temperature (PT) parallels TG, *i.e.* the loss of macromolecular organization and order coincide with the major rheological events when heating cassava starch aqueous suspensions, as deduced from observations of TG and PT data in Asaoka *et al.*, 1992; Chuzel, 1992; and with cassava flour in Eggleston *et al.*, 1993 and Defloor *et al.*, 1995 . Further correlations in Section 3.1.8 will show, for example that more crystalline samples seem to restrict the proportion of leached amylose in starch pastes.

Figure 3.11 Typical X-ray diffraction patterns in native cassava starches.



Zone B starch: 1: CG1-37, Zone A starches: 2: CG1-37, 3: MBra 897, 4: MCol 2066
and 5: MCol 22.

Table 3.7 Crystallinity of cassava starch

Samples from cultivars planted at Zone A

Cluster	Cultivar	Crystallinity (%)	Cluster	Cultivar	Crystallinity (%)
	MCol22	45.0(A)	5	MMal 1	41.4(A)
1	MCol 2066	40.7(A)			
	MBra 897	40.7(A)	6	MVen 25	44.2(A)
				MBra 1162	40.9(A)
	CG915-1	44.5(C)			
2	MMex 59	44.0(C)	7	CM3306-4	41.7(C)
	MPar 105	41.2(A)			
			8	MBra 881	42.7(A)
	Mcol 1468	45.1(C)			
3	MCR 35	40.5(C)	9	CG1118-121	40.9(C)
	MCol 1522	44.4(C)			
4	CG 1-37	43.0(C)			
	MPer 196	40.0(A)			

Samples from cultivars planted at Zone B

Cluster	Cultivar	Crystallinity (%)	Cluster	Cultivar	Crystallinity (%)
4	CG 1-37	44.3(A)	8	MBra 881	46.8(A)
	MPer 196	45.9(A)			
			10	MMal 2	47.7(A)
5	MBra 1162	47.9(A)		MNga 2	43.2(A)
6	MVen 25	44.8(A)			

3.1.6 Amylose content.

The amylose content of the starches was determined by the iodine colorimetry method (Section 2.4.6). Initial tests with standard samples of potato amylopectin and waxy rice amylopectin, yielded values of λ_{\max} of the starch- I_2 test of 555 nm and 525 nm respectively. The two standard starches composed firstly of potato amylopectin and potato amylose stained blue-purple with iodine while the second composed of waxy rice amylopectin and potato amylose stained blue-black. This latter stain resembled more closely the colour given by cassava starch and iodine. Waxy rice and cassava starch have amylopectins of relatively similar average chain lengths (23 and 25 glucose units respectively) compared with those of potato amylopectin (36 glucose units) (Hizukuri, 1985), which explains the differences in λ_{\max} between waxy rice and potato amylopectins. Therefore, waxy rice amylopectin was used in making the standard starch for this test.

The amylose content in the samples ranged from 20.4 to 26.9% (Table 3.8). Statistical analysis suggested a cultivar effect on the amylose content of starches from roots planted at both Zones A and B. The starches from Zone B roots contained a higher amylose content. The results from Zone A starches were comparable with values in starches from six cassava cultivars from India reported by Moorthy and Ramanujam, (1986) which ranged from 20.0 to 23.1% in starches extracted from 9 month old plants. The increment in amylose content in Zone B starches could be a consequence of the higher environmental temperature during growth. Such increments in amylose at higher environmental temperatures have been reported by Jenner

(1996) in Australia in research on the temperature dependence of soluble starch synthase (SSS) in wheat grains. The SSS is involved in the synthesis of amylopectin, the major component in wheat starch and it has been found to have a much lower activity in wheat starch synthesis at temperatures over 25°C. The SSS activity in starches of some other botanical origins was observed to be less dependent on temperature. If the SSS in cassava starch follows the trend of temperature dependence observed with wheat, it might explain the reduction in amylopectin (or the relative increment of amylose) detected in cassava starches from Zone B plants.

The amylose content plays an important role in the gelatinization process of some starches. However, the properties of cassava starch pastes seem to be more dominated by the amylopectin behaviour on pasting and in solution, *i.e.* high swelling power and resistance to gelation. In fact, cassava starch also leaches significant levels of amylopectin, and the solvent or continuous phase could contain equal amounts of amylose and amylopectin (Section 3.1.8), the relatively high concentration of amylopectin in the solvent phase interfering with the molecular aggregation of amylose and its gelation power. Low correlations were found in Zone A starches between amylose content and pasting temperature ($r = 0.33$, $P < 0.05^*$), the Brabender cool viscosity (values of $V_{50C,20}$ in Table 3.1) ($r = 0.29$, $P = 0.064$) and the gel strength index ($\text{Log } G'$) ($r = 0.24$, $P = 0.107$). The correlation with pasting temperature increased ($r = 0.75$, $P < 0.001^{***}$) including data of Zone B starches; there was also some negative correlation with peak viscosity ($r = -0.48$, $P < 0.01^{**}$). Since peak viscosity is an index of the swelling of the starch granules during heating

peak viscosity suggests that the amylose present could restrain the swelling of starch granules.

Table 3.8 Amylose content* of cassava starches as determined by the iodine-colorimetric test

Samples from Zone A					
Cluster	Cultivar	Amylose (% d.b.)	Cluster	Cultivar	Amylose (% d.b.)
1	MCol22	21.6±0.4	6	MCol 1684	20.7±0.2
	MCol 2066	21.1±0.4		MVen 25	22.4±0.1
	MBra 897	21.7±0.1		MThai 1	21.6±0.1
		MCol 2485		23.1±0.1	
2	CG915-1	22.5±0.1	7	MBra 1162	22.6±0.2
	MMex 59	21.2±0.3		MCol 2215	24.0±0.4
	MPar 105	22.6±0.2		CM 3306-4	22.6±0.1
3	Mcol 1468	22.7±0.8	8	CM 2772-3	23.8±0.4
	MCR 35	23.5±0.3		CM 489-1	22.9±0.3
	MCol 1522	22.0±0.4		MBra 881	22.5±0.2
4	CG 1-37	22.2±0.4	9	CG 402-11	20.4±0.2
	MVen 77	22.3±0.5		CG1118-121	22.9±0.2
	MPer 196	22.4±0.3		MCol 1132	23.2±0.3
5	CG 1141-1	22.0±0.7	10	CG 165-7	22.1±0.1
	MCol 72	24.3±0.3			
	MMal 1	23.9±0.1			

Samples from Zone B

Cluster	Cultivar	Amylose (%d.b.)	Cluster	Cultivar	Amylose (% d.b.)
4	CG 1-37	25.3±0.1	8	MBra 881	26.4±0.3
	MPer 196	25.1±0.3			
6	MVen 25	24.8±0.2	10	MMal 2	26.9±0.1
				MNga 2	26.0±0.4

* Mean of four determinations

3.1.7 Molecular structure.

3.1.7.1 Composition of native cassava starch and chain length of amylopectin by the low pressure GPC method.

The low pressure or conventional GPC method (Section 2.4.7) was used in this research for a preliminary assessment of the molecular organization of cassava starch. Eight starch samples from Zone A plants were debranched by isoamylase and the starch linear chains fractionated by GPC. Figure 3.12 presents the chromatograms of the debranched starches, showing the amylose peak (I), followed by at least three partially developed peaks of amylopectin chains (II and III). It is noticeable that fraction III has two undeveloped chain length populations. The peaks I, II, III (Figure 3.12) were distinguished as amylose, long chains of amylopectin and short chains of amylopectin, respectively, following Asaoka *et al.* (1991,1993), who identified them by the wavelengths at maximum absorbance in the iodine-starch test. As is evident from the chromatograms, fraction I peak was developed fully and separated from the other fractions, suggesting that in cassava starch there is not an intermediate material between amylose and amylopectin, as has been detected with starches from other sources, *e.g.* rice (Asaoka, *et al.*, 1986; Ong, 1994), and oats (Wang and White, 1994).

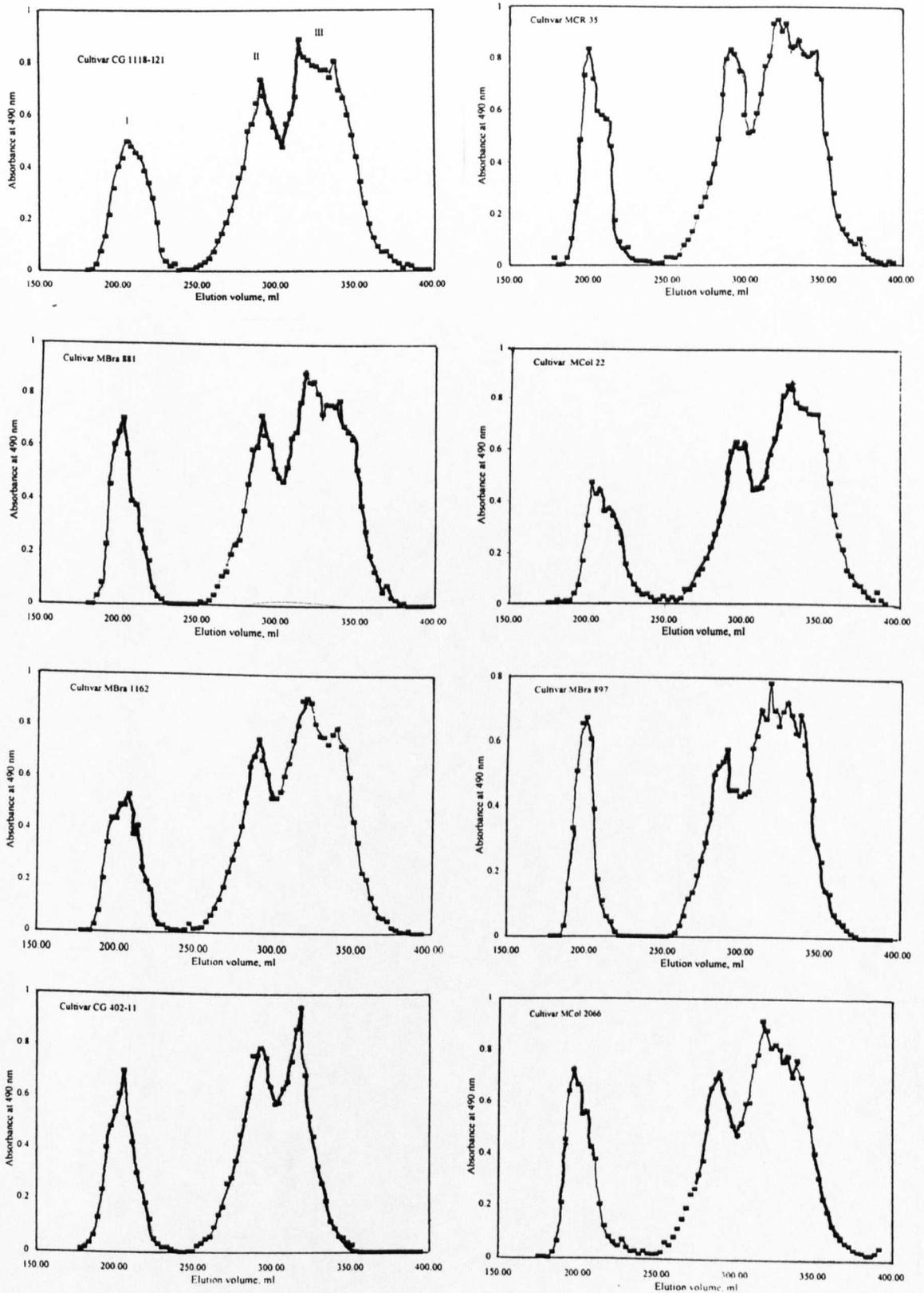
The relative proportions of fractions (Frs) I, II and III were calculated along with the chain lengths (degree of polymerization of glucose, DP) at the maxima for each fraction (Table 3.9). The range of amylose contents (Fr I) of 16.2 - 19.9% was found to agree with the values of 16.1-19.9% and 17.4 - 20.0% reported by Asaoka

et al., 1991 and 1993, respectively, using the same GPC method on starches from other cassava cultivars supplied by CIAT. The amylose content values of 20.4 - 23.5% obtained from the iodine colorimetric method with the same samples appeared higher than the values from the GPC method probably due to the contribution of long chains in the amylopectin forming a blue iodine complex. The amylopectin chain lengths of the eight starches at the peak of the long chain fractions (Fr II) ranged between 58-70 while the short chain fraction (Fr III) could be further subdivided into two populations which ranged between 21-32 and 12-19. The ratio III/II, *i.e.* the ratio by weight of the short chains to the long chains, as an index of the fine structure of amylopectin, ranged from 1.9 to 2.8.

Table 3.9 Proportion and chain length of components in cassava starch determined by the GPC technique

Clon	Fr.I (%)	Fr.II (%)	Fr.III (%)	III/ II	Chain length at peak of	
					Fr.II	Fr.III
MCol 22	16.2	22.2	61.6	2.8	58	21,12
MCol 2066	19.3	25.1	55.6	2.2	71	29,17
MBra 897	18.1	24.1	57.8	2.4	71	29,19
MCR 35	19.2	24.2	56.6	2.3	71	27,17
MBra 1162	17.8	25.5	56.7	2.2	71	27,14
MBra 881	19.2	25.6	55.2	2.2	71	29,14
CG 402-11	19.9	24.7	55.4	2.2	66	29,14
CG 1118-121	18.3	28.1	53.6	1.9	71	31,15

Figure 3.12 GPC chromatograms derived from debranched cassava starches.



The results confirmed the feasibility of the GPC procedure for isolating amylose from debranched amylopectin fractions in cassava starch. The elution patterns were comparable with those of starch from other cassava cultivars studied by Asaoka *et al.* (1991 and 1993). From the elution profiles it is evident that amylopectin has at least three populations of different chain lengths.

However, the GPC procedure was found to be a slow method of analysis, susceptible to experimental error because of the laborious chemical assay of carbohydrates in the collected fractions (a total of 80 per sample) and requiring special care to ensure a stable eluent flow rate. The technique also appeared unable to detect differences in the amylopectin structure of starches of several cassava cultivars even when grown under different conditions (Asaoka *et al.*, 1991, 1993 and Moorthy, 1994).

At this stage it was decided to continue the assessment of the molecular structure of cassava starch with the faster, more highly sensitive and reliable SE-HPLC system (Section 2.4.8).

3.1.7.2 Molecular structure of native cassava starch by the SE-HPLC-MALLS-RI method

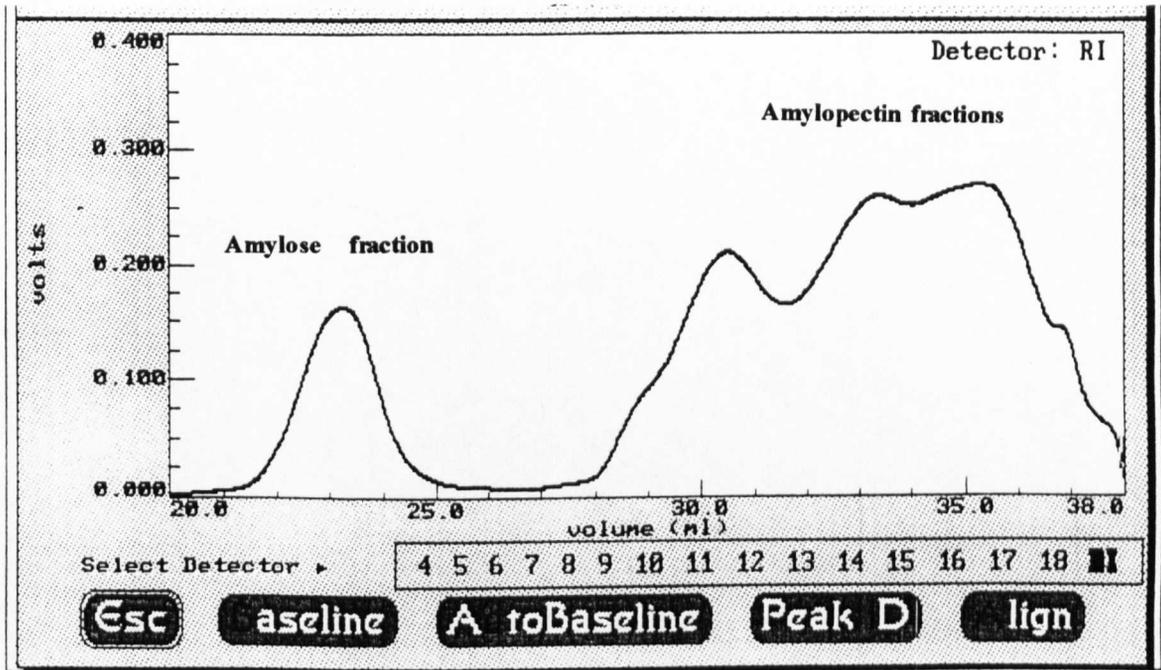
SE-HPLC profiles.

Twenty nine starch samples from Zone A plants were debranched and passed

through the SE-HPLC system (Section 2.4.8).

Tests of total carbohydrates on a selection of debranched starch solutions ready for injection into the SE-HPLC system showed that they contained between 93 to 98 % of the original starch. A typical RI-chromatogram of isoamylase-debranched cassava starch is presented in Figure 3.13. The materials occupying the highest hydrodynamic volume, i.e. with the highest molecular weight, eluted first from the the columns. These were shown to be amylose (λ_{max} of the starch-I₂ test was 630 nm), which eluted near the void volume (21.5 ml) followed by the smaller molecular weight fractions, known to derive from amylopectin. The λ_{max} average for the three broad amylopectin peaks were from the first eluted chains (longer chains) to the last ones (shorter chains) 600, 520 and 475 nm respectively. The small peaks at the tail of the elution profile were due to ammonium sulphate incorporated with the isoamylase (Hizukuri, 1985). The RI chromatograms for all the samples are presented in Figure A.1 in the Appendix. They all show a similar pattern in the amylopectin broad peaks which, in turn, were composed of multiple, unresolved peaks indicating a high degree of branching but with branches of varying chain length. A similar RI profile of cassava amylopectin RI profile was reported by Hizukuri (1985, 1986).

Figure 3.13 Typical SE-HPLC chromatogram derived from isoamylase debranched cassava starch. SE-HPLC columns: TSK G3000 PWXL, 2xAsahipak GS-320H, TSK G2500 PWXL and OLIGO PWXL



Chain length distributions in amylopectin

The unresolved amylopectin peaks in the chromatograms were analysed by dividing the elution profiles into three sections to match fractions of high molecular weight (HMW), intermediate molecular weight (IMW), and low molecular weight (LMW) and their average chain lengths obtained as described in Section 2.4.8. Figure 3.14 shows an amylopectin chromatogram sectioned as specified above.

Table 3.10 presents the chain lengths (DP), the proportions (weight% and mole%) of amylopectin chains and the A:B chain ratio of all samples. The weight percentage was calculated for each fraction from its relative area under the chromatogram. The mole percentage was calculated for each fraction from the ratio of weight% to DP_n relative to the total mole content of all fractions. The A:B chain ratio was calculated from the mole percentage figures as $LMW/(HMW+IMW)$. Fraction LMW was assumed to be composed of A chains and HMW and IMW of B chains following Hizukuri (1986). Chains in the LMW fraction were made up of the major portion of the total number of chains of the debranched amylopectins for all the samples (55-59%), followed by the mole % of the IMW (30-32%) and the HMW fractions (11-13%). The LMW and IMW chains constituted about 89% of the total chains of the amylopectins, which is in agreement with the total A and B_1 chain population (90%) reported for cassava amylopectin by Hizukuri (1986). For all samples the average chain length for the HMW fraction ranged from DP_n 46 to 49, for the IMW fraction from 21 to 23, and for the LMW fraction from 11 to 12. These values are consistent with the average DP values reported for B_2 chains (DP_n 42),

Figure 3.14 SE-HPLC chromatogram of linear chains of amylopectin (in Figure 3.13), showing fractions of low molecular weight (LMW), intermediate molecular weight (IMW) and high molecular weight (HMW).

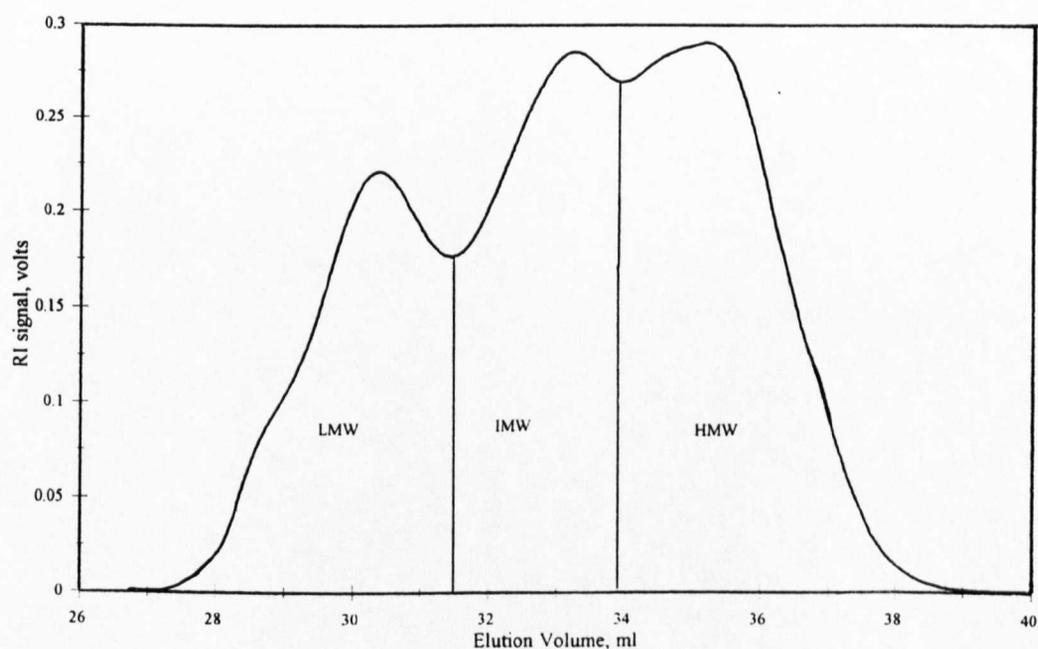


Table 3.10 Chain length and proportion of fractions HMW, IMW and LMW in isoamylase-debranched cassava amylopectins.

Starches from cultivars planted at Zone A

Cultivar	Characteristic	HMW	IMW	LMW	A:B Ratio
MCol 22	DPn	48	22	12	1.27
	DPw	50	23	12	
	Weight,%	31	35	34	
	Mole,%	13	31	56	
MCol 2266	DPn	48	22	12	1.32
	DPw	51	23	12	
	Weight,%	29	35	35	
	Mole,%	12	31	57	
MBra 897	DPn	48	22	12	1.32
	DPw	51	23	12	
	Weight,%	28	36	36	
	Mole,%	11	32	57	
CG 9151	DPn	48	22	12	1.32
	DPw	51	23	12	
	Weight,%	30	35	35	
	Mole,%	12	31	57	
MMex 59	DPn	48	22	12	1.32
	DPw	51	23	12	
	Weight,%	30	35	35	
	Mole,%	12	31	57	
MPar 105	DPn	48	22	12	1.32
	DPw	50	23	12	
	Weight,%	30	35	35	
	Mole,%	12	31	57	
MCol 1468	DPn	47	22	11	1.38
	DPw	50	23	12	
	Weight,%	30	35	35	
	Mole,%	12	30	58	

Table 3.10 (continued)

Cultivar	Characteristic	HMW	IMW	LMW	A:B Ratio
MCR 35	DPn	46	21	12	1.32
	DPw	49	22	12	
	Weight,%	31	35	34	
	Mole,%	13	30	57	
MCol 1522	DPn	47	22	11	1.38
	DPw	49	23	12	
	Weight,%	30	35	35	
	Mole,%	12	30	58	
CG1-37	DPn	47	22	11	1.38
	DPw	49	22	12	
	Weight,%	31	35	34	
	Mole,%	12	30	58	
MVen 77	DPn	48	22	12	1.32
	DPw	50	23	12	
	Weight,%	30	35	35	
	Mole,%	12	31	57	
MPer 195	DPn	48	22	12	1.43
	DPw	50	23	12	
	Weight,%	30	35	35	
	Mole,%	11	30	59	
CG1141-1	DPn	47	22	11	1.38
	DPw	50	22	12	
	Weight,%	31	35	34	
	Mole,%	12	30	58	
MCol 72	DPn	46	21	11	1.27
	DPw	49	22	12	
	Weight,%	32	35	33	
	Mole,%	13	31	56	
MMal 1	DPn	49	22	12	1.27
	DPw	51	23	12	
	Weight,%	30	36	34	
	Mole,%	12	32	56	

Table 3.10 (continued)

Cultivar	Characteristic	HMW	IMW	LMW	A:B Ratio
MCol 1684	DPn	48	22	12	1.32
	DPw	51	23	12	
	Weight,%	28	36	36	
	Mole,%	11	32	57	
MVen 25	DPn	48	22	12	1.27
	DPw	51	23	12	
	Weight,%	31	35	34	
	Mole,%	13	31	56	
MThai 1	DPn	48	22	12	1.32
	DPw	50	22	12	
	Weight,%	30	35	35	
	Mole,%	12	31	57	
MCol 2485	DPn	49	22	12	1.27
	DPw	51	23	12	
	Weight,%	30	36	34	
	Mole,%	12	32	56	
MBra 162	DPn	48	22	12	1.32
	DPw	51	23	12	
	Weight,%	29	36	35	
	Mole,%	12	31	57	
MCol 2215	DPn	48	22	12	1.22
	DPw	51	23	12	
	Weight,%	31	35	34	
	Mole,%	13	32	55	
CM 2772-3	DPn	48	23	12	1.32
	DPw	51	23	12	
	Weight,%	30	35	35	
	Mole,%	12	31	57	
CM 489-1	DPn	47	22	12	1.32
	DPw	50	23	12	
	Weight,%	30	35	35	
	Mole,%	12	31	57	

Table 3.10 (continued)

Cultivar	Characteristic	HMW	IMW	LMW	A:B Ratio
MBra 881	DPn	49	22	12	1.27
	DPw	52	23	12	
	Weight,%	29	37	34	
	Mole,%	12	32	56	
CG 402-11	DPn	48	22	12	1.38
	DPw	51	23	12	
	Weight,%	29	35	36	
	Mole,%	12	30	58	
CG 1118-121	DPn	48	22	12	1.32
	DPw	51	23	12	
	Weight,%	30	35	35	
	Mole,%	12	31	57	
MCol 1132	DPn	48	22	12	1.32
	DPw	51	23	12	
	Weight,%	30	35	35	
	Mole,%	12	31	57	
CG 165-7	DPn	48	22	12	1.32
	DPw	51	23	12	
	Weight,%	30	36	34	
	Mole,%	12	31	57	
CM 3306-4	DPn	48	22	12	1.27
	DPw	50	23	12	
	Weight,%	30	36	34	
	Mole,%	12	32	56	

B1 chains (DP_n 21) and A chains (DP_n 12) reported by Hizukuri (1986) for cassava. The A:B chain ratio ranged from 1.22-1.43; Hizukuri reported a value of 1.5 for cassava amylopectin [Yuan *et al.*(1993) found that the value of 0.89 reported for cassava by Hizukuri appears to be mistakenly calculated from the mole % data of kuzu amylopectin and that the A:B chain ratio should in fact be 1.5). The amylopectin average chain lengths in the fractions HMW, IMW and LMW appeared quite similar between cultivars. The calculation of the average chain length in the whole amylopectin gave values of DP_n 26-27 which were virtually the same between samples. Hizukuri (1985) reported a value of DP_n 26, and Hood and Mercier (1978) a value of DP_n 25. The chain length distributions, *i.e.* the A:B chain ratios, showed some variability with the type of cassava amylopectin. The A:B ratio was found to correlate loosely with the amylose content ($r = -0.41$, $P < 0.05^*$) and the initial pasting temperature ($r = -0.4$, $P < 0.05^*$). It appears that cassava starches with amylopectins of lesser degrees of branching (*i.e.* lower A:B chain ratio) have higher amylose contents and higher pasting temperature (*i.e.* longer time for viscosity development in the amylograph). To confirm these and to find other possible relationships it was decided to repeat the experimental work, with a smaller number of samples from Zone A and from Zone B. A different approach was also introduced in the analysis of the undeveloped amylopectin peaks in the chromatograms. In addition, the measurement of the absolute molecular size of cassava amylose by the laser-light-scattering system was performed in the new experiments.

Eleven starch samples from Zone A and four starches from Zone B were

retested by the isoamylase- debranching-SE-HPLC-MALLS-RI method..

The amylopectin linear chain populations were characterized by deconvoluting the chromatogram profile into the minimum number of Gaussian curves to give an adequate fit as described Section 2.4.8. The Gaussian peak fitting technique gave a better distinction between the A and B chain populations and resulted in a more effective characterization of the A:B chain ratio in the different types of cassava amylopectin.

Seven Gaussian peaks provided an excellent fit to the experimental amylopectin profile; Figure 3.15 shows an amylopectin chromatogram and the fitted Gaussian peaks, while Table 3.11 presents the chain lengths (DP_n), the proportions (weight% and mole%) of the amylopectin chain populations and the A:B chain ratios of all samples. There was no significant difference in the chain lengths between the amylopectin types; the longest chains were at DP_n 80-84 and DP_n 68-75, the intermediate chains at DP_n 47-49, and the shortest chains at DP_n 25-27, DP_n 19-20, DP_n 15, and DP_n 11-12. Peaks 6 and 7 were considered to be populations of A chains, peaks 4 and 5 populations of B_1 chains, following Hizukuri (1986) and, as in the previous analysis these contained 88-89 % of the total chains of amylopectin; peaks 1, 2 and 3 were considered to correspond to the B_4 , B_3 , and B_2 chains of Hizukuri (1986). The A chain average length between amylopectins was close to DP_n 12. This value agreed with the length of fraction A of cassava amylopectin found by Hizukuri (1986), in contrast to the views of Hood and Mercier (1978) who had

proposed an exterior chain length of 15 (length of fraction A) for cassava amylopectin.

Figure 3.15 SE-HPLC chromatogram of debranched amylopectin (in Figure 3.13) and its linear chain components resolved by the fitted Gaussians peaks.

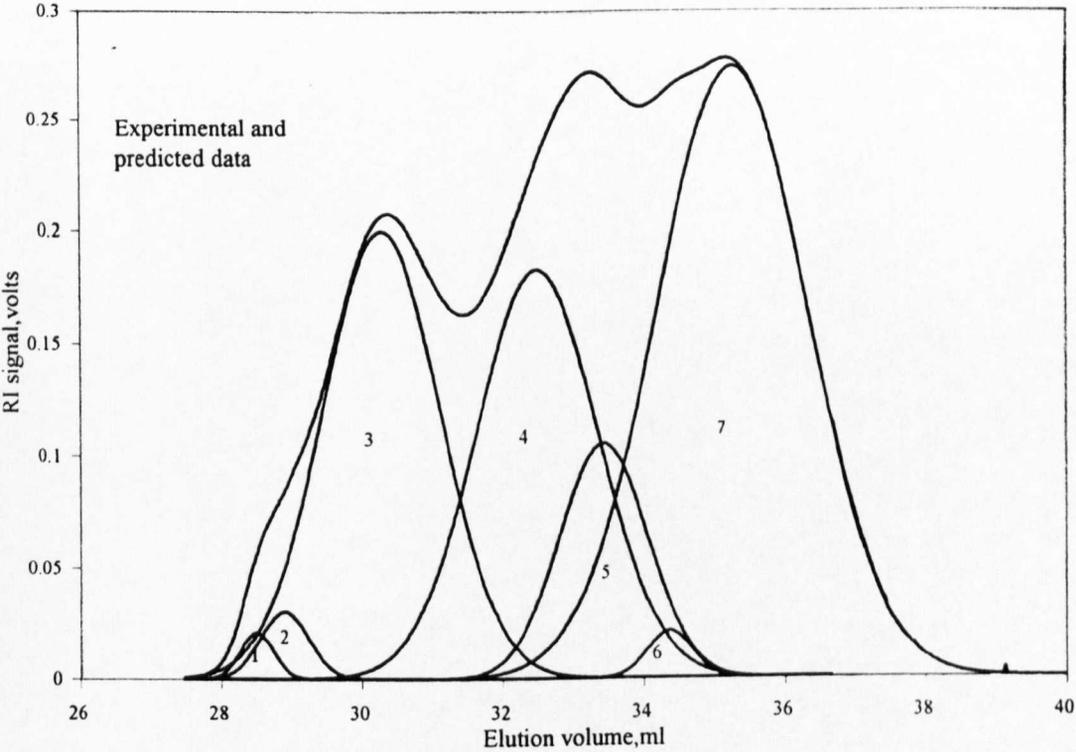


Table 3.11 Chain length and proportion of debranched cassava amylopectin components fitted with Gaussian peaks. Means of duplicate determinations.

Starches from cultivars planted at Zone A

Cultivar (Sample code)	Peak #	DP _n	Weight %	Mol %
MCol 22 (A1)	1	81.2±1.5	0.4±0.0	0.1±0.0
	2	73.7±0.1	2.5±0.3	0.6±0.1
	3	46.5±0.7	28.2±0.8	11.6±0.4
	4	27.0±0.3	13.8±0.0	9.8±0.1
	5	19.8±0.2	19.5±2.4	18.9±2.6
	6	15.1±0.3	1.3±0.2	1.7±0.3
	7	11.5±0.1	34.4±2.1	57.3±2.7
MBra 897 (A3)	1	79.7±1.3	0.7±0.0	0.2±0.0
	2	71.7±1.1	1.6±0.1	0.4±0.0
	3	48.3±0.6	27.4±2.6	11.1±1.5
	4	25.7±0.6	22.2±4.8	16.9±3.7
	5	19.2±0.2	15.5±6.5	16.1±7.3
	6	14.9±0.3	2.1±1.0	2.8±1.5
	7	11.4±0.3	30.5±5.3	52.5±6.5
MMex 59 (A5)	1	80.2±1.6	0.8±0.0	0.2±0.0
	2	71.8±1.6	1.7±0.3	0.5±0.0
	3	48.2±0.0	25.6±1.4	10.2±0.6
	4	25.5±0.7	23.7±3.9	17.9±3.4
	5	19.4±0.3	13.2±0.3	13.1±0.0
	6	14.9±0.0	1.3±0.3	1.7 ±0.4
	7	11.4±0.1	33.6±1.7	56.5±2.3
MCR 35 (A9)	1	81.8±0.7	0.6±0.3	0.1±0.0
	2	73.6±0.2	2.0±0.2	0.5±0.0
	3	48.4±1.1	25.6±0.5	10.2±0.4
	4	25.6±0.3	22.9±2.5	17.3±2.8
	5	19.8±0.2	10.3±0.8	10.0±0.5
	6	15.6±0.3	1.6±0.8	2.0±1.1
	7	11.9±0.3	36.9±3.2	59.8±3.9

Table 3.11 (continued)

Starches from cultivars planted at Zone A

Cultivar (Sample code)	Peak #	DP _n	Weight %	Mol %
MCol 1522 (A10)	1	80.7±0.3	0.5±0.4	0.1±0.1
	2	73.4±2.2	1.9±0.5	0.5±0.1
	3	47.5±0.3	27.8±2.2	11.2 ±1.1
	4	26.8±2.4	16.2±11	11.9±8.7
	5	20.1±1.0	16.9±9.9	15.9±8.8
	6	15.1±0.1	1.3±0.2	1.7±0.2
	7	11.5±0.0	35.4±1.0	58.8±1.1
CGI-37 (A11)	1	79.6±2.4	1.2±0.4	0.3±0.1
	2	70.3±2.6	2.0±0.1	0.5±0.0
	3	49.5±0.0	26.5±0.4	10.5±0.2
	4	24.7±1.2	25.9±5.4	20.3±5.1
	5	19.4±0.3	9.2±1.9	9.1±1.8
	6	15.2±0.1	2.3±1.5	2.9±2.0
	7	11.2±0.5	32.9±4.3	56.3 ±4.9
MPer 196 (A13)	1	80.3±0.3	0.7±0.4	0.2±0.1
	2	72.1±2.2	1.4±0.5	0.4±0.1
	3	48.1±0.3	25.6±2.2	10.0±1.1
	4	25.9±2.4	22.2±11	16.1±8.7
	5	19.8±1.0	11.4±9.9	10.9±8.8
	6	15.2±0.1	1.5±0.2	1.9±0.2
	7	11.6±0.0	37.2±1.0	60.4±1.1
MVen 25 (A18)	1	81.7±2.4	0.7±0.4	0.2±0.1
	2	73.2±2.6	2.3±0.1	0.6±0.0
	3	48.3±0.1	27.3±0.4	11.2±0.2
	4	26.2±1.2	20.1±5.4	15.4±5.1
	5	19.6±0.3	16.9±1.9	17.1±1.8
	6	15.1±0.1	2.2±1.5	3.0±1.9
	7	11.5±0.5	30.4±4.3	52.4±4.8

Table 3.11 (continued)

Starches from cultivars planted at Zone A				
Cultivar (Sample code)	Peak #	DP _n	Weight %	Mol %
MBra 881 (A26)	1	81.7±0.3	0.7±0.4	0.2±0.1
	2	73.2±2.2	1.6±0.5	0.4±0.1
	3	48.7±0.3	25.8±2.2	10.2±1.1
	4	25.7±2.4	23.2±11	17.4±8.7
	5	19.5±1.0	12.7±9.9	12.5±8.8
	6	15.0±0.1	1.8±0.2	2.3±0.2
	7	11.6±0.0	34.3±1.0	57.0±1.1
CG118-121 (A28)	1	81.1±2.4	0.6±0.4	0.2±0.1
	2	72.9±2.6	1.9±0.1	0.5±0.0
	3	47.8±0.1	27.4±0.4	11.1±0.2
	4	26.5±1.2	17.7±5.4	13.0±5.1
	5	19.7±0.3	16.6±1.9	16.3±1.8
	6	15.1±0.1	1.7±1.5	2.2±1.9
	7	11.6±0.5	34.0±4.3	56.8±4.9
CM3306-4 (A33)	1	80.6±0.3	0.6±0.4	0.1±0.1
	2	72.9±1.8	2.3±0.5	0.6±0.1
	3	47.9±0.1	26.1±0.9	10.4±0.5
	4	25.6±0.2	21.7±2.0	16.2±1.5
	5	19.6±0.1	12.0±0.5	11.8±0.5
	6	15.2±0.1	1.3±0.0	1.7±0.0
	7	11.6±0.1	35.9±0.4	59.1±0.6

Table 3.11 (continued)

Starches from cultivars planted at Zone B

Cultivar (Sample code)	Peak #	DP _n	Weight %	Mol %
CG1-37 (B11)	1	83.9±0.0	0.3±0.2	0.1±0.0
	2	75.9±0.4	3.8±0.6	1.0±0.2
	3	47.9±0.3	26.4±1.2	10.9±0.8
	4	25.9±1.3	19.4±7.0	14.8±5.8
	5	19.2±0.1	17.0±9.3	17.5±9.8
	6	15.0±0.4	1.3±0.7	1.7±1.0
	7	11.6±.3	31.8±4.9	54.0±6.0
MPer 196 (B13)	1	78.8±1.5	1.9±2.3	0.5±0.6
	2	68.3±7.3	1.9±2.0	0.5±0.5
	3	48.1±0.5	25.3±1.4	10.2±0.8
	4	25.5±0.9	22.9±1.6	17.5±2.0
	5	19.1±0.7	13.9±0.8	14.1±1.4
	6	14.9±0.4	1.5±0.7	2.0±1.0
	7	11.4±0.3	32.6±4.2	55.2±5.1
MVen 25 (B18)	1	79.8±6.1	0.5±0.5	0.1±0.1
	2	72.9±5.1	4.0±1.4	1.1±0.5
	3	48.3±0.6	23.5±2.3	9.4±0.7
	4	24.9±0.8	26.0±3.3	20.4±3.6
	5	19.1±0.6	12.2±2.1	12.5±2.7
	6	14.7±0.4	2.2±1.6	3.0 ±2.2
	7	11.3±0.4	31.4±6.6	53.5±8.5
MBra 881 (B26)	1	83.7±1.7	0.7±0.0	0.2±0.0
	2	74.7±1.0	2.9±0.3	0.8±0.0
	3	48.8±0.3	26.9±1.7	11.1±0.9
	4	25.0±0.7	25.8±0.1	20.7±0.8
	5	19.0±0.6	12.2±1.1	12.9±1.7
	6	14.7±0.4	2.1±1.4	2.8±2.0
	7	11.5±0.3	29.4±3.9	51.4±5.2

There was an apparent effect of the cassava amylopectin type on the relative proportions of A (DP_n 12-15) and B_1 chains (DP_n 19-27), as recorded in Figure 3.16. The change of the populations of A and B_1 chains within the different amylopectin types affected the ratios of the A: $B_{1..4}$ chain population giving a range from 1.1 to 1.63 (Figure 3.17). It was observed that Zone B starches had amylopectins with lower A:B ratios than the majority of amylopectins from Zone A starches. As indicated above, the higher environmental temperature in plants of Zone B could have reduced the synthesis of amylopectin branches as a result of the lower activity of soluble starch synthase (SSS) (Jenner, 1996) in cassava starch at higher temperatures. A possible consequence of this reduction in enzyme activity is that Zone B amylopectins would possess a relatively lower population of external chains (A chains). However, the variation of the degree of branching in amylopectins from Zone A starches was attributed to endogenous factors in the synthesis of starch in the different cassava cultivars.

The ratio of A:B chains, or the degree of multiple branching (Manners, 1985), is an important property of amylopectin. In this study the different degrees of branching between the amylopectins of cassava starch were found to be related, to some extent, to the properties of the starches during their heating in excess water. The correlations of A:B ratios with the starch solubilities and with the swelling volumes of the starches were $r = -0.58$ ($P < 0.05^*$) and $r = 0.41$ ($P < 0.05^*$), respectively. These A/B ratio relationships were more evident in the behaviour of the starches on pasting, since the starches with amylopectins of a higher degree of branching tended

Figure 3.16 Chain lengths (DP_n) and populations (mole content) in cassava amylopectins.

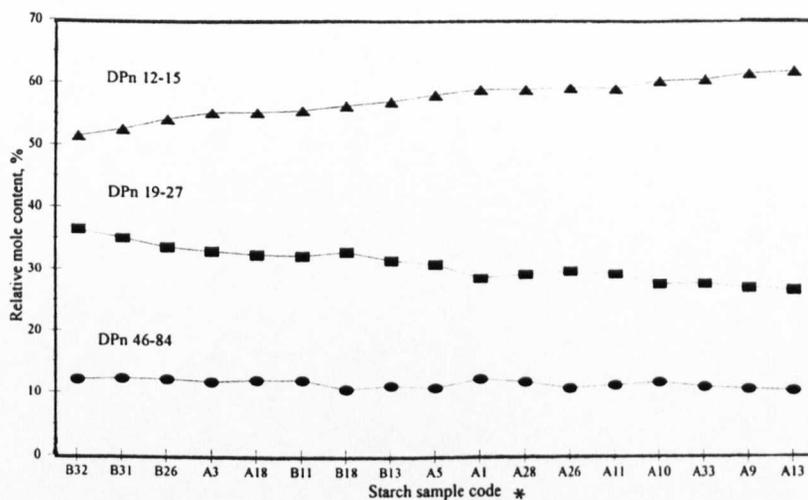
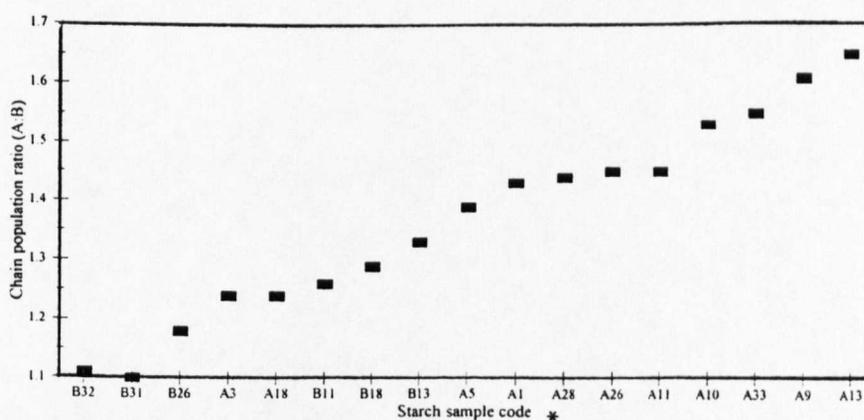
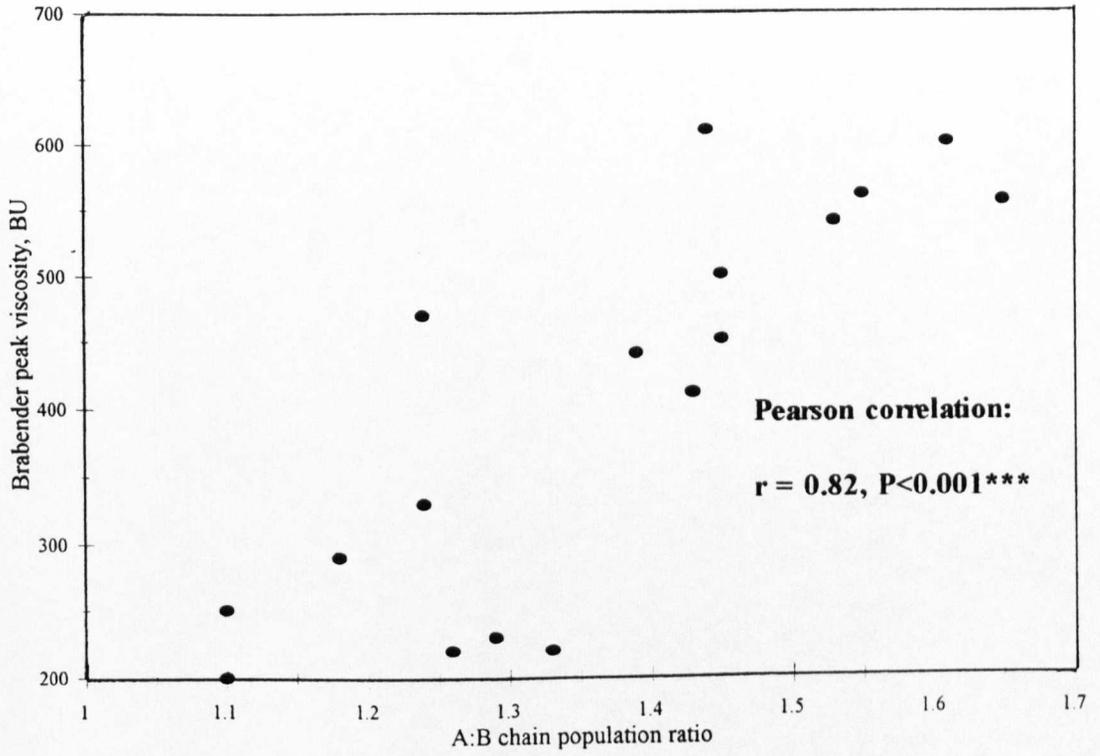


Figure 3.17 Ratio of A:B chain population in cassava amylopectins.



* Note: For identification of the sample codes see Table 3.11. The sample codes B31 and B32 correspond to the cultivars MMal 2 and MNga 2 grown in Zone B. These two starches were not listed in the Table 3.11.

Figure 3.18 Relationship of Brabender peak viscosity of cassava starch aqueous pastes and the ratio of A:B chain populations in the starch amylopectins.



to develop higher viscosities on pasting (Figure 3.18) and higher gel strengths in the cooled pastes ($\log G'$, Section 3.1.3). It has been thought that the presence of side branches in starch can affect substantially its functional properties, a branching structure favouring swelling and high viscosity (Hwang and Kokini, 1991). The A:B chain ratio also correlated positively with the degree of polymerization of amylose (page 117) ($r = 0.71$, $P < 0.01^{**}$); this relationship is not fully understood, but it can be explained from the point of view of starch synthesis. Amylose and amylopectin biosynthesis may occur simultaneously, and the activities of the enzymes responsible for chain-lengthening in amylose (insoluble starch synthase) and for branching in amylopectin (soluble starch synthase and Q-enzyme complexes) (Manners, 1989) can follow the same trends. Therefore, in cassava starches from plants under the same environmental conditions, a higher molecular weight amylose starch could be associated with a higher degree of branching in amylopectin. The relationship observed in this study between degree of branching in amylopectin and the length of the amylose chains may not hold with starches of different botanical origins, e.g. a view noted by Ong *et al.*, 1994 who found that the values of the molecular weights of amylose and A:B chain ratios of amylopectin did not correlate in starches from wheat, waxy rice, potato, cassava and sweet potato.

The starch granule size (Section 3.1.4) appeared partially correlated to the extent of branching in the amylopectin ($r = 0.5$, $P < 0.01^{**}$) and to the chain length of the amylose ($r = 0.43$, $P < 0.05^*$) (page 117), which suggests that highly branched amylopectin and longer amylose molecules are expected to occur in the larger granules

in cassava starch.

Molecular weight of cassava amyloses derived from debranched starch.

The debranched starch solutions of the previously tested samples from Zone A (11) and Zone B (4) were monitored by the multi-angle-laser-light-scattering photometer in the SE-HPLC system (Section 2.4.8). The laser light scattering results were used as a measure of the absolute molecular weight of amylose (*i.e.* the higher molecular weight material eluted first from the columns). The average DP values of the amylose peaks together with the DP distributions of the individual amyloses are presented in Table 3.12. These results show that cassava amyloses have high molecular weights (*i.e.* DP's) with molecules exhibiting a wide distribution in their sizes. In general, the molecular weight of cassava amylose is high and comparable to amyloses of potato and sweet potato, but with a wider molecular distribution (Takeda *et al.*, 1984; Hizukuri and Takagi, 1984; Takeda *et al.*, 1987). The higher temperatures experienced by Zone B plants could affect the starch synthesis (as discussed above), resulting in the amyloses from Zone B plants having lower molecular weights (DP_n 3825-4427) and a wider DP distribution than those from the same cultivars grown in Zone A (DP_n 5382-6513). The DP and the range of DP distribution of amyloses of individual cultivars in this study (DP_n and DP_w varied for different cultivars between 3825 and 6785, and from 10557 to 7170 respectively) were comparable to the value of DP_w 7710 reported by Suzuki *et al.* (1985), but higher than the DP values reported by Hizukuri *et al.* (1981) (DP_n 3390), Takeda *et al.* (1984) (DP_n 2660), Hizukuri and Takagi (1984) (DP_w 6680, with a range of DP_w

Table 3.12 Molecular properties of amylose derived from debranched cassava starch and separated and detected in the SEC-HPLC-RI-MALLS system

Cultivar	DP _w	DP _n	DP _w /DP _n	DP _w distribution ^a
Cassava cultivars from Zone A				
MCol 22	8442	5073	1.7	24375 - 2474
MBra 897	7657	4412	1.7	18613 - 1576
MMex 59	10557	6785	1.6	30507 - 3808
MCR 35	9726	6197	1.6	24242 - 2883
MCol 1522	8747	5179	1.7	22786 - 2127
CG1-37	8847	5572	1.6	23293 - 2652
MPer 196	10339	6513	1.6	29844 - 3421
MVen 25	8977	5382	1.7	24944 - 2321
CM 3306-4	9089	5704	1.6	23935 - 2721
MBra 881	10446	6328	1.7	33028 - 3657
CG1118-121	10071	6500	1.5	26238 - 3310
Cassava cultivars from Zone B				
CG1-37	8255	4228	2.0	30688 - 2127
MPer 196	8647	4427	2.0	34587 - 2536
MVen 25	7554	3825	2.0	28872 - 1948
MBra 881	7170	3900	1.9	26488 - 1843

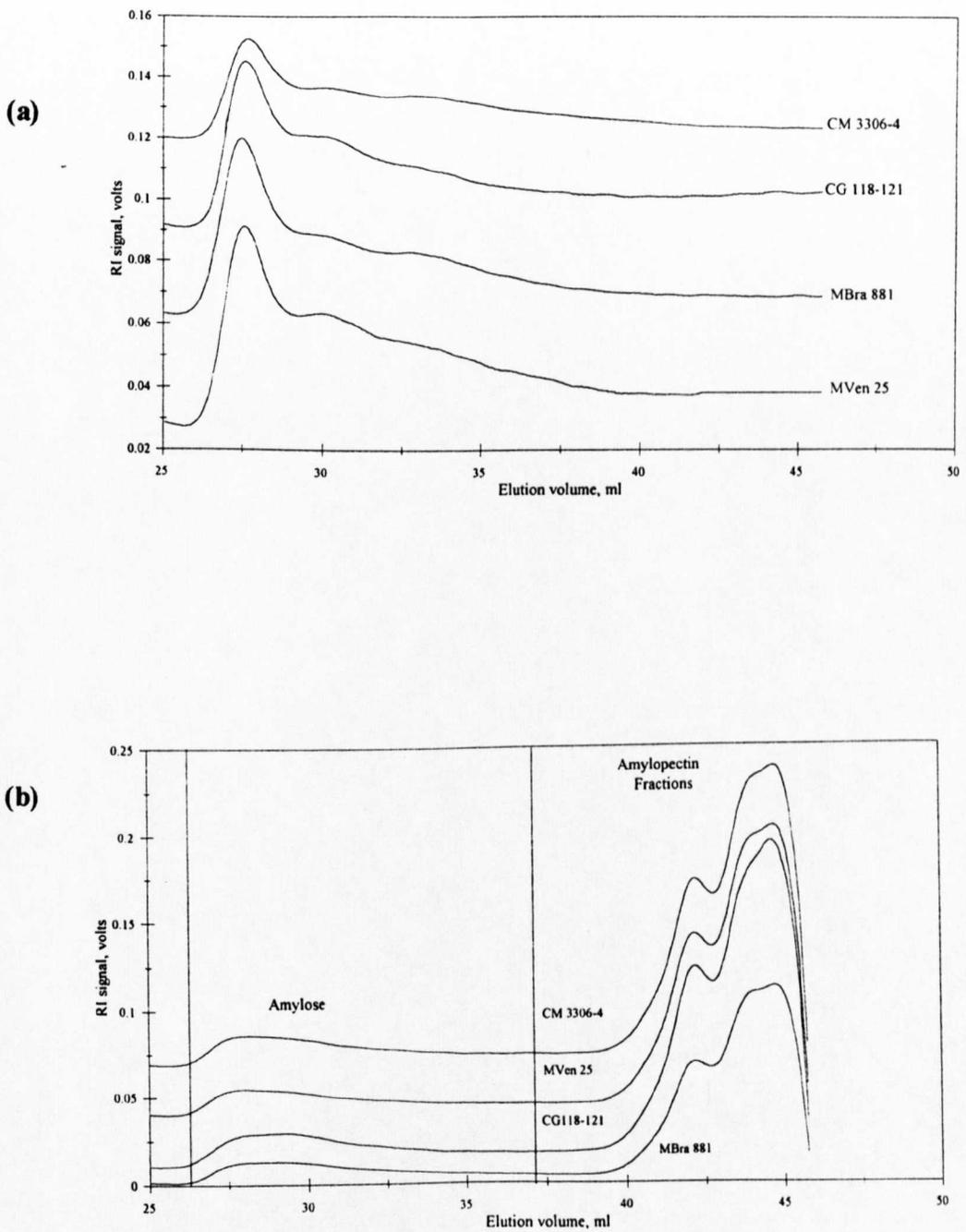
^a Each value represents 10% of amylose mass eluted from the SEC-HPLC with the highest and the lowest average molecular weight.

distribution in an individual cassava amylose of 580-22400). These researchers pre-separated amyloses using techniques which included several refining steps to ensure that the amylose was free of amylopectin, and used different techniques to determine their molecular weights e.g. Hizukuri and Takagi (1984) used preisolated amylose solutions and an HPLC size exclusion system on-line with a low-angle laser-light-scattering detector to determine DP_w , while Takeda *et al.* (1984) determined DP_n by a reducing end group analysis. They also used cassava starches of unknown cultivars, the samples were of commercial origin, which could be a mixture from more than one cultivar. Recently Ong *et al.* (1993) tested debranched cassava starch from Malaysia (commercial origin) on the same system used in this study and reported a DP_n of 3642, which falls within the range of molecular weights found in this study. The DP_n values obtained were compared with other characteristics of these starches and some interesting correlations were found. There were positive, significant correlations with the peak viscosity ($r = 0.84$, $P < 0.001^{***}$) and swelling volume ($r = 0.81$, $P < 0.001^{***}$), and negative significant correlations with pasting temperature ($r = -0.80$, $P < 0.001^{***}$) and solubility ($r = -0.79$, $P < 0.001^{***}$). It appears, therefore, that longer cassava amylose molecules were associated with starches of lower aqueous solubility and higher swelling, which resulted in higher and faster viscosity development on pasting. There was a modest correlation with the starch crystallinity ($r = -0.62$, $P < 0.01^{**}$) which could be explained assuming that the amorphous, amylose-rich domain of the starch granule is partially enhanced with longer amylose molecules and in turn the relative crystalline area is diminished.

Molecular nature of whole starch.

Studies on the molecular structure of cassava starch were carried out in the SE-HPLC-MALLS-RI system but using a different column arrangement with a larger exclusion volume (Section 2.4.8). The gel permeation system was designed to isolate amylopectin and amylose without the previous debranching treatment. The permeation volume of the SE-HPLC columns was between 27.5 ml (void volume) and 50 ml (total volume). As in the preceding experiments, eleven samples from Zone A plants were tested. The starch solutions when ready for injection into the SE-HPLC system had solubilities ranging from 54 to 76% (Table 3.13). These solubilities are a measure of the starch which is dispersed as non-aggregated amylose or amylopectin after centrifugation and filtration to exclude dispersed polymer aggregates (Jackson, 1991). The dispersed starch was determined by the phenol sulphuric acid method (Dubois *et al.*, 1956) and the solubility was calculated by dividing this quantity by the amount of starch that would have been injected into the columns if the starch were 100% soluble. The starch solutions injected into the SE-HPLC eluted between the void volume and 37 ml; an example of four elution profiles is shown in Figure 3.19a. As expected, the molecules that eluted first from the SE-HPLC columns had the largest hydrodynamic volumes and molecular weights as detected by the laser-light-scattering system. The observation of the full set of elution patterns suggested that there was no apparent difference in the molecular size distribution of the dispersed starch fractions between samples. As can be seen in Figure 3.19a, a full separation of amylose from amylopectin was not possible. Most of the amylopectin molecules eluted at the void volume but other amylopectin molecules of lower hydrodynamic volumes

Figure 3.19 SE-HPLC chromatograms of (a) whole and (b) amylose and amylopectin fractions isolated from debranched cassava starches. SE-HPLC columns: TSK PWXL G6000, G5000, G4000, G3000, and G2500.



eluted dispersed up to 37 ml (this permeation range of amylopectin was verified by injecting a pure potato amylopectin dispersion from Sigma). An explanation for this elution pattern of amylopectin is the presence of degraded amylopectin which could be created by either the breakdown of amylopectin molecules during the alkaline dispersion and solubilisation [although precautions were taken by dispersing the samples in oxygen free medium and with a low heat treatment (Section 2.4.7)] , or through the presence of a natural, wide-size range of cassava amylopectin molecules. Amylose, presumably, eluted in the same permeation range as amylopectin, but with a lower proportion of molecules near the void volume (this permeation range was verified with the elution pattern of pure amyloses isolated from debranched cassava starch solutions in this column system, Figure 3.19b). Although, the amylose molecules derived from debranched starch (the amylose peaks in Figure 3.19b) appeared at the same elution volumes as a high proportion of the amylopectin molecules in Figure 3.19a, (*i.e.* amylose molecules from debranched starch and dispersed amylopectin molecules in the whole starch occupied the same hydrodynamic volumes) the latter were of higher molecular weights when analysed by the laser light scattering system. An explanation of this is that the amylose derived from debranched starch (composed essentially the long linear chains) could have exhibited a different molecular conformation and behaviour in solution than amylopectin from the native starch (comprising of multibranched molecules). Indeed, linear and branched polymers of the same molecular weight occupy different hydrodynamic volumes. Therefore, when compared to a linear polymer, a branched polymer of the same molecular weight displays a smaller radius of gyration (Hwang and Kokini, 1991), *i.e.* free amylose and

some dispersed molecules of amylopectin could occupy the same hydrodynamic volume and yet exhibit different molecular weights. In a parallel research programme in CIRAD, France, several of the starch samples from this study were dispersed in KOH at 4° C for 7 days and injected into a different column system. Similar elution patterns were obtained again with incomplete separation of amylose from the amylopectin (Mestres, 1995).

The average molecular weights of the whole starch dispersions were measured by the MALLS system as they eluted from the SE-HPLC columns. The results are displayed in the Table 3.13. They appear different between samples, but it is difficult to determine if the difference was related to the starch type, since the samples had different molecular solubilities. There was some correlation between the weight-average molecular weight numbers (M_w) and the solubilities ($r = -0.65$, $P < 0.01^{**}$), indicating that a higher solubility was obtained by possible depolymerization of molecules during the alkaline dispersion of the starch samples.

Since all the starch samples were debranched and injected into the SE-HPLC columns for comparison with the elution profiles of the whole starches, the amyloses derived from debranched starch were examined further by the MALLS system as they eluted from the SE-HPLC columns. The values are presented in Table 3.14 and reinforce the information on the molecular weights of amylose presented in the preceding section.

Table 3.13 Molecular weight distribution of whole cassava starch, eluted in the SEC-HPLC system* and detected by the MALLS system.

Cluster	Cultivar	HPLC - Solubility**	M_w	M_n	M_w/M_n
1	MBra 897	61.6	138×10^5	93×10^5	1.5
2	MMex 59	75.9	140×10^5	75×10^5	1.9
3	MCR 35	70.2	108×10^5	61×10^5	1.7
	MCol 1522	66.2	$128(\pm 6.1) \times 10^5$	$84(\pm 15) \times 10^5$	1.3
4	CG 1-37	56.1	138×10^5	89×10^5	1.5
	MPer 196	67.8	138×10^5	89×10^5	1.5
6	MVen 25	66.3	$139(\pm 7.8) \times 10^5$	$91(\pm 6.4) \times 10^5$	1.5
7	CM 3306-4	53.8	127×10^5	78×10^5	1.6
8	MBra 881	56.7	$156(\pm 3.5) \times 10^5$	$108(\pm 4.3) \times 10^5$	1.6
9	CG1118-121	56.8	155×10^5	103×10^5	1.5

* Columns: TSK PWXL G6000, G5000, G4000, G3000, G2500.

** Amount of starch that would have been injected into the HPLC columns if the starch were 100% soluble.

Table 3.14 Molecular weight distribution of amylose derived from debranched cassava starch, separated in the SEC-HPLC system* and detected by the MALLS system.

Cluster	Cultivar	M_w	M_n	M_w/M_n
1	MCol22	1.74×10^6	0.88×10^6	1.9
	MBra 897	1.89×10^6	1.12×10^6	1.7
2	MMex 59	1.71×10^6	0.96×10^6	1.8
3	MCR 35	1.79×10^6	1.14×10^6	1.6
	MCol 1522	2.07×10^6	1.11×10^6	1.6
4	CG 1-37	1.35×10^6	0.68×10^6	2.0
	MPer 196	1.67×10^6	0.89×10^6	1.9
6	MVen 25	2.00×10^6	0.82×10^6	2.4
7	CM 3306-4	1.59×10^6	0.71×10^6	2.2
8	MBra 881	1.81×10^6	0.69×10^6	2.6
9	CG1118-121	1.65×10^6	0.68×10^6	2.4

* Columns: TSK PWXL G6000, G5000, G4000, G3000, G2500.

3.1.8 Aqueous exudates from cassava starch

Experiments were carried out to investigate the molecular composition and structure of starch released and solubilised from cassava starch granules during the pasting of aqueous starch suspensions. Starch suspensions (1%) were pasted at 95°C as described in Section 2.4.7 and the solubilised starches in the supernatants were debranched and subjected to SE-HPLC analysis. Eleven starches from Zone A plants and four from Zone B plants were investigated.

The leaching process was monitored at different stages (times) during the pasting of starch suspensions from cultivar MCol 1522 (Section 2.4.8). Figure 3.20 shows the chromatograms of debranched components of soluble starches recovered after pasting at the times indicated. Table 3.15 presents the amylose content of the soluble starch (the proportion of the amylose peaks or the first material eluted from the SE-HPLC columns), the leached amylose content in the total starch that was pasted, and the weight-average molecular weight of amylose. Amylose appeared as a major component in the leachate at the very commencement of the starch gelatinization and increased to about 69% of the starch in solution after pasting for 0.5 hr. Amylopectin had also leached profusely at 1 and 2 hr of pasting to form a starch solution with an amylose:amylopectin ratio of nearly 1:1. These results suggest that cassava starch extracted by aqueous leaching, without stirring, at 95°C for 1-2 hr could be composed of approximately equal amounts of amylose and amylopectin.

Leached starches from all the samples, extracted at 95°C for 1hr (without

Figure 3.20 SE-HPLC of linear chains of aqueous leached cassava starch extracted at 95°C at the times indicated. SE-HPLC columns: TSK G3000 PWXL, 2xAsahipak GS-320H, TSK G2500 PWXL and OLIGO PWXL.

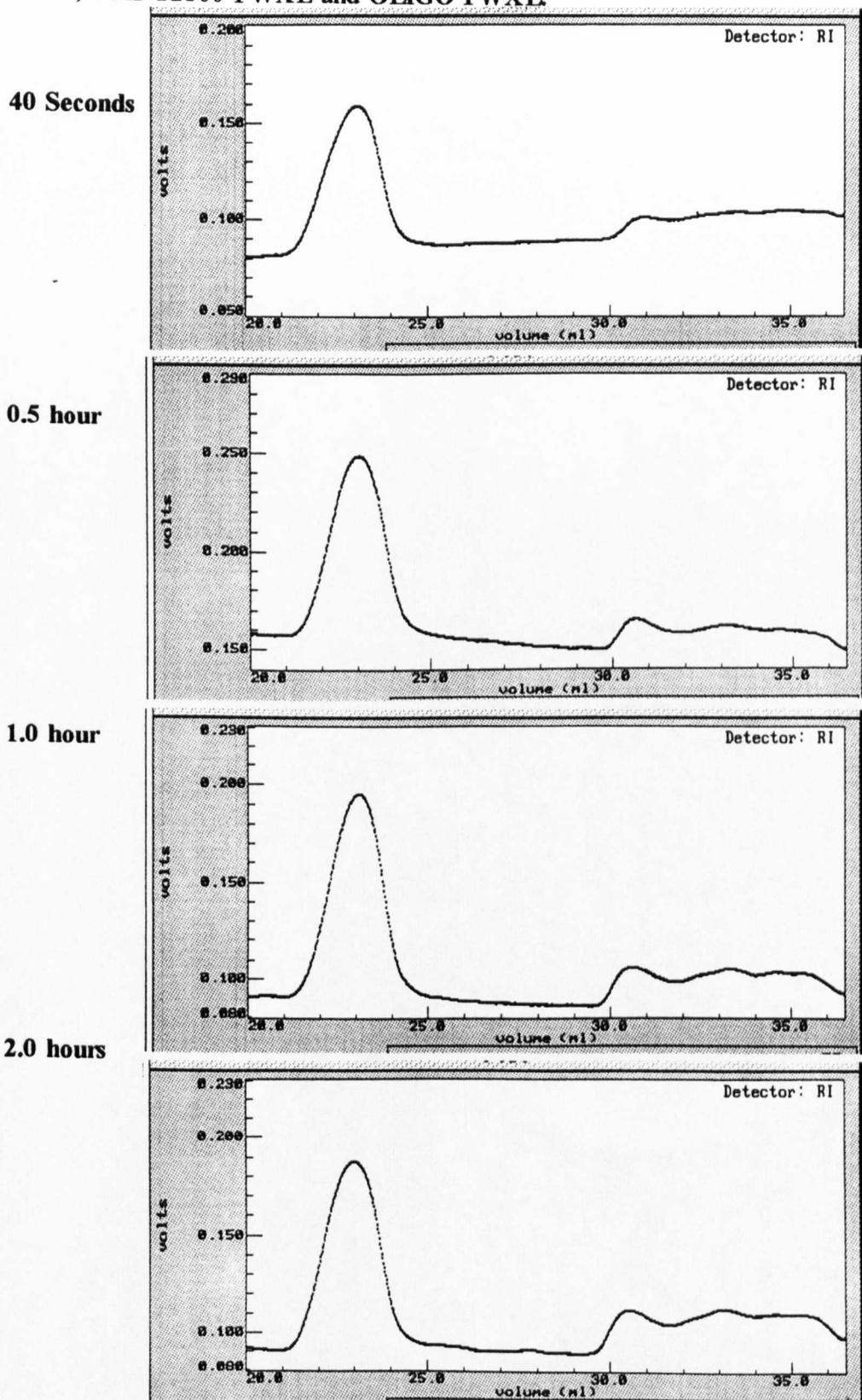


Table 3.15 Leached amylose in 1% MCol 1522 starch pastes cooked at 95 °C at different times.

Pasting time	Leached amylose		Molecular weight	
	A	B	M_w	M_w/M_n
30-40 s (starting of the granule gelatinisation)	65.8	9.4	1.5×10^6	1.4
0.5 hr	68.7	11.1	1.5×10^6	1.5
1.0 hr	60.9	10.2	1.5×10^6	1.4
2.0 hr	59.9		1.6×10^6	1.4

A: Proportion of amylose in the leached starch (%)

B: Amount of leached amylose in the pasted starch (g/100 g of starch)

stirring), were isoamylase debranched and examined by the SE-HPLC-RI-MALLS system. The amylose proportion (%) (SE-HPLC-amylose) in the leached starch and the amount of leached amylose (g/100 g of starch) in the pasted starch were obtained from the chromatograms and the content of solubilised starch in the pasted starch (Table 3.16). The facility with which amylose was released from the granules varied between starch types and production zone as can be deduced from the values of leached amylose in the pasted starch. Amylose was not always the main compound released into the solution phase when cassava starch was pasted under the conditions of this study. The proportion (%) of amylose in the total leached starch obviously depended on the amount of leached amylopectin which was also easily leached from the starch granules. The ratios of the amylose:amylopectin varied from 1:0.8 to 1:1.7 (calculated from values in the Table 3.16). The lowest amylose:amylopectin ratios were from starches taken from Zone B plants. The relatively high proportion of amylopectin dispersed in the solubilised phase of the cassava starch pastes could have played an important role in their rheological properties, e.g. some starch pastes had a weak character as shown Section 3.1.3. Nevertheless, the level of gel strength ($\log G'$) exhibited by the fresh pastes, as expected, correlated positively with the proportion (%) of amylose in the leached starch ($r = 0.73$, $P < 0.01^{**}$). It also correlated quite well with the Brabender paste viscosities, e.g. the peak viscosity ($r = 0.86$, $P < 0.001^{***}$) and "cool viscosity"- $V_{50C,20 \text{ min}}$ ($r = 0.94$, $P < 0.001^{***}$). There was also a significant negative correlation between the amount of amylose leached from the starch and the DP of amylose in the original or native starches ($r = -0.72$, $P < 0.01^{**}$), indicating that the shorter the amylose in the starch, the higher the amylose content

Table 3.16 Leached starch in 1% cassava starch pastes cooked at 95°C for 1 hr

Cluster	Cultivar	SE-HPLC-Amylose in leached starch [*] (%)	Starch solubilised in the paste (g/100 g starch)	
			Amylose [*]	Amylopectin [*]
Cassava cultivars from Zone A				
1	MCol 22	53.0±1.1	11.2±0.3	10.0±0.3
	MBra 897	51.2±2.2	11.4±0.7	10.9±0.5
2	MMex 59	51.7±2.2	9.8±0.6	9.1±0.4
3	MCR 35	58.6±4.2	9.6±1.0	6.8±0.7
	MCol 1522	58.9±2.1	10.3±0.5	7.2±0.4
4	CG1-37	53.8±2.9	10.2±0.8	8.8±0.6
	MPer 196	54.6±2.8	9.5±0.7	7.9±0.5
6	MVen 25	49.7±5.3	8.0±1.2	8.1±0.9
7	CM 3306-4	62.0±0.6	9.4±0.1	5.8±0.1
8	MBra 881	50.2±5.4	7.0±1.1	7.0±0.8
9	CG1118-121	63.6±4.6	10.8±1.1	6.2±0.8
Cassava cultivars from Zone B				
4	CG1-37	44.7±1.5	12.6±0.6	15.6±0.4
	MPer 196	46.1±0.9	12.9±0.3	15.1±0.2
6	MVen 25	43.3±0.9	13.1±0.4	17.2±0.3
8	MBra 881	52.4±2.4	11.5±0.7	10.5±0.6

* Mean of duplicate determinations.

released during the starch pasting. Low amylose as well as low amylopectin leaching was associated with high swelling of starch granules which paralleled the observations in rice starch by Reddy *et al.* (1993) who established that rice starches with low contents of leached amylose (they termed high soluble amylose equivalent) had granules that resisted swelling and disintegration.

In Section 3.1.3 it was shown that Zone B starch pastes developed the highest gel strength after storing at 4°C for 24 h, reversing the situation exhibited by the fresh pastes. As recorded above, the solubilised starch in pastes from Zone B starches had the lowest proportion (%) of amylose (or the lowest amylose:amylopectin ratios) but, in absolute terms, the pastes contained the highest amount of leached amylose, as well as amylopectin. The high proportion of amylopectin in the solubilised starch possibly interfered with and delayed the rapid reassociation of abundant amylose molecules in the fresh pastes cooled to 25 °C. However, as the pastes were cooled to 4°C and aged, the abundant amylose molecules (exhibiting the lowest degree of polymerization as noted below) finally reassociated to form the structure which resulted in pastes with high gel strengths.

The degrees of polymerization (DP_w and DP_n) of the leached amyloses (Table 3.17) measured by the laser-light-scattering system were lower than those of the native starches. The leached amyloses had a narrower range of polydispersity (DP_w / DP_n), from 1.4-to 1.5, than the previous range of 1.5 - 2.0 obtained for the corresponding native starches. Similar observations were reported with rice starch by

Table 3.17 Molecular properties of leached cassava amylose* separated from debranched leached starch and detected by the SEC-HPLC-RI-MALLS system.

(* Leached amylose in 1% starch pastes cooked at 95°C)

Cultivar	DP _w	DP _n	DP _w /DP _n	DP _w distribution ^a
Cassava cultivars from Zone B				
MCol 22	7688	5211	1.5	19116 - 2680
MBra 897	6874	4788	1.5	16453 - 2408
MMex 59	7536	5135	1.5	19356 - 2734
MCR 35	7867	5642	1.4	17810 - 2868
MCol 1522	7262	5265	1.4	16704 - 2752
G1-37	7293	5047	1.4	16592 - 2590
MPer 196	7930	5725	1.4	18088 - 3205
MVen 25	7076	5103	1.4	16225 - 2830
MBra 881	7331	5170	1.4	16758 - 2574
CG1118-121	8388	6067	1.4	19183 - 3260
CM 3306-4	8016	5685	1.4	18749 - 2936
Cassava cultivars from Zone B				
CG1-37	5498	3770	1.5	14216 - 2045
MPer 196	5359	3518	1.5	14228 - 1764
MVen 25	4371	2895	1.5	8257 - 1207
MBra 881	5266	3654	1.4	12648 - 1835

^a Each value represents 10 % of amylose mass eluted with the highest and the lowest average molecular weight.

Ong and Blanshard (1995b). As with the native starches, the leached amyloses from plants of Zone B had the lowest DPs. The DP of leached amyloses varied between samples and followed, in general, the same trend as the DP of amyloses between the native starches. It was also noticed that the leached amyloses had smaller DP's as the native starches increased in amylose content ($r = - 0.74, P < 0.001^{***}$) (a similar trend was observed with corn starch by Jackson *et al.*, 1989).

The chromatograms of the leached amylopectins showed a less distinctive multi-component configuration. The analysis of the fractionated SE-HPLC linear chain populations from the leached amylopectins showed that the small proportion of the longest B chain populations detected in the native starches in the preceding analysis ($DP_n = 68-84$, Table 3.11) were absent in the leached amylopectin from all the samples, since it eluted from 29.5 ml., and not from 27.5 ml as the amylopectins from the native starches. As a result, the average DP_n values obtained for the longer chain length fractions were in the range of 41-43 (Table 3.18) which were smaller than the values of 46-48 obtained for the fraction HMW from the native starches (Table 3.10). The DP_n values obtained for the shorter chains 22 and 11-12 (Table 3.18) were comparable to those found in the IMW and LMW amylopectin fractions (Table 3.10) from the native starches. In conclusion it is deduced that the amylopectin molecules which contain B chains as long as $DP_n 68-84$ (Table 3.14) did not leach from the starch granules under the conditions used (95 °C, 1 hr, no stirring).

In Section 3.1.4 it was suggested that the starch granule size is a factor which

partially affects the extent of leaching from the starch granule. Some experiments were carried out to investigate further the leaching components from the starch granules. Starches from cultivars MVen 25 and CM3306-4 were fractionated in samples of different granule size distributions. By successive sedimentation stages in water in a 2000 ml glass cylinder, the samples were fractionated using the settling velocity of the granules. A faster sedimentation was expected from the bigger, heavier granules. Starch Mven 25 was separated into three fractions of average granule size 9.5, 14.4, and 16.6 μm , and starch CM3306-4 into two fractions of average granule size 10.5 and 14.7 μm (Table 3.19). After cooking (for 1 hr at 95°C) starch suspensions (1%) of the size graded starch fractions, the amount of starch extracted by aqueous leaching varied according to the size of the granules (Table 3.19), showing as in Section 3.1.4 that a mass of starch with small granules could release starch more profusely through the greater surface area per unit of mass than a mass of starch composed of large granules. The intensity of starch solubilisation limited the extent of swelling of granules as is evident in Figure 3.21. This reinforces the idea suggested earlier, that besides the inherent physical binding or associative forces between molecules in each type of starch, the degree of swelling is partially controlled by the amount of starch solubilised which in turn is affected by the size of the granules in the starch mass. Finally, the composition and molecular characteristics of the leached starches (Table 3.19) did not show any relation with the amounts of leached starch.

Table 3.18 Chain lengths (DP_n) of leached amylopectin*

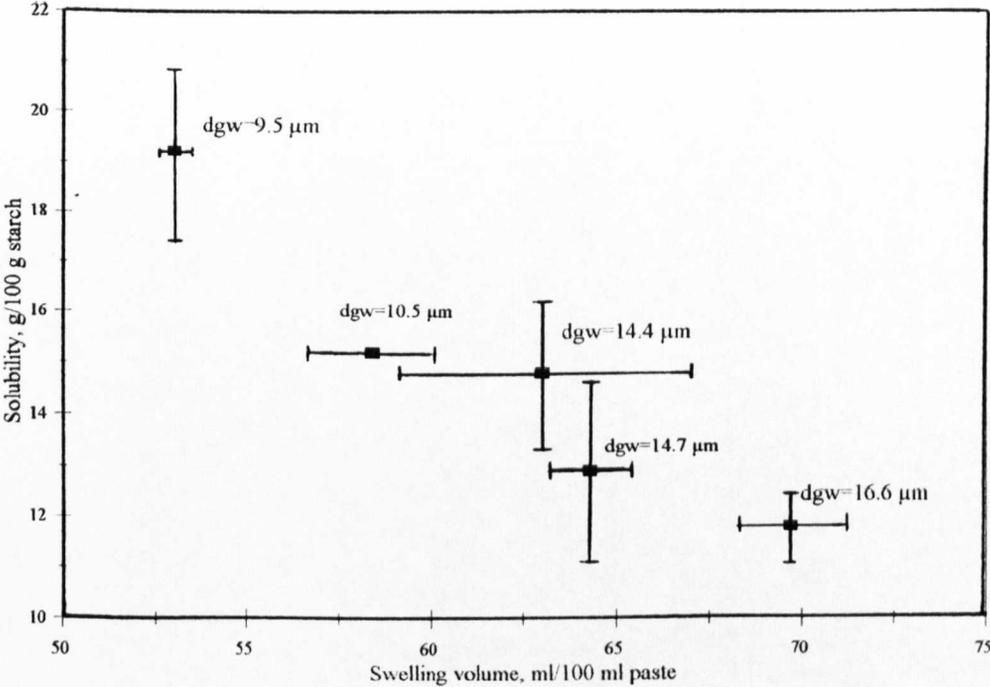
(* Leached amylopectin in 1% starch pastes cooked at 95°C)

Cultivar	Long chains	Short chains	
Cassava cultivars from Zone A			
MCol 22	43	22	12
MBra 897	42	22	12
MMex.59	42	22	11
MCR 35	41	22	11
MCol 1522	41	22	12
G1-37	42	22	12
MPer 196	41	22	12
MVen 25	42	22	12
MBra 881	42	22	12
CG1118-121	41	22	11
CM 3306-4	41	22	12
Cassava cultivars from Zone B			
CG1-37	43	21	11
MPer 196	43	22	12
MVen 25	43	22	12
MBra 881	43	22	12

Table 3.19 The effect of starch granule size (d_{gw}) on some characteristics of 1% starch pastes cooked at 95°C for 1 hr. leached starch, swelling volume, amylose:amylopectin ratio (Am:Ap) in leached starch, and chain length (DP_w) of leached amylose .

Cultivar	d_{gw} (μm)	Leached starch (g/100g)	Swelling (ml/100ml)	Am:Ap	DP_w
MVen 25	9.5	19.2±1.6	53.0±0.3	1.1	7472
	14.4	14.8±1.5	63.0±3.7	1.4	5807
	16.6	11.8±0.5	72.0±3.1	1.3	7904
CM 3306-4	10.5	15.2±0.0	58.4±1.6	1.4	7852
	14.7	12.9±1.6	64.3±1.2	1.5	8782

Figure 3.21 Solubility and swelling volume in cassava starch pastes as affected by the starch granule size.



The error bars are standard errors obtained from three replicates.

3.2 REMARKS AND CONCLUSIONS

From the results it is concluded ,

1. Cassava starches possess a range of functional properties dependent on the cassava cultivar and on the environmental temperature of the plants during growth..
2. The molecular conformation and the structure of the starch granules appear to be important factors in determining the hot-water swelling and solubility of cassava starches of different origins.
3. The viscosity development of cassava starch in hot-aqueous pasting and the mechanical properties of the pastes are determined by the degree of granule swelling, and the amounts and proportions of molecules of amylose and amylopectin dispersed in the solvent phase in the pastes.
4. The analytical results suggest that amyloses with short molecules and amylopectins with reduced branching are formed in cassava cultivars which produce starches with granule populations of small size which, in turn, during aqueous heating, release high amounts of starch molecules into the solution and result in reduced swelling and viscosity development, and *vice versa*.
5. An increase in the amylose present of cassava starch could result in a restriction of the swelling of the starch granules, an increase in the starch solubilization and an

increase in the initial pasting temperature.

6. A more crystalline granule structure in cassava starch seems also to be involved in a restriction to granule swelling during the hot- aqueous pasting.

7. High environmental temperatures during the growth of cassava plants affect the molecular and granular properties of the starch, by increasing the relative amounts of amylose, encouraging the synthesis of molecules of amylopectin which are less branched and of amylose with shorter chains, by the formation of granules of smaller size, which in turn result in starches of higher hot-water solubility, lower swelling and hence low paste viscosity.

8. These results could be useful for cassava plant breeders who may wish to develop or select potentially useful cultivars with certain attributes in their starches, and to identify regions for growing cassava at environmental temperatures which contribute to the desirable properties in the starches.

CHAPTER 4

EFFECT OF PROCESSING CONDITIONS ON THE PHYSICOCHEMICAL PROPERTIES OF CASSAVA STARCH

4.1 RESULTS AND DISCUSSION

4.1.1 Effect of natural fermentation and drying

Sour cassava starch is produced by various rural industries in Brasil and Colombia. The starch is fermented naturally and then sun dried. These two processes induce in the starch important modifications in its functional properties. Sour starch has the potential of expansion when a dough is made and oven-baked (Camargo *et al.*, 1988; Chuzel, 1993; Brabet, 1994). The functional characteristics of sour starch are thought to be the result of changes in the macromolecular conformation of the starch (Brabet, 1994). To investigate this further, cassava cultivars MCol 1522 and MVen 25 aged 14 months were processed in a rural factory in Colombia under the conditions specified in Section 2.2.2. Samples of sweet and fermented starches, sun dried or oven dried, were subjected to physicochemical assays, the results of which are presented below.

4.1.1.1 Pasting characteristics of the starch suspensions

Aqueous starch suspensions (5%, w/v) were pasted and tested in the Brabender viscoamylograph (Section 2.4.2). The pasting profiles are presented in Figure 4.1a and b and the characteristic parameters of the amylograms are included in Table 4.1. Sweet starches of each cultivar gave distinctive pasting viscosities similar to the

Figure 4.1 Brabender viscoamylogram curves of 5% aqueous pastes of sweet and fermented cassava starches from cultivars (a) MCol 1522 and (b) MVen 25.

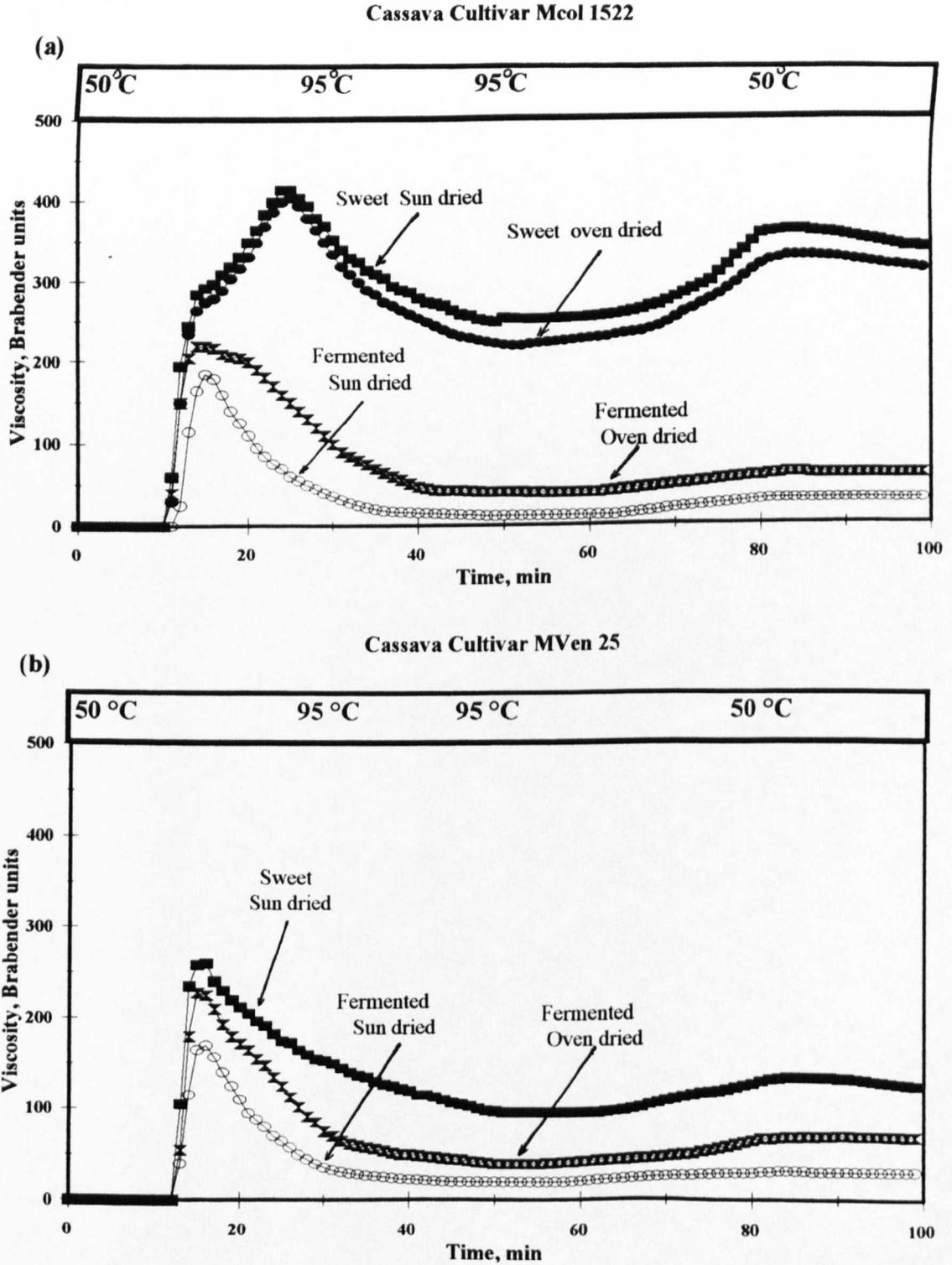


Table 4.1 Brabender pasting parameters of cassava starches processed as specified.

Starch Class	Drying Method	PT (°C)	PkV (B.U.)	V_{95°C} (B.U.)	V_{95°C,20 min} (B.U.)	V_{50°C} (B.U.)	V_{50°C,20min} (B.U.)
Starches from MCol 1522							
Sweet	Sun dried	63.6	415	353	255	258	339
Sweet	Oven dried	63.8	405	335	221	320	313
Fermented	Sun dried	65.4	185	36	12	29	29
Fermented	Oven dried	63.6	220	100	42	58	60
Starches from Mven 25							
Sweet	Sun dried	65.4	260	153	97	127	125
Sweet	Oven dried	ND	ND	ND	ND	ND	ND
Fermented	Sun dried	66.4	170	40	20	30	30
Fermented	Oven dried	65.8	228	75	40	65	69

PT: Initial pasting temperature; **PkV:** Peak viscosity; **V_{95°C}:** Viscosity at 95°C

V_{95°C,20 min}: Viscosity after 20 min. at 95°C; **V_{50°C}:** Viscosity on cooling to 50°C ;

V_{50°C,20min}: Viscosity on cooling and holding for 20 min. at 50°C.

ND: Not determined. The sample analysed was mistakenly taken from a different starch.

samples of the native starches from the same cultivars reported in Section 3.1.1., *i.e.* MCol 1522 starch, developed higher viscosities than MVen 25 starch. The fermented starches exhibited a drastic reduction in their pasting viscosities, which was more accentuated with the MCol 1522 starches. Fermented and sun dried starches gave the lowest viscosities on pasting, and their pastes did not show any setback on cooling, which was contrary to the behaviour of pastes from the sweet starches (particularly noticeable with MCol 1522 starches but limited set back with the MVen 25). These results paralleled those obtained with the same set of samples in a complementary study by Mestres (1995) using a Rapid ViscoAnalyzer. In Mestres' experiments, the oven dried samples from the fermented starches gave viscosity profiles distinct from those of fermented, sun dried starches. The reduction of the pasting viscosities in fermented starches also confirmed the results of Chuzel (1993), Larssonneur (1993) and Brabet (1994), who all observed that during the fermentation period the pasting viscosities of the starches were reduced gradually, suggesting that it was caused by a partial hydrolysis (acid and enzymic) of the starch. Fermented starches, when dried by other means than sun drying (*e.g.* oven drying, or by natural drying in the shade) were observed to exhibit about the same pasting viscosities as the wet fermented starches (Brabet, 1994) but, when they were sun dried, the pasting viscosity was reduced further as a result of changes in the macromolecular conformation.

4.1.1.2 Swelling and Solubility

The solubility and swelling in starch aqueous pastes were determined by using the same methods as with the samples of native starches in Chapter 3 (Tables 4.2 and

4.3). The results confirmed the pasting behaviour differences found in the samples. The fermented starches were much more soluble compared to the sweet samples. Unlike the pasting profiles, the solubility and swelling values showed a greater contrast between sun dried and oven dried samples from fermented starch. However, sweet starches did not show the effect of the method of drying. As recorded for acid degraded starches by Pomeranz (1991), the fermented starches probably had softer granules with a granular, micellar network, which did not resist swelling upon pasting, permitting the diffusion and dispersion of the starch components into the solvent phase. The solubilization phenomenon was enhanced in the pastes of sun dried, fermented starches, probably caused by some type of modification of the starch molecules in the partially degraded fermented starch (Brabet, 1994). On the other hand, sweet starches resisted swelling and had solubility values within the range reported for native starches from Zone A as recorded in Chapter 3. Figure 4.3 displays the grouping of the starch samples according to their starch solubility and swelling after pasting. The viscosity response upon pasting was connected, as was expected, to the extent of the starch solubilization. Therefore, for example, the highly soluble fermented and sun dried starch pastes gave the lowest maximum viscosity upon thickening (Figure 4.4).

These results confirmed the relatively novel observations of the effect of sun drying on the functional properties of fermented starches reported by Chuzel (1992), Larssonneur (1993), Brabet (1994) and Mestres *et al.* (1996). The effect of the solar radiation on fermented cassava starch has been a matter of increasing interest and it

has attracted the attention of several research groups in Colombia and France. It will be shown later in the thesis (Section 4.1.1.6) that the behaviour in hot water of the fermented samples was connected to the starch molecular structure.

Table 4.2 Aqueous solubility* (g/100g starch) of 1% cassava starch pastes cooked at 95°C for 1 hr from two cultivars processed as specified.

Cassava cultivar	MCol 1522		MVen 25	
	Sun dried	Oven dried	Sun dried	Oven dried
Sweet	16.2±0.6	16.3±0.0	19.6±0.0	20.8±0.1
Fermented	76.9±0.5	57.5±5.8	85.9±1.9	57.7±4.2

* Mean of duplicate measurements

Table 4.3 Swelling volume* (ml/100 ml paste) of 1% cassava starch pastes cooked at 95°C for 1 hr from two cultivars processed as specified.

Cassava cultivar	MCol 1522		MVen 25	
	Sun dried	Oven dried	Sun dried	Oven dried
Sweet	48.0±0.0	48.7±0.6	47.4±0.6	46.7±0.0
Fermented	10.6±0.6	29.0±4.3	8.3±1.0	30.0±3.3

* Mean of duplicate measurements

Figure 4.2 Grouping of fermented and sweet starches according to their solubility and swelling volumes.

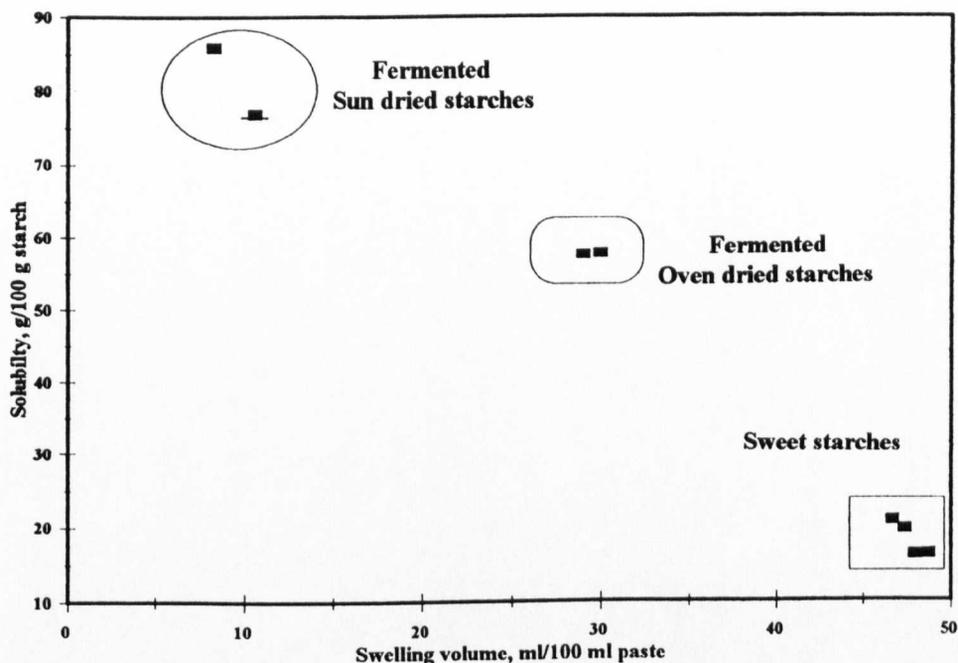
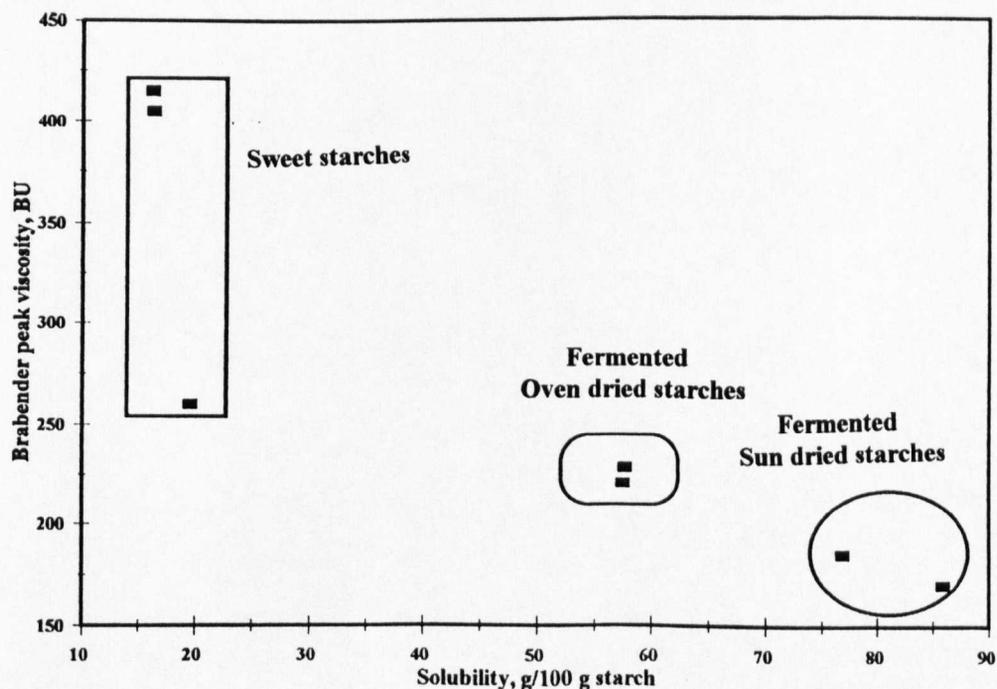


Figure 4.3 Grouping of fermented and sweet starches according to their Brabender peak viscosities and solubilities.



4.1.1.3 Mechanical properties of the starch pastes

Fresh samples from starch pastes (5% w/v), prepared according to the procedure in Section 2.4.3, were subjected to dynamic viscoelastic tests at 25 °C over a sweep frequency sweep from 0.1 to 1.0 Hz. Another set of samples were stored at 4 °C for 72 hr prior to testing.

Figures 4.4a and b and 4.5a and b show for pastes from cultivars MCol 1522 and MVen 25 the response of the storage modulus (G') to increases in the frequency of oscillation. The fresh pastes of fermented starches, both sun dried and oven dried, had lowest G' (Figures 4.4a and 4.5a), with a strong liquid-like character (*e.g.* delta values of about 44 - 55° at 0.6 Hz as in figure Figure 4.6). The fresh pastes from the sweet starches exhibited the same type of gel as the pastes from the native starches in Section 3.1.3; they had a solid-like behaviour (*e.g.* delta values of 9.5 - 9.6° for Mven 25 pastes and 21.5 -20.9° for MCol 1522 pastes).

After storing for 72 hr at 4 °C, the sweet starch pastes developed the highest gel strength (*i.e.* the highest G' and lowest frequency dependence, Figures 4.4b and 4.5b), but the pastes from the Mven 25 starches exhibited stronger gels than the pastes from MCol 1522 starches. The higher gel strength developed by MVen 25 starch can be attributed to its higher amylose content (Section 4.1.1.5) and lower molecular weight amylose (Section 4.1.1.6). Upon ageing there was an increase in the gel strength of the pastes from the fermented, sun dried starches which changed from weak, liquid gels when they were fresh to stronger elastic gel after ageing (Figures

Figure 4.4 Frequency dependence of G' in (a) fresh and (b) aged pastes of fermented and sweet starches from cassava cultivar MCol 1522.

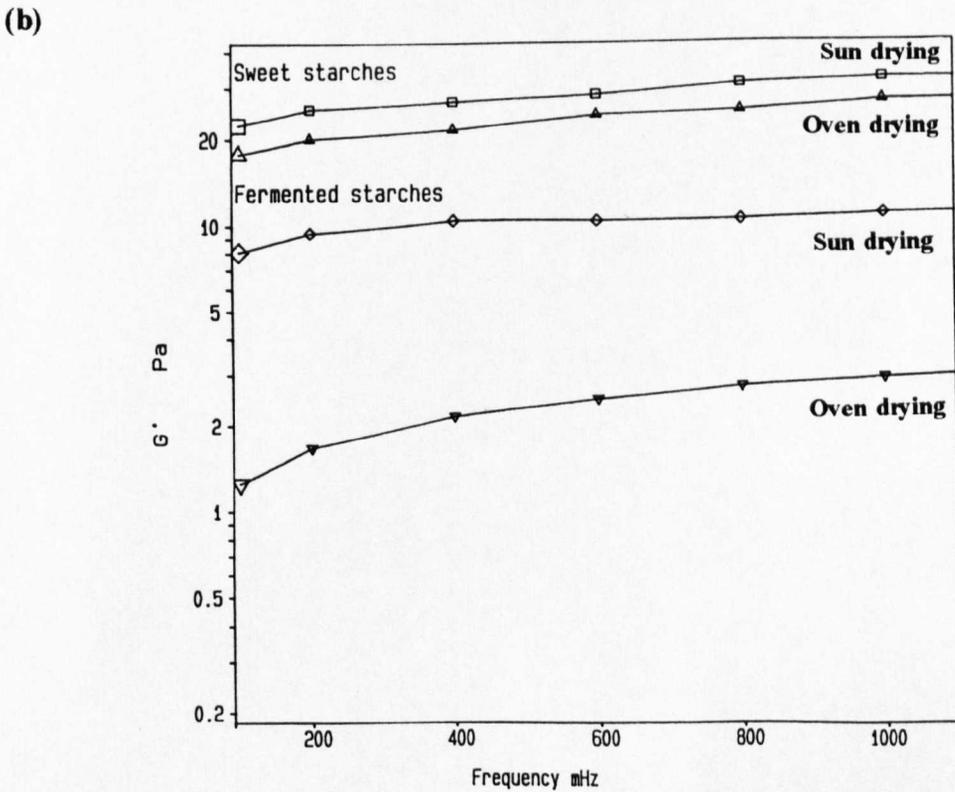
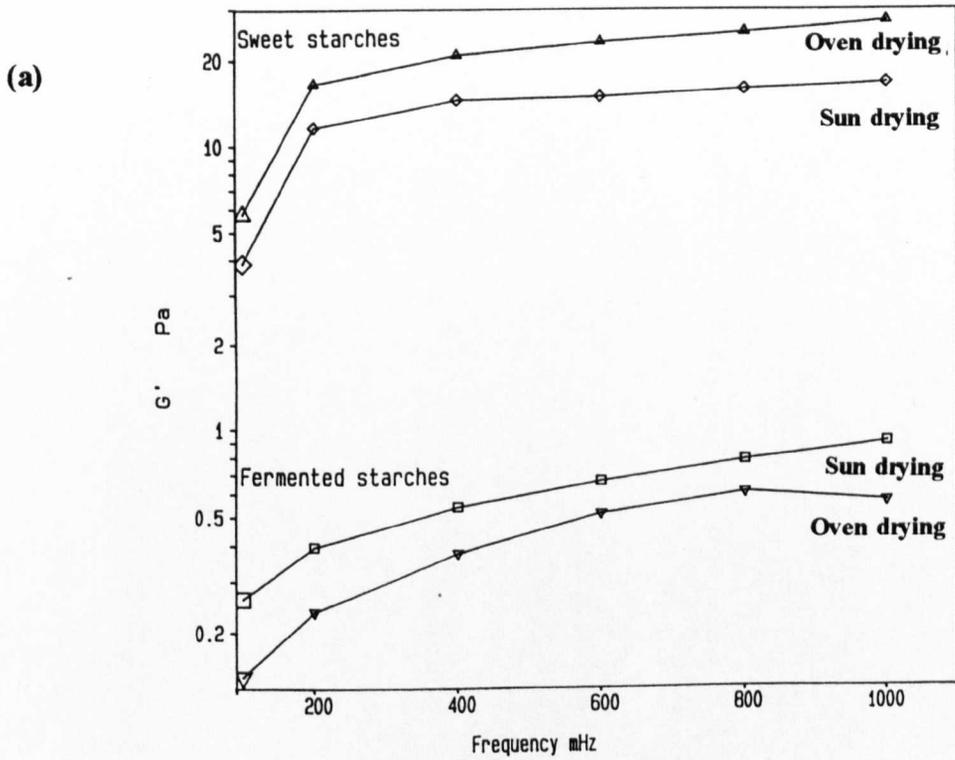


Figure 4.5 Frequency dependence of G' in (a) fresh and (b) aged pastes of fermented and sweet starches from cassava cultivar MVen 25

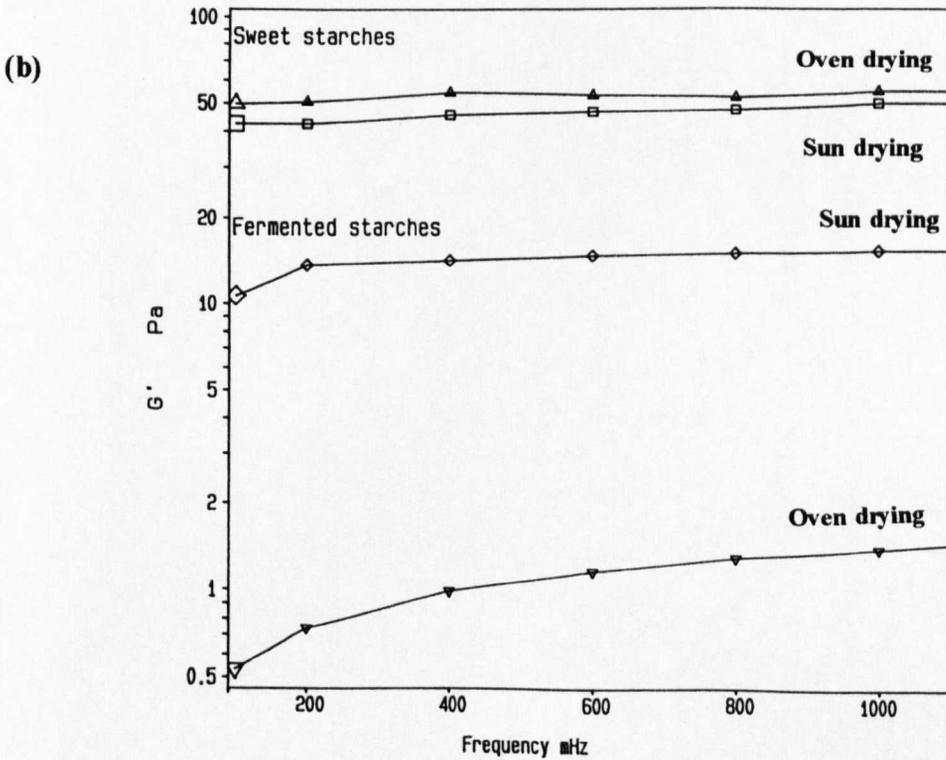
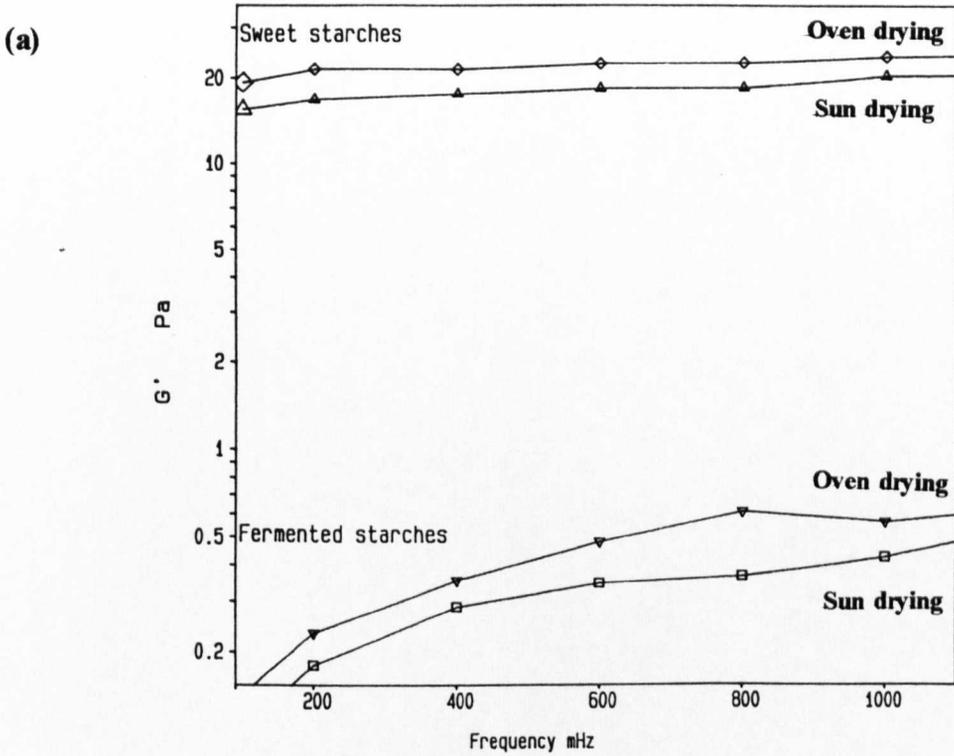
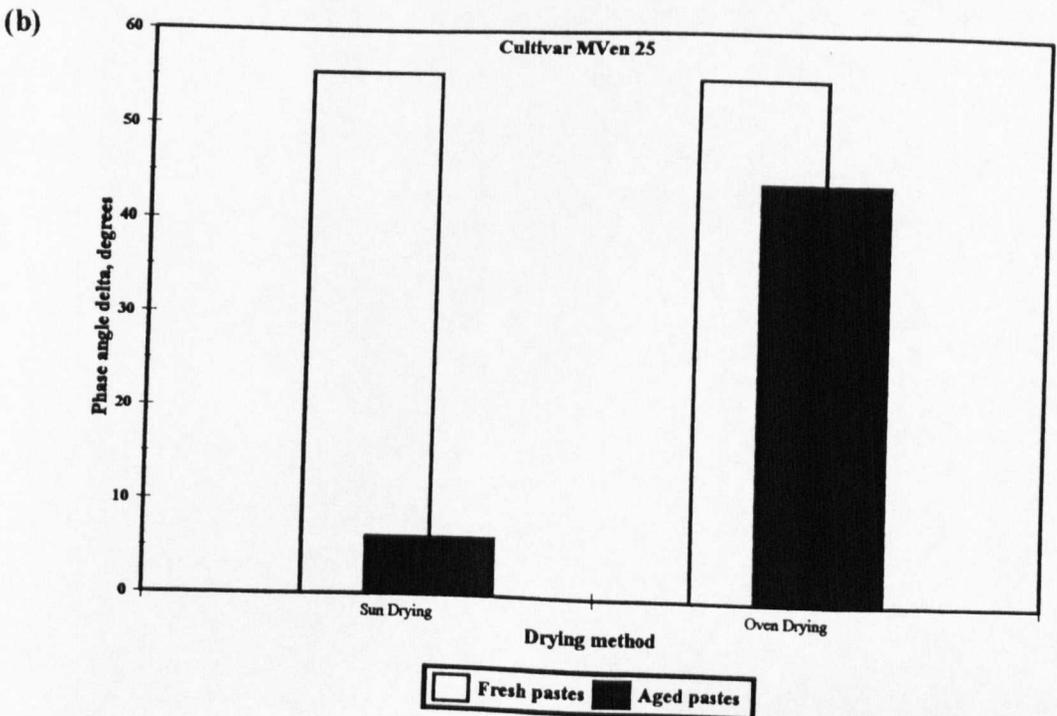
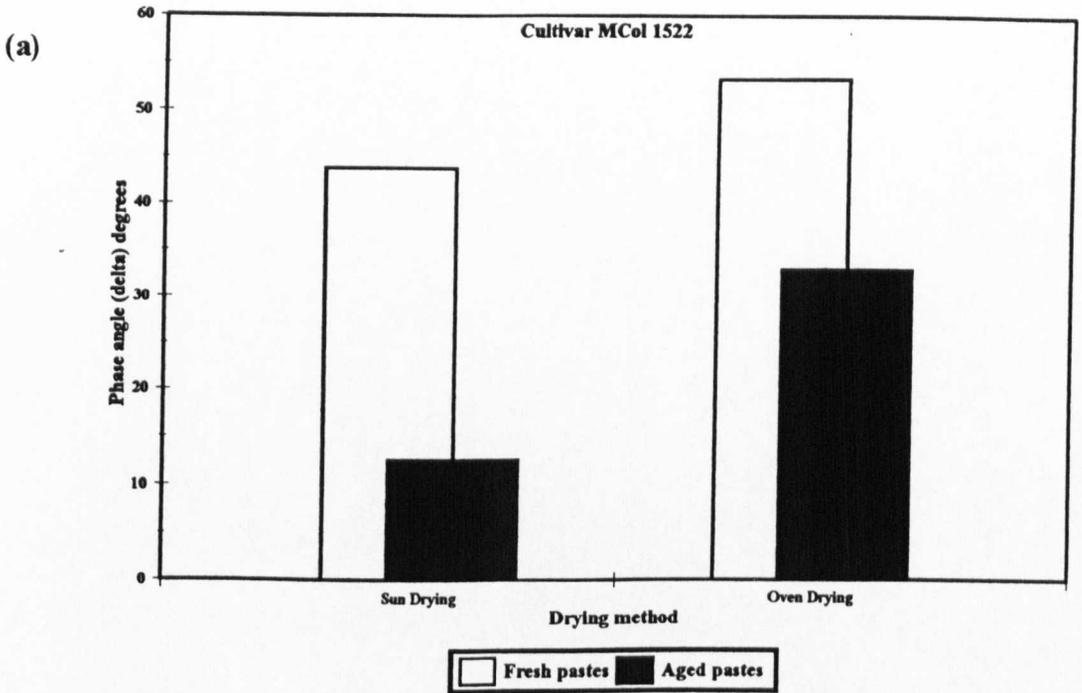


Figure 4.6 Phase angle (at 0.6 Hz and 25°C) in 5% aqueous pastes of fermented starches from cassava cultivars (a) MCol 1522 and (b) MVen 25.



4.5, 4.6 and 4.7). This change was not observed in fermented, oven dried starch pastes. When comparing the molecular structure of fermented, sun dried and oven dried starches (Section 4.1.1.6), it is probable that the presence of smaller chains of amylose, in the solvent phase of the pastes from fermented, sun dried starches could have facilitated the development of some kind of gel matrix.

4.1.1.4 Granule size and X-ray diffraction pattern in the starch powders

The geometric mean diameter of the starch granules was determined by the procedure described in Section 2.4.4. The sweet starches from Mven 25 had smaller granules than those from MCol 1552 sweet starches which paralleled the differences in the solubilities of the corresponding pastes (Table 4.2); these results agree with the general trend found between granule size and the aqueous solubilities of the starches reported in Chapter 3. It was apparent that the granule integrity was not affected by the processing conditions, since granule sizes appeared unmodified (Table 4.4). Franco *et al.*(1987) had reported a reduction in the population of the larger granules (over 15 μm) in cassava starches by enzymatic attack, but in the samples used in this study, it is clear that whatever reactions have happened during fermentation and drying, no significant erosion of the granules had occurred.

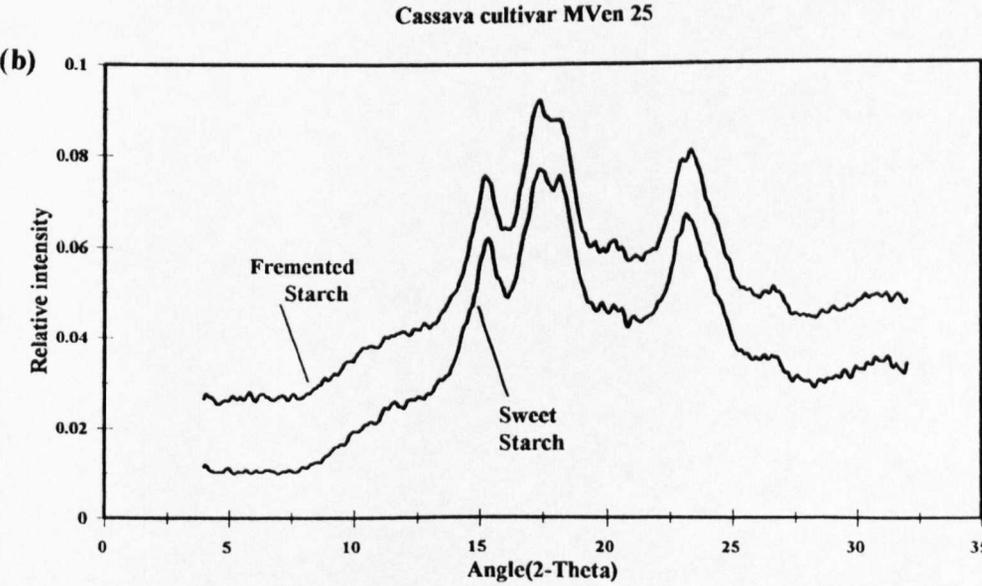
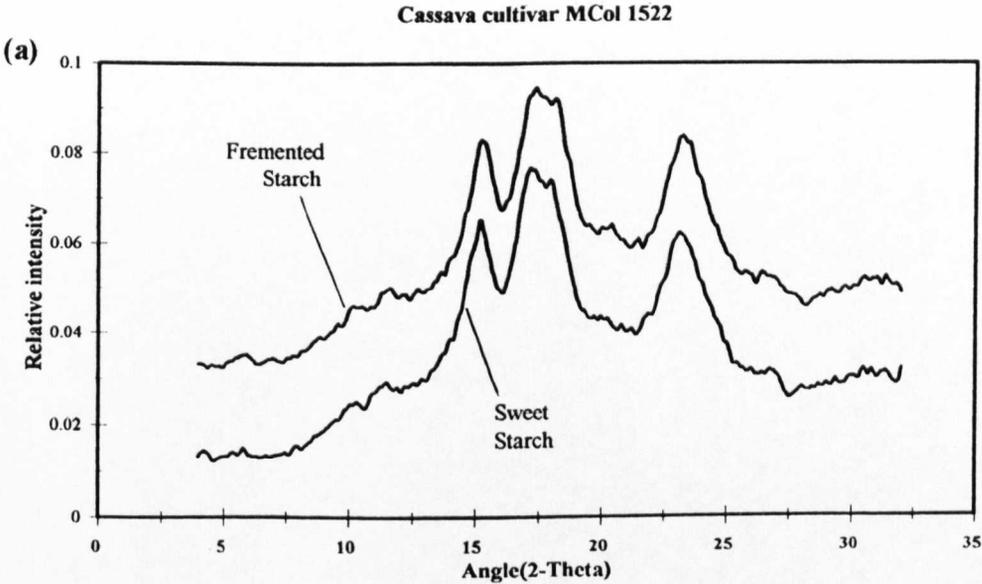
X-ray diffraction patterns (Figures 4a and b) (determined as in Section 2.4.4) revealed an apparently unaltered crystalline organization in the granules of the sweet and fermented starches. The relative crystallinity values in Table 4.4 supported the view that there had been no significant changes in the amylopectin which is the

fundamental structural element in the crystalline component of the granules. The analysis of the fine structure of the amylopectin molecules (Section 4.1.1.6) similarly did not show any detectable alteration in the lengths and distribution of the polysaccharide chains.

Table 4.4 Granule size (geometric mean diameter, d_{gw}) and relative crystallinity in sweet and fermented, sun dried cassava starches.

cultivar	MCol 1522		MVen 25	
	d_{gw} (μm)	Crystallinity (%)	d_{gw} (μm)	Crystallinity (%)
Starch class:				
Sweet	11.1	41.2	9.3	42.6
Fermented	11.0	42.5	9.1	42.8

Figure 4.7 X-ray diffraction patterns in sun dried, sweet and fermented starches from cassava cultivars (a) MCol 1522 and (b) MVen 25.



4.1.1.5 Amylose content

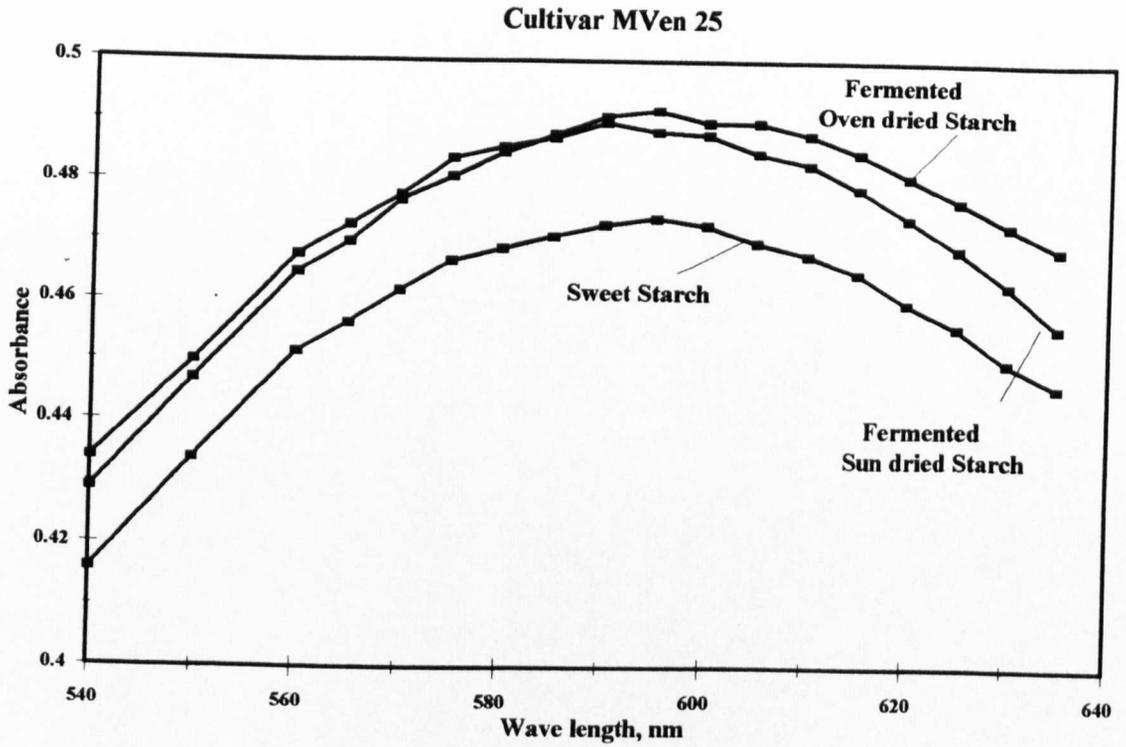
The amylose content of the samples was determined by the iodine colorimetric procedure (Section 2.4.6) and the results are presented in Table 4.5. From the amylose values obtained, there is no evidence that the amylose chains in the fermented starches could have been shortened to the point of reducing the blue value of the iodine-amylose complex. In fact, if any changes had occurred to the whole molecular structure of the fermented starches, they were undetectable by the starch-iodine staining reaction since the λ_{\max} of the starch- I_2 samples of sweet and fermented starches from MVen 25 appear virtually the same as in Figure 4.8.

Table 4.5 Amylose content (% d.b.)¹ of cassava starches, factory processed as specified. Mean of duplicate determinations

Cassava cultivar	MCol 1522		MVen 25	
	Sun dried	Oven dried	Sun dried	Oven dried
Starch class:				
Sweet	21.7±0.0	21.4±0.0	22.8±0.2	23.1±0.0
Fermented	21.2±0.0	21.8±0.0	23.4±0.1	23.7±0.0

1. Amylose content as determined by the iodine colorimetric method

Figure 4.8 Spectra of the starch-iodine complex determined in dispersions of sweet and fermented starches from cassava cultivar MVen 25.



4.1.1.6 Molecular structure

Starches debranched by the isoamylase method were analysed by the SE-HPLC-RI-MALLS system as previously described (Section 2.4.5). Figures 4.9a and b and 4.10a and b present the SE-HPLC chromatograms. There are two distinctive features in the elution profiles:

(1) The amylose peaks (Figures 4.9b and 4.10b) of the fermented starches eluted were delayed with respect to the amyloses derived from the sweet starches, especially when fermented and sun dried. This suggests that the amylose molecules of the fermented starches were smaller than those from sweet starches. This was confirmed from the laser light scattering system results by the determination of their absolute weight average molecular weights (M_w) values, which are presented beside the amylose peaks in the Figures 4.9a and 4.10a;

(2) The elution profiles of the amylopectin fractions had essentially the same pattern, although in the fermented, sun dried starches the amylopectins had a small population of chains (indicated by arrows in the Figures 4.9a and 4.10a), which eluted approximately between 26.7 and 28 ml. These were believed to be caused by degraded chains derived from the degraded amyloses; in fact the relative proportion of the amylose peaks in the SE-HPLC profiles decreased from the sweet to the fermented, sun dried starches (from 16.5 to 13.6% in the MCol 1522 starches and from 19.3 to 13.1%). Therefore, it appears that the amylose molecules were degraded, more extensively in the fermented and sun dried samples, but that the amylopectin fine structure was unmodified. However, it does not mean that some of the

Figure 4.9 (a) SE-HPLC chromatograms derived from isoamylase-debranched sweet and fermented starches from cassava cultivar MCol 1522 and (b) a close-up of the amylose peaks to enhance the differences in their elution position .

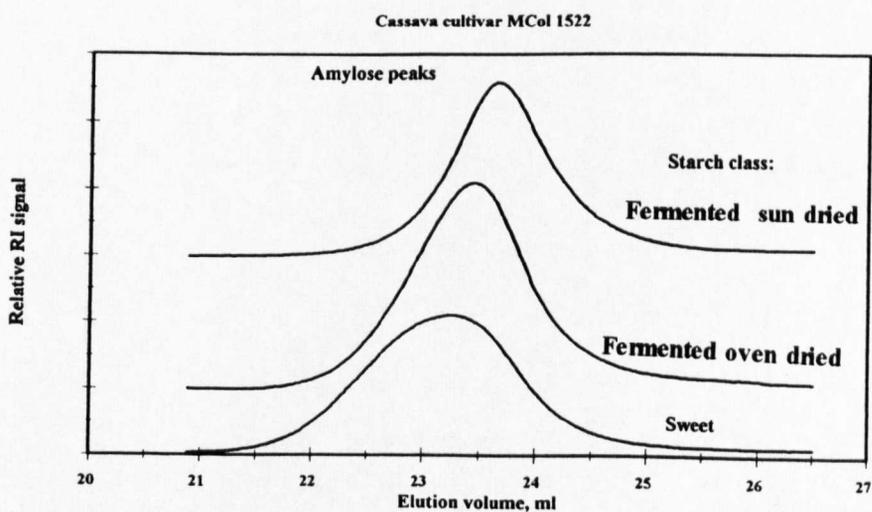
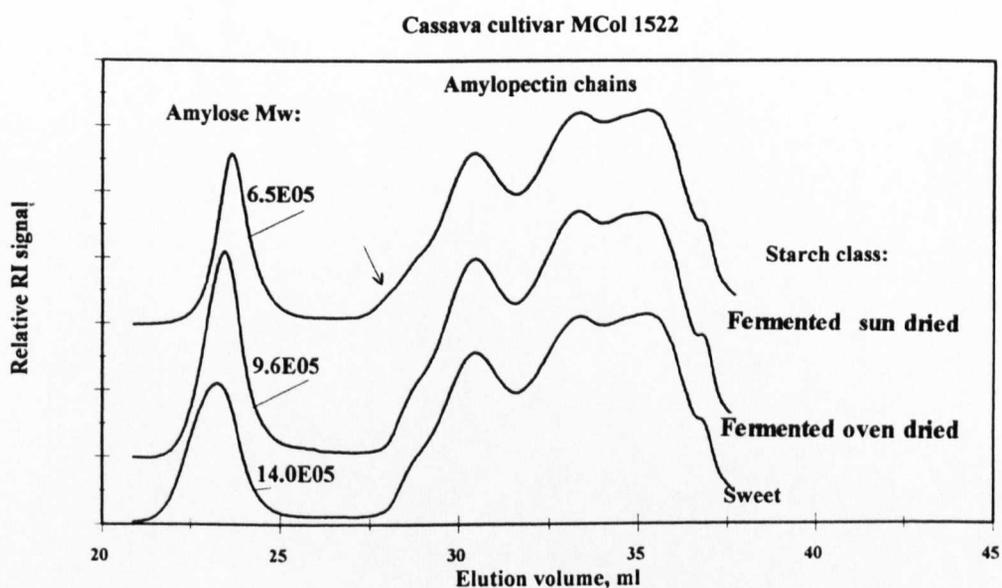
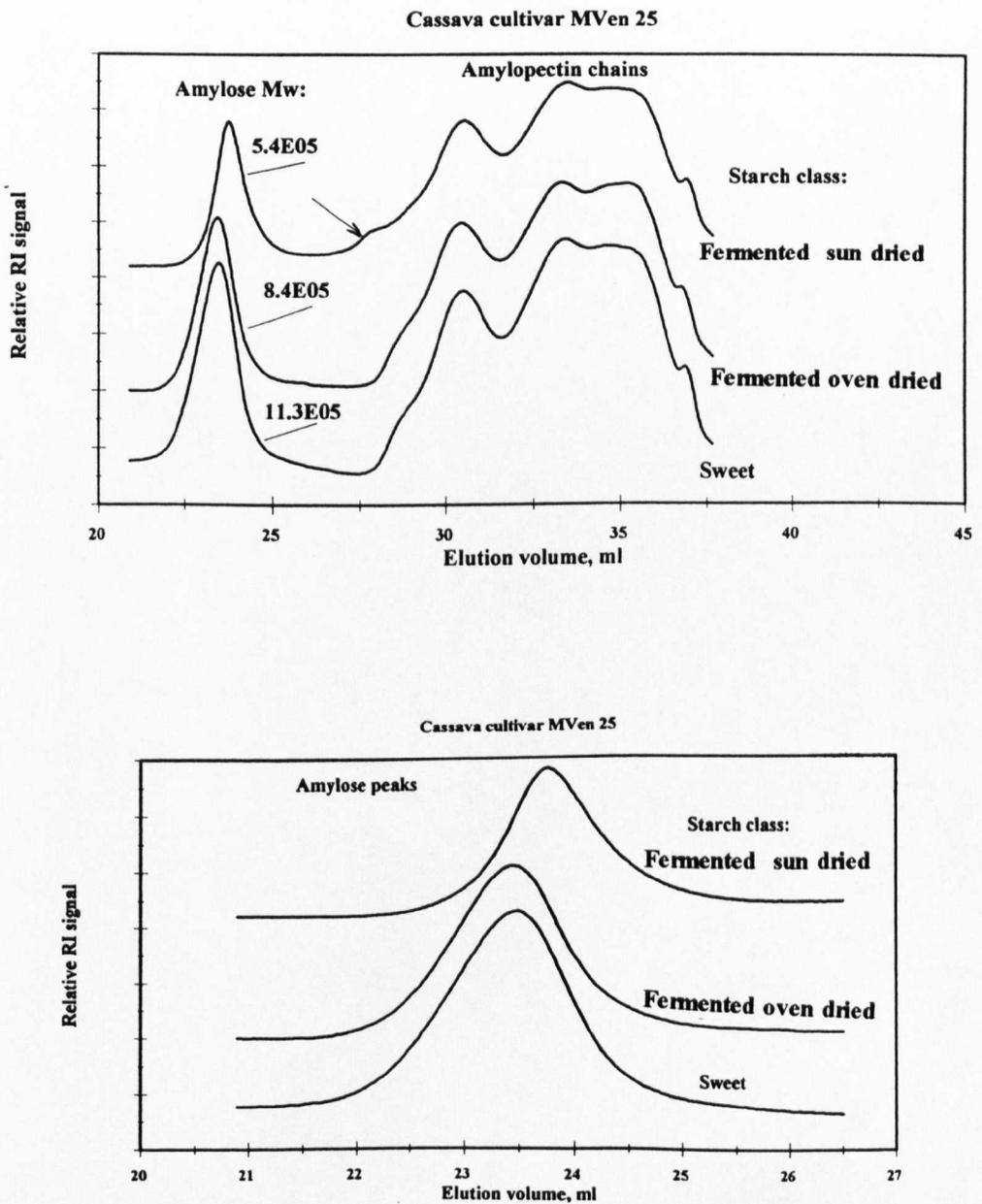
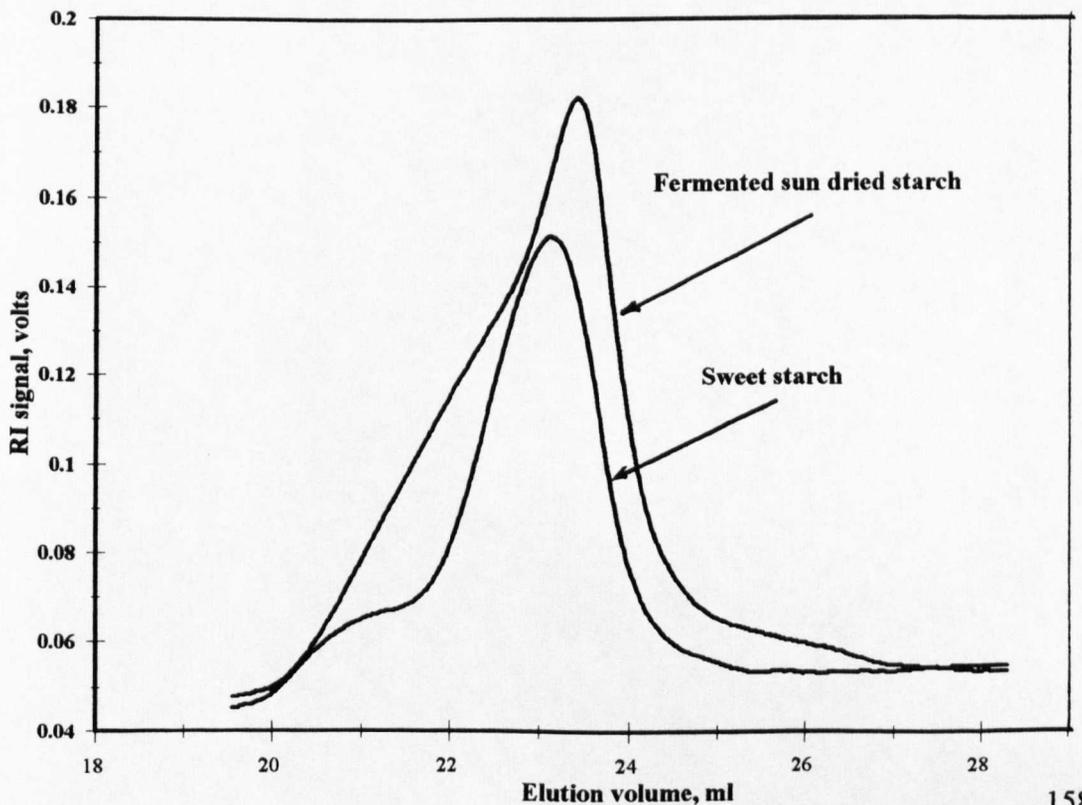


Figure 4.10 SE-HPLC (a) chromatograms derived from isoamylase-debranched sweet and fermented starches from cassava cultivar MVen 25 and (b) a close-up of the amylose peaks to enhance the differences in their elution position .



macromolecules could not have been split into pieces which nevertheless need not have disturbed the integrity of the racemose structure in the amylopectin. It is evident that the whole population of macromolecules has changed in the fermented, sun dried starches by comparing the SE-HPLC chromatograms of the two aqueous starch solutions shown in Figure 4.11 (the starches were leached in water at 95 °C for 1 hr). The weight average molecular weight of the entire population, as detected by the MALL system, confirmed the suspected change in the whole polymer, since the values were 2.2×10^7 and 1.7×10^7 in a sweet and a fermented, sun dried starch respectively.

Figure 4.11 SE-HPLC chromatograms derived from the materials in the soluble phase of 1% aqueous pastes of sweet and fermented, sun dried starches from cultivar MCol 1522 .



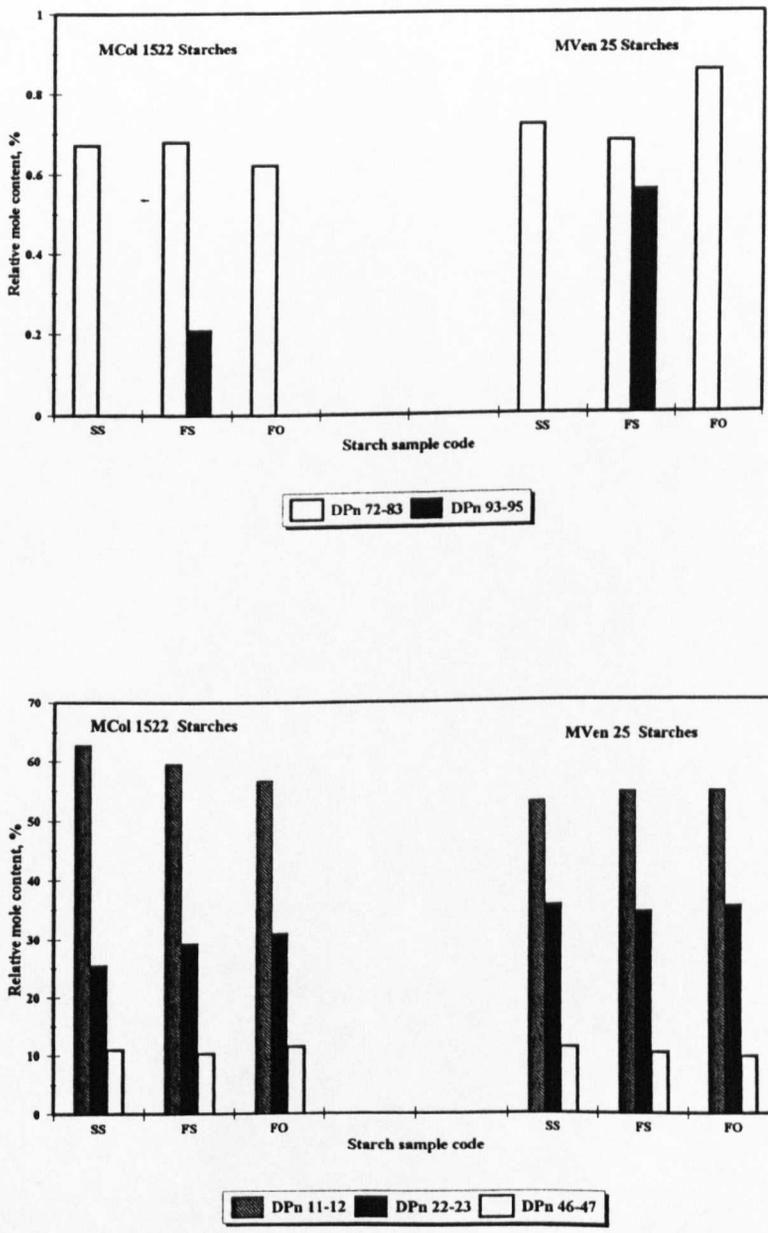
From the observations above, the amylopectin fine structures of sweet and fermented starches appeared similar. The amylopectin chromatograms were investigated further by using the analytical technique of fitting Gaussian peaks to the amylopectin chromatographic data (Section 2.4.8). The resolved populations of amylopectin chains corresponding to the Gaussian peaks were grouped and identified as A and B₁₋₄ chains following Hizukuri (1986); their lengths and mole % are presented in Table 4.6 and in Figure 4.12. It is evident that the B₄ chains (although a very low population) in the fermented sun dried starches had the longest lengths (DP_n 94 -95) but, as shown previously, the presence of degraded amylose could artificially inflate the length of the B₄ chains. When the ratios of A/B₁₋₄ chain populations (mole %) were calculated, it was observed that in the amylopectins from MCol 1522 starch, the ratios were smaller in the fermented starches than in the sweet sun dried starch, *i.e.* 1.69, 1.47 and 1.31 in the sweet sun dried starch, fermented sun dried starch and fermented oven dried starch respectively. The reduction in the A/B ratio in amylopectins could be associated with a relative increase in the B₁ chain population (DP_n 22-23 as can be seen in Figure 4.12) caused by the addition of chains from the breakdown of the larger B chains when the starch was fermented and dried. As in Chapter 3, the results here show that the A and B₁ components comprise about 85-90% of the chain populations in amylopectin. The A/B ratio is therefore more sensitive to changes in the relative populations of the A and B₁ chains. With the MVen 25 starches, no significant changes were detected in the A/B ratio between the sweet and fermented starches. However, compared to the amylopectin in the MCol 1522 starch, the A/B₁₋₄ ratio was smaller in the amylopectins of MVen 25 starches

(i.e. 1.11, 1.18 and 1.19, respectively in the sweet, sun dried starch, fermented sun dried starch and fermented, oven dried starch), as it was with the corresponding native starches in Chapter 3.

Table 4.6 Distribution of chain lengths (DP_n) in amylopectins derived from starches processed at the shown conditions.

Starch Class	Drying Method	Fraction					
		Whole	A	B1	B2	B3	B4
Starches from MCol 1522							
Sweet	Sun dried	26	11	23	47	74	82
Sweet	Oven dried	27	11	23	47	74	81
Fermented	Sun dried	27	11	23	47	76	94
Fermented	Oven dried	27	11	23	47	76	83
Starches from Mven 25							
Sweet	Sun dried	26	11	22	46	74	82
Fermented	Sun dried	28	11	22	46	72	95
Fermented	Oven dried	26	11	22	47	72	80

Figure 4.12 Chain lengths (DP_n) and populations (relative mole content) in amylopectins of sweet, sun dried (ss), fermented, sun dried (fs) and fermented, oven dried (fo) starches from cassava cultivars MCol 1522 and MVen 25..



4.1.1.7 Aqueous starch exudates

The starch exudates extracted by pasting 1% starch suspensions at 95 °C for 1 hr were reacted with iodine (2ml of 0.2% I₂ solution with 5 ml of 0.8-0.9 % starch aqueous solutions from the solvent phase in the pastes) and the spectra of the starch-iodine complex were determined (Figures 4.13 a-c). The peaks of maximum absorbance, λ_{max} , in the spectra revealed differences in the composition of the leached starches. The pastes from the sweet starches displayed an amylose rich solvent phase ($\lambda_{max} > 640$ nm), while those from the fermented starches had a relatively higher proportion of amylopectin in solution. The fermented, sun dried starch pastes had the highest amylopectin content in solution ($\lambda_{max} = 595$ nm for MCol 1522 starch solution and $\lambda_{max} = 590$ for MVen 25 starch solution). Since amylopectin is the major component in cassava starch, the pastes from highly soluble, fermented sun dried starches are expected to have solvent phases with a high proportion of solubilised amylopectin.

The previous results were confirmed by the SE-HPLC analysis. The starch components exuded in MCol 1522 pastes, after debranching and size exclusion chromatography, gave the results shown in Figures 4.14a-c. As with the native starches in Chapter 3, about equal amounts of amylose (Am) and amylopectin (Ap) (Ap/Am ratio of about 0.8/1 in the solubilised starch) were leached from the sweet starch. In contrast, a substantial amount of amylopectin was leached from the fermented starches in accord with their higher solubilities, leading to an Ap/Am ratio of about 6.2/1 in the solvent phase of their pastes (estimated from the chromatogram peak area in

Figure 4.14) The fermented sun dried starch had higher amylopectin solubility (about 67 g/100g starch) than the fermented, oven dried starch (about 50 g/100g starch); these figures were estimated from the total starch solubility in Table 4.2 and from the ratio of Ap/Am areas in the chromatograms in Figure 4.14. The M_w 's of the dissolved amyloses in the pastes were calculated from the light scattering data as 11.8×10^5 , 4.6×10^5 and 3.5×10^5 , respectively from sweet, fermented, oven dried and sun dried starch. As expected, the M_w 's of the aqueous - leached amyloses were lower than the corresponding M_w 's of the whole amyloses analysed in Section 4.1.1.6 (Figure 4.9a). Furthermore, these results parallel the differences between the M_w of the whole amylose peaks in Figure 4.9a, confirming the effect of fermentation and drying on the molecular structure of cassava starch.

Figure 4.13 Spectra of the starch-iodine complexes determined in the soluble phase of 1% starch pastes from (a) sweet starches, and fermented starches from cultivars (b) MCol 1552 and (c) MVen 25.

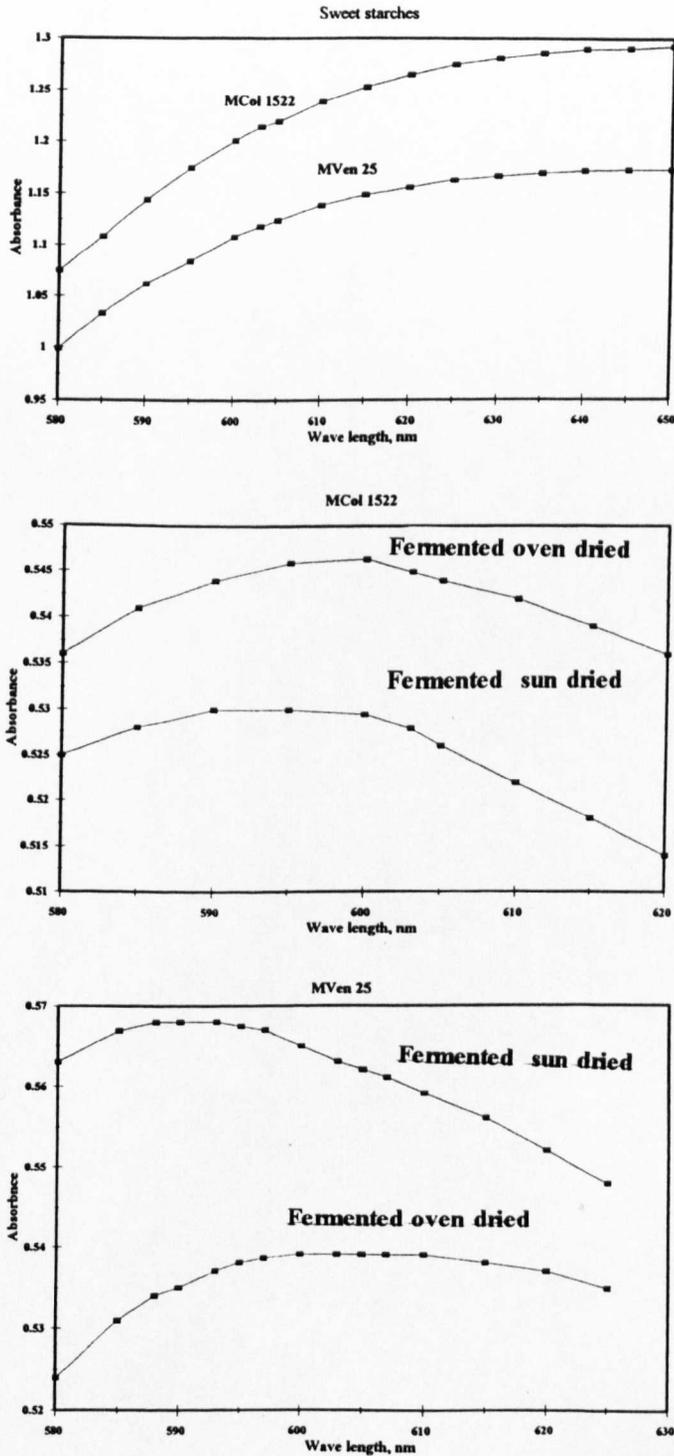
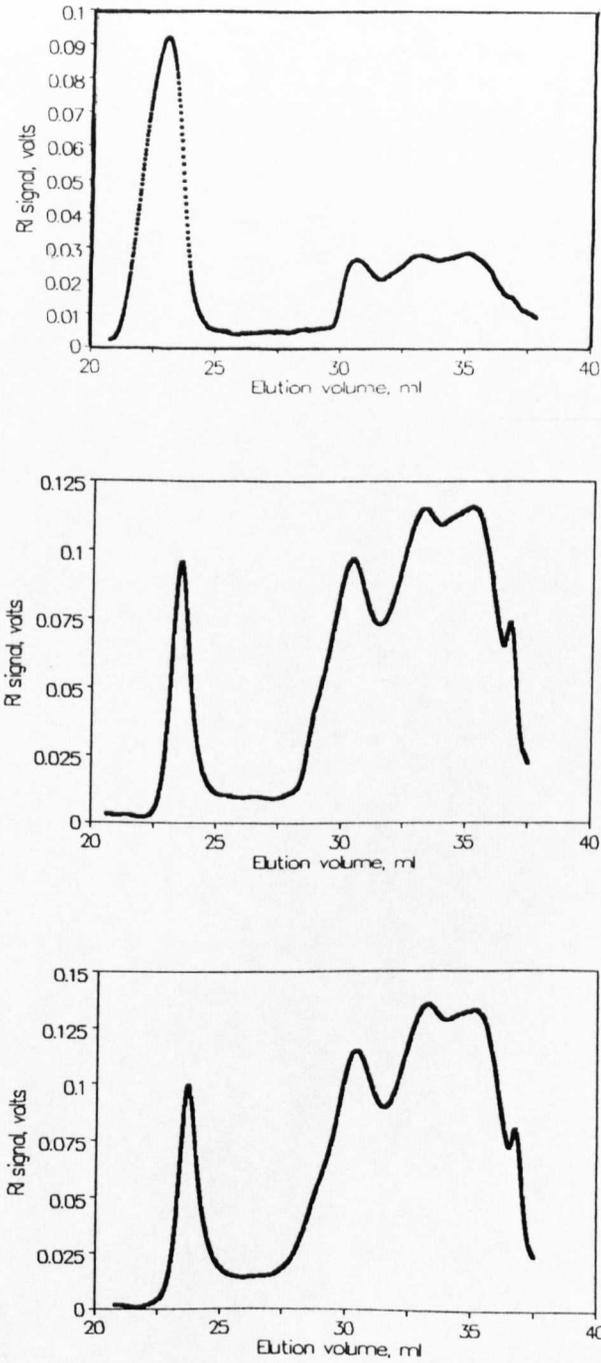


Figure 4.14 SE-HPLC chromatograms derived from debranched polysaccharides found after aqueous leaching (at 95 °C for 1 hr) of (a) sweet starch, (b) fermented, oven dried starch, and (c) fermented, sun dried starch .



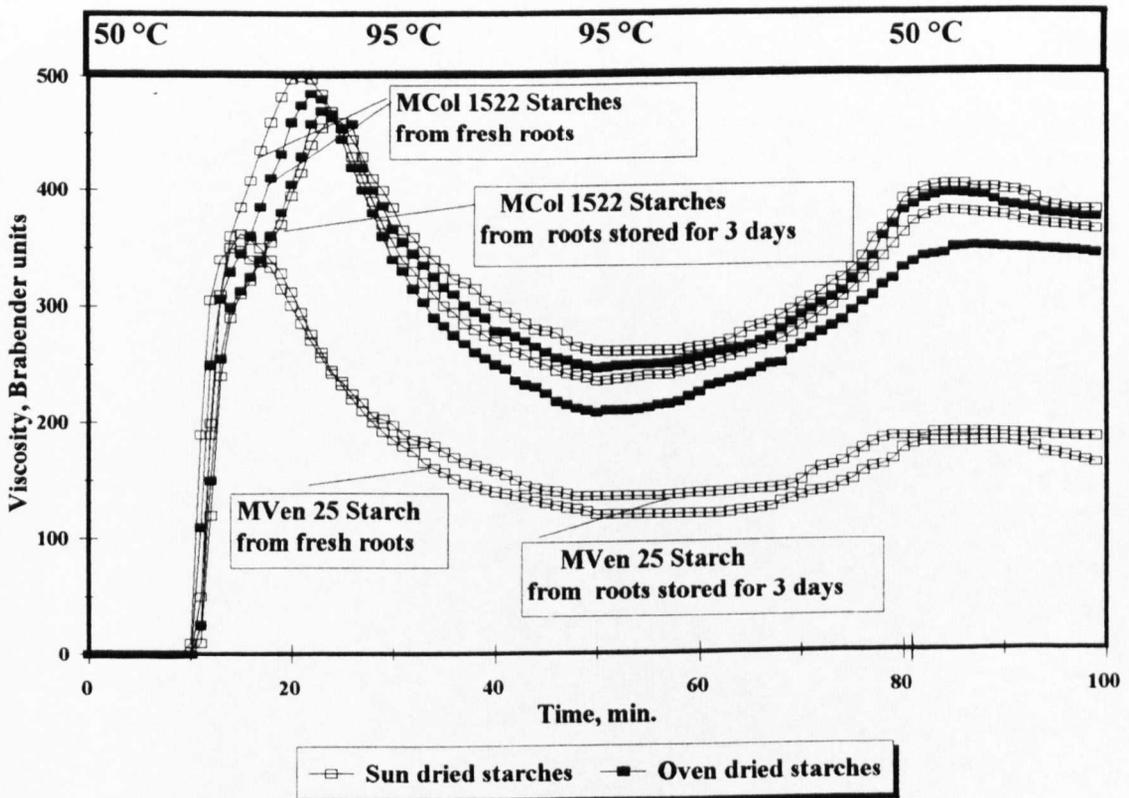
4.1.2 Effect of storage of roots for 3 days prior to the starch extraction

If the roots are not processed soon after harvest, postharvest physiological deterioration may occur which results in the production of a number of phenolic compounds, within 48 hr from harvest (Rickard, 1985, 1995). To determine whether the properties of starch may be affected if extracted from deteriorating roots, the effect of delaying processing by 3 days was examined under laboratory conditions. Laboratory-extracted (Figure 2.2) starch samples from fresh roots and from roots stored for 3 days (Figure 2.3) were assessed by a series of physicochemical tests. The roots, from MCol 1552 and MVen 25 cultivars, were from the same harvest as those which had been factory - processed and reported in the preceding section.

4.1.2.1 Pasting viscosities, solubilities and swelling volumes

The viscograms from the Brabender pasting tests (Figure 4.15) show that with the Mcol 1522 starches prepared from fresh roots, the viscosity increased sharply and had slightly higher peak viscosities but lower cold viscosities (viscosities at 50°C upon cooling) than samples from roots stored for 3 days. Starches from MVen 25 appeared quite comparable in their pasting viscosities (Figure 4.15) but, in general, it was observed that the two types of starch from fresh and stored roots (in both MCol 1522 and MVen 25 cultivars) followed a similar viscosity pattern as they were pasted. The solubility of the starch and the swelling volume of the undissolved granules in 1% starch pastes (Table 4.7, 4. 8) showed no recognisable effect of a 3 day delay in the processing of the roots.

Figure 4.15 Brabender viscoamylogram curves of 5% aqueous pastes of starches extracted from fresh roots and from roots stored for 3 days of cassava cultivars MCol 1522 and MVen 25



The only noticeable difference in the above results was that arising from the type of cassava cultivar. MCol 1522 starches had lower solubilities and hence higher granular swelling volumes than MVen 25 starches, and consequently developed higher paste viscosities. This cultivar effect is in agreement with the results obtained from the factory-extracted starches investigated in Sections 4.1.1.1 and 4.1.1.2. Furthermore, this cultivar effect also parallels the differences exhibited by the same (MCol 1522 and MVen 25) from other experimental plots and at a different season for the studies reported in Chapter 3.

Table 4.7 Aqueous solubility (g/100g starch) of cassava starch from two cultivars processed under defined conditions.

Cassava cultivar	MCol 1522		MVen 25	
	Sun dried	Oven dried	Sun dried	Oven dried
From fresh roots	14.7±0.0	13.5±0.7	19.5±0.3	17.3±0.0
From stored roots	14.0±0.4	13.2±0.4	18.5±0.0	19.9±0.3

Table 4.8 Swelling volume (ml/100 ml paste) of cassava starches from two cultivars processed at the shown conditions.

Cassava cultivar	MCol 1522		MVen 25	
	Sun dried	Oven dried	Sun dried	Oven dried
From fresh roots	52.6±0.6	54.7±1.3	50.0±0.0	53.0±0.0
From stored roots	51.4±1.3	52.6±0.6	48.7±1.3	46.7±0.0

4.1.2.2, X-ray diffraction pattern, crystallinity, granule size and amylose content.

The X-ray diffractograms were very similar (Figure 4.16a and b). None of the properties presented in Table 4.9 provided evidence that the starches from roots stored for 3 days were different from those of fresh roots. However, MCol 1522 starches had lower amylose content than MVen 25 starches, The higher amylose content in MVen 25 starches could have been responsible for limiting the swelling (as was suggested with the native starches investigated in Section 3.1.6) of the starch granules and the development of paste viscosities.

4.1.2.3 Molecular structure.

Some debranched starch samples were analysed by the SE-HPLC technique. Figure 4.16 shows virtually identical SE-HPLC chromatograms and amylose peaks of similar M_w for MCol 1522 starches from fresh and roots stored for 3 days. The amylopectin fractions in the chromatograms were resolved by the Gaussian peak fitting procedure (Section 2.4.8), and the chain lengths, along with the mol % of the chain populations and the A/B₁₋₄ chain population ratio, were calculated (Table 4.10). Compared to the starch from fresh roots, the A/B₁₋₄ ratio of the starch from roots stored for 3 days had apparently decreased. The observation of a slightly faster increase of viscosity and a higher peak viscosity in the pastes from fresh roots of MCol 1522 noted above, could be related to the higher population of A chains in the amylopectin of this starch compared to that from starch prepared from roots stored .

Figures 4.16 X-ray diffraction patterns in starches extracted from fresh roots and from roots stored for 3 days of cassava cultivars (a) MCol 1522 and (b) MVen 25.

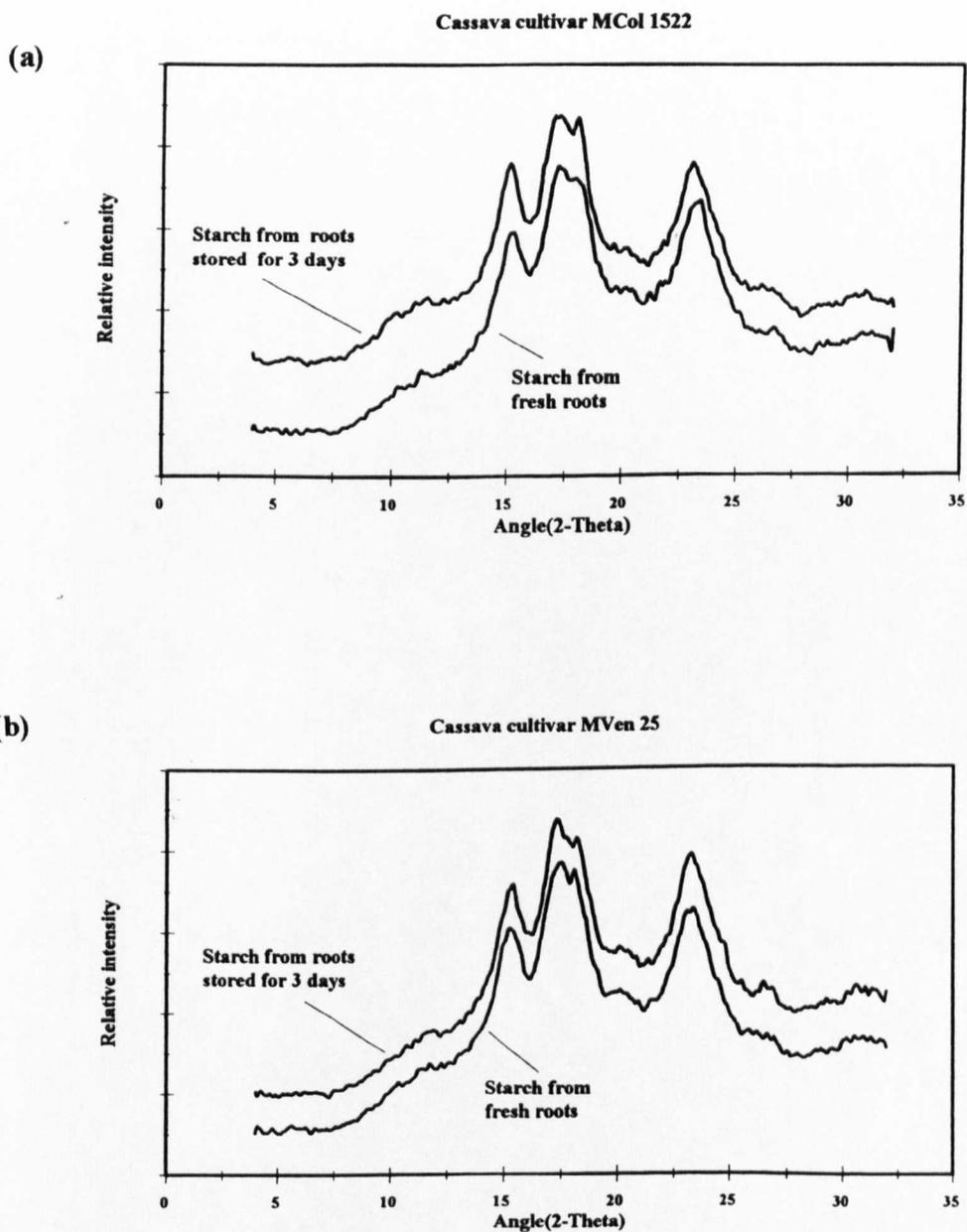


Figure 4.17 SE-HPLC chromatograms derived from debranched starches extracted from (a) fresh roots and from (b) roots stored for 3 days of cassava cultivar MCol 1522.

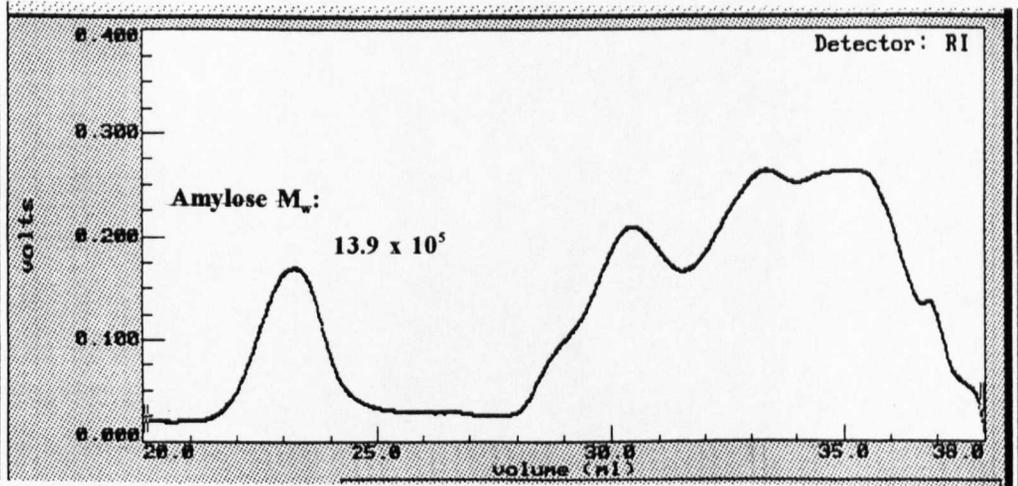
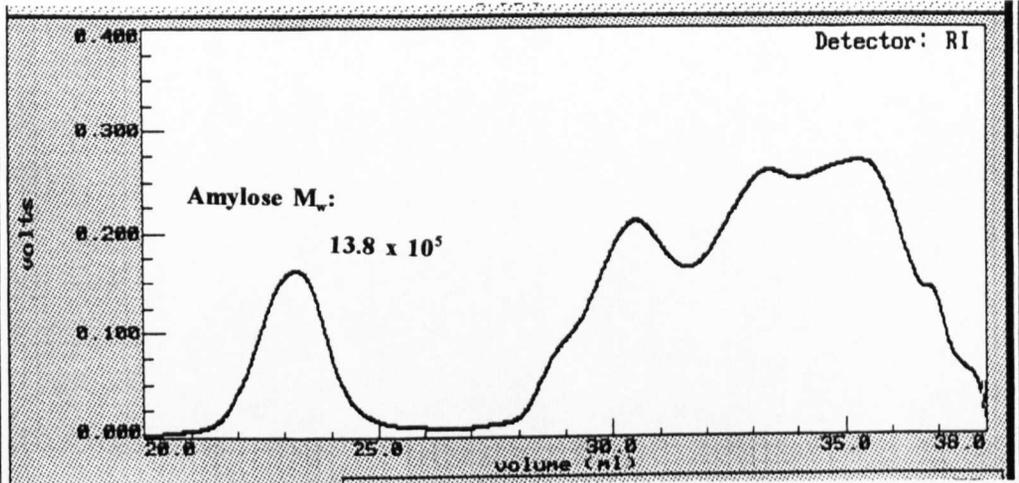


Table 4.9 Geometric mean diameter (d_{gw}), geometric standard deviation (s_{gw}), crystallinity, and amylose content of starch granules from two cultivars processed under defined conditions.

Cassava cultivar	MCol 1522		MVen 25	
	Fresh roots	Stored roots	Fresh roots	Stored roots
d_{gw} (μm)	10.5	10.2	10.3	10.9
s_{gw}	1.56	1.58	1.57	1.55
Crystallinity (%)	41.8	41.3	42.9	41.9
Amylose (%db) *	21.9 \pm 0.0	21.3 \pm 0.0	24.0 \pm 0.0	24.2 \pm 0.3

* Mean of duplicate determinations

Table 4.10 Distribution of the chain lengths (DP_n) of amylopectins of cassava starches from MCol 1522 processed from fresh roots and roots stored for 3 days.

Fraction	Whole	A	B1	B2	B3	B4	A/B ₁₋₄
<u>From fresh roots:</u>							
Chain length	26	11	24	47	71	78	
Mol %	100	64.6	25.2	9.2	0.79	0.17	1.83
<u>From stored roots:</u>							
Chain length	26	11	23	48	72	80	
Mol %	100	57.7	32.0	9.6	0.47	0.17	1.36

4.1.3 A comparison of laboratory and factory extracted starches

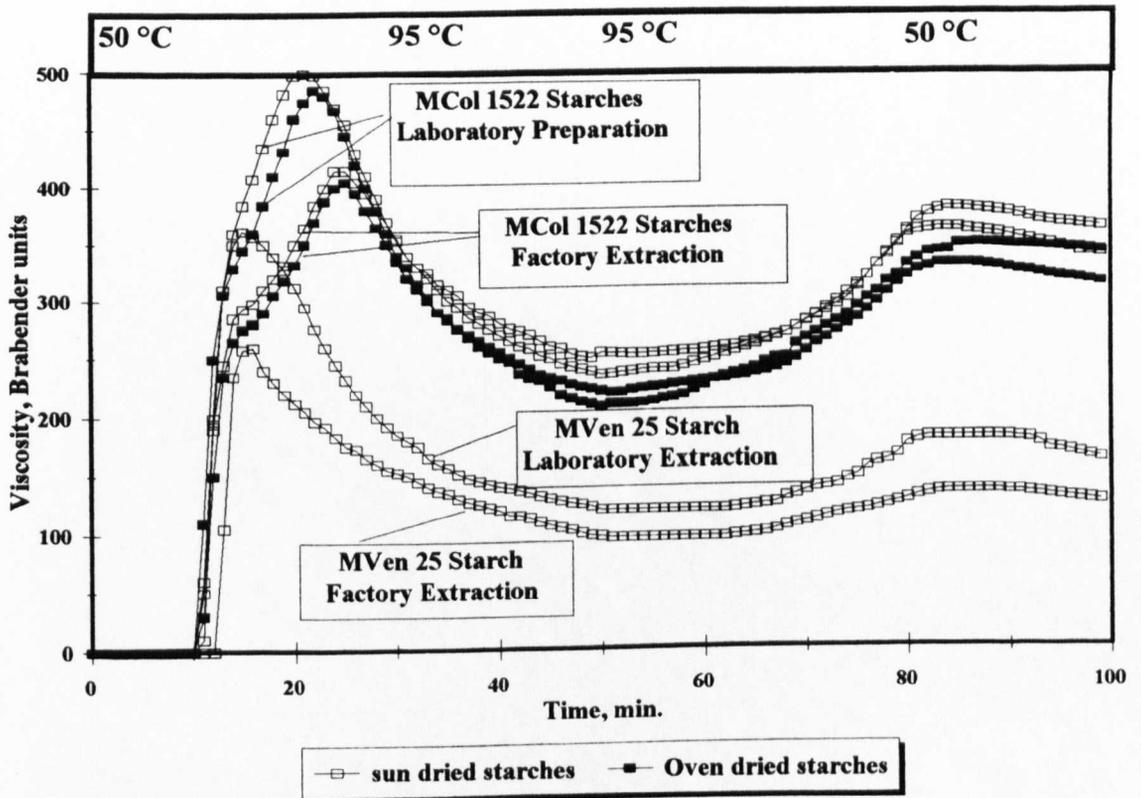
To determine the influence on starch quality of processing conditions in the rural factory, the physicochemical properties of factory prepared sweet starches in Section 4.1.1 and laboratory starches extracted from fresh roots in Section 4.1.2 were compared.

There were no perceptible differences in molecular structure between the factory extracted starches and laboratory extracted starches. The starch granule crystallites also appeared unaffected.

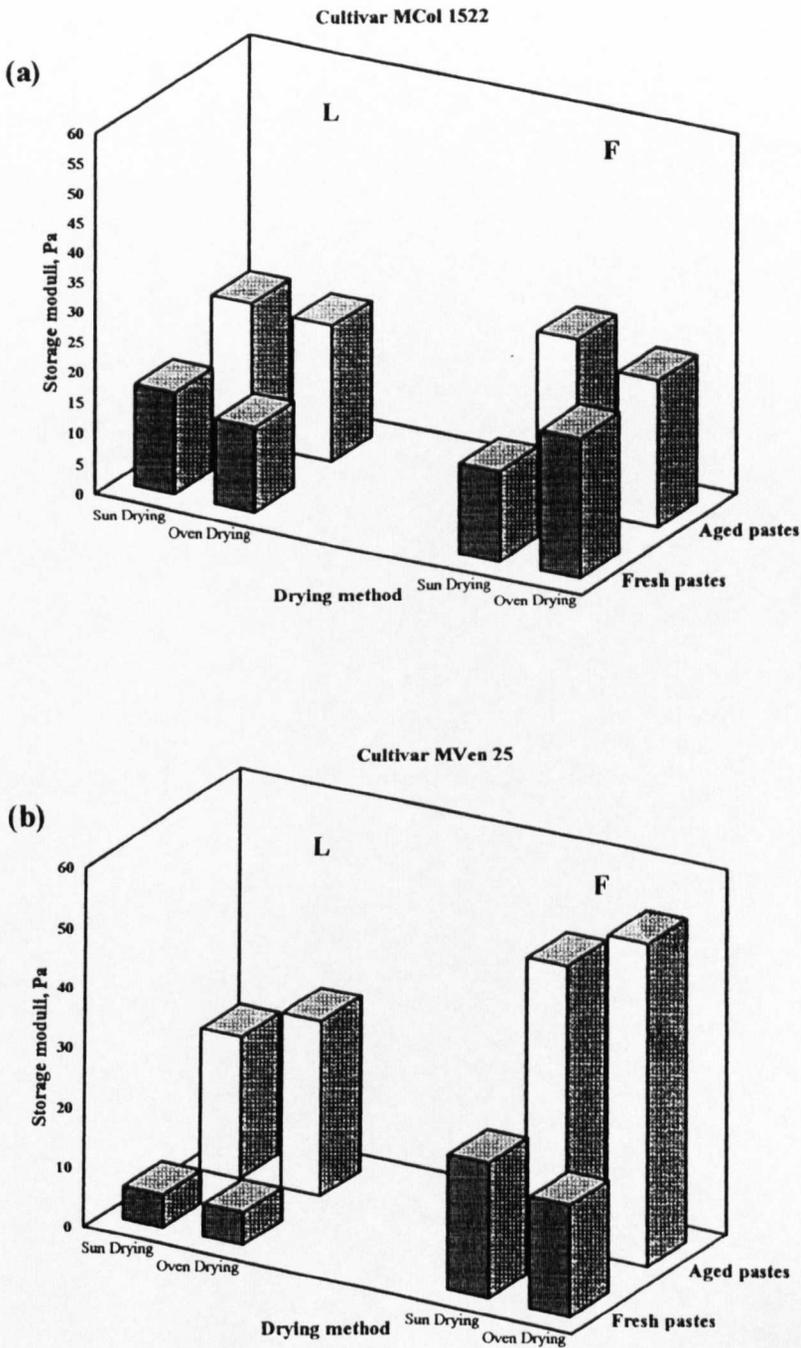
Pastes from laboratory starch from both cultivars MCol 1522 and MVen 25 had slightly higher Brabender viscosities than those prepared from factory extracted starches (Figure 4.18). The aqueous starch solubilities were slightly higher in factory starches (Tables 4.2) than in laboratory (Table 4.7). It is possible that the slightly reduced paste viscosities and increased solubilities in factory prepared starches were a sign that fermentation had already begun during the sedimentation stage of processing (Figure 1.5). Starch functional behaviour appeared unaffected by the method of drying used.

However, the aged pastes from factory starches developed higher gel shear moduli than those from laboratory starches (Figure 4.19). This effect was particularly noticeable with MVen 25 starches.

Figure 4.18 Comparison of Brabender viscoamylograms curves of factory-extracted sweet starches and laboratory-prepared native starches from cassava cultivars MCol 1522 and MVen 25.



Figur 4.19 Comparison of storage moduli (at 0.6 Hz and 25 °C) in 5% aqueous starch pastes from the laboratory-extracted samples (L) and from the factory-extracted samples (F) for cultivars (a) MCol 1522 and (b) MVen 25.



It was noted that fresh cooled pastes from factory starches exhibited a pink coloration. This was probably due to the presence of skin (periderm) residues which contains anthocyanins (NRI, Dr. J. Wenham private communication). Laboratory starch pastes from MVen 25 were more opaque than those from MCol 1522, possibly due to the the MVen 25 starches having a higher amylose content than the MCol522 starches. The faster reassociation of amylose as part of the retrogradation phenomenon results in a corresponding increase in opacity.

A high concentration of amylose in solution, and maybe some compounds released from the root peels could have intensified the retrogradation and aggregation of molecules in solution, resulting in the high gel shear moduli in aged pastes prepared with factory extracted starches from MVen 25.

4.2 REMARKS AND CONCLUSIONS

The factory-prepared samples from the sweet and fermented starches were also assayed for lactic acid content and baking expansion properties at CIRAD (Montpellier, France) and the results have been reported by Mestres (1995). The average values of lactic acid content and baking expansion (Tables 4.16, 4.17) showed that the fermented starches had elevated amounts of acetic acid as a product of the natural fermentation, especially in the fermented oven dried starches. The fermented, sun dried starches were the only samples with a significant baking power. Compared to the MVen 25 starch , the fermented, sun dried sample from the MCol 1522 starch

Table 4.11 Lactic acid content (mg/g dm) of cassava starch from two cultivars processed at the shown conditions.

Cassava cultivar	MCol 1522		MVen 25	
	Sun dried	Oven dried	Sun dried	Oven dried
Sweet	< 1.0	< 1.0	< 1.0	< 1.0
Fermented	3.4	5.5	6.1	7.5

From Mestres (1995)

Table 4.12 Baking expansion property, specific volume (cm³/ g dm), of cassava starches from two cultivars processed at the shown conditions.

Cassava cultivar	MCol 1522		MVen 25	
	Sun dried	Oven dried	Sun dried	Oven dried
Sweet	5.5	3.5	4.5	3.0
Fermented	13.0	5.5	10.5	5.0

From Mestres (1995)

had better expansion and a lower content of acetic acid. These results confirmed the reports from other research work on natural fermentation of cassava starch by Larssonneur (1993) and Brabet (1994) who both demonstrated that sun drying imparted to the fermented starches an expansion capacity upon oven baking. It seems that the U.V. solar radiation catalyzed some chemical reaction involving the mildly modified fermented starch and other compounds from the fermentation process or microbial metabolites such as acetic acid and hemicelluloses (Brabet,1994).

From the results it is concluded,

1. Fermented, oven dried starches, which did not expand when a dough was made and oven baked (Mestres, 1995), were found to have suffered molecular degradation when compared to the sweet starches. They were, therefore, more soluble in hot water than the sweet starches, and their pastes had a reduced viscosity compared to their sweet counterparts. The molecular degradation in the fermented oven dried starches was the result exclusively of the fermentation process, since Brabet (1994) demonstrated that the paste viscosities of wet fermented starches were comparable to those of oven dried, fermented starches. During the fermentation, lactic acid was produced and amylolytic activity was observed which suggests that the starch was modified by acid hydrolysis and/or enzymic action (Brabet, 1994).

2. Fermented, sun dried starches, which had the ability to expand when a dough was

oven baked (Mestres, 1995), were found to have suffered greater molecular degradation than the fermented, oven dried starches. They were highly soluble in hot water and possessed the lowest pasting viscosities. This provides strong evidence that the molecular structure of the fermented starches was further modified during sun drying. The origin and the mechanism of the molecular degradation during the sun drying needs to be investigated further, but it is believed to be the result of an oxidative process activated by the solar U.V. radiation. Wet, fermented starch has the potential of being modified by solar radiation (Brabet, 1994) as it has the products of fermentation (*e.g.* lactic acid) which apparently participate in a chemical reaction with the starch during sun drying. Furthermore, the results of research on the effects of antioxidants on starches in progress in this laboratory, may suggest that the products in the wet fermented starch (*e.g.* water, lactic acid and hemicelluloses) plus U.V. radiation could initiate free radicals and/or superoxide ion ($\cdot\text{O}_2^-$) that then cause an oxidative reductive reaction leading to the depolymerization of the starch polysaccharides (Hashim *et al.*, 1992, Paterson *et al.*, 1994, and Valles-Pamies, 1996). Further research in this area is under consideration.

3. The pastes of fermented, sun dried starches were rich in amylopectin in the solvent phase. Compared to the weak, liquid-like pastes from fermented, oven dried samples, the pastes from fermented, sun dried starches developed some gel strength upon ageing which, it was believed, was due to the more rapid reassociation of shorter amylose chains detected in their solvent phases. The above observations could help in understanding why the fermented, sun dried starches expanded when a dough was

oven baked. Mestres (1996), incidentally, noted that amylopectin extracted and purified from both sweet and sour starches puffed during the final drying at 100 °C in the extraction procedure, whereas purified amylose did not puff which suggests that amylopectin could be responsible for the gas retention and expansion and associated with baking. Hence, since fermented, sun dried starch in this study had the ability to solubilize substantial amounts of amylopectin in hot water, the dough made with this starch could produce an amylopectin rich continuous phase when oven-baked, leading to the retention of gas bubbles generated on cooking and to the final expansion of the dough. On cooking, the presence of shorter amylose chain in the continuous phase of the doughs made with fermented starches would contribute to building a more rigid structure in the cooked dough on cooling. There is still the question as to why the dough made of MVen 25 fermented sundried starch did not expand to the extent of the MCol 1522 dough (Table 4.17). A possible explanation is that the polysaccharides in the MVen 25 fermented sun dried starch had been subjected to a higher molecular breakdown (Figure 4.10), In consequence, the more highly degraded amylopectin in solution would not resist expansion to the degree exhibited in the the MCol 1522 dough. This possibility arose from the observation of the results of Camargo *et al.* (1988), where an expansion during baking obtained with medium-acid treated starches disappeared progressively as starch depolymerization increased with an extended duration of the acid treatment. It was concluded that the acid hydrolysis must be very limited otherwise expansion would be reduced. Since the paste viscosity of the MVen 25 sweet starch was lower than the MCol 1522 counterpart, it is possible that MVen 25 is more prone to the modifications during the fermentation and sun drying and

consequently to suffer greater depolymerization and a reduced baking expansion.

4. Storing the roots for 3 days prior to processing slightly affected the paste viscosity, but not the swelling of the granules nor starch solubility in hot water. Other properties such as the starch composition, granular and molecular structure were also found to be unaltered. The quality of sweet starch appeared to be affected by the processing conditions employed (Section 2.2.2). Compared to laboratory prepared starches, factory extracted sweet starches had pastes with a pink visual appearance, as a result of contamination, and showed some signs that fermentation had already begun during the sedimentation stage of processing.

CHAPTER 5

EFFECT OF PROCESSING CONDITIONS ON THE PROPERTIES OF CASSAVA FLOUR

5.1 RESULTS AND DISCUSSION

Two cassava cultivars, CM3306-4 and MVen 25, were processed to produce flour with the experimental conditions described in Section 2.3.1 (Figure 2.4). Cultivar CM3306-4 was planted in two different years. The first harvest date was in October 1992 and the second in September 1993. The cultivar MVen 25 was only harvested in September 1993. All plants were harvested after 10 months of growth.

5.1.1 Drying of the cassava chips

Figures 5.1 and 5.2 show the moisture and temperature-time curves for the cassava chips from each drying experiment at air temperatures of 40, 60 and 80 °C. The moisture content and the temperature are average measurements of the entire bed of cassava chips in the drying chamber, determined in representative samples of chips collected at different depths in the drying bed. According to the results obtained, by the time the bed of chips reached temperatures over 50 °C (for drying conditions at 60 and 80 °C) their moisture contents were about 15 - 13%. Therefore the starch in the chips could not have undergone gelatinization since the water available was too low for the starch granules to have suffered a change in their structural order when the chips temperatures were over 50 °C (which is the case for granules for cassava

Figure 5.1 Drying curves of cassava chips from cultivar CM3306-4 produced in 1992.

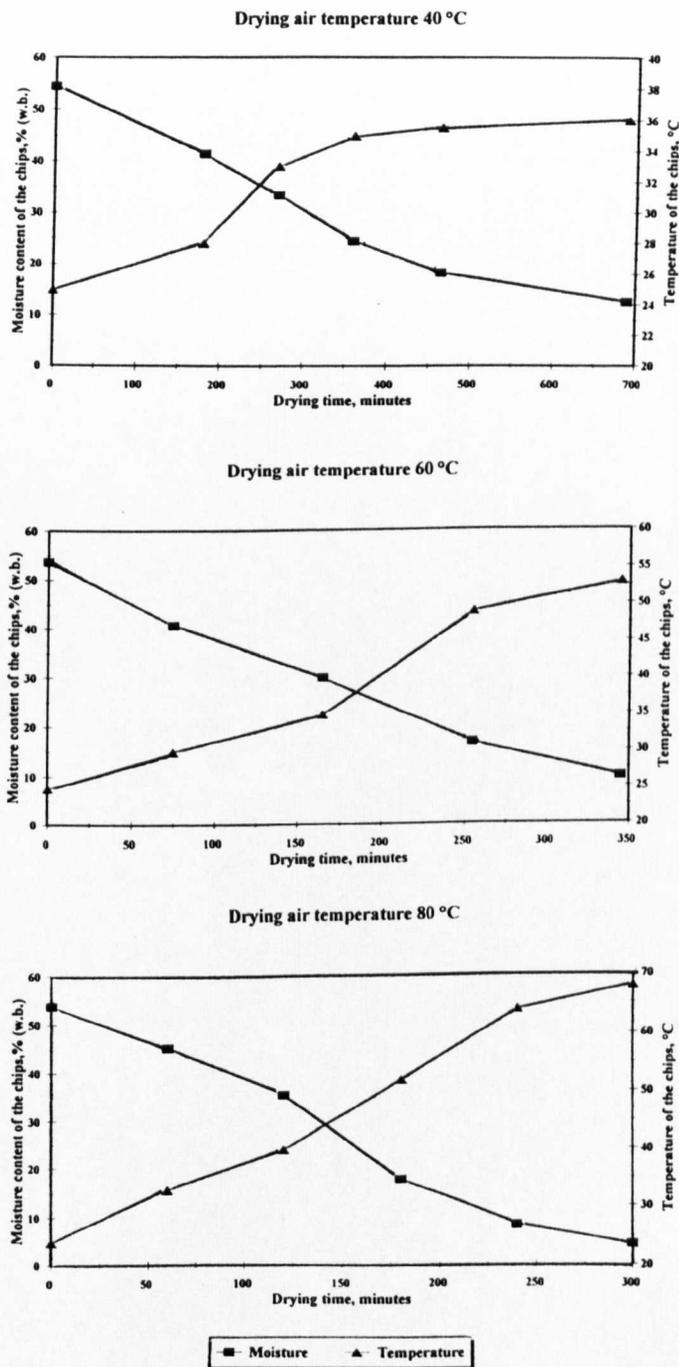
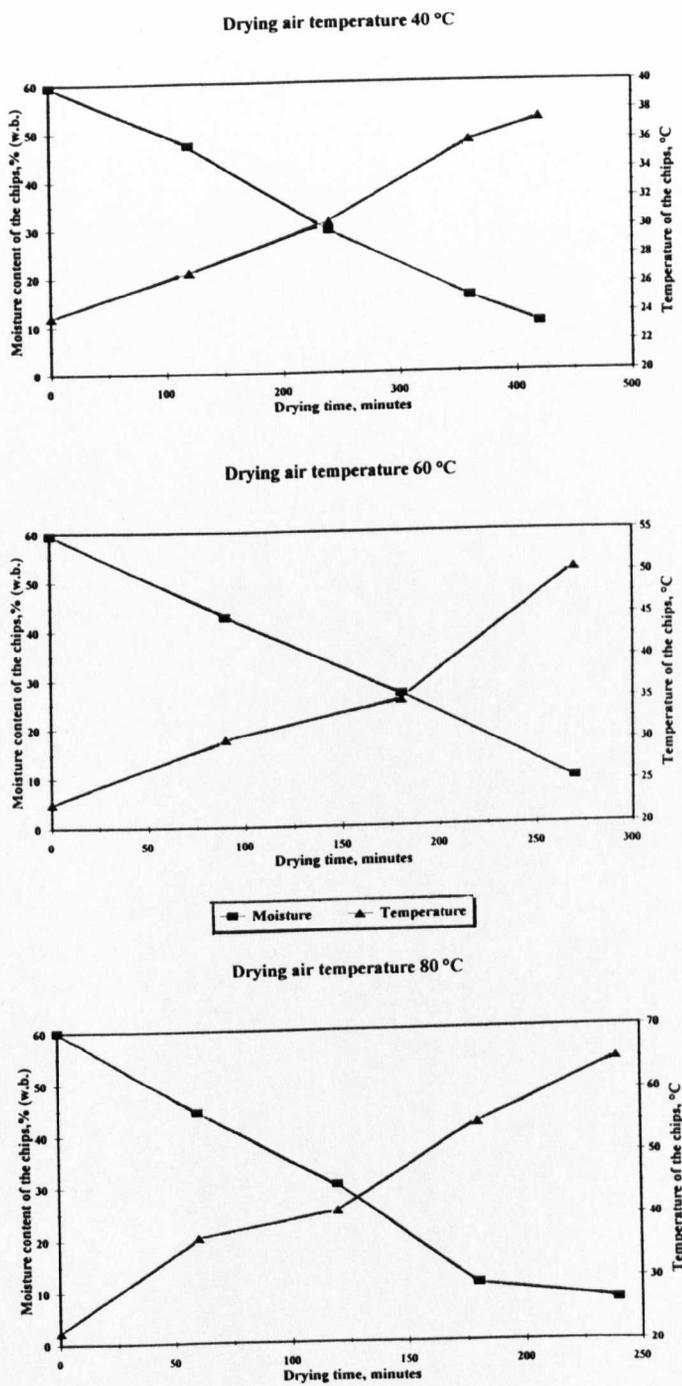


Figure 5.2 Drying curves of cassava chips from cultivar CM3306-4 produced in 1993.



starch in excess water, Asaoka *et al.*, 1992). However, during drying at temperatures above 40 °C, individual chips may have developed internal gradients of moisture and temperature which are not reflected in the mass-average moistures and temperatures observed in Figures 5.1 and 5.2. Furthermore, the layer of chips touching the floor of the drying chamber (where the inlet of the drying air is located) could have dried rapidly on the surface and then have slightly scorched at the highest drying temperature of 80 °C. Scorching of the surface of some chips could have reduced the moisture diffusion inside the chips and the internal temperature could have been raised to a sufficient level to cause starch gelatinization. These observations are made because it will be seen below that the pasting viscosities of the flour were found to be affected by the drying temperature of the chips.

5.1.2 Particle size distribution in the flours

Samples of ground chips from each mill (roller, hammer, pin and paddle mill, whose characteristics and operating conditions are given in Section 2.3.1) were passed through a 250 µm laboratory sieve. These sieved materials, which were considered flours, had a particle size distribution (from a sieving analysis with sieves of sizes 250 µm, 212 µm and 150 µm and 106 µm) which varied with the type of mill used (Figures 5.3 and 5.4). The roller mill produced the coarsest flour particles and the paddle mill the finest flour particles. The hammer-milled and pin-milled flours had about the same particle size distribution. The predominant proportion of particle size in the flours was below 106 µm, which was between 60 and 92 %. Two types of flours with different granule sizes were subjected to analysis, Flour M which passed

Figure 5.3 Particle size distribution in flours from cassava chips dried at 40, 60 and 80 °C and milled by: roller, hammer, pin and paddle mills. Flours were prepared from cultivar CM3306-4 produced in 1992

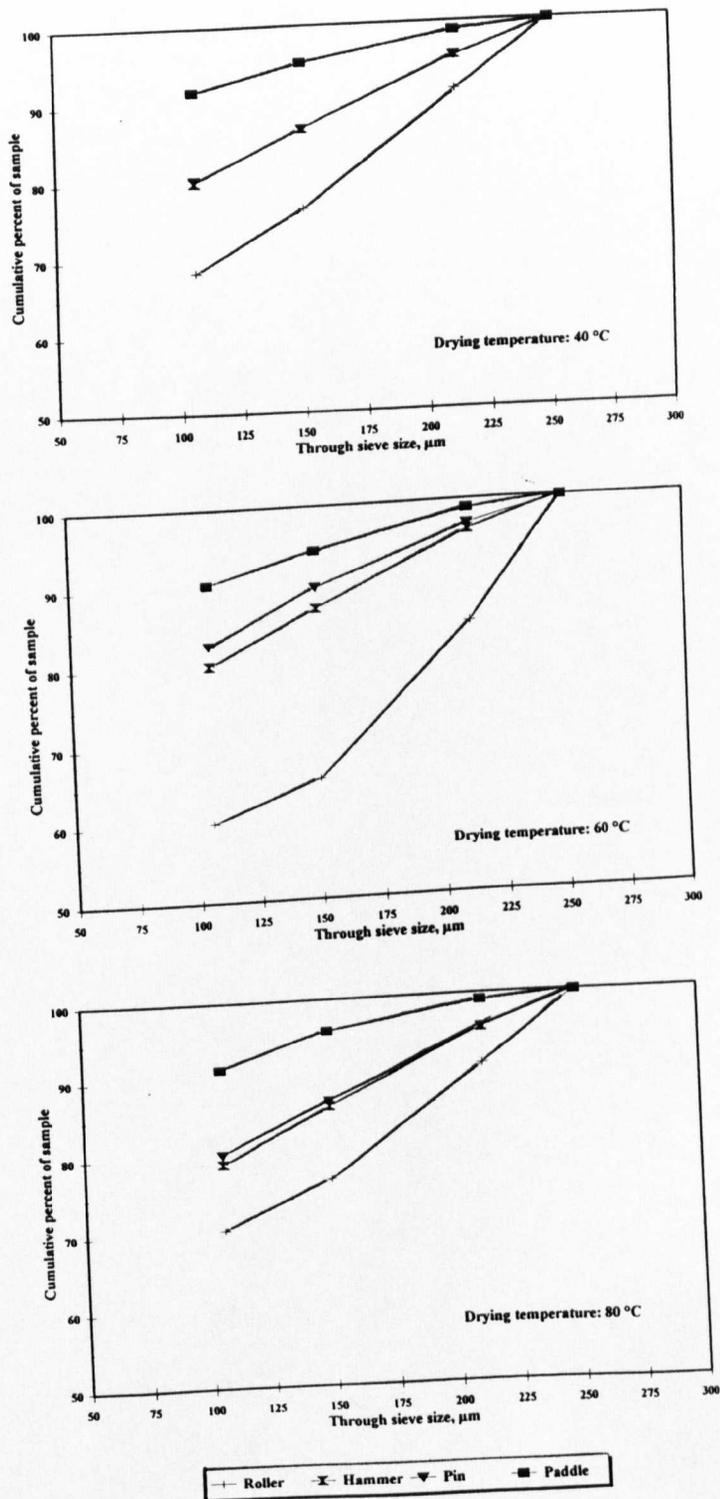
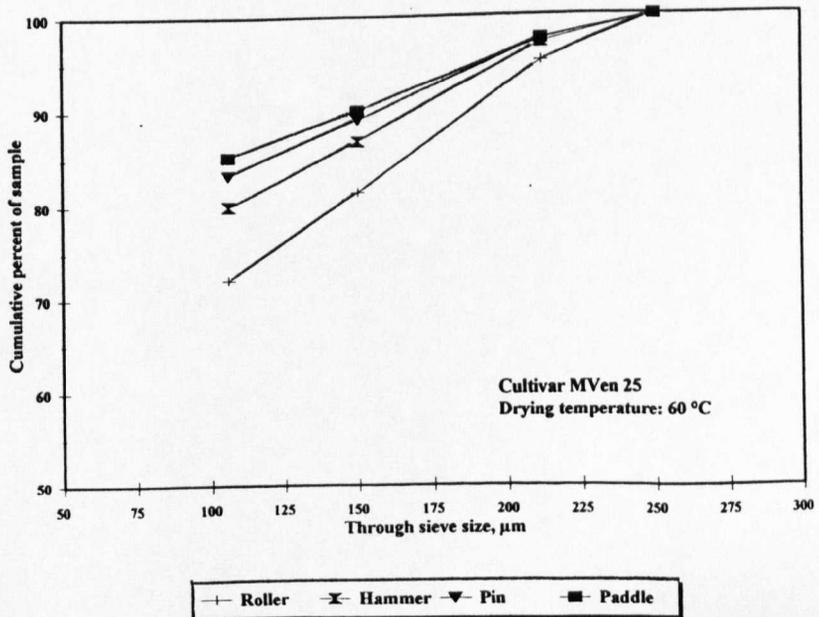
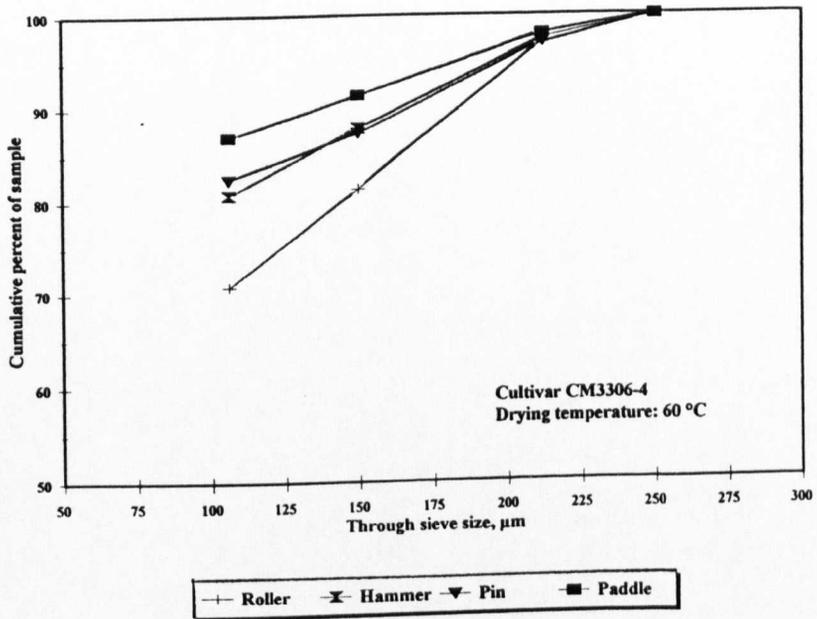


Figure 5.4 Particle size distribution in flours from cassava chips dried at 40, 60 and 80 °C and milled by: roller, hammer, pin and paddle mills. Flour were prepared from cultivar CM3306-4 produced in 1993.



through the 250 μm sieve and Flour N which passed through the 106 μm sieve.

5.1.3 Pasting characteristics

Flour suspensions at 6% concentration were tested in a Brabender viscoamylograph using the conditions as previously described (Section 2.5.2). From the Brabender viscograms, several profiles were selected and other plots prepared to demonstrate the changes in the pasting characteristics of the flours in relation to the processing procedures (Figures 5.5 - 5.10). From the viscograms several important values were determined which are summarized, together with the contents of starch, crude fibre and ash, for all samples in Tables 5.1 - 5.4.

The viscosity profiles of cultivar CM3306-4 Flour M samples from the harvests of October 1992 and September 1993 are given in Figures 5.5 and 5.6 respectively. The viscosity curves show that all the flours produced from cassava chips dried at 60 and 80 °C were distinguishable from those flours produced from chips dried at 40 °C (Figure 5.6). The majority of flour samples from chips dried at 80 °C exhibited the fastest and highest increase in viscosity. The samples from chips dried at 60 °C developed faster and higher viscosities than samples from the drying tests at 40 °C. In the pin-milled and paddle milled flour samples, the effect of the chip drying temperature on the pasting properties of the flours, observed during the swelling phase in the viscograms, is not clearly defined after reaching the maximum viscosity (*i.e.* after the granular disruption of the starch). However, with roller milled samples the effect of drying temperature was obvious throughout pasting. From the

Figure 5.5 Brabender viscograms of pastes from cassava flours prepared from chips dried at 40, 60 and 80 °C and milled by: roller, hammer, pin and paddle mills.

Samples are from Flour M prepared from cultivar CM3306-4 produced in 1992.

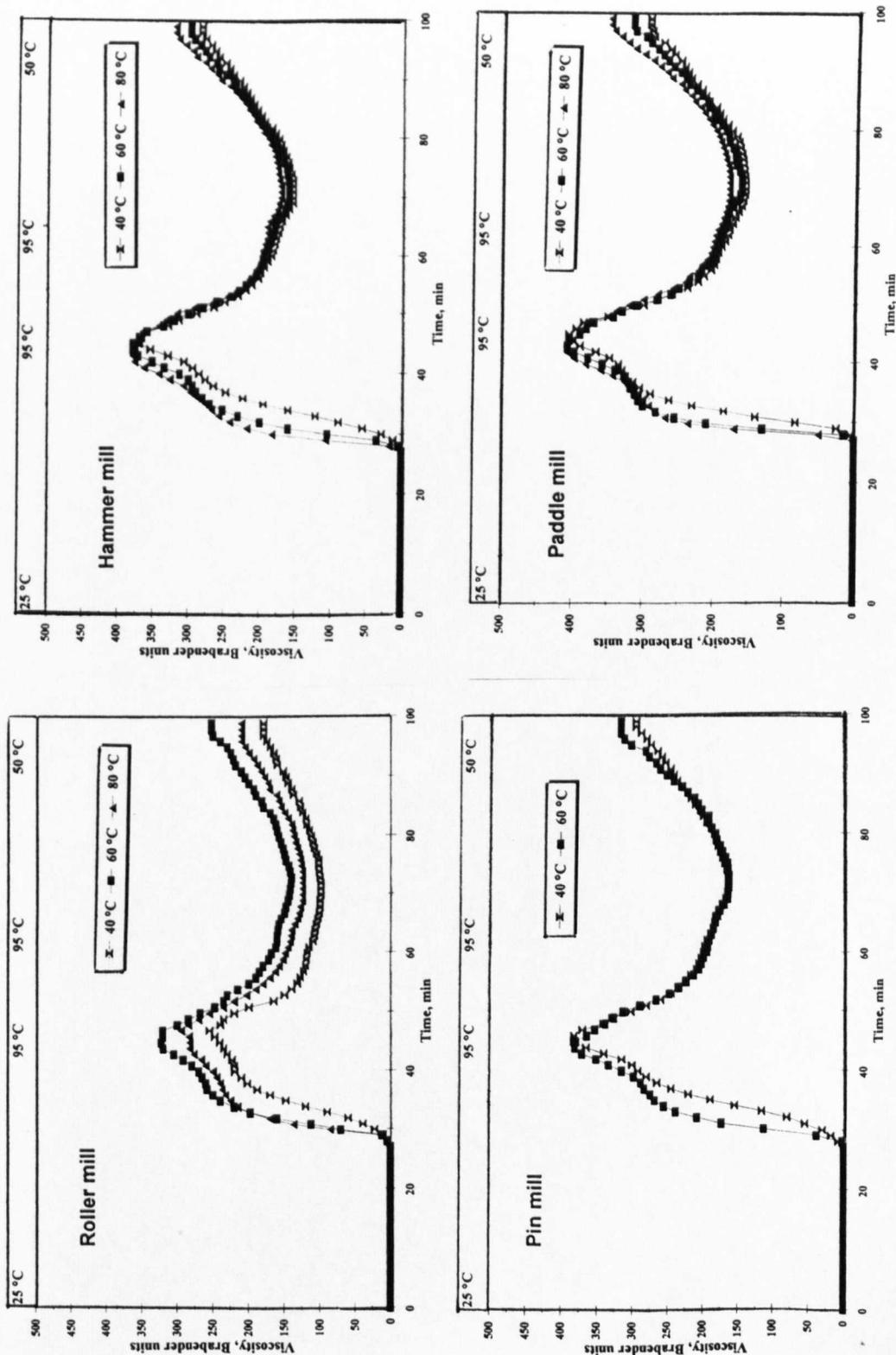
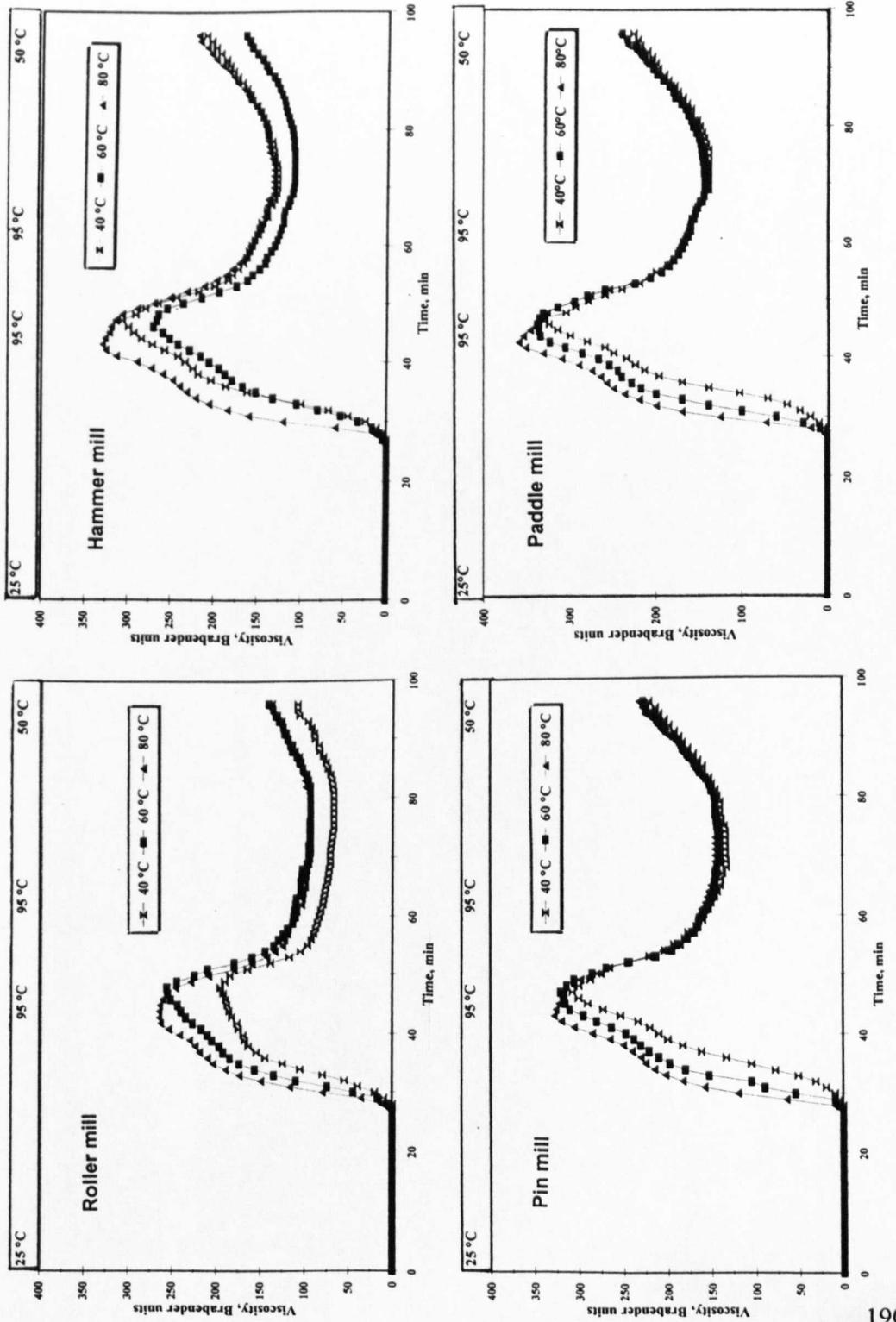


Figure 5.6 Brabender viscograms of pastes from cassava flours prepared from chips dried at 40, 60, and 80 °C and milled by: roller, hammer, pin and paddle mills.

Samples are from Flour M prepared from cultivar CM3306-4 produced in 1993.



results it seems that there are other factors which can play an important role in the pasting characteristics of the flours. The method of milling was found to affect particle size distribution in the flours and could possibly have influenced the proportion of mechanically damaged starch. It is possible that a higher proportion of damaged starch occurred in the finest ground flour from the paddle and pin mills and this could have influenced granular disruption on pasting, reducing the effect of drying temperature. The combined effects of the drying temperature and milling method on the Brabender pasting characteristics of the flours are exemplified with in Figures 5.7 and 5.8. Paddle milled flour samples from chips dried at 80°C and 60 °C tended to take the shortest time to reach the peak viscosities (*i.e.* lower values of ease of cooking) and have higher cool viscosities (*i.e.* viscosities at 50 °C upon cooling) than the flours from roller milled chips dried at 40 °C. It was clear that the drying temperature at 80 °C and to some extent at 60 °C slightly altered the properties of the flours, possibly by causing gelatinization of the starch in a proportion of the mass of chips as previously explained.

Figure 5.9 shows more clearly the effect of the milling procedure used on the pasting characteristics of Flour M samples produced from chips dried at 60 °C . Paddle milled and pin milled flours had higher paste viscosities than hammer and roller milled flours. The other Flour M samples from chips dried at 40 °C and 80 °C always showed that roller-milled flours had the lowest paste viscosities but the hammer and pin milled flours exhibited the same pasting characteristics, both having lower paste viscosities than the paddle milled flours (Table 5.1 and 5.2). Flour N samples

Figure 5.7 Brabender pasting data: ease of cooking and cool viscosity in pastes from flours prepared from chips dried at 40, 60 and 80 °C and milled by: roller, hammer, pin and paddle mills. Samples are from Flour N prepared from cultivar CM3306-4 produced in 1992.

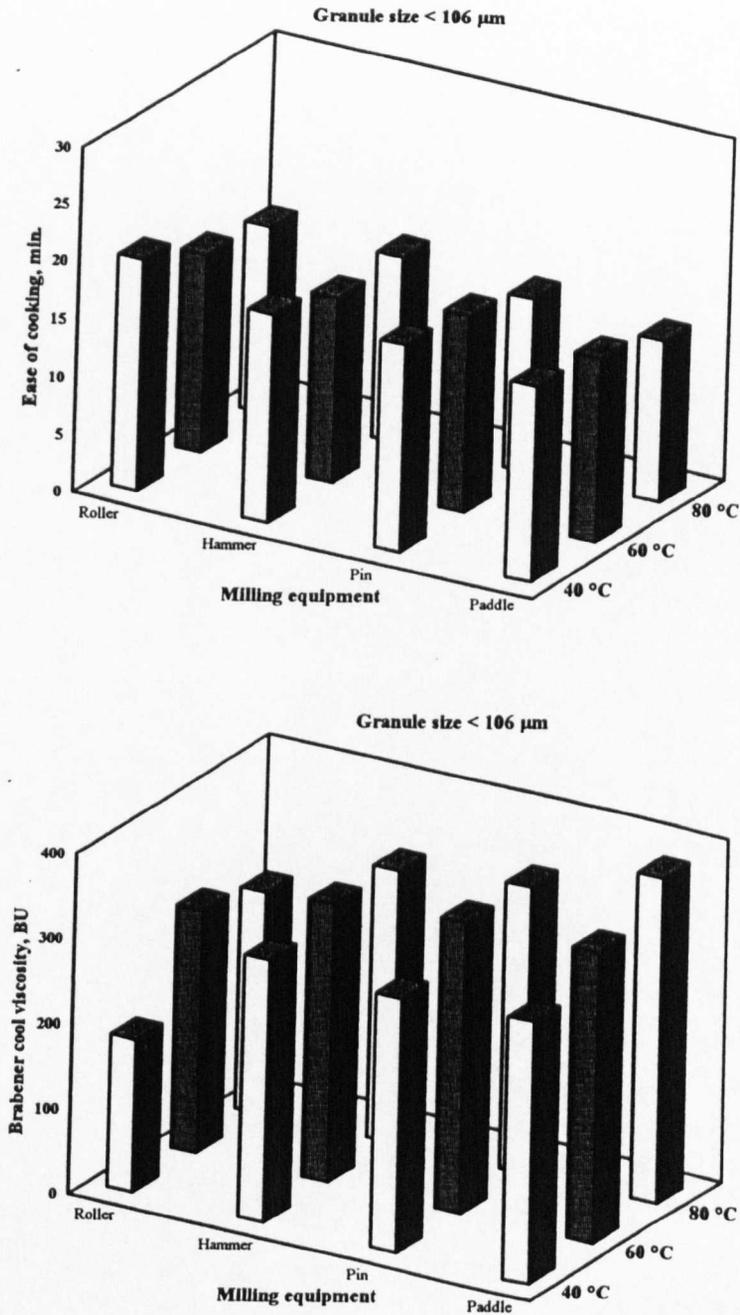
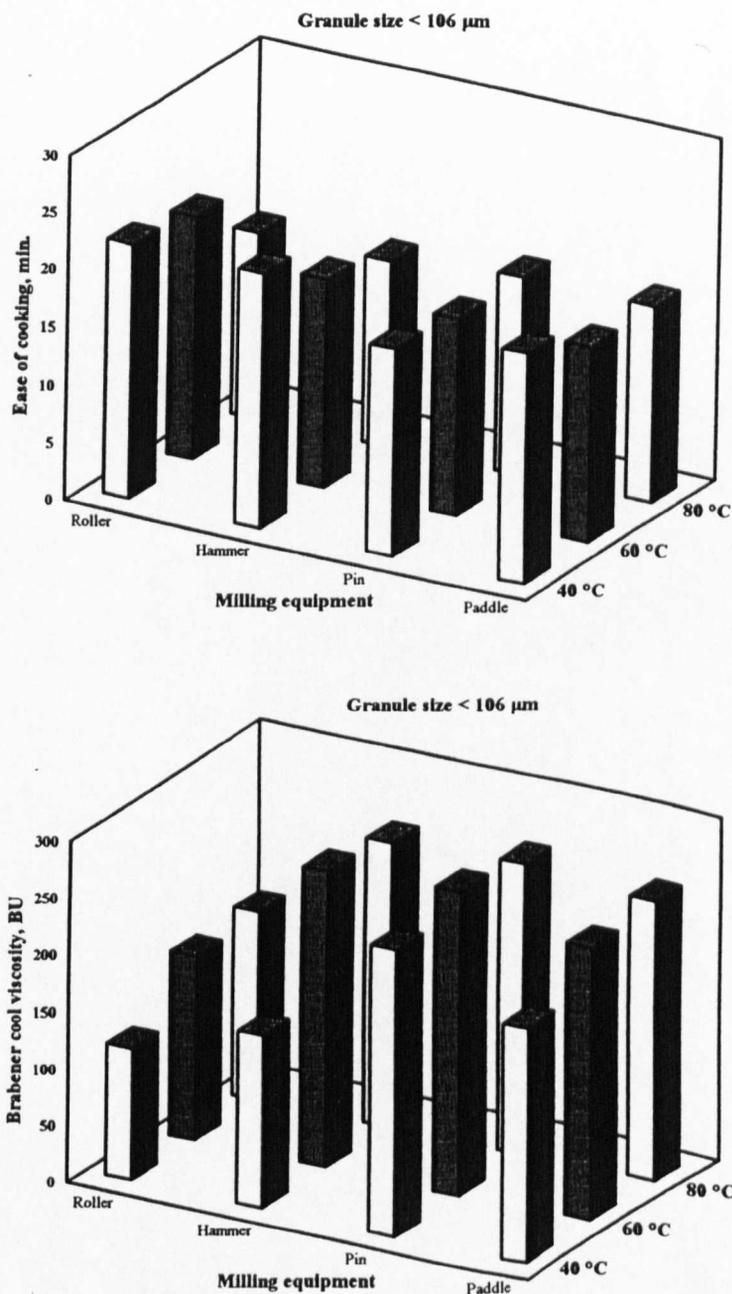


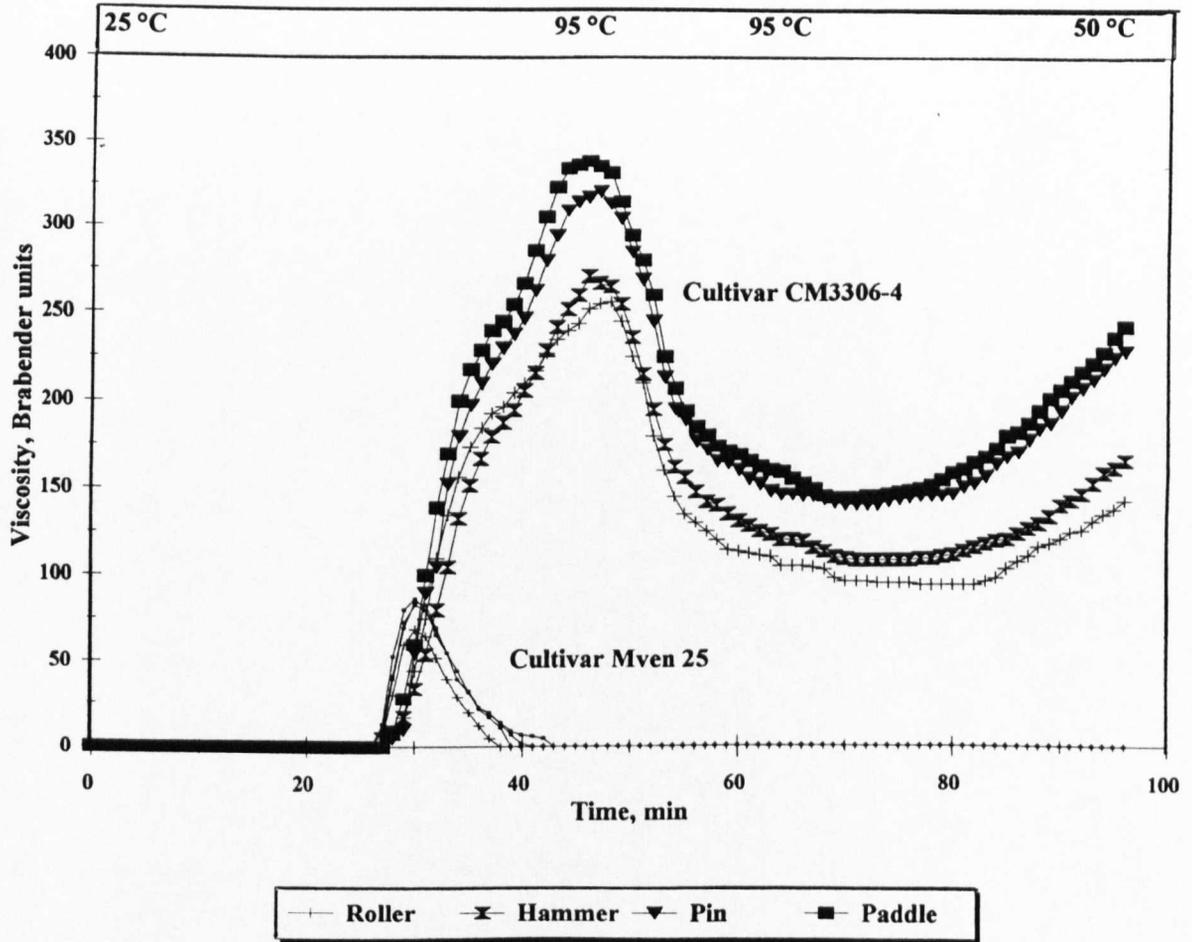
Figure 5.8 Brabender pasting data: ease of cooking and cool viscosity in pastes from flours prepared from chips dried at 40, 60 and 80 °C and milled by: roller, hammer, pin and paddle mills. Samples are from Flour N prepared from cultivar CM3306-4 produced in 1993.



confirmed the same effects of milling procedure as exhibited by the Flour M samples (Tables 5.1 and 5.2). As shown above, the particle size distribution in the flours depended on the milling equipment, with the finest ground flours being produced in the paddle mill. The finest flours would be expected to have a high proportion of mechanically damaged starch, as reported for wheat flour by Sullivan *et al.*, 1960 and Sosulski *et al.*, 1988, for rice flour by Nishita and Bean (1982), and an increased area of particles per unit of mass as suggested for cassava flour by Raja *et al.* (1978). Fine flours have been reported with higher water absorption than the coarse flours from cassava (Raja *et al.*, 1978) and Sefa-Dedeh, 1989) and rice (Nishita and Bean, 1982). This could explain the higher pasting viscosities in the finely ground flours compared to the coarsely ground flours.

All samples of MVen 25 flours exhibited very limited pasting properties as compared with the viscosity profiles of flours from cultivar CM3306-4 (Figure 5.9, Tables 5.2 and 5.3). This difference is essentially attributable to the endogenous properties of CM3306-4 and MVen 25 starches. In Chapter 3, it was shown that starch extracted from the MVen 25 cultivar had lower viscosity profiles than CM3306-4 starch, but this difference was enhanced in the flour samples which contained around 79-92 % starch and other compounds that could influence the pasting properties. Fibre and ash were determined in all samples (Tables 5.1, 5.2, 5.3, 5.4) and their recorded amounts suggest that they should be considered as factors which might affect the pasting behaviour of the flour suspensions. In general, the ash content was about the same level in all the samples, but it was observed that the fibre content

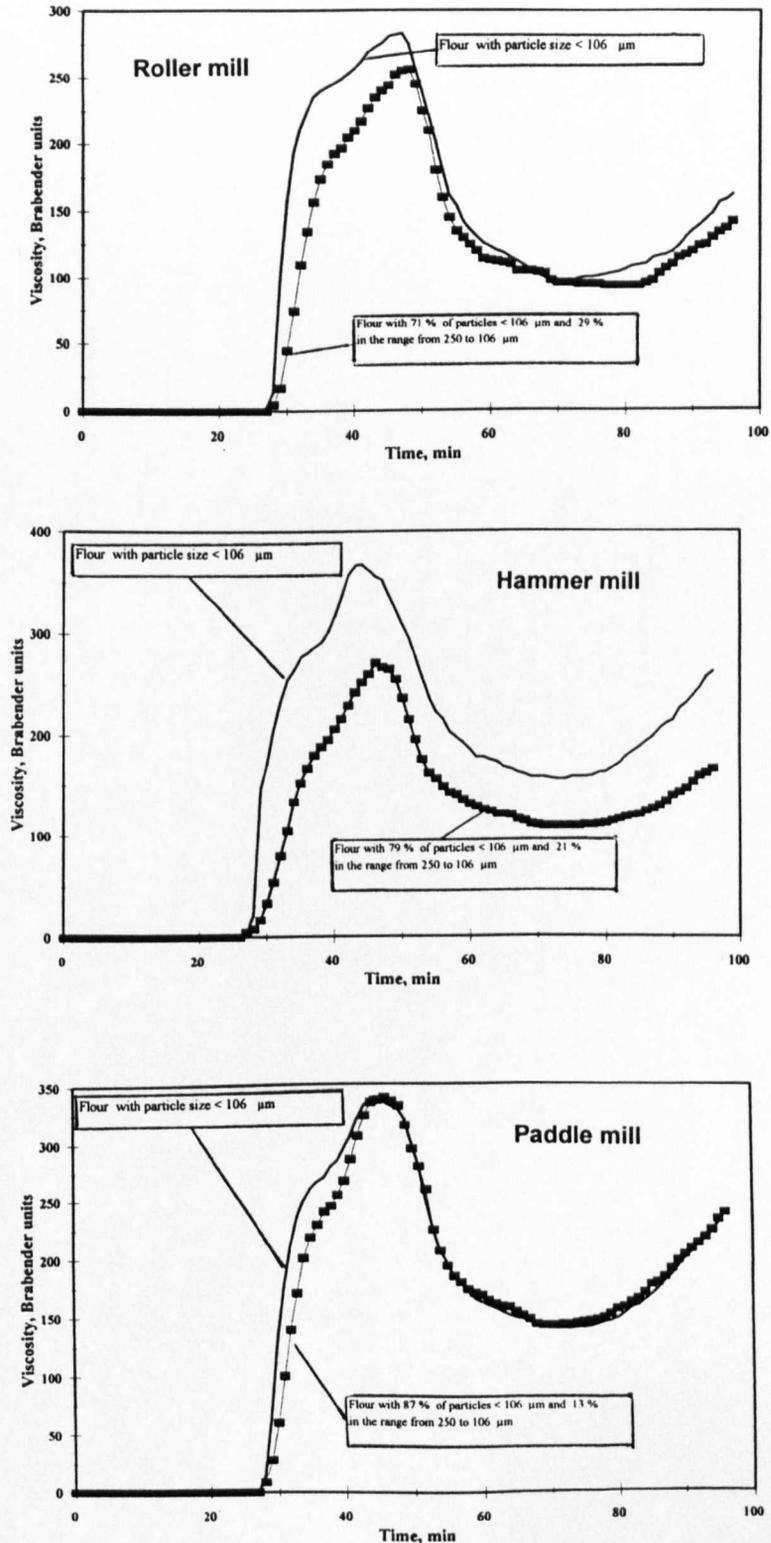
Figure 5.9 Brabender viscosograms of pastes prepared from cassava flours milled by: roller, hammer, pin and paddle mills. Flours were obtained from chips dried at 60 °C and cultivars CM3306-4 and MVen 25 produced in 1993.



(crude fibre and total dietary fibre) was lower in the more finely sieved ($< 106 \mu\text{m}$) Flour N samples compared with the Flour M samples ($< 250 \mu\text{m}$). Together with the reduced fibre content in the Flour N samples the concentration of starch in these flours was also increased as can be seen in Table 5.1. Flours prepared from cultivar CM3306-4, produced in 1992, had lower crude fibre than those produced in 1993. This difference in fibre content between harvests could partially explain the higher pasting properties exhibited by the 1992 flours. It appears to be that the presence of the fibre in the flours is a barrier to the free swelling and hence to viscosity development upon pasting. Compared to the viscosity profiles of cassava starch pastes in Chapter 3 (Figure 3.1), the cassava flour pastes exhibited a pronounced, two stage swelling as observed by the sharp inflection in the viscosity curves during heating. This phenomenon is attributable to the presence in the flours of compounds others than starch, such as fibrous materials. This discussion of the effect of the fibre content in the flours on their pasting characteristics does not apply to MVen 25 flours which had lower fibre content than those from CM3306-4 produced in 1993. As observed above, the primary factor that limited the pasting of MVen 25 samples was their starch which is naturally different to the CM3306-4 starch.

The pastes of Flour N samples had higher viscosities than Flour M samples (Figure 5.10) which could be attributed to the reduced amount of fibre and smaller particle size in the finely sifted Flour N samples. As shown in Figure 5.10, the difference between the viscosity profiles was dependent on the particle size distribution in the flours.

Figure 5.10 Brabender viscograms of pastes prepared from cassava flours with different particle size distribution milled by: roller, hammer, pin and paddle mills. Flours were obtained from chips dried at 60 °C and cultivar CM3306-4 produced in 1993.



5.1.4 Physical testing of doughs

The feasibility of incorporating cassava flour into wheat bread at levels of 10-20 % has been demonstrated (Ciaccoco, *et al.*, 1978; CIAT *et al.*, 1988; Almazan, 1990; Eggleston *et al.*, 1993a). The rheological properties of doughs made of wheat-cassava flour blends have also been reported (Ciaccoco, *et al.*, 1978 and Eggleston *et al.*, 1993). The incorporation of cassava flour at any level in wheat flour was found to reduce the development time and dough stability when compared with 100 % wheat doughs. Cassava flours from different cultivars were found not to have a marked effect on the dough properties. The effect of different drying and milling conditions for the production of cassava flours on the rheology of doughs of wheat-cassava flour blends has not previously been reported.

A Brabender farinograph was used for testing the effect of incorporating cassava flour samples on wheat flour dough quality with a 20 % level of cassava flour (Section 2.5.4). A sample of flours prepared from cultivars harvested in September 1993 were selected for evaluation. Figure 5.11 shows the typical Brabender farinograph (a) for a 100 % wheat dough and (b) for the composite wheat-cassava dough. The required farinograph data: water absorption (WA), dough development time (DT), dough stability (ST) and dough degree of softening (DS) were obtained (Table 5.5). The substitution of wheat for cassava flour affected the dough performance in the farinograph as compared with the 100 % wheat dough. With the exception of the flour blend made with pin milled cassava flour, WA values for all the other blend were less than for the 100% wheat dough, and the DT's were similar.

The flour blend made with pin milled cassava flour gave WA's quite similar to the 100% wheat flour but slightly lower DT's which was probably caused by the presence of the more damaged starch granules which absorbed more water and faster. Cassava flours of different cultivars mixed with wheat flour have also exhibited lower DT's than the 100% wheat flour doughs (Eggleston *et al.*, 1993a). This is considered to be due to the lower gluten strength caused by the addition of cassava flour and also indicates that water uptake by the various components present in cassava flour is faster. DT (time of mixing of the developed dough before it begins to breakdown or to lose strength) for all blends was considerably lower than in the wheat sample. The DS (the amount of strength reduction after 12 minutes of mixing) was for all blends higher than in the control, indicating an overall weakening of the doughs by cassava flour substitution.

By comparing the farinograph data for both cultivar it was concluded that the processing conditions during the cassava flour preparation were not reflected in the dough rheology characteristics, at least at the level of the cassava flour substitution used (20%) in the blends. However, as this test does not involve any heating of the samples it may not be a true reflection of the potential uses of these flours for food

Figure 5.11 Brabender farinograms of (a) a wheat dough and (b) a wheat-cassava blend dough.

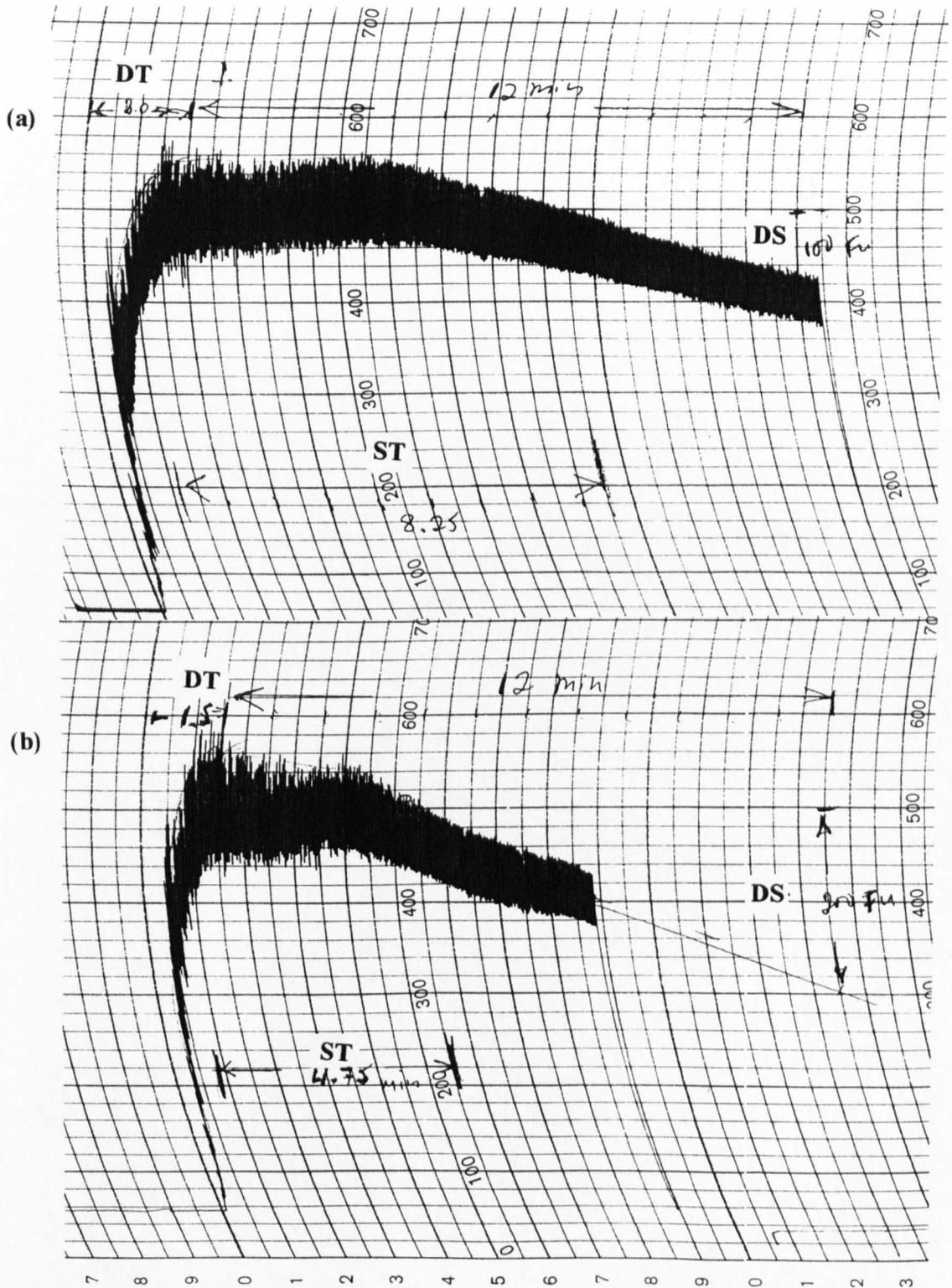


Table 5.1 Properties of cassava flours processed as specified.

Cultivar CM3306-4 produced in 1992

MILLING EQUIPMENT:	HAMMER			ROLLER			PIN			PADDLES		
DRYING TEMPERATURE:	40°C	60°C	80°C	40°C	60°C	80°C	40°C	60°C	80°C	40°C	60°C	80°C
<u>Flour composition</u>												
Flour M:												
Starch (%d.b)	83	82	79	85	83	81	84	82	81	88	86	82
Crude Fibre (%d.b)	0.9	0.8	1.0	1.4	1.0	1.6	1.2	0.7	1.3	1.0	1.2	1.3
Ash (%d.b)	1.5	1.7	1.7	1.8	1.8	1.8	1.5	1.6	1.7	1.6	1.5	1.4
Flour N:												
Starch (%d.b)	87	85	83	86	86	86	86	86	85	92	91	87
Crude Fibre (%d.b)	0.6	0.8	0.4	0.4	0.5	0.6	1.1	0.8	1.1	0.6	0.8	1.1
Ash (%d.b)	1.4	1.5	1.5	1.7	1.3	1.6	1.5	1.5	1.7	1.5	1.3	1.5
<u>Pasting temperature. °C</u>												
Flour M	65.5	65.5	65.5	65.5	65.5	65.5	64.0	65.5	65.5	65.5	65.5	65.5
Flour N	65.5	65.5	65.5	65.5	65.5	65.5	65.5	64.0	65.5	65.5	65.5	65.5
<u>Maximum viscosity</u>												
Flour M	371	380	380	255	323	295	380	380	340	408	410	410
Flour N	385	420	380	285	350	327	387	405	360	400	430	425
<u>Viscosity at 95 °C</u>												
Flour M	365	365	366	251	321	289	377	363	340	390	380	385
Flour N	375	387	367	284	338	320	377	380	358	380	390	385
<u>Viscosity after 20 min. at 95 °C</u>												
Flour M	172	175	185	102	152	131	202	183	239	168	180	190
Flour N	177	190	185	119	160	152	177	190	228	170	185	200
<u>Viscosity at 50 °C after cooling</u>												
Flour M	285	300	322	180	252	210	292	313	319	295	318	350
Flour N	308	327	320	180	285	260	297	340	335	305	340	380
<u>Ease of cooking</u>												
Flour M	19	17	16	20	18	20	19	17	16	17	16	15
Flour N	18	16	16	20	17	16	18	17	15	17	16	14
<u>Gel instability</u>												
Flour M	199	205	185	153	171	164	178	197	101	240	230	220
Flour N	208	230	195	166	190	175	210	215	132	230	245	225
<u>Gelification Index</u>												
Flour M	113	125	137	78	100	79	90	130	80	127	138	160
Flour N	131	137	135	61	125	108	120	150	107	135	155	180

Notes: Ease of cooking = time to maximum viscosity - time to initiation of pasting

Gel instability = maximum viscosity - viscosity after 20 min. at 95 °C

Gelification Index = viscosity at 50 °C after cooling - viscosity after 20 min. at 95 °C

Flour M: Flour with particles below 250 µm in size

Flour N: Flour with particles below 106 µm in size

Table 5.2 Properties of cassava flours processed as specified

Cultivar CM3306 produced in 1993

MILLING EQUIPMENT:	HAMMER			ROLLER			PIN			PADDLES		
DRYING TEMPERATURE:	40°C	60°C	80°C	40°C	60°C	80°C	40°C	60°C	80°C	40°C	60°C	80°C
<u>Flour composition</u>												
Flour M:												
Crude Fibre (%d.b)	3.0	3.3	3.0	2.8	3.0	2.9	3.4	2.8	2.7	3.3	3.1	3.3
Ash (%d.b)	1.8	2.1	2.2	2.6	2.7	2.8	2.0	2.0	2.2	2.2	2.1	2.3
Flour N:												
Crude Fibre (%d.b)	2.1	1.5	2.3	1.3	1.8	2.2	1.9	2.0	2.1	1.9	0.7	1.9
Ash (%d.b)	2.4	2.4	2.6	2.5	2.7	2.9	2.8	2.8	2.8	2.6	2.1	2.5
<u>Pasting temperature °C</u>												
Flour M	66.2	65.2	64.8	66.4	65.9	65.1	64.8	64.8	64.8	65.5	66.3	64.8
Flour N	65.2	65.5	65.5	64.0	64.8	64.0	65.5	61.8	64.8	65.5	65.5	65.1
<u>Maximum viscosity</u>												
Flour M	307	271	330	196	256	265	307	321	328	334	339	360
Flour N	250	368	372	220	283	309	339	341	365	304	339	365
<u>Viscosity at 95 °C</u>												
Flour M	300	265	320	173	245	265	300	315	317	320	337	330
Flour N	204	355	344	196	266	293	338	281	340	245	330	340
<u>Viscosity after 20 min. at 95 °C</u>												
Flour M	140	119	141	75	105	100	139	145	147	152	152	155
Flour N	106	165	160	82	106	116	167	150	160	139	149	160
<u>Viscosity at 50 °C after cooling</u>												
Flour M	211	165	219	107	142	138	220	227	232	228	241	244
Flour N	152	261	250	116	162	165	252	268	255	206	240	246
<u>Ease of cooking</u>												
Flour M	20	20	17	20	21	17	19	20	16	20	20	17
Flour N	22	18	16	22	21	16	18	17	17	20	17	17
<u>Gel instability</u>												
Flour M	167	173	194	121	160	179	168	176	181	182	201	205
Flour N	144	203	212	138	177	193	172	191	205	165	190	205
<u>Gelification Index</u>												
Flour M	71	46	63	32	37	38	81	82	85	76	89	89
Flour N	44	96	90	34	56	49	85	118	95	67	91	86

Notes: Ease of cooking = time to maximum viscosity - time to initiation of pasting

Gel instability = maximum viscosity - viscosity after 20 min. at 95 °C

Gelification Index = viscosity at 50 °C after cooling - viscosity after 20 min. at 95 °C

Flour M: Flour with particles below 250 µm in size

Flour N: Flour with particles below 106 µm in size

Table 5.3 Properties of cassava flour processed as specified

Cultivar MVen 25 produced in 1993

MILLING EQUIPMENT:	HAMMER			ROLLER			PIN			PADDLES		
DRYING TEMPERATURE:	40°C	60°C	80°C	40°C	60°C	80°C	40°C	60°C	80°C	40°C	60°C	80°C
<u>Flour composition</u>												
Flour M:												
Crude Fibre (%d.b)	2.5	2.3	2.3	2.2	2.0	2.5	2.3	2.3	2.9	2.5	2.6	3.0
Ash (%d.b)	2.3	2.0	1.8	1.3	2.0	2.3	1.8	2.2	2.0	2.2	2.0	1.6
Flour N:												
Crude Fibre (%d.b)	1.2	1.2	1.9	1.3	1.2	1.3	1.5	1.2	1.3	1.1	1.4	1.5
Ash (%d.b)	1.4	1.5	1.4	1.1	1.5	1.4	1.7	1.4	1.7	1.8	2.0	1.6
<u>Pasting temperature °C</u>												
Flour M	64.8	64.9	64.5	64.8	64.8	64.8	64.8	65.1	64.8	64.8	64.3	64.3
Flour N	64.8	64.8	64.8	64.8	64.9	64.9	64.9	64.0	64.8	64.8	64.8	64.8
<u>Maximum viscosity</u>												
Flour M	93	85	86	74	69	71	80	83	82	87	87	82
Flour N	119	115	109	125	118	116	88	97	93	100	105	103
<u>Viscosity at 95 °C</u>												
Flour M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flour N	ND	ND	ND	ND	ND	ND	3	ND	ND	ND	ND	ND
<u>Viscosity after 20 min. at 95 °C</u>												
Flour M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flour N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<u>Viscosity at 50 °C after cooling</u>												
Flour M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flour N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<u>Ease of cooking</u>												
Flour M	4	4	4	4	4	5	4	4	4	4	4	4
Flour N	4	4	4	4	4	4	5	5	5	4	4	4
<u>Gel instability</u>												
Flour M	93	85	86	74	69	71	80	83	82	87	87	82
Flour N	119	115	109	125	118	116	88	97	93	100	105	103
<u>Gelification Index</u>												
Flour M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flour N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Notes: Ease of cooking = time to maximum viscosity - time to initiation of pasting

Gel instability = maximum viscosity - viscosity after 20 min. at 95 °C

Gelification Index = viscosity at 50 °C after cooling - viscosity after 20 min. at 95 °C

Flour M: Flour with particles below 250 µm in size

Flour N: Flour with particles below 106 µm in size

ND: not detected

Table 5.4 Total dietary fibre in cassava flours processed as specified.

Flours prepared from cultivars produced in 1993

MILLING EQUIPMENT	ROLLER			HAMMER			PIN			PADDLES		
	40°C	60°C	80°C	40°C	60°C	80°C	40° C	60° C	80°C	40°C	60°C	80°C
From cultivar CM33006-4												
Flour M:												
Fibre (%d.b)	7.2	6.6	6.6	6.6	6.9	6.1	8.2	7.2	7.1	7.4	6.6	6.0
Flour N:												
Fibre (%d.b)	4.7	4.6	4.6	4.6	4.5	4.4	5.7	5.5	4.8	4.7	4.6	4.2
From cultivar MVen 25												
Flour M												
Fibre (%d.b)	5.9	5.6	5.0	5.7	5.2	5.9	6.2	6.1	6.3	6.3	6.2	6.2
Flour N												
Fibre (%d.b)	3.3	3.3	3.6	4.1	4.3	4.3	4.9	4.8	4.5	4.6	4.5	4.6

Flour M: Flour with particles below 250 µm in size

Flour N: Flour with particles below 106 µm in size

Table 5.5 Farinograph dough data for cassava-wheat flour blends (20 % of cassava flour). Cassava flours prepared from cultivars produced in 1993.

MILLING EQUIPMENT	PADDLE			HAMMER			ROLLER	FN
DRYING TEMPERATURE:	40°C	60°C	80°C	40°C	60°C	80°C	60°C	60°C
Cassava-wheat flour blends:								
<u>Cassava cultivar CM33006-4</u>								
Flour M:								
WA (ml/100 g. blend)	60.2	60.3	60.6	60.6	60.4	60.7	60.9	63.0
DT (min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	1.5
ST (min)	4.8	5.0	5.0	5.0	4.7	5.2	5.5	5.3
DS (FU)	210	200	190	200	180	200	170	180
Flour N:								
WA (ml/100 g. blend)		61.2		60.2			60.2	63.4
DT (min)		2.0		2.0			1.5	1.5
ST (min)		5.3		4.4			4.5	5.5
DS (FU)		180		220			200	170
<u>Cassava cultivar MVen 25</u>								
Flour M:								
WA (ml/100 g. blend)	61.3	61.1	61.6					
DT (min)	2.0	2.0	2.0					
ST (min)	4.8	4.3	5.0					
DS (FU)	190	200	190					
Flour N:								
WA (ml/100g. blend)	61.3							
DT (min)	2.0							
ST (min)	4.8							
DS (FU)	190							
<u>Wheat flour (100%)</u>								
WA (ml/100 g. blend)	63.3±1.0							
DT (min)	2.0±0.0							
ST (min)	8.3±0.3							
DS (FU)	100± 0.0							

Flour M: Flour with particles below 250 µm in size

Flour N: Flour with particles below 106 µm in size

5.2 REMARKS AND CONCLUSIONS

1. Both the processing conditions and the cultivar used in the preparation of cassava flour affected the properties of the flours. The drying temperatures used influenced the viscosities of the flour pastes. The method of milling, and sifting modified the pasting viscosities of the flours, the particle size distribution and the amount of fibrous material present in the samples. The type of cultivar affected the viscosities of the flour pastes and the fibre content present in the samples.

2. It is evident that the thermal treatment during the drying of cassava chips at temperatures as high as 80 °C modifies the starch which enhances the flours swelling upon pasting. The effect of the drying temperature is less evident on the cool paste viscosities of the finely ground flours.

3. The method of milling affects the fineness of the flours and probably the proportion of damaged starch in the flours which results in the finely ground flours giving higher paste viscosities than coarsely ground flours. Sifting the flours results in an increase of the paste viscosities of the finely sifted flours with smaller particle size, less fibre content and higher starch content.

4. The pasting characteristics of cassava flour depend mainly on the cultivar utilized. The flour from cultivar MVen 25 had very limited paste viscosity properties. Moreover, in other investigations flour from MVen 25, performed worse than flours from other cultivars in wheat-cassava breads (CIAT *et al.*, 1988). Cassava cultivars

which produce flours with high paste viscosities were considered to perform better in wheat-less breads than cultivars whose flours had low paste viscosity (Eggleston, 1992).

5. Dough physical properties (Brabender farinograph dough results) of wheat-cassava flour blends (containing 20 % cassava flour substitution) were not greatly influenced by the processing procedures used on the cassava flours. Finely ground and sifted flours could resemble 100% "strong" wheat flour doughs in water absorption and have faster dough development.

6. The results of this study indicate that some degree of control of the functional properties can be achieved by selecting the drying temperature, the milling procedure and controlling the particle size distribution. Therefore, processing procedures are important considerations in the production of cassava flours for different end-products uses.

To obtain a greater understanding of the implication of processing on end-product potential it is recommended that cassava flours prepared under the processing conditions discussed in this thesis are tested in the production of different foods in which cassava flours could be incorporated. However, from the reports of other research workers, it is to be expected that the finer cassava flours will perform better in composite wheat-cassava bread (Hudson and Ogunzua, 1974 and Nishita and Bean, 1982). Finely sieved fractions of cassava flours were found to perform in a similar

way to cassava starch in wheat-cassava breads (Hudson and Ogunzua, 1974). Cassava starch has been shown to give better results than cassava flour in wheat-cassava bread making which is attributed to the presence of fibre in the flour interfering with the formation of the gas-retainer-gluten-film that develops during baking (Hudson and Ogunzua, 1974; Ciacco and D' Appolonia, 1978). Coarser cassava flours could be suitable for the preparation of wheat-less bread as observed with rice flour (Nishita and Bean, 1982). Coarser flours could also be used for direct cooking purposes, being somewhat less sticky, as observed by Raja *et al.* (1978), while the finer flours may be used for processing in whole starchy products.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

6.1 INTRODUCTION

Cassava starch and flour, as will be clear from the literature survey in Chapter 1, are increasingly being used in tropical countries by a variety of industries. At this stage, industry has not clearly defined the attributes of these raw materials which are most appropriate to their particular application. We may safely assume, however, that following the pattern of developed countries companies will increasingly lay down quality specifications for the starch or flour which will require the application of considerable skills and knowledge in the growth of the cassava plant and its subsequent extraction and manipulation. It is certainly true that industrial companies from the developed countries are progressively applying such specifications via their purchasing departments.

A fundamental question, therefore, is how far can the grower and processor manipulate the characteristics of the starch granule or flour to achieve the wide variety of potential applications that these particular products could fulfil. In addressing this issue, it is also important to remember the reverse question, namely " how readily is it possible to obtain a product of identical specification, when it is extracted from a different cultivar and grown under different conditions ?".

6.2 HOW FAR CAN WE MANIPULATE VARIABILITY OF THE PROPERTIES OF CASSAVA STARCH ?

(a) Extent of variation

The fundamental source of variation lies in the genetic constitution of the various cassava cultivars and from the results of this study it can be seen that considerable variation in the functional properties can be achieved by selecting cultivars, even when they are grown in the same environment. For example, within the starches from 29 cultivars grown in Zone A, it is possible to have a range of temperatures of initiation of paste formation from 61.5 to 67.6 °C and to obtain maximum viscosities from 320 to 610 BU in pastes of 5% starch concentration. However, there is every reason to believe that genetic engineering of the cassava genome could lead to much greater differences, along the lines already attained in pea starches.

Superimposed upon this background is the effect of environment during the growth and maturation of the starch granules. The temperature at which the plants are grown has substantial effects, e.g. a change in the environmental temperature in Zone A from 23 to 31 °C in Zone B could cause in MPer 196 starch the pasting temperature to rise from 62.2 to 72.1 °C and the maximum pasting viscosity to drop from 555 to 220 BU. It would be interesting to investigate how intense would be the change in that same cultivar grown at an average environmental temperature of 17 °C which was reported by Cook (1985) in the Andean regions. Would such cultivars

grown in those cold conditions produce starches of even lower pasting temperatures and higher paste viscosities than those from Zone A ?

Some further manipulation of the viscosity behaviour of starches can be effected by fermentation and sun drying. Such 'sour' starches are more soluble in hot water than the 'sweet' starches and their pastes have a reduced viscosity compared to their 'sweet' counterparts. There is good evidence that such fermented, sun dried starches have suffered molecular degradation.

(b) Does the variability lie in molecular or granular structure ?

The rheological properties which are the principal interest in this thesis arise largely from the thermally induced gelatinization of starch granules in an aqueous environment. In practice the rheological behaviour will represent the interaction of two factors: (a) the degree of swelling of the gelatinized starch granules and (b) the viscosity of the intergranular matrix. One view may hold that the differences in the rheological properties are the consequence of differences in the size, shape and concentration of the constituent molecules from cultivar to cultivar in the native starch granule. Another view would emphasize that the behaviour of the system reflects the architecture of the granule; with such a perspective, granules may have the same constituent molecules but assume a significantly different organization. In other words the same bricks can build a cathedral or a cottage *i.e.* the building blocks are the same but the end products are very different.

The evidence that has emerged has shown that the amylopectin molecules are substantially similar in the length of their constituent chains but there are significant differences in the A/B ratio which may reflect the general morphology of the molecule. Starches from the B Zone had A/B ratios from 1.1 to 1.33 while the starches from the A Zone had ratios from 1.25 to 1.65. Such results are in accord with the evidence that a high A/B ratio is associated with high swelling. In contrast, the amylose is quite different in molecular size from the various cultivars and this is significant in subsequent behaviour. It is particularly interesting that cassava cultivars having granules of small size contain amylose of short molecular weight and amylopectins with reduced branching; such small granules on heating in water release high amount of starch molecules into the solution which result in reduced swelling and viscosity development. The converse situation is also true. The results parallel in those of Geddes *et al.* (1965) who found that smaller granules of potato starch contained small molecules of amylose but the granules had higher gelatinization temperature. It is increasingly clear that small granule size is associated with a reduced DP of amylose molecules and an enhanced temperature of gelatinization, irrespective of the superimposed effects of cultivar, environment or maturity.

Since small size granules apparently permit a more extensive release of molecules from the interior of the granules on gelatinization, we may conclude that granule architecture and size are ultimately significant in the viscosity behaviour of such systems. Such a view is further endorsed by the observation that a more crystalline granule structure in cassava starch appears to restrict granule swelling

during the hot aqueous pasting and, therefore the ultimate observed viscosity. The crystallinity of granules of a given cultivar can be enhanced by growing the plant at higher temperatures as is evident from comparing the crystallinity of starches extracted from the same cultivar grown in Zones A and B.

Where starches are being used as components of bread doughs, the modification of the molecules by fermenting and sun drying is particularly important and the ability to assist in the dough expansion process is a consequence of molecular degradation having occurred, which is not the case in the fermented oven dried starches. Such pastes of fermented, sun dried starches are rich in amylopectin in the solvent phase. It was therefore surprising that Mestres (1996) found that amylopectins which had been extracted from purified 'sweet' and 'sour' starches both puffed on drying at 100 °C whereas purified amylose did not demonstrate such behaviour and therefore we can conceive of a situation where the actual behaviour of the whole gelatinized granule will be controlled by the degree of disintegration of the amylopectin.

(c) How does processing change the properties of cassava starch ?

The processing which has been considered in this thesis has related, firstly to the conversion of 'sweet' starch into 'sour' starch and its subsequent manipulation by either sun or oven drying, and secondly to the preparation of native starch by a factory procedure. The results of investigations have shown that fermentation is important in the conversion of 'sweet' into 'sour' by either acid hydrolysis and/or enzymatic action

and the less well defined modification and molecular degradation by sun drying is probably the consequence of an oxidative reductive reaction. The consequences of changes in molecular size of the starch macromolecules have important implications for the baking quality of 'sour' starch. In terms of its utilization as an additive to bread the molecular depolymerization should be just enough to produce on aqueous heating a dispersion and solubilization of starch molecules sufficient to produce an amylopectin-rich solvent phase without excessive molecular degradation.

There is little doubt that the amylopectin is responsible for this property of expansion of 'sour' starch. Confirmatory results have emerged from the study of 5% liquid-viscous pastes produced by gelatinization of fermented sun dried starch ('sour' starch); these exhibited after a short period of cold storage an increase in shear modulus G' and a decrease in the phase angle, indicating that aggregation of amylopectin and/or amylose molecules occurred creating a network in the solvent phase. In sharp contrast, the fermented, oven dried starches did not exhibit this change in the mechanical properties and therefore there appears to be strong evidence that in 'sour' starch pastes the dispersed modified molecules of amylopectin and amylose do have the ability to aggregate in a continuous network.

The presence of non-starchy products (peel and fibre residues) in cassava starch influences its functional properties by restricting the swelling and paste viscosity development upon gelatinization, and modifying the appearance of the pastes. The intrinsic characteristics of cassava starch must be preserved by extracting starch from

peeled roots and controlling the filtration and refining operations.

6.3 CAN THE PROPERTIES OF CASSAVA FLOUR BE CONTROLLED BY THE PROCESSING PROCEDURE ?

The results have shown evidence that the processing conditions used in the preparation of the flours influenced the particle size distribution, the amount of fibrous materials and the flour pasting in hot water. Physical testing of doughs made of wheat and cassava flour blends was not greatly influenced by the processing procedures. The thermal treatment during the drying of the cassava chips at the highest temperature certainly caused gelatinization of the starch which resulted in flours with enhanced swelling upon pasting. The mechanical treatment during milling of the dry chips influenced the particle size, and probably the extent of damage to starch granules in the resulting flours; as a result, the finely ground flours gave the highest paste viscosities. The presence of fibre in the flour seemed to restrict its swelling during the aqueous pasting by either a direct interference or a relative reduction of the starch available for gelatinization in the flours. Obviously, the finely sifting flours with smaller particle sizes, less fibre and higher starch content gave high paste viscosities. Therefore it is clear that some degree of control of the functional properties of cassava flours can be achieved by selecting the drying temperature, the milling procedure and the particle size distribution.

6.4 CONCLUSION

This study has traced the origin of the behavioural characteristics of native cassava starches to differences in their molecular and granular structures. The starch properties varied between cassava cultivars and were influenced by the temperature of the environment during the growth of the plants. The effect of the environment was so marked, that the selection of a zone for growing cassava should be of great importance when certain properties are desired in the starches. It was confirmed that native amylose molecules of cassava possess long chains, similar in sizes to those of potato. The pastes of cassava starch, like potato, have abundant amylopectin in the solvent phase and therefore they exhibit the character of weak gels. (Chapter 3). The specific functional characteristics of 'sour' starch are the result of molecular modifications caused during the processing. The extraction of starch from unpeeled roots and the inadequate filtration and refining procedures influenced the paste viscosities and the aqueous solubility of native cassava starches. The starch extracted from roots stored for about 3 days suffered very slight changes in the pasting viscosities, but others properties remained unmodified (Chapter 4). Cassava flour properties were influenced by the conditions of preparation. Drying temperature, milling procedure and particle size could be selected and controlled to give cassava flours of desired functional properties (chapter 5).

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APPENDIX

Figure A.1 SE-HPLC chromatograms derived from debranched cassava starches. SE-HPLC columns: TSK G3000 PWXL, 2xAsahipack GS-320H, TSK G2500 PWXL and Oligo PWXL.

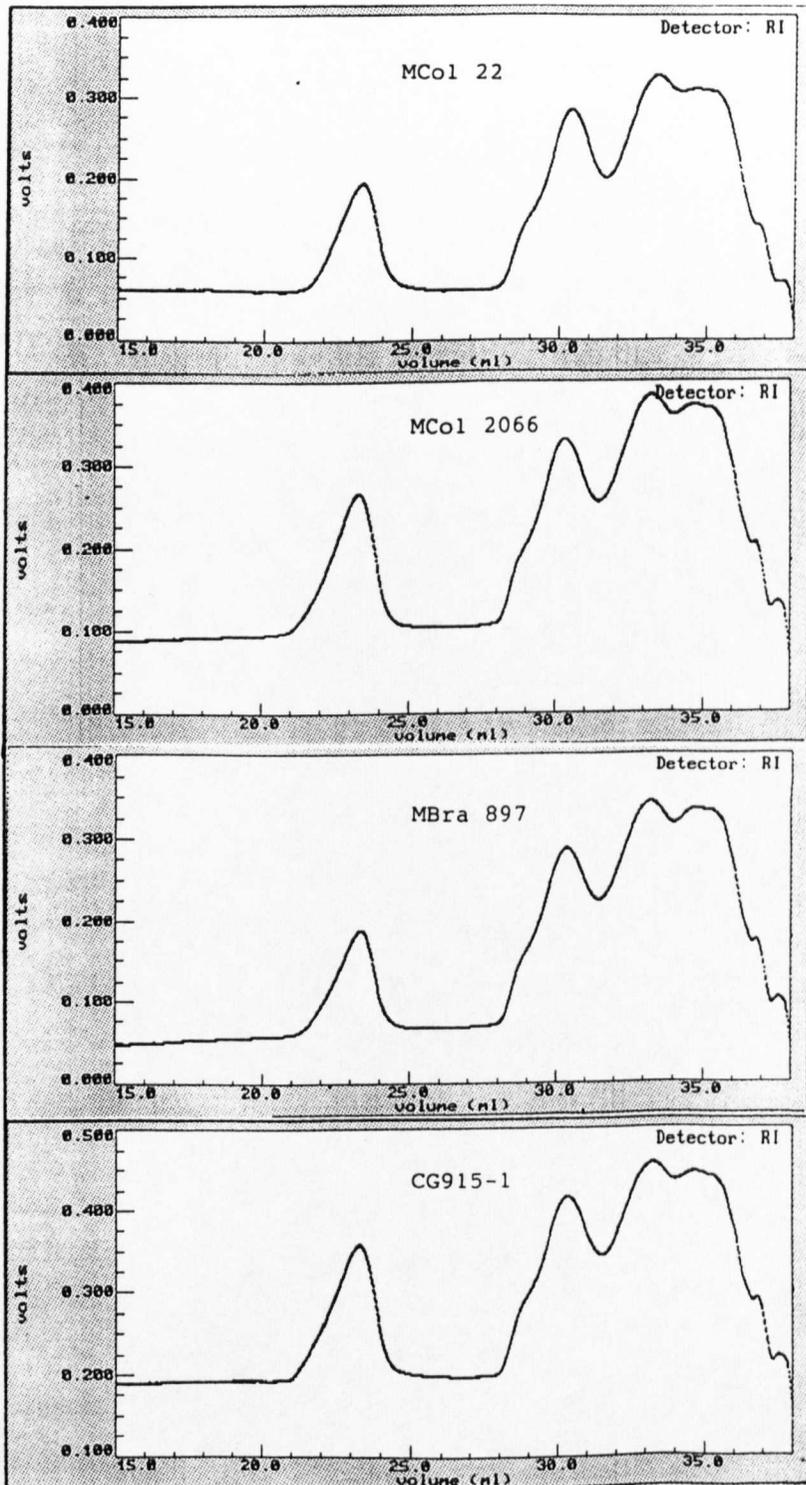


Figure A.1 (Continued)

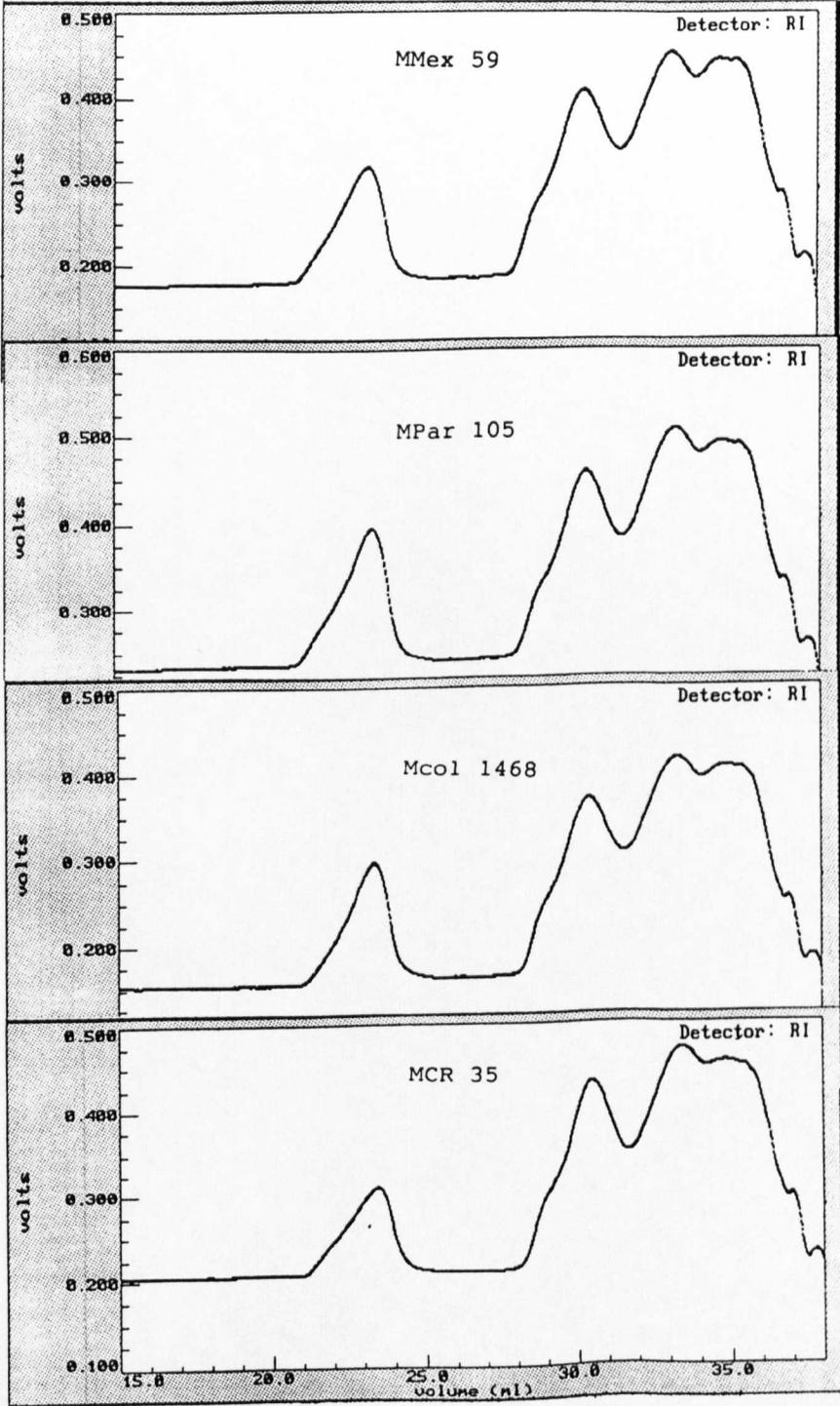


Figure A.1 (Continued)

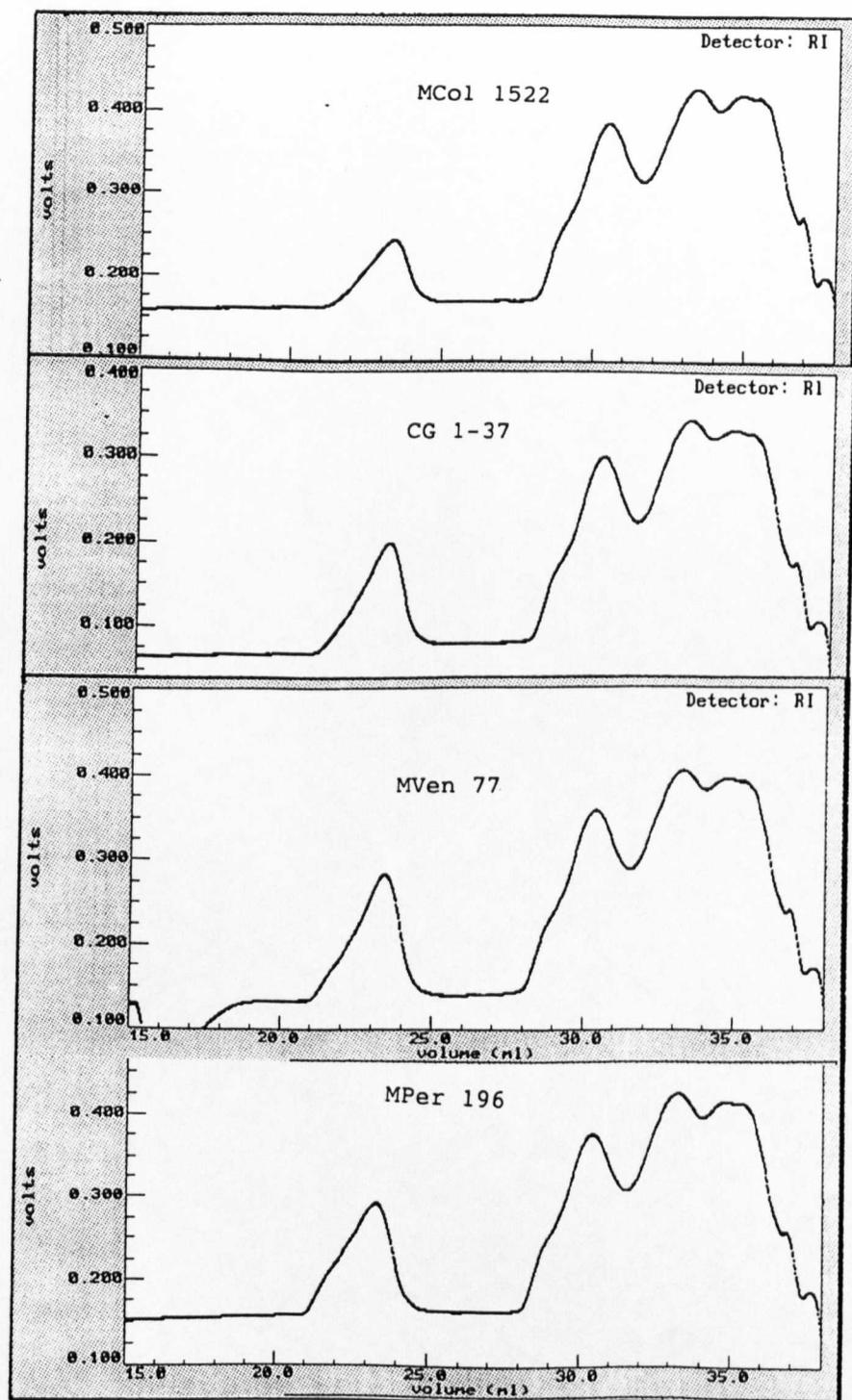


Figure A.1 (Continued)

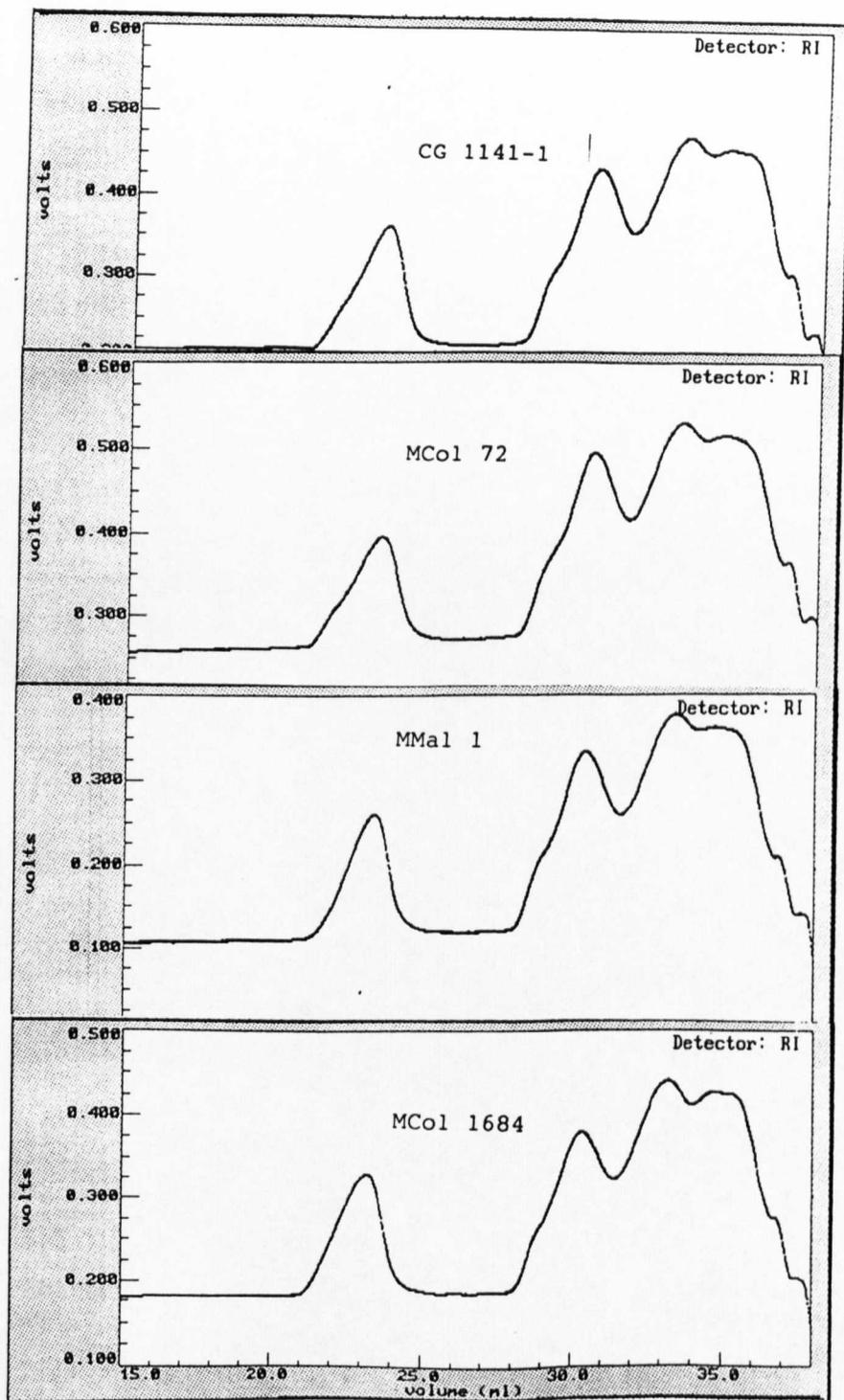


Figure A.1 (Continued)

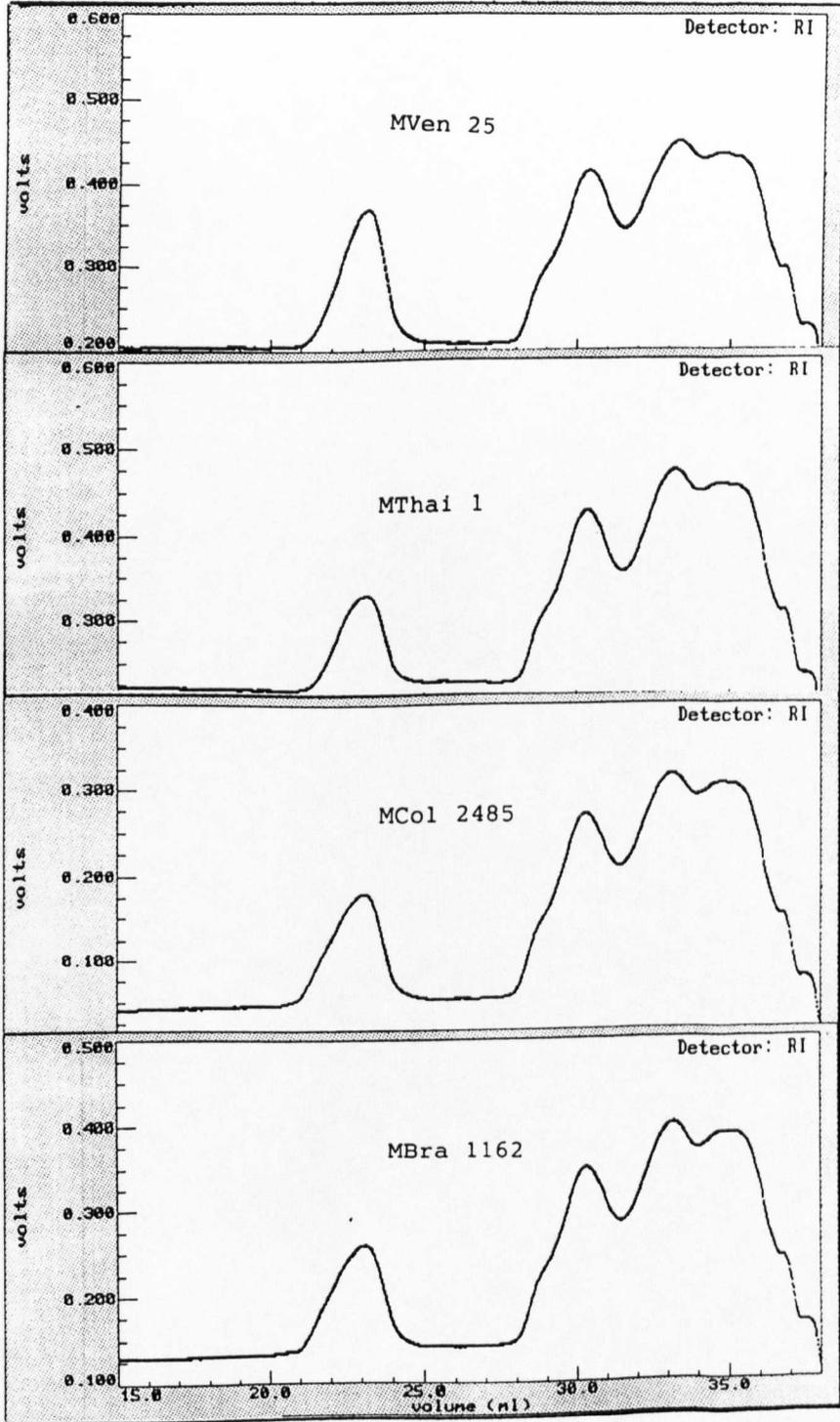
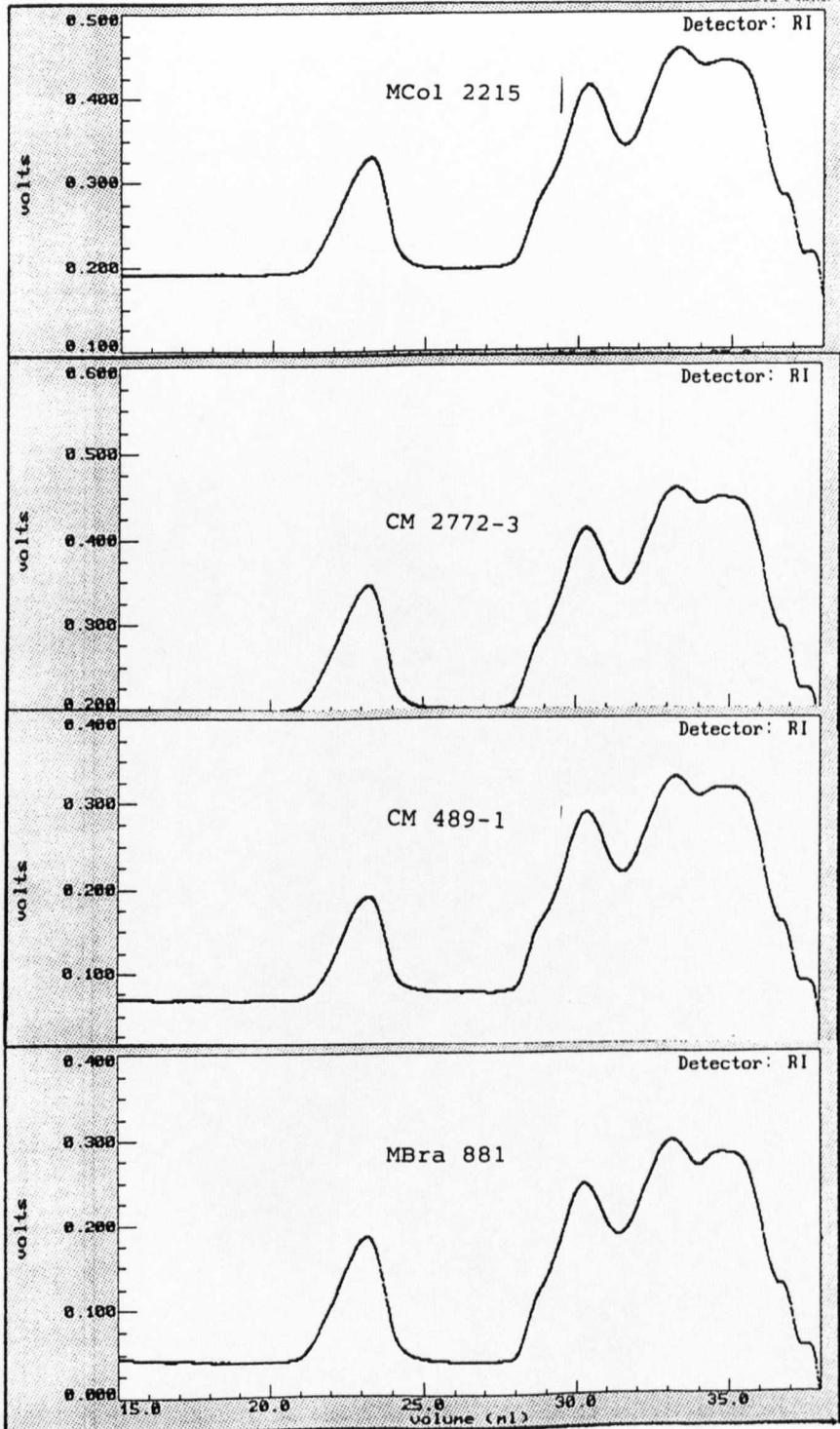


Figure A.1 (Continued)



Scientific contributions and publications

(a) Paper presentation

In International Cassava Meeting. CIAT, Cali, Colombia, entitled: Appraisal of the influence of variety and processing on the physico chemical and functional properties of cassava starch and flour.

(b) Poster presentation

In Starch: Structure and Function Conference, 15-17 April 1996, Churchill College, Cambridge, U.K., entitled: The effect of the growth ecozone upon the functional, structural and physico-chemical properties of cassava starch: A case of structure-function relationships.

(c) Publications to be submitted

(i) Effects of cultivar, growth environment and processing on the gelatinization properties of cassava starch.

(ii) Structural and physico-chemical properties of starches from cassava and relationships with their functional properties.

(iii) The molecular composition and structure of cassava starch: A comparison of sweet and fermented starches of particular functional behaviour.

(iv) The physi-cochemical properties of cassava starch: A comparison of laboratory and factory extracted starches.

(v) The impact of processing on the properties of cassava flour.