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Starch structure and some properties of cocoyam (*Xanthosoma sagittifolium* and *Colocasia esculenta*) starch and raphides

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Abstract

Studies were conducted on the structure and rheological properties of three cocoyam varieties [*Xanthosoma sagittifolium* (red-flesh), *Xanthosonia sagittifolium* (white-flesh) and *colocasia esculenta*] starches and raphides in an attempt to characterize them. The microstructures of starch granules and raphide sizes, which were obtained from the distal, middle and apical sections of the raw cocoyam samples, were examined using a light microscope. Evaluations of the rheological properties of the cocoyam species were also conducted, using a Brabender viscoamylograph. No distinct variation was observed in starch granule sizes of the two *Xanthosoma* species. Starch granules sizes in the ranges of 0.74–1.19 and 0.74–1.10 μm were obtained for the *Xanthosoma* species (red-flesh) and *Xanthosoma* species (white-flesh), respectively. Significantly smaller sizes (0.05–0.08 μm) of starch granules were observed for *Colocasia esculenta*. Microstructures of raphide sizes which were obtained from the distal section of the cocoyam species showed significant differences ($P \leqslant 0.05$) among the varieties, with the *Colocasia* species having more pronounced needle-like structures. Evaluation of the rheological properties of the cocoyam flours, using the Brabender viscoamylograph, showed considerable variations among the varieties, especially between the *Xanthosoma* species and the *Colocasia* species. The *C. esculenta* showed lower hot paste viscosity but higher thermal stability than the *Xanthosoma* species. Peak viscosity was highest in the *X. sagittifolium* (red-flesh) variety while the white-flesh variety showed the least tendency to retrogradation. © 2002 Published by Elsevier Science Ltd.

Keywords: *Xanthosoma sagittifolium*; *Colocasia esculenta*; Starch structure; Rheological properties; Starch and raphides

1. Introduction

Starch occurs in all higher plants as semi-crystalline granules of polymers of glucose units which are genetically linked to linear (amylose) and branched (amylopectin) structures. The starches of major economic importance are those found in root crops and the seeds of cereals (Carr, Sufferling, & Poppe, 1995). Starch chains, in native granules, are regularly designed in micellar forms which are the basis of their crystalline structure. The granules show diversity of shapes and the sizes are often uniquely characteristic of the cultivar or variety from which the starch is isolated. Esau (1965) reported on the extensive nature of morphological variation of starch grains, and their use in identification and characterization of starchy plants. Even though starch structures of most plant species have been evaluated using different analytical procedures, very little is known of their characteristics in defining varietal differences among the plants species. Particle size data have therefore become useful in providing information about the structural characteristics of plants and the effects of processing on granular starch products. Light microscopes have been extensively used to elucidate the microstructural properties of starch. Banks, Greenwood, and Muir (1973) reported that starch granules of different plant species possess radial symmetry, exhibit well-defined rings or lamellae, are optically anisotropic and have a well defined birefringence pattern. Baker and Whelan (1982), in their studies on the submicroscopic morphology of protoplasm, showed that the intensity of birefringence in starch varied with cultivars and mutant varieties of the same plant. These observations were associated with the structure-ordering amylopectin component of starch.

Raphides are thin elongated crystal-containing cells which taper off at their ends and store calcium oxalates. Their presence in food substances has been associated with acridity (Esau, 1965). In spite of the fact that their structures have been elucidated and widely reported (Esau, 1965; Sakai, 1979), little effort has been made in

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using their morphological properties for varietal classification. In this study, an attempt was made to characterize the selected cocoyam varieties using the cross-sectional measurements of the raphide sizes under an optical microscope.

A typical method for characterizing starch paste viscosity is the Brabender viscoamylograph test, in which viscosity is plotted against time during a standard cycle of heating with continuous stirring. Banks et al. (1973) reported that the paste characteristics of starch are affected by the amylose:amylopectin ratio, granule diameter, distribution and physicochemical properties. Paste properties have been studied to tailor functional characteristics to desired applications in food product development and also to identify starches with unique characteristics for possible new uses. Among the tropical root crops, only potatoes, and to some extent cassava starches, have received extensive studies of their paste characteristics. Some studies have also been done on the rheological properties of *Dioscorea dumetorum* species (Afoakwa & Sefa-Dedeh, 2002). However, very little information is available on the rheological properties of the various cocoyam varieties consumed in West Africa.

The purpose of this study was therefore to characterize the three cocoyam [*Xanthosoma sagittifolium* (red-flesh), *Xanthosoma sagittifolium* (white-flesh) and *Colocasia esculenta*] varieties, based on their starch structure, raphide sizes and rheological properties.

2. Material and methods

2.1. Materials

2.1.1. Preparation of fresh cocoyam samples

Fresh samples of two varieties of *X. sagittifolium* (red and white varieties) cormels and one variety of *C. esculenta* var *esculenta* corms were, respectively, harvested from local farms at Akyem-Begoro and Anyinam in the eastern region of Ghana and transported to the laboratory for the study. Within 2 days of harvest, the samples were peeled and the edible portion cut into three sections, representing the distal, middle and apical parts of the cormels. A sample ratio, based on weight, was used to divide each cormel into three parts, i.e. a ratio of 2:3:1 for the distal, middle and apical sections. The earlier ratio was arrived at by examining the colour variation across the cormel. Portions of the raw sample of the sections were blended and used for the analysis. The other portions were dried at 50 °C for 24 h using the air oven method. The dried samples were subsequently milled into flour in a hammer mill (Christy and Norris Co., USA) to pass through a 4-mm sieve. The flour products were kept in sealed polyethylene containers for analysis.

2.1.2. Experimental design

A 3×3 factorial experimental design was used and the principal factors were:

1. Type of cultivar: X *sagittifolium* (white-flesh), X *sagittifo1ium* (red-flesh) and *C. esculenta*
2. Cormel section: distal, middle and apical

Samples were then analyzed for starch and raphide structure as well as the rheological properties of the cocoyam starch.

2.2. Methods

2.2.1. Studies on the starch structure

2.2.1.1. Sample preparation. The three cocoyam varieties were peeled and tissues of dimensions 7.55×5×5 mm were sectioned from the inner portions of the middle section using a dissection blade. The slices were cut along the transverse section.

2.2.1.2. Fixation and dehydration. A formal saline fixative was prepared, according to the procedure described by Mahoney (1973). Ten millilitres of formalin (4% of 40% commercial formalin) were taken and 0.9 g of pure sodium chloride (AnalaR-grade) added. Distilled water (100 ml) was added and the resulting solution stirred; the solution (formal-saline) was adjusted to pH of 7.6 with dilute hydrochloric acid and used as the fixative. The cut tissues were placed in the fixative for 24 h, washed with distilled water and dehydrated through a graded series of aqueous alcohol (50, 70, 90%, and absolute ethanol) the dehydrated tissues were cleared or de-alcoholized in an antemedia (toluene) for 2 h.

2.2.1.3. Embedding. The procedure of Peacock (1966) was used for the embedding process. The cleared samples were impregnated in molten paraffin wax–benzene (50/50) mixture for 1 h. Samples were transferred to molten paraffin wax of melting point 58 °C for a further 1 h and finally embedded in molten paraffin wax in a mould. The mould was transferred into cold water to solidify the wax.

2.2.1.4. Sectioning. Sections of tissues (8 μm) were cut using the laboratory scale sledge microtome (Erma Inc., Tokyo, Japan). Egg albumin solution was warmed on a hot plate sufficiently to soften but not to melt the paraffin and any fold in the section flattened out. Water was drained off and the slide left on the hot plate to dry.

2.2.1.5. Staining and examination. The staining procedure of Fowel (1962) was followed. The fixed tissues on the slides were immersed in xylene to remove the wax. The de-waxed samples were passed through the series of

ethanol; absolute ethanol, 90 and 70 °C. After this treatment the samples were stained in Safranin 0 for 10 min, washed again in the ethanol series (50, 70, 90%, and absolute) and counter-stained in Fast Green for 2 min. The samples were then cleared in clove oil and mounted in Euparal (Flatters and Garvett Ltd, UK). Examinations of starch granular size, shape and raphide structure were done using a TMS-F light microscope (Nikon Co., Tokyo, Japan).

2.2.2. Viscoamylograph determination

The cooked paste viscosities of 10% (dry matter basis) slurries were determined using the Brabender viscoamylograph (Viscograph PT 100, Brabender Instrument Inc., Duisburg, West Germany) equipped with a 700 cmg sensitivity cartridge. The samples were heated at 1.5 °C/min to 95 °C, held at this temperature for 30 min, cooled at 1.5 °C/min to 50 °C and held for 30 min at 50 °C. The pasting temperature, viscosity at 95 °C, viscosity at 95 °C after holding for 30 min, viscosity at 50 °C and viscosity after holding for 30 min at 50 °C were measured. Paste stability and set-back ratios were calculated.

3. Results and discussion

3.1. Structural properties of cocoyam starch and raphides

3.1.1. Microscopy of starch granule shapes and sizes

The endospermic starches of the three cocoyam varieties were studied using a light microscope. The light microscope provides a useful and rapid means for determining the structural properties of native starch. The microscopic examination revealed differences in starch granule shapes. The starch granules of the *Xanthosoma* species were oval- to kidney-shaped, with the small sizes appearing roughly spherical. The shape of the *C. esculenta* var *esculenta* starch appeared to be 5- to 6-sided polygonal. The differences in starch granule shapes have also been known to be greatly influenced by the environment of growth (Moss, 1976). Table 1 shows the statistical evaluation of starch granule sizes obtained from the sections (distal, middle and apical) of each of the varieties studied. The *X. sagittifolium* (red-flesh) variety showed a progressive decrease in starch granule size from the distal section (1.19 μm ± 0.27) to the apical section (0.74 μm ± 0.27). Even though the sizes were in a close range, there were considerable variations with respect to dominant size at each section. The starch granule sizes obtained at the sections of the *X. sagittifolium* (white-flesh) variety were found to be smaller than those of the *X. sagittiolium* (red-flesh) variety. For the two *Xanthosoma* species, the largest starch granule size was found at the distal section of the red-flesh variety and the middle of the white-flesh variety.

In all, the starch granule sizes at the sections of the two *Xanthosoma* species did not show wide variations. These results were in agreement with the observations of the Lauzon and Kawabata (1988). Starch granule sizes of *C. esculenta* var *esculenta* differed considerably from the two *Xanthosoma* species. The granules were much smaller (0.25 μm ± 0.06) in size and showed a closer range than the *Xanthosoma* species. The characteristically small starch granule sizes of *Colocasia* species have been reported (Purseglove, 1988). Frequency distributions of the starches studied are presented in Figs. 1–3. For the *X. sagittifolium* (red-flesh) variety, the frequency distribution (Fig. 1) showed a gradual decrease in granular size from the distal to the apical section. Over 70% of the granules had sizes in the range of 1.00–1.5 μm at the distal, 0.75–1.25 μm at the middle and 0.5–1.00 μm at the apical sections (Fig. 1). In the *X. sagittifolium* (white-flesh) variety, 70% of the granules had sizes in the range of 0.5–1.00 μm at the distal, 0.75–1.25 μm at the middle and 0.3–1.00 μm at the apical sections (Fig. 2). Whereas a gradual decrease in trend was observed for the frequency distribution of starch granule sizes in the red-flesh variety, the white-flesh

Table 1
Descriptive statistics of starch granules sizes obtained from three cocoyam varieties (μm)

Variety	Section	Mean	S.D.[a]	Mode	Range	Min	Max
Xanthosoma sagittifolium (red-flesh)	Distal	1.19	0.27	1.50	1.13	0.50	1.63
	Middle	1.13	0.26	1.25	1.25	0.50	1.75
	Apical	0.74	0.27	0.50	1.00	0.30	1.30
Xanthosoma sagittifolium (white-flesh)	Distal	0.75	0.26	0.50	1.25	0.25	1.50
	Middle	1.10	0.26	1.13	1.40	0.40	1.80
	Apical	0.74	0.23	0.75	0.97	0.28	1.25
Colocasia esculenta	Distal	0.23	0.08	0.25	0.45	0.05	0.05
	Middle	0.25	0.06	0.25	0.40	0.10	1.05
	Apical	0.08	0.05	0.05	0.20	0.05	0.25

[a] S.D., standard deviation.

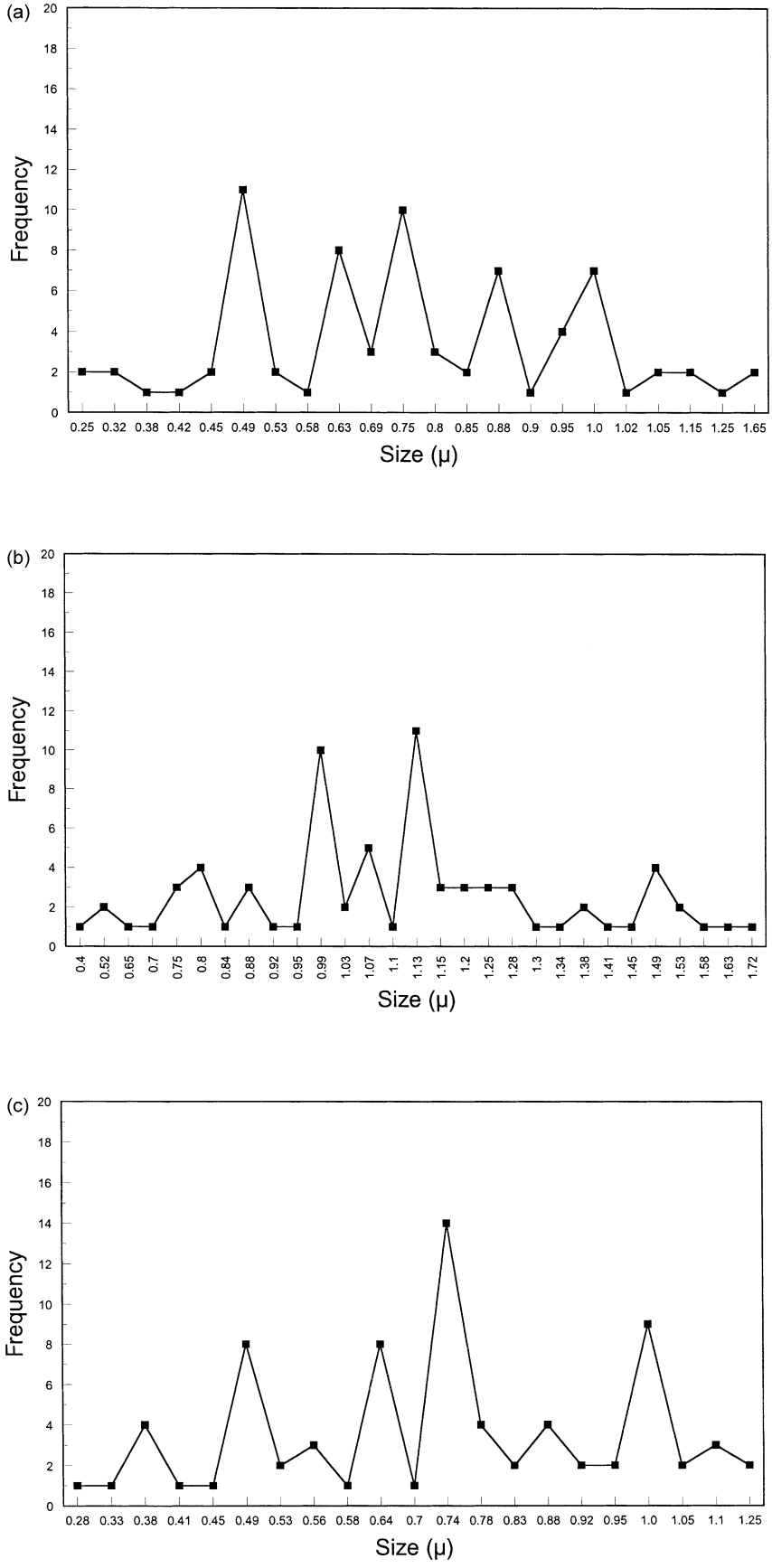

Fig. 1. Plot of frequency distribution of starch granule sizes of distal (A), middle (B) and apical (C) sections of *Xanthosoma sagittifolium* (red-flesh) variety.

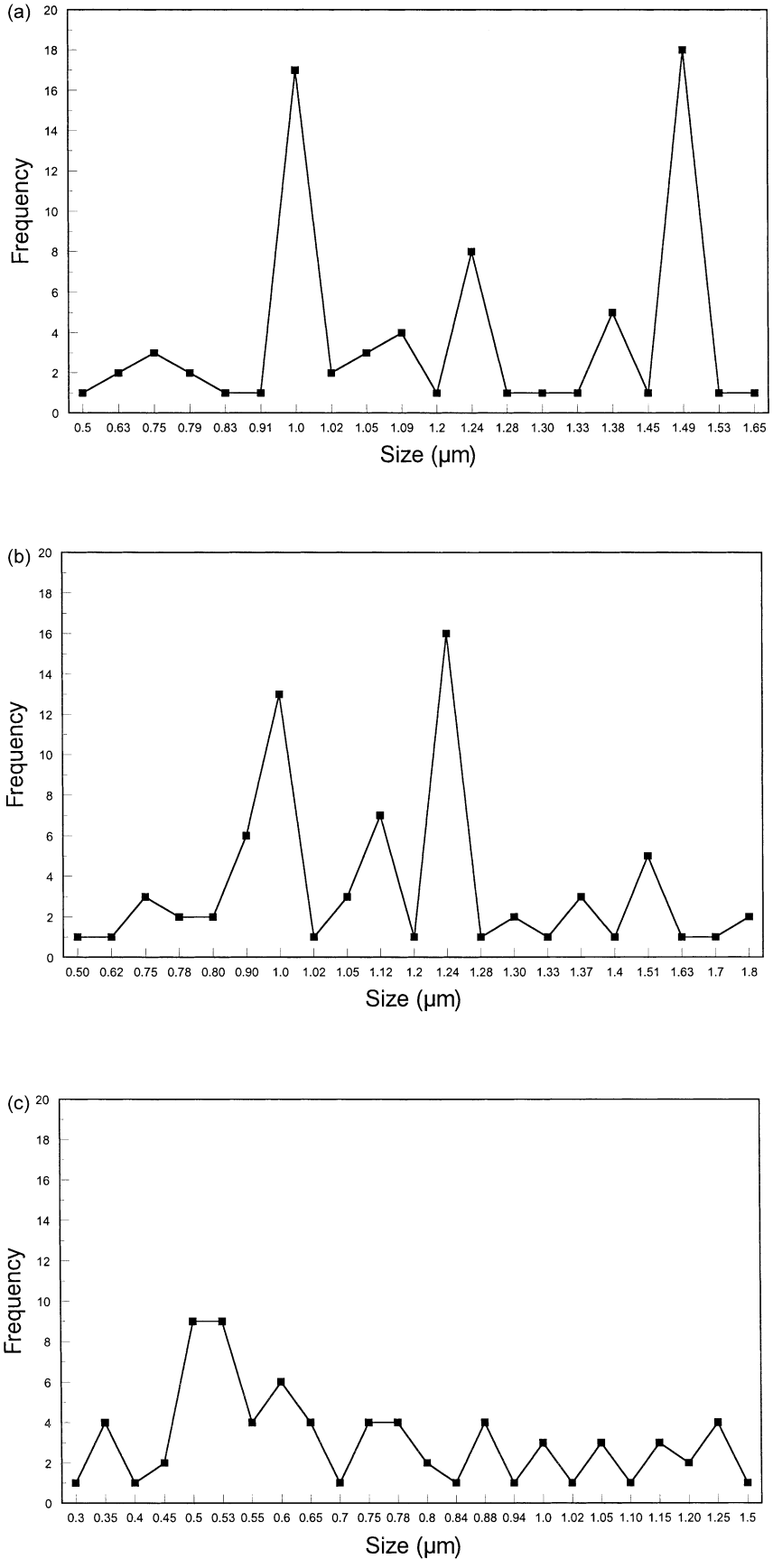

Fig. 2. Plot of frequency distribution of starch granule sizes of distal (A), middle (B) and apical (C) sections of *Xanthosoma sagittifolium* (white-flesh) variety.

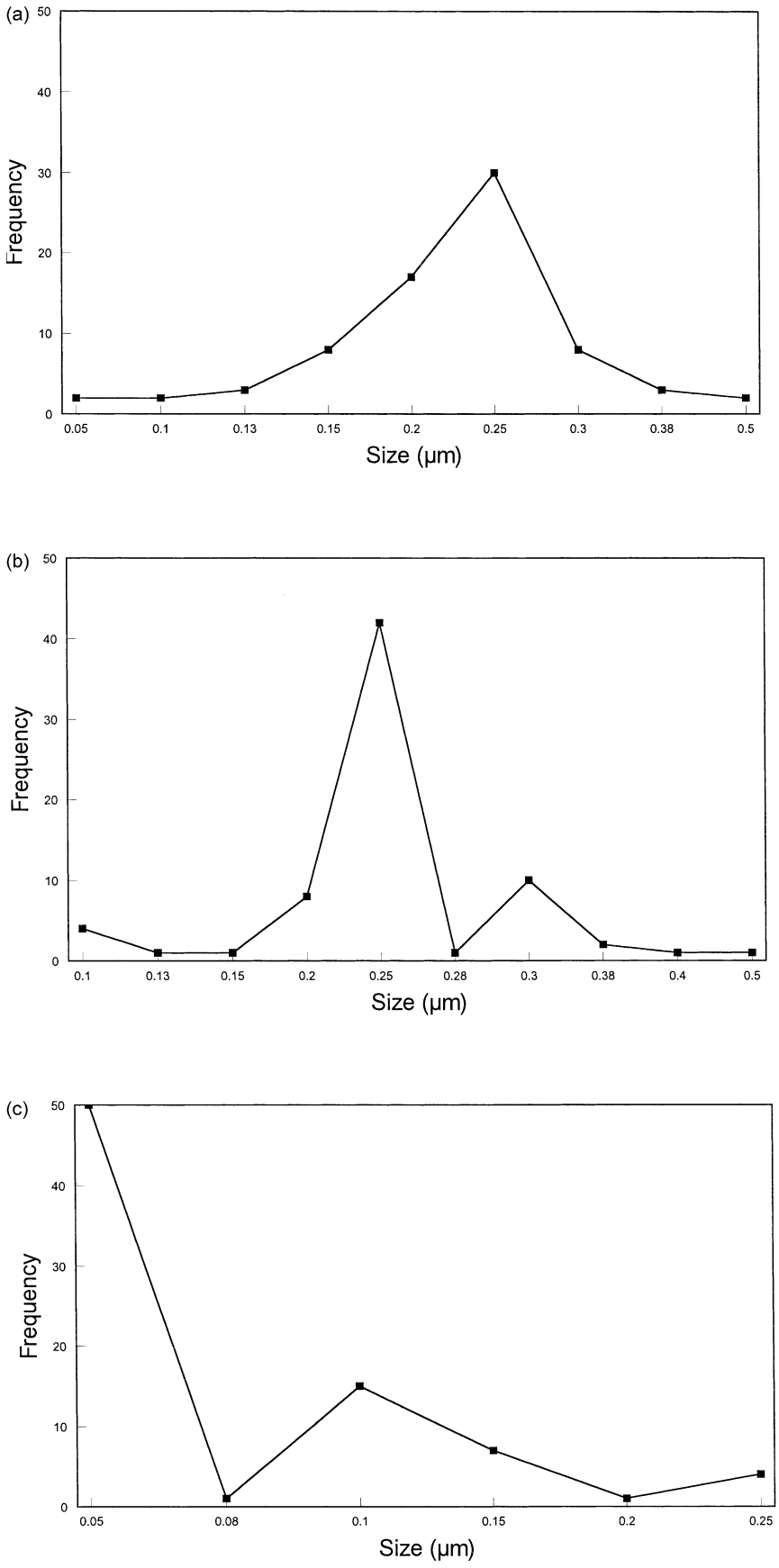

Fig. 3. Plot of frequency distribution of starch granule sizes of distal (A), middle (B) and apical (C) sections of *Colocasia esculenta*..

variety showed an increase in granule sizes from the distal to the middle and a decrease in the apical sections. The *X. sagittifolium* (white-flesh) variety showed slightly smaller starch sizes than the red-flesh variety. The variation could be due to the differences in the maturity of the cormels at harvest and genetic factors. The results obtained for the *C. esculenta* var *esculenta* differed considerably from those of the *Xanthosoma* species. Values obtained for the highest frequency distribution (70%) of starch granule sizes at the section of the *Colocasia* species were, 0.25, 0.25 and 0.05 μm at the distal, middle and apical sections, respectively. From the results obtained, it is clear that the distributions of the starch granule sizes were greatly influenced by the species investigated. The influences of plant species and maturity stages on size, shape and birefringence properties of starch granules have been reported (Manners, 1974). The mean granule sizes of starch obtained for the *Colocasia* and the *Xanthosoma* species in the study were lower than values obtained for cassava (1.8–27.8 μm) and other root crops (Eggleston, Omoaka, & Arrowshegbe, 1993; Whister, Bemiller, & Paschall, 1984). The characteristically small starch granule sizes of cocoyam have been associated with higher digestibility than other starchy crops (Onayemi & Nwigwe, 1987). Such particle size data are useful in distinguishing differences among starchy species as well as for monitoring changes or modifications due to processing.

3.1.2. Microscopy of cocoyam raphides

The cross-sectional measurements of cocoyam raphides investigated are presented in Table 2.

The aim of this study was to examine raphide structures at all sections of the samples evaluated. However, some of the middle and apical sections did not contain raphides; therefore only values obtained at the distal section could be compared. Fig. 4 provides drawings of raphides as observed under the microscope. Wide variations were observed for the raphide lengths (maximum and minimum) among the varieties. The raphides in the *Xanthosoma* species were shorter and thicker than those the of *Colocasia* species. As sharper reduction in thickness, indicating a pronounced needle-like structure, was observed for *X. sagittifolium* (red-flesh) and *C. esculenta* than for *X. sagittifolium* (white-flesh) variety. These differences in the morphological features of raphides have been reported to influence the degree of acridity in cocoyams (Sakai, 1979). The higher level of acridity in *Colocasia* species than the *Xanthosoma* species could be partly due to the sharpness and size of its raphides. Sakai (1979) has reported that irritations caused by ingestion of cocoyam foods could be related to mechanical effect caused by the needle-like nature of the raphides.

3.2. Rheological properties

The performance of cocoyam varieties in food preparations depends on the cooking properties of their flours. This property has been used to provide qualitative information on the formulation of food products. The rheological properties of flour samples of the three cocoyam varieties were studied using a Brabender viscoamylograph. All the sections showed distinct peak viscosities, with the middle section being more pronounced. With the *X. sagittifolium* (white-flesh) variety, the cooking trends observed were quite similar to those of the red-flesh variety except that the white-flesh had greater thermal stability and higher set-back viscosities. The *C. esculenta* showed lower hot paste viscosity but higher thermal stability than *Xanthosoma* species (Table 4). The viscosities of the *Xanthosoma* species during cooking were higher than the peak viscosities. Table 3 shows the viscoamylograph indices of the distal, middle and apical sections of the cocoyam varieties investigated.

The pasting temperature for the varieties ranged from 78.3 to 79.8 °C, with pasting temperatures being highest in the *X. sagittifolium* (red-flesh) variety and lowest in *C. esculenta*. This index is characterized by the initial change in viscosity due to the swelling properties of the starches. The implication is that the variety with the lowest pasting temperature (*C. esculenta*) will be easier to cook and require less heat for gelatinization to start. The pasting temperatures of the cocoyam varieties investigated were slightly higher than the mean range of 68–75 °C reported for cassava (Safo-Kantanka & Asare, 1993) and relatively lower than the values of 80.2–80.4 °C reported for trifoliate yam *D. dumetorum* tubers (Afoakwa & Sefa-Dedeh, 2002).

Table 2
Cross-sectional measurements of cocoyam raphides at the distal sections of three cocoyam varieties (μm)

Variety	Section	Raphide length		Raphide width	
		Maximum	Minimum	Maximum	Minimum
Xanthosoma sagittifolium (red-flesh)	Distal	375.0	320.0	12.5	4.0
Xanthosoma sagittifolium (white-flesh)	Distal	123.0	110.0	11.0	11.0
Colocasia esculenta	Distal	656.0	590.0	7.5	3.0

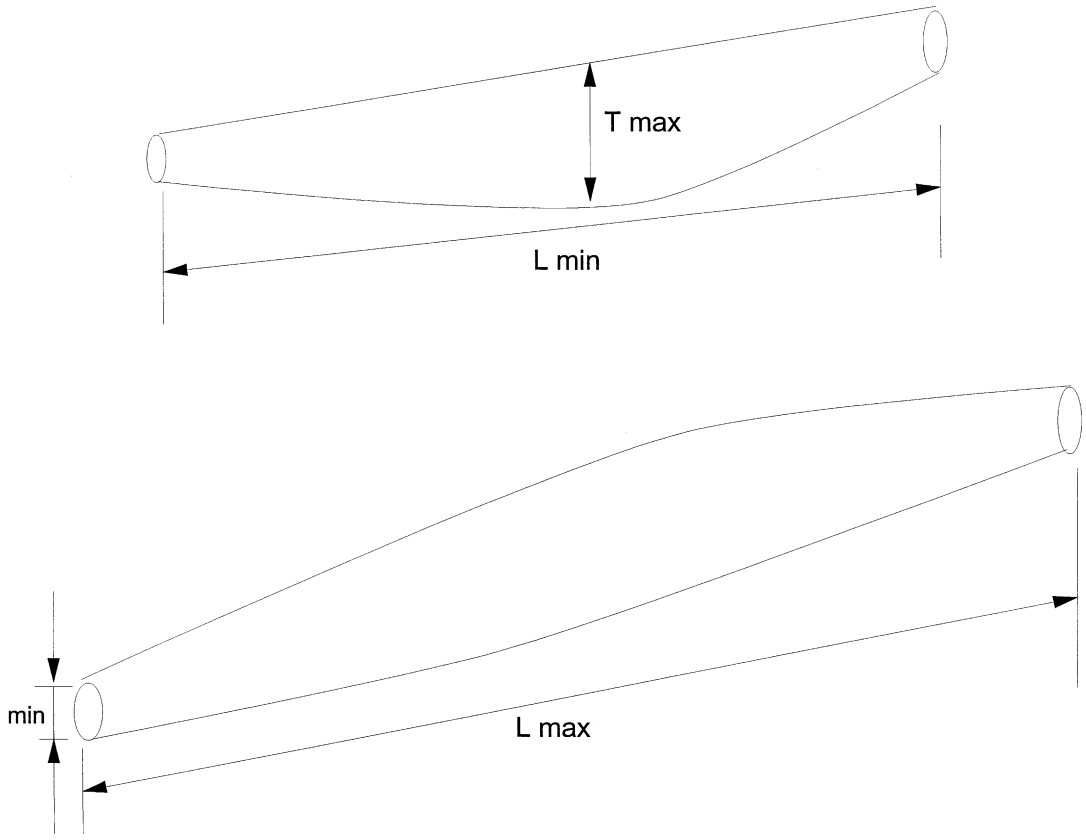

Fig. 4. Raphides as observed under the microscope.

Table 3
Amylograph indices of three cocoyam samples

Variety	Section	Pasting temperature (°C)	Peak viscosity (BU)	Viscosity at 95° C (BU)	Viscosity at 95° C Hold (BU)	Viscosity at 50° (BU)	Viscosity at 50° C Hold (BU)
Xanthosoma sagittifolium (red-flesh)	Distal	79.5	1080	960	525	1060	1165
	Middle	79.1	1370	1090	680	1180	1310
	Apical	79.8	930	920	720	1220	1514
Xanthosoma sagittifolium (white-flesh)	Distal	78.3	782	780	658	1290	1590
	Middle	78.5	1020	1000	780	1490	1760
	Apical	78.8	980	950	678	1250	1620
Colocasia esculenta	Distal	77.8	990	780	480	730	735
	Middle	77.9	925	735	535	880	840
	Apical	77.7	960	710	505	800	800

Peak viscosity was higher in the *X. sagittifolium* (red-flesh) variety with values ranging from 930 to 1370 Brabender Units (BU) than the *X. sagittifolium* (white-flesh) which had 980–1020 BU and *C. esculenta* var *esculenta*, with values between 925 and 990 BU. Wide variations in peak viscosities among the sections of the varieties were observed. The short time taken for the samples to reach their peak viscosities indicates that the starch granules had relatively low resistance to swelling and would be expected to swell rapidly and become susceptible to shear-induced disintegration. The paste at 95 °C showed only a slight viscosity drop. The middle sections of the *Xanthosoma* species and the distal section of the *Colocasia* spp. gave the highest viscosities at 95 °C. Sharp viscosity drops during the holding time (30 min) at 95 °C were observed for all the varieties. This illustrates the stability or breakdown of the paste during cooking. Stability ratios are shown in Table 4. The stability ratio ranged from 0.49 to 0.84. A high ratio usually indicates the attainment of relatively stable

Table 4
Paste set-back and stability ratios

Variety	Section	Stability ratio	Set-back ratio
Xanthosoma sagittifolium (red-flesh)	Distal	0.49	2.22
	Middle	0.49	1.94
	Apical	0.77	2.1
Xanthosoma sagittifolium (white-flesh)	Distal	0.84	2.24
	Middle	0.76	2.26
	Apical	0.69	2.39
Colocasia esculenta	Distal	0.48	1.53
	Middle	0.58	1.57
	Apical	0.53	1.58

viscosity with respect to time. High stability ratio for *X. sagittifolium* (white-flesh) variety implied that the cooked paste of the variety could better withstand shear at high temperatures. The stability ratios of the other two cocoyam species were distinctly lower. The stability in viscosity of the *X. sagittifolium* (white-flesh) variety is a desirable property since it gives a short non-cohesive paste, suitable in many food and industrial applications. The differences observed in the stability ratio may be due to the influence of agronomic climate conditions or heritable transfer of paste characteristics (Banks et al., 1973).

The viscosity after cooling the paste to 50 °C reflects the retrogradation tendency or set-back of the cooked pastes. The high retrogradation property of the pastes of the *Xanthosoma* species has been ascribed to high degree of association of starch molecules caused by the strong tendency for hydrogen bond formation between hydrogen groups on adjacent starch molecules. Properties such as staling in bread, skin formation, paste gelling and loss of clarity in prepared starch pastes have been associated with retrogradation. The paste from the *Xanthosoma* species underwent substantial increase in viscosity during cooling while the *C. esculenta* var *esculenta* showed a slight viscosity increase. This can be seen from the high set back ratios of the *Xanthosoma* species than the *C. esculenta* var *esculenta* (Table 4). The viscosity after 30 min at 50 °C measures the stability of paste as it might be used. Of the three varieties the *C. esculenta* var *esculenta* showed the greatest stability and the *X. sagittifolium* (white-flesh) the least.

4. Conclusion

Variations in starch granule sizes were found between the two *Xanthosoma* species. This observation may be associated with the genetic control of starch deposition in the two cultivars. The *Colocasia* species did not show any significant variation in starch granule sizes. However, marked differences were observed between the *Xanthosoma* species *and Colocasia* species with regard to their starch structures. This feature is distinct and aids the characterization of the different cultivars of cocoyam. The raphides in the two *Xanthosoma* species were similar. However, they differed significantly from the *Colocasia* species, which is indicative of the fact that raphides of *Xanthosoma* species and *Colocasia* species differ in structure. The rheological properties of the three cocoyam varieties were significantly different ($P \leqslant 0.05$) from each other. Maximum peak viscosity was obtained in the red-flesh variety of *Xanthosorna* spp. while the *C. esculenta* showed the minimum viscosity. The high starch stability ratio of the whiteflesh variety of *Xanthosoma* spp. is a novel functional attribute for cocoyam product development.

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