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A Research Note

Alterations in Cell Wall Constituents of Yams *Dioscorea dumetorum* and *D. rotundata* with Maturation and Storage Conditions. Relation with Post-Harvest Hardening of *D. dumetorum* Yam Tubers

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ABSTRACT

Hot water-insoluble cell wall polysaccharides of immature and mature *Dioscorea dumetorum* and *D. rotundata* yam tubers have been characterized in enzymatically destarched flours and were mainly constituted of cellulose followed by hemicellulosic polymers of xylose, galactose, mannose and arabinose. Immature tubers of both species contained a higher proportion of cell wall carbohydrates than mature ones essentially because of their low starch content. Storage of mature tubers of both species induced an increase of cell wall polysaccharides content particularly for *D. dumetorum*. The strong hardening of *D. dumetorum* yam during storage was characterized by the deposition of a xylose-containing polymer and of additional cellulose and by the lignification of the tubers.

INTRODUCTION

YAMS are widely grown in Western Africa and constitute an important source of starch for the energy requirements of the indigenous populations and it has even been suggested that yams provide more protein than is often appreciated (Coursey and Haynes, 1970). Cultivation of *Dioscorea dumetorum* is widespread in Western Cameroon and to a lesser extent in the south-eastern part of Nigeria. Yields obtained from this species are three to seven times higher than other species currently cultivated, mainly *D. rotundata* (Treche and Guion, 1979). Moreover *D. dumetorum* possesses a relatively high protein content and a favorable nitrogen/energy balance. Finally the tubers from this species have shapes more suited to mechanical harvesting than other African yams. Therefore, this species of yam is potentially suitable for intensive cultivation in Cameroon and could be developed in the future in other western African countries.

The storage ability of this yam is however restricted by the severe hardening of tubers which occurs between 2 and 16 wk after harvest and renders them unsuitable for human consumption. Therefore, only freshly collected tubers can be consumed locally and technological transformation of *D. dumetorum* must be carried out promptly after harvest. Former studies (Treche and Delpeuch, 1979) suggested that during hardening, parenchyma cell walls thickened and cell wall carbohydrate content determined by overall methods increased.

The purpose of the present investigation was to study variations in cell wall components occurring during maturation and post-harvest storage of *D. dumetorum* in comparison to *D. rotundata* which is not affected by this hardening phenomenon. Hot water-insoluble cell wall polysaccharides and lignin were analyzed using starch-free residues obtained by destarching the yam flours. Post-harvest hardening of *D. dumetorum* was further characterized by light microscopy examination.

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EXPERIMENTAL

Materials

The yams *Dioscorea dumetorum* (cv. Jakiri) and *D. rotundata* (cv. Oshei) were grown at Bambui (Cameroon) in 1976. Samples of 10 tubers were harvested at random 4 months after planting (immature tubers) and 6 months after planting (mature tubers). Furthermore samples of mature tubers of both species were stored for 4 months in a well aerated barn, the minimum and maximum temperatures being 18 and 31°C, the relative humidity rising to approximately 100% during the night and 62% at midday during the period of storage. No growth of moulds was observed. Hardening of *D. dumetorum* tubers was observed but not in *D. rotundata* at the end of the storage period. This hardening was characterized not only by a visible hardness but by the rough and fluffy aspect of a tuber section as opposed to the smooth and moist aspect of the freshly harvested mature one.

Immature, mature tubers (immediately after harvesting) and mature tubers which were stored for 4 months were peeled, cut into small cubes, dehydrated under vacuum (40°C) and finally ground to pass a 0.2 mm sieve.

Reagents

Aspergillus niger amyloglucosidase (50 UI/mg) (Merck, Germany); Supelcoport (100–120 mesh) with 3% SP 2340 (Supelco Inc.); metahydroxydiphenyl (Eastman Kodak Co); kraft lignin Indulin (Westvaco, S.C.); all other reagents were of an analytical grade.

Preparation of starch-free residues

Yam flours were extracted by boiling in 80% ethanol (2x30 min), then removal of starch from extracted samples was done by treatment with fungal amyloglucosidase after autoclaving as described by Thivend et al. (1972) for starch determination. The entire procedure was repeated once on the crude destarched flours to ensure complete removal of starch. Finally starch-free residues were dried by acetone and used for further analyses.

Neutral sugars analysis

Cell wall polysaccharides of starch-free residues were hydrolysed according to Saeman et al. (1954) by pretreatment with 72% sulfuric acid (26N) at 20°C for 2.5 hr then diluted to 2N and heated at 100°C for 2.5 hr. Cell wall polysaccharides were also hydrolyzed with 2N trifluoroacetic acid (TFA) at 120°C for 1.5 hr (Albersheim et al., 1967; Selvendran et al., 1979). Individual monosaccharides were converted to their alditol acetates (Sawardeker et al., 1965) and analyzed by gas-liquid chromatography with a column (180 x 0.2 cm) of 3% SP 2340 coated on Supelcoport (100-120 mesh) at 225°C (nigtrogen flow: 20 ml·min^{-1}). Meso-inositol was used as the internal standard.

Acidic sugars analysis

Starch-free residues were treated with concentrated sulfuric acid at 20°C for 10 min, samples were then placed in a water-ice bath and water was slowly added over a period of approximately 15 min to obtain a final acid concentration of 72% (El Rayah Ahmed and Labavitch, 1977). After suitable dilution with water, uronic acids were analyzed by metahydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) using galacturonic acid as standard.

Lignin analysis

Lignin was measured by acetyl bromide procedure (Morrison, 1972) with the following modifications (Monties and Rambourg, 1978): phenolic substances bound to the lignin were by alkali [illegible]

Table 1—Cell wall carbohydrates and lignin content of Dioscorea dumetorum and D. rotundata yam tubers

Samples	Maturation and storage conditions	Sugars[abc] Ara	Xyl	Man	Gal	Glu	Uronic acids	Total sugars	Lignin[a]
D. dumetorum	Immature	0.18	0.46	0.19	0.21	3.10	0.23	4.37	0.39
	Mature	0.05	0.28	0.07	0.21	1.98	0.15	2.74	0.30
	Stored (Hardened)	0.08	1.04	0.08	0.33	3.00	0.29	4.82	0.72
D. rotundata	Immature	0.11	0.29	0.36	0.15	2.49	0.11	3.51	0.29
	Mature	0.03	0.09	0.06	0.11	1.19	0.13	1.61	0.21
	Stored (Unhardened)	0.08	0.14	0.11	0.28	1.61	0.18	2.40	0.47

[a] Percent of dry matter of peeled tubers.
[b] Expressed as anhydropolymeric form.
[c] Data from Saeman hydrolysis.

ester linkages were removed by treating starch-free residues with 2N NaOH for 1 hr at 35°C; then lignin determination was conducted in the usual way on the alkali extracted cell walls (20 mg) using a calibration curve (1–10 mg) obtained with kraft lignin.

Light microscopy examination

Small cylinders (L=6 cm; ϕ = 0.6 cm) were obtained using a cork borer by piercing through the middle portion of unhardened and hardened mature tubers of *dumetorum* species. Fixation was carried out in a formalin/calcium mixture and inclusion in a paraffin embedding medium. Specimens of a thickness around 7μ were treated with a 12% sodium hypochlorite solution for 5 min in order to empty the parenchyma of some of its starch which hampered observation. They were then passed in a 2% acetic acid solution followed by the coloring agent carmine/iodine green (Treche and Delpeuch, 1979).

RESULTS & DISCUSSION

AFTER ELIMINATION of the low molecular weight intracellular compounds (free sugars, phenolics) by extraction with ethanol, starch elimination using a widely accepted enzymatic method for starch analysis (Thivend et al., 1972) was adopted. Completion of starch removal was checked by light microscopy examination of residues under polarized light or after coloration with I_2/KI. Hot water soluble hemicelluloses were eliminated simultaneously with starch removal and were not studied in the present paper.

Neutral and acidic sugars as well as lignin contents of starch-free residues were estimated and expressed as percentage of dry matter of whole peeled tubers (Table 1). Glucose was the major monosaccharide constituent in all studied samples. This sugar might arise from cellulose and/or from hemicellulosic polymers (Ring and Selvendran, 1978). After treatment of starch-free residues with trifluoroacetic acid (Albersheim et al., 1967) which does not hydrolyse the $\beta(1\rightarrow4)$ links of cellulose (Ring and Selvendran, 1978), negligible amounts of glucose were obtained. Therefore glucose occurring in cell walls of all studied yam tubers samples originated from cellulose contrary to Hanh and Rasper (1974) who reported the presence of high amounts of hemicellulosic glucose in hot water insoluble cell wall polysaccharides of *Dioscorea alata* and *D. rotundata*. Insufficient removal of starch could be responsible for their high values. Hemicellulosic polymers were constituted of xylose, galactose, mannose and arabinose in decreasing importance. Mannose content must be carefully interpreted as some mannose might be produced by epimerization of glucose under strongly acidic conditions of the Saeman procedure (Saeman et al., 1954; Selvendran et al., 1979). Uronic acids originating from pectic substances (galacturonic acid) and/or from hemicelluloses (glucuronic acid) were not individually determined but were present in significant amounts in all tubers.

Immature tubers from both species were characterized by higher proportion of cell wall carbohydrates than mature ones as indicated by total sugar content and also by individual monosaccharide levels. Ketiku and Oyenuga (1973) showed that the percentage of starch in tubers of *D. rotundata* doubled between 4 and 6 months of growth which could explain the corresponding proportional decrease in cell wall carbohydrate content. Furthermore during maturation, moderate changes occurred in the relative distribution of sugar constituents in walls of tubers of both species: arabinose and mannose decreased whereas galactose content increased. Cellulose content of tubers increased very slightly between 4 and 6 months of growth. Therefore, maturation of tubers *D. dumetorum* and *D. rotundata* was characterized by slight variations in hemicellulosic polymers whereas cellulose remained rather constant.

Storage of mature tubers of both species for prolonged periods induced an increase in cell wall carbohydrates but to a lesser extent in *D. rotundata* (∿50%) than in *D. dumetorum* (∿76%) taking into account the storage weight losses (∿10%) (Treche and Guion, 1979). Apart for a strong increase in galactose content, increases in other sugar constituents were moderate in *D. rotundata* as opposed to *D. dumetorum* in which a striking augmentation in xylose content (∿4 fold) was observed. Moreover cellulosic glucose was also markedly increased during storage of *D. dumetorum* tubers. Therefore the hardening process of *D. dumetorum* yam tubers during storage seemed to be closely related to a deposition in cell walls of a xylose containing polymer which could be of the xylan type and to the appearance of additional cellulose. *D. rotundata* seemed to be affected by a similar alteration of cell wall carbohydrates when stored but to a lower extent which could explained that no hardening was visible. Moreover *D. rotundata* species contained lesser amounts of wall polysaccharides as percent of dry matter as compared to *D. dumetorum*. Lignin was detected in immature and mature tubers of both species and originated most likely from xylem elements (Table 1). Xylem elements were stained dark green as opposed to surrounding parenchyma cells whose thin walls were stained pink in the case of mature tubers by the carmine/iodine green reagent. After a long period of storage (16 wk) lignin content of both species increased strongly. In the case of *D. dumetorum* parenchyma cell walls were stained green showing a lignification of this tissue which is one of the characteristic features of the hardening of this species.

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