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Changes in Carbohydrate Contents of Germinating Cowpea Seeds

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ABSTRACT

A decrease in the concentration of total carbohydrates was observed during 144 hours' germination of three cowpea varieties. However, whilst starch, raffinose, stachyose and verbascose levels decreased, the reducing sugars, including glucose and fructose, increased. Starch was the major carbohydrate and about 50% of it was hydrolysed after 144 hours' germination. Changes also occurred in the concentration of the unavailable carbohydrates. Lignin contents increased slightly while hemicelluloses and celluloses decreased. The above-mentioned changes, occurring during germination, appear to have a favourable effect on the nutritional value of cowpea varieties.

INTRODUCTION

Cowpeas, a grain legume or pulse and commonly referred to in Nigeria as beans, are an important source of dietary protein. They can contribute up to 80% of the total dietary protein intake in some parts of Nigeria (Luse & Okwuraiwe, 1975).

The cowpea grains are prepared in a variety of ways and are consumed either singly or in combination with staple foods such as rice, yam or plantain. Although the traditional processing methods are mainly soaking, decortication and conversion to a paste product before cooking, another processing method that is fast gaining popularity among

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Nigerians is the cooking of the already germinated grains. Germinated legumes are believed to be nutritionally superior because they are more easily digested and have a higher vitamin content (Chen *et al.*, 1975; Subbulakshmi *et al.*, 1976). The effects of this processing method on the nutritional and eating quality of some cowpea varieties, popularly consumed in Nigeria, have to date received little attention.

This paper reports the results of an investigation into changes in the quantitative distribution of the carbohydrates of cowpea (*Vigna unguiculata*), during the early stages of germination.

MATERIALS AND METHODS

Three varieties of cowpea, 'Ife brown', 'Adzuki' and 'Farv-13', were employed in this study. The seeds were obtained from the National Cereals Research Institute, Moor Plantation in Ibadan, Nigeria. They are local accessions of beans developed through selection from accessions collected from parts of Nigeria. The dry cowpea grains, about 40 g, were first washed with mercuric chloride to remove surface contamination. They were then soaked overnight in water and allowed to germinate in sterile petri dishes lined with wet filter paper at 27 °C. Seeds and seedlings were removed at 0, 48 h, 96 h and 144 h intervals, rinsed again with mercuric chloride and water, then freeze-dried and ground to a fine powder.

Analytical procedure

The ethanol-soluble sugars were extracted from the legume flour at room temperature (27 °C) by repeated shaking with 80% ethanol and the extracts were pooled. The ethanol was evaporated from the pooled extracts under vacuum at 40 °C and quantitative analysis of the sugars present was accomplished by paper chromatography using ethylacetate–pyridine–water (8:2:1 v/v) as solvent. Sugars were detected by spraying with *p*-anisidine hydrochloride reagent (Mukherjee & Srivastavata, 1952). Oligosaccharides were separated on Whatman No. 3 paper by developing the chromatogram for 4 h using propanol–ethanol–water (7:1:2) and constituent sugars were determined by the phenol–sulphuric acid method (Dubois *et al.*, 1956). Starch and total sugars were estimated as glucose equivalents (McCready *et al.*, 1950)

and reducing sugars were determined using 3,5-dinitro salicyclic acid (Bernfeld, 1954). Pentosans were precipitated as phloroglucinol derivatives and estimated gravimetrically (AOAC, 1970). Unavailable carbohydrates of cell wall origin were estimated by acid hydrolysis (Southgate, 1969) after which total sugars in hydrolysates were estimated (Dubois *et al.*, 1956). Sugars identified on paper chromatograms were quantified. Fructose, glucose and sucrose were determined by the combined enzymatic and chemical methods of Johnson *et al.* (1964).

RESULTS

Carbohydrate compositon of germinated and ungerminated cowpea varieties

The total available carbohydrates in the legume varieties studied increased when germinated for either 48, 96 or 144 h. Reducing sugars increased at the expense of starch and non-reducing sugars (Table 1). A slight increase in the level of pentosans was also found.

Ethanol—Soluble sugars

The sugars present in the 80% ethanol extracts of the samples are shown in Table 2. In all ungerminated varieties, glucose and fructose were only present in traces while sucrose ranged between 2·06 and 2·83 g/100 g. Raffinose content varied from 1·23 to 1·80 g/100 g while stachyose varied from 1·30 to 1·80 g/100 g and verbascose content was 0·60, 0·65 and 0·85 g/100 g for Farv-13, Adzuki and Ife brown, respectively. During germination, the concentrations of glucose and fructose increased considerably while sucrose reached a maximum after 48 h and thereafter gradually decreased in content. The oligosaccharide concentration decreased very rapidly in all varieties and, in Ife brown and Adzuki, verbascose completely disappeared after 48 h and, in Farv-13, after 24 h. At the termination of germination, no trace of stachyose, raffinose or verbascose was evident in any of the germinated varieties.

Unavailable carbohydrates

Qualitative analysis of the water-soluble polysaccharides and hemicellulose revealed the presence of hexoses and pentoses. However, the

TABLE 1

Carbohydrate Composition of Germinated and Ungerminated Cowpea Varieties[a] (Grams per 100 g of Seed)

Legume varieties	*Duration of germination (h)*	*Total available carbohydrates*[c]	*Soluble carbohydrates*			*Pentosans*	*Starch*[d]
			Total	*Reducing*	*Non-reducing*[b]		
Ife brown	0	58·2	16·8	2·7	14·1	4·2	30·3
	48	75·2	20·3	6·7	13·6	4·4	37·6
	96	65·2	23·0	11·8	11·2	4·8	25·4
	144	62·3	27·6	17·7	9·9	5·5	20·0
	Mean	65·2	21·9	9·9	12·2	4·7	28·3
	Standard deviation	±7·2	±4·6	±6·5	±2·0	±0·6	±7·5
Adzuki	0	78·1	23·6	3·8	19·8	4·9	43·9
	48	73·3	24·0	5·5	18·5	5·0	33·7
	96	69·8	26·7	12·3	14·4	5·6	25·8
	144	66·3	29·3	19·5	10·8	6·0	19·3
	Mean	71·9	25·9	10·3	15·9	5·4	30·7
	Standard deviation	±5·0	2·7	7·2	±4·1	±0·5	±10·6
Farv-13	0	66·5	18·0	2·5	15·5	3·5	37·6
	48	68·4	21·6	6·9	14·7	3·8	31·0
	96	65·9	26·4	16·3	10·1	4·2	22·5
	144	65·6	30·1	23·0	7·1	5·0	17·1
	Mean	66·6	24·0	12·2	11·9	4·1	27·1
	Standard deviation	±1·3	±5·3	±9·2	±4·0	±0·7	±9·1

[a] Estimations based on chemical analysis (duplicate samples).

[b] Expressed as the difference between total and reducing sugars.

[c] Sum of soluble carbohydrates, pentosans, starch and ethanol-soluble sugars.

[d] Values expressed as glucose.

TABLE 2
Ethanol-Soluble Sugars in Germinated and Ungerminated Cowpea Varieties[a]
(Grams per 100 g of Seed)

Legume varieties	*Duration of germination (h)*	*Glucose*	*Fructose*	*Sucrose*	*Raffinose*	*Stachyose*	*Verbascose*
Ife brown	0	0·20	0·46	2·25	1·80	1·30	0·83
	48	1·80	1·10	7·55	1·34	0·60	0·54
	96	2·50	1·86	6·50	0·95	0·15	—
	144	3·00	2·20	4·00	—	—	—
	Mean	1·9	1·41	5·08	1·03	0·51	—
	SD	±1·2	±0·78	±2·40	±0·76	±0·58	—
Adzuki	0	0·26	0·33	2·06	1·23	1·15	0·65
	48	1·13	1·00	6·70	0·75	0·70	0·27
	96	3·00	2·60	5·90	—	0·20	—
	144	3·30	3·75	4·65	—	—	—
	Mean	1·92	1·92	4·83	—	0·51	—
	SD	±1·47	±1·55	±2·03	—	±0·52	—
Farv-13	0	0·19	0·48	2·83	1·45	1·80	0·60
	48	2·20	1·50	6·96	0·60	0·76	—
	96	2·95	2·77	6·33	0·36	0·35	—
	144	3·50	4·30	5·60	—	—	—
	Mean	2·21	2·26	5·43	0·60	0·73	—
	SD	±1·44	±1·65	±1·82	±0·61	±0·78	—

[a] All analyses were carried out in duplicate and the results are expressed on a dry matter basis.
SD = Standard deviation.

TABLE 3

Hemicelluloses, Celluloses and Lignins in Germinated and Ungerminated Cowpea Varieties[a] (Unavailable Carbohydrates.) (Grams per 100 g of Seed)

Legume varieties	*Duration of germination (h)*	*Water-soluble polysaccharides*[b]			*Hemicelluloses*			*Celluloses*[c]	*Lignins*
		Hexoses	*Pentoses*	*Total*	*Hexoses*	*Pentoses*	*Total*		
Ife brown	0	4·66	2·90	7·56	6·87	1·42	8·29	2·08	0·93
	48	4·40	2·80	7·20	6·50	1·20	7·70	1·75	1·00
	96	4·00	2·59	6·59	6·00	1·00	7·00	1·60	1·15
	144	3·85	2·40	6·25	5·80	0·90	6·70	1·53	1·33
	Mean	4·23	2·67	6·90	6·29	1·13	7·42	1·74	1·10
	SD	±0·37	±0·22	±0·59	±0·48	±0·23	±0·71	±0·24	±0·18
Adzuki	0	3·90	2·97	6·87	5·32	0·54	5·86	1·39	0·89
	48	3·60	2·70	6·30	5·10	0·50	5·60	1·10	1·30
	96	3·50	2·70	6·20	4·83	0·45	5·28	0·94	1·50
	144	3·00	2·55	5·55	4·50	0·42	4·92	0·88	2·20
	Mean	3·50	2·73	6·23	4·94	0·48	5·42	1·08	1·47
	SD	±0·37	±0·17	±0·54	±0·35	±0·05	±0·41	±0·23	±0·55
Farv-13	0	4·50	2·00	6·50	5·88	0·90	6·78	3·23	0·80
	48	4·25	1·80	6·05	5·75	0·90	6·65	2·90	0·95
	96	3·90	1·75	5·65	5·70	0·65	6·35	2·81	1·22
	144	3·44	1·50	4·94	5·30	0·50	5·80	2·59	1·50
	Mean	4·02	1·76	5·78	5·66	0·74	6·40	2·88	1·12
	SD	±0·46	±0·21	±0·66	±0·25	±0·20	±0·44	±0·27	±0·31

[a] Constituents reported were assayed by chemical analysis of duplicate samples and the results are expressed on a dry matter basis.
[b] Water-soluble polysaccharides were determined after the removal of starch and ethanol-soluble free sugars.
[c] Expressed as glucose.
SD = Standard deviation.

hemicellulose hydrolysates contained more hexoses while the water-soluble polysaccharides contained more pentoses and, in all cases, pentose contents in the water-soluble polysaccharides more than doubled the concentration in the hemicelluloses (Table 3). During germination, all cell wall components, with the exception of lignin, decreased in content. Farv-13 recorded the highest cellulose level and, even though germination effected a slight reduction in content, values obtained for the germinated and ungerminated samples of this variety greatly exceeded the corresponding values in Ife brown and Adzuki. Lignin levels were, however, comparatively low, especially in ungerminated Farv-13 (0·80%), and levels in the germinated samples did not exceed a maximum of 2·20%, obtained in Adzuki after 144 h of germination.

DISCUSSION

Germination resulted in a marked increase of total available carbohydrates, coupled with qualitative alterations in the make up of individual constituents. While total soluble carbohydrates increased with successive stages of germination, starch contents decreased rapidly and, being the principal carbohydrate, resulted in an overall decrease in total carbohydrate content. A comparison of the amounts of reducing sugars with those of non-reducing soluble carbohydrates shows that, by the sixth day (144 h) of germination, reducing sugars formed the bulk of the total soluble carbohydrates.

During the early phases of germination, it appeared that enzymatic hydrolysis of starch was initiated. McCready *et al.* (1950) had observed that the rapid depletion of starch in germinating seeds was due to the action of microbial α and β amylases which hydrolyse amylose and amylopectin to simple sugars. Making similar observations, Jenning & Morton (1963) explained that the increase in soluble sugar content indicated that starch hydrolysis by amylases increased the pool of precursor compounds, especially glucose, fructose and, to some extent, sucrose which are maintained at a relatively high concentration during seed germination. It is noteworthy that the patterns of carbohydrate utilization, especially of starch, during germination were similar for all samples. However, the slight variations in magnitude and rate of utilization observed could have resulted from genetic differences between the varieties of cowpea. This is consistent with the observation (Matheson

& Saini, 1977) that the pattern of reserve carbohydrate hydrolysis during germination varies with the legume type and variety.

The increase in glucose and fructose contents agrees with the observations of Hsu *et al.* (1976) that the appearance of glucose and fructose during soybean germination originates during the hydrolysis of starch and the oligosaccharides. Stachyose and verbascose were the predominant oligosaccharides in the ungerminated varieties but these sugars very rapidly decreased as germination progressed. Stachyose disappeared after 96 hours' germination while verbascose was completely absent in Farv-13 after 48 h. Abrahemsen & Sudia (1966) reported a nearly complete depletion of stachyose and raffinose in germinating soybean by 48 h and Kawamura (1964) reported the disappearance of these sugars between days 5 and 6 of growth. These observations suggest that oligosaccharides are not only hydrolyzed very rapidly during germination but are also utilized for respiration (Meyer & Mayber, 1966), incorporated into cell walls or translocated to the growing embryo (Meyer & Anderson, 1952).

Germination also had considerable effects on the concentration and characteristics of the water-soluble polysaccharides, hemicelluloses, celluloses and lignins. While lignin contents increased slightly, all the other unavailable carbohydrates of cell wall origin decreased. Some of these changes, including the decrease in oligosaccharide sugar, brought about by germination, may have a favourable impact on the digestibility, the flatus-forming property and other nutritional qualities of these legumes.

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