Changes in the Major Constituents of Millet (*Pennisetum americanum*) during Germination

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The quantitative and qualitative changes in carbohydrates, proteins, and lipid materials during the germination of millet have been studied. A two-stage metabolism was exhibited during germination. It was observed that starch content decreased during germination and this coincided with an increase in soluble carbohydrates and proteins. The high lipid content of the grain was reduced to about one-third in 72 h. The possible role of these primary metabolite changes in the development of millet for malting is discussed.

Millet (*Pennisetum americanum* L. Leeke) is one of the most nutritive cereals consumed (U.S. Agency for International Development, 1971). An estimated 3.8 million metric tons of millet is cultivated annually in the Savanna regions of Nigeria as a source of dietary carbohydrates.

Millet is also used for the production of local alcoholic beverages (Oke, 1977). Commercial malters reject millet because the relatively low carbohydrate and the high lipid content would contribute to poor head retention and organoleptic properties of the beer (Zeurcher, 1971). The desire to use local cereals as malting material in the industries has enhanced the importance of millet in the Nigerian economy. It is therefore important to carry out investigations into the changes in the major storage reserves of millet with a view of modifying the grain into an adequate malting material.

MATERIALS AND METHODS

Millet (*P. americanum*) grains were obtained from the local market. Grains were surface sterilized with 1% sodium hypochlorite and then steeped at 4 °C for 6 h in sterile distilled water. The hydrated grains were germinated at 22 °C in the dark (Aisien and Ghosh, 1978) for 0, 6, 12, 15, 18, 21, 24, 36, 48, 72, and 96 in 9-cm Petri dishes lined with distilled water–saturated Whatman's No. 1 filter paper. Three germinations were carried out, each involving 100 whole and sound intact grains. Germinated grains were kilned at 45 °C for 24 h and then ground to pass a 20-mesh screen.

Carbohydrate Determination. Starch and 80% ethanol soluble carbohydrates were isolated and estimated by the method of Hansen and Moller (1975). Paper chromatography (Whatman No. 1) was used to resolve and identify the soluble sugars employing propan-2-ol–water (4:1 v/v) and 1-butanol–pyridine–water (2:2:1 v/v/v) as developing solvents. The sugars were located with 2 mL of saturated silver nitrate in 400 mL of acetone.

For quantitative analysis of the soluble sugars, the 80% ethanol soluble extracts were deionized by cation (Dowex 50, H^+) and anion (Amberlite Ir-45, free base) exchange resin chromatography. The resulting extracts were converted to trimethylsilyl (Me_3Si) derivatives (Tharanathan et al., 1976; Wankhede and Tharanathan, 1976) and analyzed by GLC with a Varian 2700 gas chromatograph fitted with flame ionization detector and glass column (180 × 0.2 cm i.d.) packed with 3% OV-17 on Chromosorb W, 60–80 mesh. The temperature of the column was programmed to run from 130 to 320 °C at 6 °C min^{-1} with a final holding period of 10 min. The carrier gas was 40 mL of nitrogen min^{-1}. An Autolab minigrator was used for quantitative evaluation of the peaks.

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Protein Analysis. The ground samples were refluxed with 80% ethanol for the isolation of soluble nitrogen. Total proteins were then estimated as nitrogen (William, 1964) on the ethanol-extracted residue. Soluble proteins were extracted with 0.85% NaCl, centrifuged at 3000*g* for 15 min, and estimated by the biuret method (Ohnishi and Barr, 1978).

Determination of Lipids. Total lipid content was determined as fatty acid methyl esters by the method of Morrison et al. (1980). Heptadecanoic acid was used as an internal standard. The concentrated hexane extracts of the fatty acid methyl esters were chromatographed isothermally (180 °C) on a 2.1 m × 6 mm (i.d.) glass column packed with HI-EFF 1,4-butanediol succinate polyester on AW-DMCS Chromosorb W (80–100 mesh) by using a Pye Unicam GCD gas chromatograph equipped with a flame ionization detector. Oxygen-free nitrogen was used as the carrier gas at a flow rate of 50 mL min^{-1}. Results were calculated as milligrams of fatty acid methyl esters per milligram of dry tisue after applying the appropriate corrections (Morrison et al., 1980).

All experiments were in triplicate.

RESULTS AND DISCUSSION

Observations show that millet has a short germinating period (indicated by the appearance of the radicle within 15–18 h); thereafter, there is a period of rapid growth.

Figure 1 shows the changes in the hydrolysis of starch and the development of soluble carbohydrates during the germination of millet. The starch content decreased during germination and this coincided with increases in soluble carbohydrates. The same general trend has been reported for sorghum (Aisien and Ghosh, 1978) and groundnuts (Wankhede et al., 1977). It is especially noteworthy that as in barley malt (Greenwood and Thompson, 1959), starch degradation in millet is limited even though germinated millet (Opoku and Uraih, 1982; Opoku et al., 1982) and barley (Greenwood and Thompson, 1959) and known to contain large quantities of α-and β-amylases.

The composition of the soluble carbohydrates (Figure 2) reveals that raffinose is the major soluble sugar in the resting grain. This sugar is quickly metabolized during steeping and early germination. The increase in glucose and sucrose during germination is maximum at 48 h, while the maltose content remained almost constant except that there was obvious metabolism during the first 24 h of germination. It has been demonstrated in several reports that sucrose and raffinose are utilized during the germination process of barley to provide energy for the process (James, 1940; MaCleod, 1957; Palmer, 1969). The results presented from this investigation are consistent with these earlier findings.

The 10% protein levels were found to undergo striking changes concurrent with the changes in carbohydrate concentration. Figure 3 shows that millet grains rapidly

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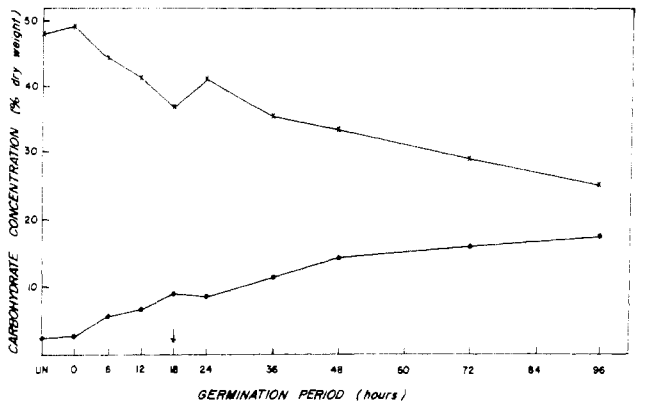

Figure 1. Development of soluble carbohydrates (●) and hydrolysis of starch (×) in germinating millet grains. UN = unsteeped seeds. ↓ = time at which seed germinated and seedling growth began.

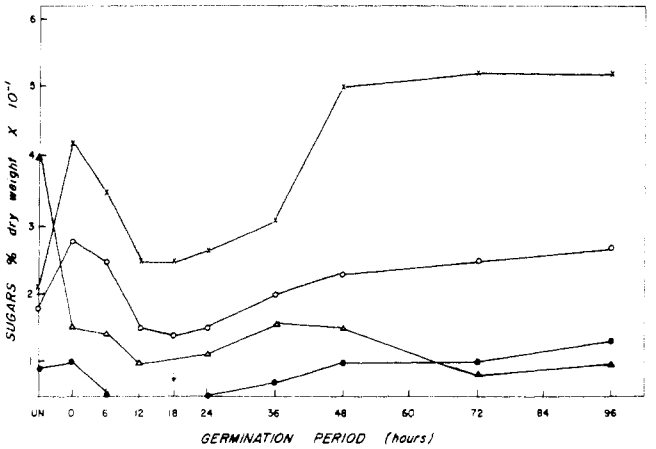

Figure 2. Changes in the composition of soluble sugars during the germination of millet. Sucrose (×); raffinose (Δ); glucose (○); maltose (●). UN = unsteeped grains. ↓ = time at which seed germinated and seedling growth began.

lose proteins until the 18th hour of germination; thereafter the protein concentration begins to increase.

There is little degradation of lipid materials during the first 18 h of germination (Figure 3). In fact, there is an apparent increase in lipid content, reaching a maximum at 18 h. This could be because other food reserves are preferentially metabolized so that lipids form the major part of the remaining materials. Catabolism of stored lipid possibly starts at about 18 h, leading to an absolute decrease in total lipids during growth. This catabolism of lipids coincides with the accumulation of sucrose (see Figure 2). It may be inferred that there is a possible conversion of lipid materials to carbohydrates (Beevers, 1961; Kornberg and Beevers, 1957) since the photosynthetic apparatus in the etiolated seedlings is not functional.

The fatty acids levels are shown in Table I. Linoleic acid was predominant throughout the period of germination, and similar to cassava seedlings (Nartey and Moller, 1973) no fatty acid was preferentially metabolized.

The striking feature of the degradation of storage materials in millet is that there seems to be a two-stage metabolism. In the first phase proteins and starch are broken down to products that are utilized within the 18-h germinating period. Stored lipids and a portion of starch are rapidly metabolized during the postgermination period of growth (18–72 h). It is possible that the hydrolytic products of proteins (from the first stage) are used for synthesis of new cellular materials and enzymes that are involved in the breakdown of lipids and carbohydrates in the second

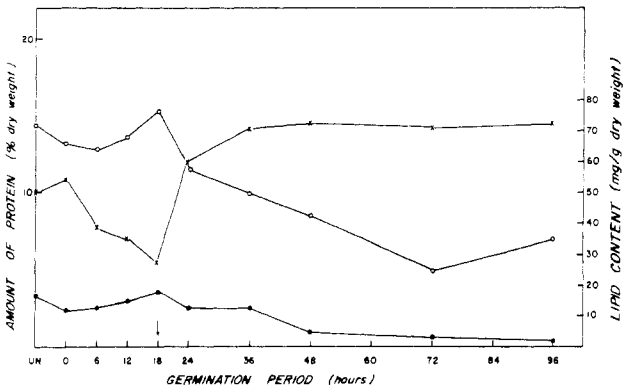

Figure 3. Changes in protein [total (×); soluble (●)] and lipid (○) materials during the germination of millet. UN = unsteeped grains. ↓ = time at which seed germinated and seedling growth began.

Table I. Changes in Fatty Acid Composition of Millet Seeds during Germination[a]

time, h[b]	lipid quantity, mg/g dry wt	fatty acid, % total fatty acids[c]					
		14:0	16:0	18:0	18:1	18:2	18:3
UN	70.2	1.75	19.61	2.25	29.10	54.42	1.87
0	66.1	1.97	18.97	1.99	29.44	45.48	2.15
6	62.3	1.77	19.20	2.11	29.47	45.62	1.77
12	67.3	1.76	19.40	1.96	29.45	45.18	1.76
18	77.2	1.72	19.47	1.93	30.08	44.97	1.83
24	58.7	1.88	18.95	2.01	29.13	45.58	2.14
36	57.7	1.91	18.23	1.89	30.00	45.79	2.18
48	43.6	2.15	19.26	1.99	29.43	45.06	1.93
72	25.4	2.21	19.40	2.09	29.34	45.07	1.87
96	35.2	2.22	19.13	1.94	29.15	45.56	1.80

[a] Seeds were germinated in the dark. Trace quantities (i.e., less than 0.5%) of 12:0 and 20:0 fatty acids were present in all samples. [b] UN = unsteeped seeds. [c] Notation for fatty acids: number of carbon atoms:number of double bonds.

stage. It also suggests that as in barley (Macleod and Palmer, 1966) there is transfer of the hydrolyzed products to the growing embryo.

It is apparent therefore that millet grains should be germinated for 72 h for malt making, at least for the high lipid content to be lowered (to about 2–3% as it occurs in barley; Harris, 1962) and starch to be hydrolyzed to fermentable sugars. Nevertheless, in 72 h, the first leaves have appeared and the resulting material may not be suitable as brewing malt. Yet the high protein content in such a product may represent a nutritionally and economically useful asset to the developing countries (Opoku et al., 1981). For millet to be used in the making of brewing malt, genetic improvement programs should aim at selecting a strain that will metabolize stored lipids preferentially during the 18-h germinating period. It is worthwhile mentioning here that the native brewers germinate millet for 4–5 days. The resulting beer is, however, thick and does not foam. A different approach to utilizing millet in beer making is therefore to develop suitable technology that could "thin" the 72 h grown millet malt during brewing to internationally acceptable standards.

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Nutrient and Cardenolide Composition of Unextracted and Solvent-Extracted *Calotropis procera*

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Solvent extraction of *Calotropis procera* results in a hydrocarbon fraction potentially valuable as a fuel or chemical feedstock. The data presented indicate that residues extracted with solvents have a value as a fibrous animal feedstuff. The nutrient and cardenolide composition of unextracted, hexane-extracted, and hexane and methanol extracted plant and individual plant component residues from *C. procera* were determined. All residues were compared with respect to proximate analysis, amino acid profile, in vitro digestibility, and mineral and cardenolide composition. Hexane extraction of the plant residues generally enhanced the nutrient composition but did not greatly reduce cardenolide levels. Hexane and methanol extracted residues, however, were somewhat lower in nutrient content but essentially cardenolide free. The presumptive nutrient value of extracted residue from *C. procera* supports cultivation of this plant species as a potential fuel and feed resource.

Calotropis procera (Art.) R. Br. (Asclepeadaceae) is a large broadleaf evergreen which grows abundantly in arid and semiarid regions of the world without irrigation, fertilizer, pesticides, or other agronomic practices. This plant is very resistant to fire and coppices profusely (Little et al., 1974; Mahmound et al., 1979a; Saxena and Singh, 1976; Karschon, 1970). Although widely distributed, little commercial use for this plant species has been reported (Vasudevan et al., 1981).

The use of plants as renewable hydrocarbon sources or chemical feedstocks has been reported (Buchanan et al., 1978a,b; Nemethy et al., 1979; Calvin, 1980, 1982). Recently harvested *C. procera* biomass was solvent extracted with hexane and methanol and also demonstrated to be a rich source of hydrocarbons (Erdman and Erdman, 1981) that was comparable to extractables from *Euphorbia lathris* (Nemethy et al., 1979; Calvin, 1980). Although hydrocarbon extraction from *C. procera* has been demonstrated, secondary uses for the extracted residues have not been reported.

The principal toxic components in *C. procera* are cardenolides (Watt and Breyer-Brandwijk, 1965; Brüschweiler et al., 1969a,b; Biedner et al., 1977; Garg, 1979; Nelson et al., 1981; Seiber et al., 1982). Cardenolides are 5βH,14β-hydroxy α,β-unsaturated γ-lactones and are widely distributed in the plant kingdom as glycosides. In small doses they tend to slow and strengthen the heart beat, but excessive doses can cause the heart to stop (Standen et al., 1969). Any proposed cultivation or processing scheme to utilize *C. procera* must consider these highly toxic compounds.

Limited feeding trials of unextracted residues or latex from *C. procera* were shown to cause death in sheep fed 5–10 gm/kg of body weight (Mahmoud et al., 1979a,b), while cattle were unaffected (Canella et al., 1966). Oral administration of *C. procera* flower extract was shown to cause widespread testicular necrosis, reduced levels of protein, RNA, and sialic acid in tissues, and liver damage in the gerbil *Meriones hurrianae* (Garg, 1979). Accidental contact of plant latex with the human eye caused a temporary acute inflammatory reaction and intense photophobia, which subsided with medical treatment (Biedner et al., 1977). The objectives of this work were to determine the nutrient and cardenolide content of unextracted and solvent-extracted *C. procera* residues.

MATERIALS AND METHODS

C. procera plant (aerial portion, ≤2 m in height) and individual plant components were collected in the southern coastal region of Puerto Rico. The components were oven dried (75 °C), Wiley milled, and Soxhlet extracted with

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