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Changes in fatty acid, simple sugar, and oligosaccharide content of cowpea (*Vigna unguiculata*) flour as a result of soaking, boiling, and fermentation with *Rhizopus microsporus* var. *oligosporus*

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Changes in fatty acid, simple sugar, and oligosaccharide content of cowpea flour as a result of soaking, boiling, and fermentation with *Rhizopus microsporus* var. *oligosporus* were investigated. The major fatty acids in flour made from both nonfermented and fermented cowpeas were linoleic, palmitic, and linolenic. With the exception of lower amounts of lauric, palmitic, linoleic, and linolenic acids and slightly higher amounts of lignoceric acid, the remaining fatty acids in flour made from soaked cowpeas were essentially unchanged from the control flour made from cowpeas that were not soaked, boiled or fermented. Soaking followed by boiling generally increased amounts of fatty acids in flour compared to the control. *Rhizopus microsporus* did not utilize lipid during 24 h of fermentation. Soaking decreased the amount of sucrose and stachyose by 39% and 18.4%, respectively, whereas soaking followed by boiling caused decreases of 58.3% and 49.3%. Complete elimination of sucrose, raffinose, and stachyose was achieved after 15 h of fermentation. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Cowpeas (*Vigna unguiculata*), also known as black-eyed peas, Southern peas, and crowder peas, are an important grain legume in East and West African countries as well as in other developing countries (Dovlo *et al.*, 1976; McWatters, 1983; Phillips & McWatters, 1991; Prinyawiwatkul *et al.*, 1994). Despite their potential as an inexpensive source of protein, energy, and B vitamins, cowpeas are underutilized in the USA and other industrialized countries, largely due to their inconvenient form when used as an ingredient in food preparation and the presence of certain antinutritional factors and indigestible components.

Considerable interest has developed in expanding the use of cowpeas in the form of meal and flour (McWatters, 1990; Prinyawiwatkul *et al.*, 1996*b*). A simple technology for preparing cowpea flour for use as a functional ingredient in food products would stimulate increased consumption of this legume (Prinyawiwatkul *et al.*, 1996*b*). Dry milling technology which yields cowpea flour that retains functional and nutritional properties has been developed in our laboratory (McWatters *et al.*, 1988; Phillips *et al.*, 1988). Attempts to further enhance the quality of cowpea flour have employed a wide range of technologies, such as germination (Vaidehi *et al.*, 1985; Padmashree *et al.*, 1987; Nnanna & Phillips, 1988; Malleshi *et al.*, 1989; Obizoba, 1989), fermentation (Djurtoft & Jensen, 1977; Zamora & Fields, 1979*a*,*b*; Schaffner & Beuchat, 1986*a*,*b*; Lu & Sanni-Osomo, 1988; Prinyawiwatkul *et al.*, 1996*a*,*b*), γ-irradiation (Dario & Salgado, 1994), and α-galactosidase treatment (Somiari & Balogh, 1993).

One of the earliest attempts to ferment cowpeas was a process developed for preparing a soysauce-like product (Bowen, 1767). In a search for pulses to be used as substrates for making fermented products in countries where soybeans are not locally available, cowpeas were identified as a potential alternative (Djurtoft, 1982). Recognizing similarities in size, color, and composition of soybeans and other legume seeds, it is possible that fermented products resembling tempeh and natto, both typically made from soybeans, could be prepared from cowpeas (Beuchat *et al.*, 1985). Although fermentation

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can be achieved by allowing a lengthy succession of events caused by naturally occurring microorganisms, the use of a pure starter culture of a mold (*Rhizopus microsporus*) under controlled conditions was found to be efficient for the preparation of fermented peanut flour (Prinyawiwatkul *et al.*, 1993*a*) and fermented cowpea flour (Prinyawiwatkul *et al.*, 1996*a,b*).

Several biochemical changes are known to occur during fermentation of legumes and cereals. Although *R. microsporus* has been reported to exhibit strong carbohydrase and lipase activities (Sorenson & Hesseltine, 1966; Wang & Hesseltine, 1966; Murata *et al.*, 1967; Wang *et al.*, 1968; Beuchat & Worthington, 1974; Souser & Miller, 1977; Fardiaz & Markakis, 1981; Mitchell *et al.*, 1988; Nahas, 1988; Agosin *et al.*, 1989; Nout & Rombouts, 1990; de Reu *et al.*, 1994), biochemical changes caused by these enzymes during fermentation of cowpeas have not gained much attention. The objective of this study was to determine changes in fatty acid, simple sugar, and oligosaccharide content of cowpea flour as a result of soaking, boiling, and solid-substrate fermentation using *R. microsporus* var. *oligosporus* as a starter culture.

MATERIALS AND METHODS

Preparation of fermented cowpea flour

Cowpea seeds

Dry cowpeas (cultivar 'White Acre', 1993 crop), obtained from Southern Frozen Foods (Montezuma, GA, USA), were used to prepare fermented cowpea flour. Upon receipt, cowpeas were visually inspected and defective seeds were discarded. Cowpeas were stored at 7°C and 60% relative humidity until utilized.

Fermented flour

Cowpeas (1.75 kg) were soaked in tap water at a ratio of 1:6 (cowpeas:water, w/w) at room temperature (approx. 25°C) for 24 h. Seeds were then boiled in the same soak water for 45 min, drained, immediately cooled to 25–30°C, and uniformly inoculated with a commercial dried powder *R. microsporus* var. *oligosporus* starter culture (Tempeh Lab, Summertown, TN, USA) at a ratio of 1:200 (starter:cooked cowpeas, w/w). The inoculated seeds (1-kg batch) were placed in perforated Zip-loc® vegetable bags (a gallon size, 26.8 cm×27.9 cm; DowBrands L.P., Indianapolis, IN, USA). Bags were placed on a wire mesh screen and fermented at 30°C for 0 (inoculated but dried immediately), 15, 18, 21, and 24 h. Fermented cowpeas were then oven-dried at 60°C for 13 h and finely ground through a 1-mm screen in a Thomas-Wiley Laboratory Mill (Model 4; Arthur H. Thomas Co., Philadelphia, PA, USA). Cowpea flours were placed in Zip-loc® freezer bags, sealed, and stored at −18°C until used. Two fermentation batches were prepared.

Fatty acid analysis

Direct transmethylation

Direct transmethylation of lipid from cowpea flour was performed according to the procedure described by Dahmer *et al.* (1989), with modifications. Flour samples (approx. 1 g) were weighed (±0.1 mg) and placed into glass test tubes. Three milliliters of a methanol–benzene–H_2SO_4 (75:25:3, v/v/v) mixture containing heneicosenoic acid methyl ester ($C_{21:0}$) were added to each sample. Heneicosenoic acid methyl ester was used as an internal standard (approx. 1 mg g^{-1} flour) to more accurately determine fatty acid methyl ester (FAME) content in the samples. Each tube was tightly sealed with a Teflon-liner cap, thoroughly mixed, and placed into a water bath at 90°C for 3 h. Care was taken to assure no leakage occurred, as indicated by the volume of methanol–benzene–H_2SO_4 mixture. Tubes were cooled with tap water to room temperature and 3 ml of petroleum ether were added. Samples were mixed and allowed to stand undisturbed for about 1 min for phase separation. Two milliliters of distilled water were gently added and the mixture was immediately centrifuged (1800*g* for 1 min at approx. 25°C). If a gel-like emulsion formed after centrifuging, tubes were gently shaken to break the gel and 1 ml of petroleum ether was added followed immediately by centrifugation (1800*g* for 1 min). The top layer of petroleum ether–benzene–FAME extract was carefully collected with a capillary pipet and filtered through a glass funnel into a 5-ml vial. The funnel was packed with glass wool topped with anhydrous Na_2SO_4 to remove residual water. Samples were re-extracted twice with 1 ml of petroleum ether and the ether extracts were likewise filtered and combined with the first extract. The petroleum ether–benzene–FAME extract was partially evaporated under oxygen-free nitrogen. All of the FAME preparations were performed under a hood. Two extractions were performed for each treatment and each fermentation batch. Samples were stored at −18°C until analyzed. Before gas-chromatographic analysis, 0.7 ml of iso-octane was added to all FAME extracts and thoroughly mixed.

Gas chromatographic analysis

Fatty acid content was analyzed using a Hewlett-Packard 5790A gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector and a HP 3390A integrator (Hewlett-Packard). A DB-225 fused silica open tubular column (30 m×0.25 mm i.d.) with a 0.15-μm film thickness (J&W Scientific, Folsom, CA, USA) was used. The initial column temperature was programmed at 180°C, held for 10 min, then increased at 4°C min^{-1} to 220°C and held for 15 min. The injector and detector port temperatures were maintained at 200°C and 250°C, respectively, and helium was used as a carrier gas at a linear velocity of 19.4 cm min^{-1} at 200°C. Fatty acids were identified by comparing their retention times with an external standard mixture containing lauric ($C_{12:0}$),

myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), arachidic ($C_{20:0}$), *cis*-11-eicosenoic ($C_{20:1}$), behenic ($C_{22:0}$), and lignoceric ($C_{24:0}$) acid methyl esters (Sigma Chemical Co., St. Louis, MO, USA). Fatty acid contents were quantified based on peak areas of known concentrations of respective standards obtained under identical conditions. Recovery (%) of the internal standard was also taken into consideration. The sample volume injected was 0.5 µl. Seven injections of each sample extract were done.

Sugar analysis

Flour samples (approx. 10 g) were weighed (±0.01 g) and placed into 150-ml centrifugal glass bottles. One milliliter of 20% α-lactose monohydrate (free of glucose) solution was added to each flour sample. Lactose was used as the internal standard because it yielded a single peak that did not interfere with other sugars and is not present in cowpea flour. Sugars were extracted using 40 ml of chloroform–methanol (1:1, v/v). After 30 min of intermittent stirring, 20 ml of filtered deionized water were added and the mixture was stirred for 5 min, then centrifuged at 15 300*g*, 25°C, for 30 min. The aqueous layer was collected and three drops of octanol (anti-foaming agent) were added. The extract was flash-evaporated under vacuum at 50°C; concentrated extract (approx. 5 ml) was made to 10 ml with filtered, deionized water, then filtered (0.2-µm Nylon Acrodisc-13; Gelman Science, Ann Arbor, MI, USA) before high-performance liquid chromatographic (HPLC) analysis. Two extractions were performed for each treatment and each fermentation batch. The HPLC system consisted of a SCL-6A system controller module, LC-6A solvent delivery pump (Shimadzu Corp., Kyoto, Japan), a 771 refractive index detector set at 0.5×10^{-3} RI units (Micromeritics, Norcross, GA, USA), a 220 mm×4.6 mm i.d. amino-spheri-5 column (Brownlee Labs, Santa Clara, CA, USA) with a NH_2 guard column, a 3390A Hewlett-Packard integrator, and Rheodyne injector valve (Model 7215, 20-µl loop; Rheodyne Inc., Catati, CA, USA). The mobile phase was a mixture of acetonitrile and filtered deionized water (70:30, v/v) containing 0.01% tetraethylenepentamine; the flow rate was 1.5 ml min^{-1}. Sugars were identified by comparing retention times with those of a standard mixture containing xylose, fructose, galactose, sucrose, maltose, lactose, raffinose, and stachyose (Sigma). Sugar contents were quantified based on peak areas of known concentrations of respective sugar standards obtained under identical conditions. The sample volume injected was 10 µl. Three injections of each sample extract were done.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA). Tukey's studentized range test (HSD) was performed for post-hoc multiple comparisons.

RESULTS AND DISCUSSION

Fatty acid content

Distribution of fatty acids (Table 1) did not show drastic differences among flours made from control, soaked, soaked/boiled, or fermented cowpeas. Linoleic ($C_{18:2}$), palmitic ($C_{16:0}$), and linolenic ($C_{18:3}$) acids are dominant fatty acids in cowpea seeds and the levels of these fatty acids are variety dependent (Mahadevappa & Raina, 1978; Ologhobo & Fetuga, 1983; Ukhum, 1984; Piergiovanni *et al.*, 1990). The major fatty acids in flours made from both nonfermented and fermented cowpeas were linoleic ($C_{18:2}$) ranging from 34.2% to 37.4%, palmitic ($C_{16:0}$) ranging from 25.8% to 26.9%, and linolenic ($C_{18:3}$) ranging from 15.7% to 17.3% (derived from Table 1), compared to the mean values of 31.7% linoleic, 25.1% palmitic, and 18.8% linolenic reported for ten Nigerian cowpea varieties (Ologhobo & Fetuga, 1983). Cowpea flours also contained approximately 6% stearic ($C_{18:0}$) and 7–8% oleic ($C_{18:1}$) acids (derived from Table 1). While legumes generally contain numerous minor acids with 14–18 carbons (Kuemmel, 1964), the presence of eicosenoic ($C_{20:1}$), behenic ($C_{22:0}$) and lignoceric ($C_{24:0}$) acids in the cowpea flours (Table 1) is a rather unusual characteristic (Ologhobo & Fetuga, 1983). Of the total fatty acids, unsaturated fatty acids constituted about 60%, compared with 40% for saturated fatty acids (Table 1). Linoleic acid was dominant among unsaturated fatty acids, whereas palmitic acid was the dominant saturated fatty acid.

Although various aspects of fermented legumes and cereals have been studied, information about changes in lipid and fatty acid content of cowpeas fermented with *R. oligosporus* or *R. microsporus* var. *oligosporus* is lacking. It is not understood whether changes in the content of fatty acids occur during soaking, boiling or fermentation. Results in Table 1 show changes in the content of fatty acids in cowpea flour at various stages during fermentation. With the exception of relatively lower amounts of lauric, palmitic, linoleic, and linolenic acids and slightly higher amounts of lignoceric acid, the remaining fatty acids in lipid of flour made from soaked cowpeas were essentially unchanged from the control. Soaking, followed by boiling, increased the amount of fatty acids in cowpea flour compared to the control sample, except for lauric acid which decreased considerably. Changes in fatty acid content during soaking and boiling may not be the result of an actual increase or decrease in fatty acid weight. Increase in fatty acid content was more likely due to a loss of water-soluble substances during soaking (Mulyowidarso *et al.*, 1991) and boiling, consequently accounting for proportionate increases in unaltered constituents. The relative percentage (Table 1) of saturated fatty acids in flour made from fermented cowpeas was comparatively lower than that in the control flour. This was due primarily to a decrease in lauric ($C_{12:0}$) acid as a result of soaking and boiling seeds before fermentation. Comparison of the fatty acid content of flour made from soaked/boiled

Table 1. Changes in individual, saturated, and unsaturated fatty acid content (mg per 100 g) in cowpea flour as a result of soaking, boiling, and fungal fermentation[a]

Fatty acid	Treatment							
	Control	Soaking	Soaking/boiling	Fermentation time (h)				
				0	15	18	21	24
Lauric	74.6a	21.9b	6.5c	7.1c	4.3c	8.0c	5.5c	7.0c
($C_{12:0}$)	(0.1)	(6.0)	(0.2)	(2.1)	(0.8)	(0.5)	(0.4)	(1.3)
Myristic	2.9c	2.8c	3.5c	3.5c	6.2bc	6.4bc	11.3a	9.9ab
($C_{14:0}$)	(0.3)	(0.2)	(0.1)	(0.1)	(0.9)	(0.6)	(2.2)	(2.3)
Palmitic	596.0b	567.5b	697.7a	694.0a	738.3a	740.2a	759.7a	754.5a
($C_{16:0}$)	(36.8)	(27.4)	(1.9)	(10.1)	(5.9)	(21.6)	(20.8)	(5.1)
Stearic	130.2d	131.3d	157.5c	164.0bc	173.6abc	172.1abc	175.5ab	182.7a
($C_{18:0}$)	(7.4)	(7.6)	(0.3)	(3.6)	(0.8)	(3.3)	(1.0)	(3.1)
Oleic	160.4c	158.0c	196.3b	218.9ab	229.0a	226.0a	229.7a	232.5a
($C_{18:1}$)	(11.5)	(9.6)	(1.5)	(6.2)	(0.7)	(1.6)	(2.2)	(5.7)
Linoleic	765.0b	746.0b	939.8a	1005.1a	1033.7a	1014.0a	1017.2a	1010.7a
($C_{18:2}$)	(50.6)	(40.3)	(1.6)	(29.5)	(11.6)	(27.9)	(30.9)	(3.8)
Linolenic	370.3b	353.7b	450.7a	436.4a	452.3a	450.5a	457.3a	439.7a
($C_{18:3}$)	(27.8)	(19.1)	(4.9)	(10.3)	(3.1)	(22.1)	(19.8)	(6.8)
Arachidic	29.0b	29.3b	34.2a	34.5a	36.0a	35.4a	36.3a	36.4a
($C_{20:0}$)	(1.4)	(1.7)	(0.2)	(0.1)	(0.2)	(0.5)	(0.2)	(0.3)
Eicosenoic	6.3cd	6.0d	7.5bc	8.0ab	8.6ab	8.3ab	8.1ab	8.9a
($C_{20:1}$)	(0.5)	(0.1)	(0.3)	(0.3)	(0.3)	(0.1)	(0.4)	(0.2)
Behenic	64.8c	65.1bc	72.4ab	73.2a	75.7a	74.9a	77.2a	76.9a
($C_{22:0}$)	(1.9)	(4.0)	(1.0)	(0.1)	(0.5)	(1.5)	(1.9)	(0.7)
Lignoceric	38.2b	44.1ab	42.5ab	42.0ab	46.3ab	47.8ab	46.3ab	50.7a
($C_{24:0}$)	(0.8)	(4.2)	(3.5)	(3.1)	(4.0)	(0.8)	(1.9)	(1.7)
Saturated[b]	935.7b	862.0b	1014.3a	1018.3a	1080.4a	1084.8a	1111.8a	1118.1c
[%]	[41.8]	[40.5]	[38.9]	[37.9]	[38.5]	[39.0]	[39.4]	[39.8]
Unsaturated[c]	1302.0b	1263.7b	1594.3a	1668.4a	1723.6a	1698.8a	1712.3a	1691.8a
[%]	[58.2]	[59.5]	[61.1]	[62.1]	[61.5]	[61.0]	[60.6]	[60.2]
Total fatty acid	2237.7b	2125.7b	2608.6a	2686.7a	2804.0a	2783.6a	2824.1a	2809.9
	(137.5)	(120.3)	(11.2)	(61.1)	(26.6)	(77.9)	(77.8)	(14.7)

[a]Dry weight basis. Numbers in parentheses refer to standard deviations of two replications (duplicate extractions of each replication and seven injections of each extraction). Mean values in a row not followed by the same letter are significantly different ($P = 0.05$).
[b]Includes $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$, and $C_{24:0}$.
[c]Includes $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:1}$.

cowpeas with that of flour made from soaked/boiled cowpeas fermented did not show a substantial difference. This indicates that the addition of starter culture did not contribute to the increase in fatty acid content in flour.

R. microsporus has been reported to exhibit strong lipase activity, hydrolyzing triacylglycerides to yield free fatty acids which accumulate to various levels depending upon fermentation conditions (Beuchat, 1986). Recent published reports on changes in fatty acid content of legumes fermented with *R. microsporus* indicate a lack of general agreement (Djurtoft & Nielson, 1989; Nout & Rombouts, 1990). Comparison of information is difficult because of differences in types and forms of substrates, properties of microorganisms, and fermentation conditions. In this study, myristic, palmitic, stearic, oleic, and lignoceric acids increased as a result of fermentation and generally increased with increased fermentation time (Table 1). Lauric, linoleic, linolenic, arachidic, eicosenoic, and behenic acids were essentially unchanged during 24 h of fermentation. This would indicate that *R. microsporus* did not utilize cowpea lipid as a primary source of energy and carbon during rapid growth, particularly between 21 and 24 h of fermentation. The disappearance of free fatty acids in soybean tempeh after active growth of *R. microsporus* (30°C for 24 h) was noted by de Reu *et al.* (1994). The fact that fatty acids disappear only after active growth implies that they are not the preferred carbon source (de Reu *et al.*, 1994). No changes were observed in the pattern of fatty acids in tempeh made from soaked/dehulled/boiled cowpeas fermented with *R. microsporus* at 31°C for 24 h (Djurtoft & Nielson, 1989). Our results and those reported by de Reu *et al.* (1994) and Djurtoft & Nielson (1989) did not, therefore, substantiate the observations of Sorenson & Hesseltine (1966), Beuchat & Worthington (1974), and Nout & Rombouts (1990), who reported that *R. microsporus* possibly utilizes lipid as a source of carbon and energy during fermentation, although no evidence of preferential utilization of specific free fatty acids in peanut was observed (Beuchat & Worthington, 1974). This apparent discrepancy can be rationalized. Sources of energy and carbon for mold growth during fermentation are variable depending upon availability and utilizable forms of nutrients present in substrates. Soybeans and peanuts contain as high as 28% and 50% fat, 25–33% and 13% carbohydrate, and 0.2–0.9% and 4.0% starch, respectively. Cowpeas, on the other hand,

contain very low fat (approx. 2%) but high amounts of starch (31–48%) and total carbohydrate (56–68%). The low fat content of cowpea flour may have limited its utilization by *R. microsporus*, causing a shift toward utilization of starch and other carbohydrate breakdown products as a source of carbon and energy during fermentation.

Various optimum pH and temperature conditions for lipase activity of *R. microsporus* have been reported: for instance, at pH 7.0 and 40°C (Souser & Miller, 1977) and at pH 6.5 and 25°C (Nahas, 1988). Whether the fermentation conditions employed in this study were optimal for lipase activity is not known. However, these conditions (initial substrate pH of 6.73 and incubation at 30°C for up to 24 h) promoted rapid mold growth with profuse mycelium formation after 21 h of fermentation.

Lipids are minor components of cell walls of fungi, ranging from 1% to 10% of the cell wall dry weight. In general, palmitic and stearic acids are the most abundant fatty acids in fungal cell walls and myristic acid is present only in trace amounts (Ruiz-Herrera, 1992). Shaw (1966) reported that palmitic, stearic, oleic, linoleic, and linolenic acids were the major fatty acids in mycelia of *Rhizopus* spp. An intermediary effect of fungal fatty acid content on the substrate fatty acid profile was reported (Quinn *et al.*, 1975). Since the initial total fatty acid content in the control cowpea flour was low (approx. 2.2%), the fatty acid profile may have reflected the presence of both fungal and cowpea fatty acids as indicated by increased myristic, palmitic, stearic, and oleic acid contents. Unfortunately, a generalization cannot be made from this study because the experimental design did not permit distinction between fatty acids originating from cowpeas and fungal mycelia.

Simple sugar and oligosaccharide content

The total carbohydrate content of flours made from fermented and nonfermented cowpea seeds ranges from 59.5% to 63.5% (Prinyawiwatkul *et al.*, 1996*a*). Starch is the most abundant carbohydrate in cowpea, while sugars represent only a small percentage (Longe, 1980). An extensive review of literature indicates that the three principal oligosaccharides present in mature cowpea seeds are verbascose, stachyose, and raffinose. Most researchers (Akpapunam & Markakis, 1979; Sosulski *et al.*, 1982; Onigbinde & Akinyele, 1983; Abdel-Gawad, 1993) have reported that stachyose is present in the largest concentration in cowpeas, followed by raffinose and a smaller amount of verbascose. However, Longe (1980) reported that verbascose is the most abundant sugar, followed by stachyose and raffinose. Stachyose (3.43%), sucrose (2.97%), and raffinose (1.24%) are the predominant sugars in nonfermented (control) cowpea flour (Table 2). Verbascose was not detected in the control flour even after a 35-min elution time. Akpapunam & Markakis (1979) reported sugars from 13 American cowpea varieties to have 3.4% stachyose, 2.2% sucrose, 1.2% raffinose, and 0.9% verbascose (dry weight basis), while 2.7% stachyose, 1.6% sucrose, 0.7% raffinose, and 3.6% verbascose were reported for 20 Nigerian cowpea varieties (Longe, 1980). Differences in oligosaccharide content of cowpeas are most likely due to differences in variety (Onigbinde & Akinyele, 1983).

Soaking and boiling are essential preliminary processes in the preparation of fermented cowpeas. Hydration and softening of seeds occur, thus facilitating leaching of seed components into soak water (Mulyowidarso *et al.*, 1991). Leaching of carbohydrate components from cowpeas during soaking and boiling

Table 2. Changes in simple sugar and oligosaccharide content (g per 100 g) in cowpea flour as a result of soaking, boiling, and fungal fermentation[a]

Sugars/oligosaccharides	Treatment							
	Control	Soaking	Soaking/boiling	Fermentation time (h)				
				0	15	18	21	24
Xylose	0.21 (0.03)	0.17 (0.03)	0.15 (0.02)	0.08 (0.00)	0.08 (0.01)	0.04 (0.02)	0.15 (0.01)	0.68 (0.03)
Fructose	0.44 (0.09)	0.13 (0.03)	0.35 (0.10)	0.35 (0.08)	0.58 (0.12)	0.46 (0.12)	ND	0.08 (0.01)
Glucose/galactose	0.03 (0.02)	0.10 (0.01)	0.02 (0.01)	0.04 (0.01)	ND	ND	ND	0.13 (0.04)
Sucrose	2.97 (0.68)	1.81 (0.26)	1.24 (0.09)	1.26 (0.13)	ND	ND	ND	0.26 (0.11)
Maltose	ND	0.51 (0.12)	ND	ND	ND	ND	ND	0.33 (0.05)
Raffinose (A)	1.24 (0.40)	1.74 (0.38)	1.42 (0.40)	1.40 (0.41)	ND	ND	ND	ND
Stachyose (B)	3.43 (0.29)	2.80 (0.33)	1.74 (0.21)	1.52 (0.09)	ND	ND	ND	ND
A&B	4.67	4.54	3.16	2.92	ND	ND	ND	ND
[% reduction]	[0.0]	[2.8]	[32.3]	[37.5]	[100]	[100]	[100]	[100]

[a]Dry weight basis. Numbers in parentheses refer to standard deviations of two replications (duplicate extraction of each replication and three injections of each extraction). ND, not detectable.

has been previously demonstrated (Onigbinde & Akinyele, 1983; Akinyele & Akinlosotu, 1991; Abdel-Gawad, 1993; Somiari & Balogh, 1993). In our study, soaking (24 h, 25°C) alone decreased the amount of sucrose and stachyose in seeds by 39% and 18.4%, respectively (Table 2). Soaking followed by boiling for 45 min decreased the amount of sucrose by 58.3% and stachyose by 49.3%. Abdel-Gawad (1993) reported that soaking (12 h, 25°C) or soaking followed by boiling for 60 min decreased oligosaccharide (raffinose and stachyose) contents of flour made from nondecorticated cowpeas by 27.6% and 58.9%, respectively, compared to 2.8% and 32.3% in our study (Table 2). A decrease in sucrose, raffinose, and stachyose was greater for flour made from soaked cowpeas than flour made from unsoaked cowpeas, both boiled for 60 min (Abdel-Gawad, 1993). In addition, autoclaving (10 p.s.i., 20 min) has a greater effect on the reduction of oligosaccharide content than does traditional cooking for 60 min.

The mechanism by which oligosaccharide content is decreased during soaking and boiling is not clear (Somiari & Balogh, 1993). Although leaching has been suggested, Mulyowidarso *et al.* (1991) proposed that hydration may activate enzymes in soybeans, causing endogenous degradation of starch and other polysaccharides into smaller molecular weight products that diffuse into soak water (Lo *et al.*, 1968; Wang *et al.*, 1979). An increase in raffinose and glucose/galactose content after soaking and in fructose content after boiling (Table 2) indicates that changes in sugar contents of cowpeas are not solely accounted for by leaching. Wang *et al.* (1979) and Mulyowidarso *et al.* (1991) noted that the amount of stachyose, raffinose, sucrose, and fructose in soybeans decreased during soaking, even in the presence of antibiotics that completely inhibited microbial growth in soak water. Consequently, the decrease in stachyose content (Table 2) was likely due to hydrolysis, induced by endogenous enzyme activity during soaking and by heat during boiling (Worthington & Beuchat, 1974), to simple disaccharides and monosaccharides. The increase in raffinose and glucose/galactose content after soaking and in fructose after boiling could have resulted from the breakdown of stachyose, as indicated by the gradual decrease in stachyose content (Table 2). The increase in maltose content after soaking may have resulted from endogeneous β-amylase activity which broke down starch and dextrins to maltose.

Although oligosaccharide content has been reported to decrease with increased cooking time and bean:water ratio (Ku *et al.*, 1976; Silva & Braga, 1982), an attempt to use excessive water for soaking and boiling was not made in this study to avoid losses of other water-soluble nutrients. Hesseltine (1965) suggested that, to prevent loss during soaking and boiling, water could be added in an amount to hydrate the beans thoroughly, thus leaving less water to be discarded after cooking. In this study, a ratio of 1:6 (cowpeas:water, w/w) was used. The amount of water remaining after soaking was sufficient for a further boiling step. Using the same soak water for boiling did not adversely affect mold growth during fermentation.

Although practices such as dehulling, soaking, cooking, and enzyme treatment could reduce oligosaccharide content of cowpeas, reductions have been inconsistent or insufficient to suggest a significant reduction in flatus activity. Evidence of reduction in cowpea oligosaccharides as a result of germination (Nnanna & Phillips, 1988) and natural fermentation has been reported (Zamora & Fields, 1979*a*; Akinyele & Akinlosotu, 1991). The effect of solid-substrate fermentation on simple sugar and oligosaccharide content is shown in Table 2 and Fig. 1. Complete elimination of sucrose, raffinose, and stachyose was observed in flours made from cowpeas fermented for at least 15 h at 30°C. This indicates that sucrose, raffinose, and stachyose were completely utilized by *R. microsporus*. These results are not in agreement with those reported by Sorenson & Hesseltine (1966) and Nout & Rombouts (1990), who observed that *R. microsporus* cannot utilize sucrose, raffinose, or stachyose as a sole source of carbon and energy during fermentation. Worthington & Beuchat (1974), however, reported that *R. microsporus* could utilize small amounts of stachyose in peanuts only after 68 h of fermentation, but did not utilize sucrose and raffinose. These conflicting observations can be rationalized as was the fatty acid profile addressed earlier. Because there was no evidence of a decrease in fatty acid content during 24 h of fermentation and there was a depletion of sucrose, raffinose, and stachyose after 15 h of fermentation, it is likely that soluble sugars are the primary source of carbon and energy for *R. microsporus* during fermentation of cowpeas.

R. microsporus was reported to utilize carbohydrates in wheat as an energy source during fermentation (Wang *et al.*, 1968). The fact that wheat contains low fat (1.9%) and high carbohydrate (76.5%) content would support the analogy made in our study; that is, *R. microsporus* can utilize carbohydrate as a source of carbon when lipid is not sufficiently available for growth. Further evidence supporting this hypothesis was given by Mitchell *et al.* (1988), who observed that *R. microsporus* exhibited strong amylolytic activity as demonstrated by growth on cassava starch as a sole source of carbon and energy. Gelatinization of starch improved growth of *R. microsporus* (Mitchell *et al.*, 1988). In our study, boiling soaked cowpeas for 45 min caused starch to gelatinize, making it a more readily utilizable source of carbon. Fardiaz & Markakis (1981) noted that *R. microsporus* utilizes sucrose, raffinose, and stachyose of peanuts, but at a slow rate. Using a mixed culture of *Neurospora sitophila* and *R. microsporus* practically eliminated oligosaccharides associated with flatulence (Fardiaz & Markakis, 1981).

The presence of flatulence-causing oligosaccharides (raffinose and stachyose) continues to pose problems with regard to complete nutritional utilization of cowpeas. The digestion problem is due to the absence of α-galactosidase in humans. When present in high

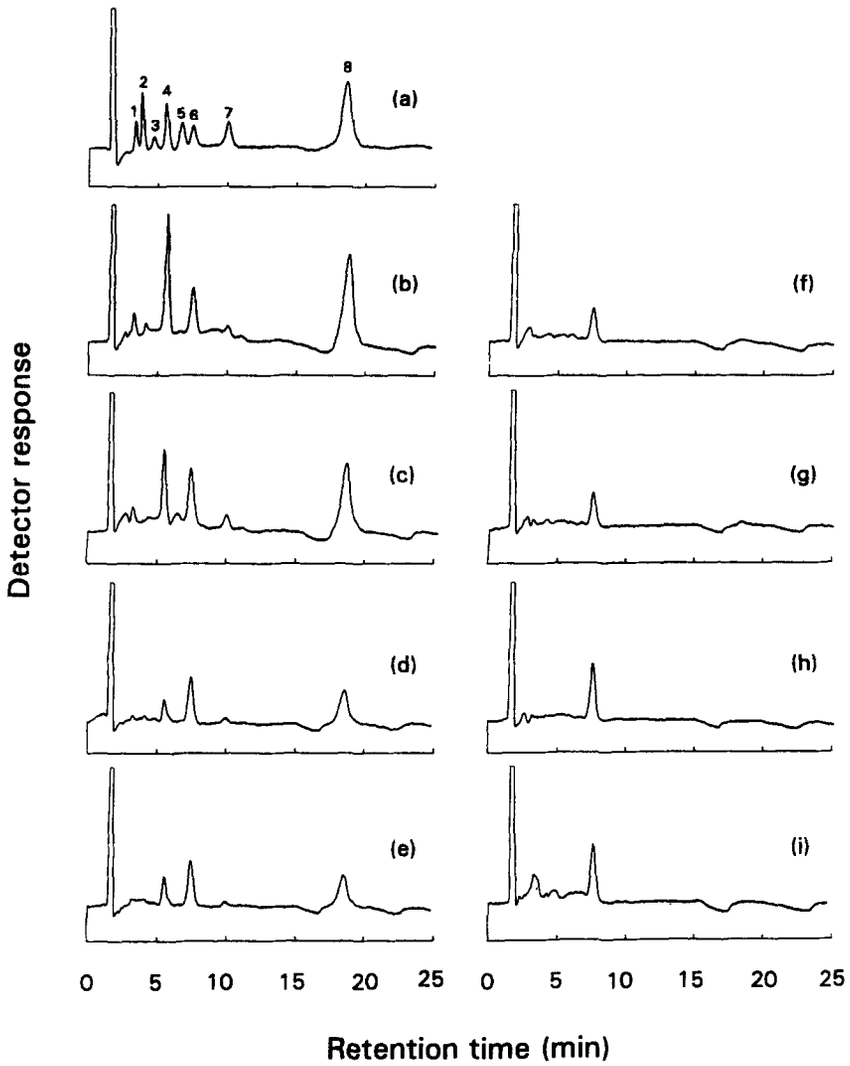

Fig. 1. Chromatograms of standard sugars (a) and sugars extracted from flour made from control (b), soaked (c), and soaked/boiled (d) cowpeas, and from cowpeas fermented for 0 h (e), 15 h (f), 18 h (g), 21 h (h), and 24 h (i). Numbers 1–8 designate xylose, fructose, glucose/galactose, sucrose, maltose, lactose, raffinose, and stachyose, respectively.

concentrations in the small intestine, these sugars increase osmotic pressure which in turn causes diarrhea. In the lower gut, raffinose and stachyose are rapidly degraded by bacterial α-galactosidase with subsequent gas production. Results from this study show that solid-substrate fermentation was effective in eliminating flatulence-causing oligosaccharides in cowpea flour and is more effective than soaking and boiling. Because of this absence of flatulence-causing oligosaccharides, flour made from fermented cowpea would be a highly acceptable ingredient for incorporating into food products. Cereals and legumes are the main sources of nutrients for weaning children in developing countries (Aguilera & Lusas, 1981; Malleshi *et al.*, 1989). Because of their availability and popularity, cowpeas are an option for use in weaning foods in Africa (Boeh-Ocansey, 1989). Weaning foods containing only cowpea flour or mixtures of cowpea and other legume flours have been formulated (Okaka & Potter, 1979; Malleshi *et al.*, 1989; Almeida-Dominguez *et al.*, 1993). Incorporating oligosaccharide-free cowpea flour into weaning food products undoubtedly contributes to increased digestibility in young children. Fermented, partially defatted peanut flour with enhanced functional properties was prepared (Prinyawiwatkul *et al.*, 1993*a*) and successfully incorporated into extruded snack products (Prinyawiwatkul *et al.*, 1993*b*). Cowpea flour prepared using a similar procedure would be anticipated to impart modified functional and sensory properties to food products. Potential applications of the oligosaccharide-free cowpea flour in bakery and snack foods and in meat extenders are numerous and need further investigation.

CONCLUSION

This study demonstrated that flour essentially free of flatulence-causing oligosaccharides can be successfully prepared from fermented, nondecorticated cowpeas using a solid-substrate fermentation process and *R. microsporus* var. *oligosporus* as a pure starter culture. Scale-up production of this flour is feasible and would stimulate increased utilization of cowpeas.

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