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EFFECT OF OLIGOSACCHARIDES ON INTESTINAL MICROFLORAL POPULATION IN RATS FED PROCESSED COWPEA DIETS

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Target Audience: Animal Scientists; Nutritionists, Plant Breeders and Toxicologists.

ABSTRACT

Autoclaved, cooked, germinated and soaked cowpea diets fed to adult-male rats of the wistar strain for three weeks, gave average intestinal microflora population of 9.41, 7.50, 0.32 and 17.55 x 10^7/g cecal content respectively. Oligosaccharide content and microbial population in autoclaved, cooked, germinated and soaked cowpea seeds had correlation coefficient values of 0.56, 0.57, 0.91 and 0.27 respectively. Germinated seeds gave the least microflora population due to starvation of the microbes because of low oligosaccharide contents. The reverse was the case for soaked seeds.

Key words: Oligosaccharides, processing, microbes, correlation.

DESCRIPTION OF PROBLEM

Cowpea seeds, like other legumes, are commonly consumed in virtually all homes in the developing countries mainly because they are cheap sources of protein (1) and when supplemented with cereal grains, they form a good combination.

Apart from the protein content, cowpeas have been found to contain some carbohydrates (2) which are not enzymatically digested and absorbed in the small intestine (3). The protein content of legume seeds has been much emphasized at the expense of the other major component, carbohydrates (4).

However, variable amounts of oligosaccharides including raffinose, stachyose and verbascose are found in most legume seeds (5). In addition, it has been reported that certain constituents of navy beans encouraged fermentative action of anaerobic spore-forming micro-organisms like *Clostridium per-frigens* in the dog intestine (6,7).

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Food consumed could elicit intestinal microbial activity when it contains substances that cannot be digested (8) but which bacteria located in the large intestine can digest (9), thus making the undigested or unavailable portion of the food (mainly oligosacharides) substrate for microbial activity (3, 9, 10). This therefore led to the main thrust of this study which was aimed at establishing whether or not a relationship existed between oligosaccharide content and intestinal microfloral population in rats fed processed cowpea varieties.

MATERIALS AND METHODS

The International Institute of Tropical Agriculture, Ibadan, supplied the main experimental materials used in this study which included different varieties of cowpea.

Cowpea Seed Processing

The different cowpea varieties were processed as follows:

(a) *Cooking:* A pressure cooker was employed to cook raw cowpea seeds at 105°C for 30 min followed by drying at 65°C for 48h, milled and stored in screw-capped bottles at 4°C.

(b) *Soaking:* Raw cowpea seeds were soaked in distilled water for 24h, rinsed thrice with distilled water, dried at 65°C for 48h, milled and stored in screw-capped bottles at 4°C.

(c) *Autoclaving:* The raw cowpea seeds were airdried, milled and autoclaved at 105°C and 15psi pressure for 30 min (11). Milling before autoclaving was to ensure that a greater proportion of the antinutritional factors, especially lectin, was removed before being fed to rats to avoid inducement of small intestinal microflora over-growth.

(d) *Germination*: Seeds were immersed in 25% sodium hypochlorite solution for two minutes to kill all pathogenic organisms that could be present and then spread thinly on a tray. Seeds were sprayed with distilled water every 24h at 25°C according to the method of some workers (12). Germinated seeds were dried at 65°C for 48h, milled and stored in screw-capped bottles at 4°C.

Estimation of Oligosaccharides

The oligosaccharides were extracted from cowpea flour with 70% ethanol and then concentrated in a rotary evaporator at 40°C. The resulting syrup was made up to 25cm^3 volume with distilled water and thereafter subjected to paper chromatography by spotting the extracts along the short edge of a 46 x 57mm Whatman No. 1 filter paper next to authentic raffinose and stachyose (13). Areas corresponding to each sugar were cut, eluted with distilled water and colorimetrically quantified (12).

Rat Feeding Trials:

Adult male rats of the Wistar strain were randomly distributed into five groups of eight rats per group such that the average initial weights of the groups ranged from 96.71 ± 2.68g to 98 ± 0.95g. Each group was housed in the same metabolic cage and rats were fed *ad libitum* for 21 days while distilled water was also supplied. One group was placed on a basal protein-free diet (Table 1) while the remaining groups were placed on experimental diets which were constituted by replacing 45% of maize starch in the basal ration with autoclaved, cooked, germinated or soaked cowpea varieties (14). At the end of the experiment, the rats were starved for 4 hours after which three rats were slaughtered from each group. The gastrointestinal tract above the stomach, close to the anus of each rat, was cut after removing adhering tissues.

Microbiological Assay:

Colony count method of determining the number of viable organisms in 1g of cecal sample was employed (15).

Statistical Analysis:

Results were subjected to randomized complete block analysis of variance and treatment means were compared by using Duncan's Multiple Range Test (16).

Table 1: Percentage Composition of basal diet

Ingredients	%
Maize starch	65.75
Glucose	5.00
Sucrose	10.00
Non-nutritive cellulose	5.00
Vegetable oil	10.00
Mineral/Vitamin Premix	1.00
Oyster shell	0.50
Bone Meal	2.50
Salt (Nacl)	0.25
	100.00

RESULTS AND DISCUSSION

Average oligosaccharide contents in autoclaved, cooked, germinated and soaked samples, as contained in Table 2, were 2.16, 2.15, 0.26 and 4.79g/100g DM respectively. The values were significantly different ($p < 0.05$). As for the intestinal microflora population, rats that consumed diets containing

autoclaved, cooked, germinated and soaked cowpea seeds gave average values of 9.14, 7.50, 0.32 and 17.55 x 10^7/g cecal content respectively. This microbial population differed significantly ($p < 0.05$). Germination was the most effective processing method in reducing oligosaccharides content while soaking was the least effective method. Correspondingly, germinated seeds gave the least microflora population while soaked seeds gave the highest microflora population which confirmed the report that oligosaccharides serve as a substrate for intestinal microbial activity (2). A very strong positive correlation ($r = 0.91$) between oligosaccharide content and microbial population in germinated seeds and a very weak positive correlation ($r = 0.27$) observed in soaked sampled further lend support to the claim that oligosaccharide serve as a substrate to the intestinal micro-organisms. The weak relationship between oligosaccharide content and microbial population in soaked seeds is a deviation which suggests that other factors might be responsible for microbial proliferation apart from oligosaccharides. Since this deviation was not prominent in autoclaved and cooked seeds, it follows that the other suspected factors are peculiar to soaked seeds.

Table 2: Effect of Oligosaccharide Contents (g/100g DM) on Intestinal Microflora Population (x 10^7/g cecal content) in Processed cowpea varieties

	Autoclaving		Cooking		Germination		Soaking	
Cowpea Variety	Oligosac	Micro-flora Popula tion	Oligosac	Micro-flora popula-tion	Oligosac.	Micro-flora popula-tion	Oligo-sac.	Micro flora popu-lation
IT 84 E-1-108	2.16	8.82	2.17	6.61	0.22	0.24	5.27	20.03
IT 82 D-889	1.92	7.77	1.56	7.82	0.18	0.29	4.47	16.22
IT 81 D-1137	3.13	11.03	2.63	8.52	0.37	0.43	5.59	19.35
IT 82 E-16	2.20	10.80	2.73	8.80	0.40	0.39	5.27	16.22
Ife Brown	1.38	8.61	1.68	5.74	0.13	0.25	3.36	15.23
Mean	2.16$_a$	9.41^a	2.15$_a$	7.50^b	0.26$_b$	0.32^b	4.79$_c$	17.55^c
r	0.56		0.57		0.91		0.27	

a,b,c, = Means followed by different letters or with different superscripts are significantly different ($P<0.001$).

Reduction in oligosaccharide content obtained in the germinated seeds has been ascribed to enzymes present in both dormant and germinating seeds and these have been reported to be responsible for the hydrolysis of oligosaccharides to their constituent sugars (10). It was also demonstrated that a rapid and complete removal of sucrose stimulated oligosaccharide hydrolysis and subsequent utilization by germinating seeds (10). As a result of these activities, only a small quantity of oligosaccharides was available in the germinated samples to be utilised by the intestinal microbes.

This study has revealed that Ife Brown and IT 82D - 889 were the cowpea varieties that encouraged starvation of the microbes due to low oligosaccharide contents in germinated seeds.

CONCLUSION AND APPLICATIONS

In view of the outcome of this investigation, it can be concluded that:

1. Germination was the most effective processing method that reduced oligosaccharide contents in cowpea seeds.
2. There was a concomitant reduction in intestinal microbial population as the level of oligosaccharide reduced in the cowpea seeds.
3. The very weak positive correlation between oligosaccharide content and microflora population ($r = 0.27$) in soaked samples is an indication that there are other factors responsible for the thriving of the microbes as soaking could not remove an appreciable amount of the oligosacharides.
4. Ife Brown and IT 82D - 889 cowpea varieties encouraged microbial starvation.

The results obtained can be applied:

5. In plant breeding programmes because cowpea varieties with low levels of oligosaccharide can be selected for subsequent multiplication.
6. By increasing the acceptability of cowpea among individuals who experience abdominal disturbance after a cowpea meal.

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