EFFECT OF COWPEA SEED PROCESSING ON INTESTINAL MICROFLORAL POPULATION OF RATS

D.B. OKE, O.O. TEWE⁺ AND B.L. FETUGA⁺⁺

Department of Animal Production, Ogun State University, Ago-Iwoye, Nigeria

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ABSTRACT

Five cowpea varieties were subjected to autoclaving, cooking, germination and soaking. Soaked samples gave the highest intestinal microfloral population with autoclaved and cooked cowpea seeds having intermediate microbial count values although cooking was more effective than autoclaving in reducing microbial population. Germinated samples gave very little microbial population. Intestinal microfloral population was positively correlated ($r=0.85$, $P<0.001$) to oligosaccharide concentrations. In the raw cowpea varieties, a good number of the antinutritional factors worked complementarily depending on the processing method with the oligosaccharides and thus had significant effects in promoting microbial growth. Varietal and processing methods greatly influenced microbial population ($P<0.001$).

Key words: Autoclaving, cooking, soaking, microfloral population

INTRODUCTION

Consumption of legume grains has been observed to elicit gaseous conditions due to the presence in the diet substances that man cannot digest but which his bacteria can, with the food providing gas formation (Calloway, 1973). This situation exists in normal individuals with alpha-galactosidase present in legumes and with milk sugar in lactase-deficient persons. The microorganism, *Clostridium perfringens* has been shown to be the primary source of gas, and it is the main intestinal anaerobe (Bressani, 1973). Certain oligosaccharides such as raffinose and stachyose present in legume seeds (Aspinwall

et al., 1967; Steggerda, 1961; Murphy, 1964 and Rackis *et al.*, 1970) are not digested by intestinal enzymes but accumulate in the large intestine (Subba Rao and Desikachar, 1964 and Rackis, 1975) where they undergo fermentation to produce the gases.

Banwell *et al.*, (1983) had shown that phytohaemagglutinin facilitated bacterial proliferation in the small intestine. The polymicrobial small intestinal bacterial overgrowth associated with increased adherence of bacteria to the mucosal surface caused by lectin was postulated to have provided the requisite conditions for microbial proliferation. This development seems to complement the role of oligosaccharides in accentuating microfloral multiplication. The fact that the various processing methods have been shown to influence elimination of the oligosaccharides from cowpea seeds, has necessitated this study. Therefore, this investigation aims at looking at the effect seed processing would have on intestinal microfloral population and also identify other factor(s) that may affect microbial growth.

MATERIALS AND METHODS

The cowpea varieties used for this study are described in Table 1. They were supplied by IITA, Ibadan.

TABLE 1: DESCRIPTION OF COWPEA VARIETIES

Variety	Coat Colour	Coat texture
IT 84E-1-108	Cream	Smooth
IT 82D-889	Red	Smooth
IT 61D-1137	White	Rough
IT 84E-16	Red	Smooth
Ife Brown	Brown	Rough

Processing of Cowpea Seeds:

All the cowpea varieties were autoclaved, cooked, germinated and soaked in accordance

⁺ Department of Animal science, University of Ibadan, Ibadan, Nigeria

⁺⁺ Glaxo Nigeria Ltd. Apapa, Lagos, Nigeria

with the procedures outlined below.

Autoclaving:

Cowpea seeds which had been air-dried were milled and the flour autoclaved at 105°C and 103.5kPa pressure for 30 minutes. Autoclaved samples were dried at 65°C for 48hr and then milled.

Cooking:

Raw cowpea seeds were cooked with a pressure cooker at 105°C for 30 minutes, dried for 48hr at 65°C and then milled.

Germination:

Seeds were immersed in 2% sodium hypochlorite solution for two minutes to kill all pathogenic organisms that they may contain and then spread thinly on a tray. Every 24hr, seeds were sprayed with distilled water at 25°C according to the method of Ikeda *et al.* (1984). Germinated seeds were dried at 65°C for 48 hr and then milled.

commencement of the feeding trial were randomly distributed into seven groups of six rats each such that the average initial weights of the groups ranged from 96.71±2.68g to 98.96±.95g. Each rat was housed individually in separate metabolic cage. One group was placed on a basal protein free diet (Table 2), another on a casein diet while the other groups were placed on experimental diets such that processed or raw cowpea constitutes 45% by replacing casein and part of the maize starch (Hedin and Adachi, 1962). Urine and faeces were collected on a daily basis and stored in screw-capped plastic containers at 4°C until when bulked according to rats and diets. Distilled water and feed were supplied *ad libitum* throughout the 14-day experimental period.

Dissection procedure:

Four hours after removing the diets from the cages, three rats were slaughtered (cutting

TABLE 2: PERCENTAGE COMPOSITION OF PROTEIN-FREE AND CASEIN DIETS

INGREDIENTS	DIET	
	Protein-Free	Casein
Casein	-	18.00
Corn Starch	65.75	63.25
Glucose	5.00	-
Sucrose	10.00	-
Non-nutritive cellulose	5.00	5.00
Vegetable oil	10.00	10.00
Vitamin/Mineral premix [†]	1.00	1.00
Oyster shell	0.50	0.50
Bone meal	2.50	2.50
Salt	0.25	0.25
	100.00	100.00

[†] Vitamin A 7500 IU, Choline chloride 15.0mg, Vitamin D₃ 1000IU, Vitamin B₁₂ 30mg, Vitamin E 1 IU, Cobalt 0.2mg, Vitamin K 2 mg, Copper 2.0mg, Riboflavin 4 mg, Iodine 1.2mg, Pantothenic acid 8mg, Zinc 50.0mg, Niacin 20mg, Manganese 80.0mg, Iron 25.0mg

Soaking:

Raw cowpea seeds were soaked with distilled water for 24hr rinsed thrice with distilled water, dried at 65°C for 48hr and milled.

Rat feeding trials:

Adult male rats of the wister strain weighing between 95g and 110g at the

of jugular vein) from each group and their gastrointestinal tracts were exposed by a midline incision. Ligation of the g.i.t above the stomach and as close to the anus as possible was made and the entire structure was cut after removing adhering tissue.

Microbiological assay:

Colony count method of determining the

number of viable organisms in 1g of cecal sample as described by Harrigan and McCance (1966) was used.

Estimation of the antinutritional factors and Oligosaccharides:

The antinutritional factors of the cowpea varieties used in this study had been previously determined (Oke *et al.*, 1995). Oligosaccharides were extracted using the method of Akpapunam and Markakis (1979) and quantified using Dubois *et al.* (1956) method.

RESULTS AND DISCUSSION

Table 3 provides the summary of the effect of processing on intestinal microfloral population in rats fed with different cowpea varieties. Estimation was made in $\times 10^7$ microbes/cecal content, hence, all values that are given below would have this unit of expression. In the raw state, Ife Brown with 17.92 had the lowest number of microbes while IT 81D - 1137 with 22.99 had the highest microbial population. The mean was 20.97. Soaked samples gave 15.23 as the lowest microbial population in Ife Brown while the highest microbial count was recorded in IT 84E-1- 108 and the average was 17.55. In germinated samples, microbial population ranged from 0.24 in IT 84E-1-108 to 0.43 in IT 81D-1137 with a mean of 0.32. Cooked samples gave values ranging from 5.74 in Ife Brown to 8.80 in IT 82E-16. The average was 7.50. Variety IT 82D-889 had the lowest microbial count of 8.61 while IT 81D - 1137 gave the highest value of 11.03 in the autoclaved samples. An average of 9.41 was obtained. Relative to raw cowpea diets, with a view to knowing the effectiveness of each processing method in reducing the microbial population, percentage intestinal microfloral population was calculated. On the average, these values were 44.87, 35.65, 1.52 and 82.99% for autoclaving, cooking, germination and soaking respectively (Table 4). Intestinal microfloral population was influenced by variety and processing methods ($P < 0.001$). Differences between varietal means may not be unconnected with genetic variation because

the ability of a variety to encourage intestinal microfloral growth partly depended on the content of oligosaccharides. For the fact that oligosaccharides are indigestible, they could be taken as belonging to the antinutritional factors family.

In the raw cowpea varieties, a good number of the antinutritional factors (ANF) played almost equal but significant roles in supporting microbial growth. This is exemplified by the result of stepwise regression analysis (Table 5). Raffinose, stachyose, trypsin inhibitor (TI), lectin, tannic acid, HCN and phytic acid contributed 20.68, 20.91, 5.27, 20.30, 8.34, 20.24 and 4.26% respectively to the microfloral population (MFP). The direction of contribution of each of these ANF was revealed by their correlation coefficient values (Table 6). This shows that while tannic acid discouraged microbial growth, other ANF encouraged microbial growth. This could be due to the fact that tannic acid did not provide the requisite conditions for microbial proliferation (Banwell *et al.*, 1983) and possibly served as an antimetabolite to the microbes and could impair their nutrient utilisation. The poor contribution made by TI and phytic acid in encouraging microbial growth may be due to the provision of microbial requisite conditions for microbial proliferation. It is possible that the proportion of indigestible protein that resulted from TI and phytic acid forming complex with protein was small. Therefore, the amount of these indigestible products serving as substrates to the microbes would be small, hence, TI and phytic acid would have very little contribution in enhancing microbial growth. The contribution of raffinose and stachyose on the one hand, and lectin and HCN on the other hand, to enhance microbial growth could be taken as being complementary.

The significant ($P < 0.001$) positive correlation coefficient values exhibited by these substances serve as attestation to this complementary action.

In the autoclaved samples, stepwise regression analysis results revealed that raffinose, stachyose, tannic acid, HCN and

TABLE 3: INTESTINAL MICROFLORAL POPULATION IN RATS FED DIFFERENT VARIETIES OF PROCESSED COWPEA ($\times 10^7$ /g CECAL CONTENT)

Cowpea Varieties	PROCESSING									
	Auto Claving	Cooking	Germination	Soaking	Raw	Mean				
IT 84E-1-108	8.82	6.61	0.24	20.03	21.88	11.52				
IT 82D-889	7.77	7.82	0.29	16.22	20.60	10.54c				
IT 81D-1137	11.03	8.52	0.43	19.35	22.99	12.46a				
IT 82E-16	10.80	8.80	0.39	16.22	21.48	16.67b				
Ife Brown	8.61	5.74	0.25	15.23	17.92	9.55d				
Mean	9.41u	7.51v	0.32x	17.55y	20.97z					
$\pm$ SE	± 0.37	± 0.33	± 0.02	± 0.55	± 0.49					

Each value is a mean of three determinations

a,b,c,d = Varietal means followed by different letters are significantly different ($P < 0.001$)

u,v,x,y,z = Processing means followed by different letters are significantly different ($P < 0.001$).

TABLE 4: PERCENTAGE MICROFLORAL POPULATION WITH REFERENCE TO RAW COWPEAS

Cowpea Varieties	PROCESSING			
	Auto Claving	Cooking	Germination	Soaking
IT 84-1-108	40.31	30.21	1.10	91.54
IT 82D-889	32.72	37.96	1.41	78.74
IT 81D - 1137	47.98	37.06	1.87	84.17
IT 82E-16	50.28	40.97	1.82	75.51
Ife Brown	48.05	32.03	1.40	84.99
Mean	44.87c	35.65b	1.52a	82.99d
SE	± 1.42	± 1.14	± 0.08	± 1.59

Each value is a mean of three determinations

a,b,c,d = Processing means followed by different letters are significantly different ($P < 0.001$).

TABLE 5: STEPWISE REGRESSION OF OLIGOSACCHARIDES AND ANTINUTRITIONAL FACTORS WITH INTESTINAL MICROFLORAL POPULATION

Coefficient of determination	Dependent IMBRCP	Variable IMPACP
Due to raffinose	20.68	28.98
Due to stachyose	20.91	20.06
Due to trypsin inhibitor	5.27	0.00
Due to lectin	20.30	0.00
Due to tannic acid	8.34	25.12
Due to HCN	20.24	8.82
Due to phytic acid	4.26	17.02

IMPRCP = Intestinal microfloral population of raw cowpeas

IMPACP = Intestinal microfloral population of autoclaved cowpeas.

COWPEA PROCESSING VS INTESTINAL MICROFLORA

phytic acid contributed 28.98; 20.06, 25.12, 8.82 and 17.02% respectively to the MFP. The direction of contribution of each of these factors was shown by their *r* values. Following the same sequence, *r* values were 0.74, 0.61, -0.12, 0.42 and 0.05 respectively. Like in the raw samples, tannic acid still served as an anti-nutrient to the microbes. Phytic acid was negligibly positively correlated to MFP which may be due to the formation of protein-phytate cation complex during processing thus decreasing mineral bioavailability (Saio *et al.*, 1968; Okubo *et al.*, 1976; Omosaiye and Cheryan, 1979). The unavailable minerals could impair microbial growth (O'Dell and Savage, 1960). The oligosaccharides tend to act complementarily with HCN in enhancing microbial growth as shown in the raw samples despite its low contribution (8.82%).

Generally, diets containing autoclaved cowpea gave less MFP than the raw samples. This result is in conformity with the findings of Kakade and Borchers (1967) that heated navybeans produced only $\frac{1}{3}$ - $\frac{1}{2}$ as much as the raw beans which may be ascribed to

reduction in oligosaccharide content of autoclaved samples. Cooking was more effective than autoclaving in reducing MFP which may not be unconnected with the reduction, by cooking, in the level of the substance that provided the requisite condition for microbial proliferation. Autoclaving usually involves heating a substance in a confined environment such that little or no steam escapes except at the end of the procedure. Some of the HCN would have condensed during autoclaving thus increasing its level in the autoclaved samples. During cooking, some steam escaped and some HCN would have escaped also, thus reducing its level in the cooked samples. Therefore, cooking would be expected to be more effective than autoclaving in reducing MFP. Germination was the most effective method of processing in reducing MFP. This could be ascribed to the complete removal of raffinose and significant removal of stachyose by germination. Hence, their removal from the diet would starve the microbes thereby leading to reduction in their population as a result of reduced growth. Soaking was the least effective in reducing

TABLE 6: PREDICTION EQUATIONS AND CORRELATION COEFFICIENTS RELATING INTESTINAL MICROFLORAL POPULATION WITH ANTI-NUTRITIONAL FACTORS AND OLIGOSACCHARIDES.

Dependent Variable	prediction equation		Correlation coefficient
IMPRCP IMPACP	$Y = 11.58 + 5.94x - 0.57x^2$ $y = -2.23 + 24.60x - 11.01x^2$	Raffinose	0.96 0.74
IMPRCP IMPACP	$Y = 4.49 + 6.56x - 0.55x^2$ $y = 9.08 - 1.66x + 1.19x^2$	Stachyose	0.96 0.61
IMPRCP IMPACP	$y = 11.62 + 4.71x - 0.48x^2$ $y = 3.89 - 8.40x - 2.80x^2$	HCN	0.94 0.42
IMPRCP IMPACP	$y = 19.38 + 0.04x + 0.001x^2$	Trypsin inhibitor	0.49
IMPRCP IMPACP	$y = 5.44 + 0.46x - 0.003x^2$	Lectin	0.91
IMPRCP IMPACP	$y = 34.64 - 80.20x + 108.70x^2$ $y = 19.54 - 70.60x + 110.20x^2$	Tannic acid	-0.22 -0.02
IMPRCP IMPACP	$y = -126.00 + 0.63x - 0.001x^2$ $y = 139.90 - 0.78x + 0.001x^2$	Phytic acid	0.37 0.05

IMPRCP = Intestinal microfloral population of raw cowpeas.

IMPACP = Intestinal microfloral population of autoclaved cowpeas.

Correlation coefficient values exceeding 0.51, 0.64 and 0.76 are significant at 5%, 1% and 0.1% respectively.

MFP which may be due to the inability of this processing method of reduce the oligosaccharides' levels in the soaked samples. It is concluded that germination was the most effective method of processing to remove the oligosaccharides. Some antinutritional factors acted complementarily with the oligosaccharides in encouraging intestinal microbial growth.

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