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Full-Length Research Paper

Effects of Different Fermentation Methods of Pigeon Pea (*Cajanus Cajan*) on the Haematological and Pathological Parameters of Wistar albino rats

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ABSTRACT: This research was on the effects of different fermentation methods of Pigeon pea on the haematological and pathological parameters of Wistar albino rats. Pigeon pea *(cajanus cajan)* was subjected to different fermentation methods and treatments for 72 hours. The sample was divided into six parts and the following treatments and fermentation method were employed; solid state, back slope, hot water, cold water, 2% potassium bicarbonate treated sample, unfermented pigeon peas and commercial animal diet used as control. Haematological and pathological analyses were carried out on the wistar albino rats after 28 days feeding. The results of the haematological parameters showed that there was significant difference ($P<0.05$) in the rats fed with fermented samples when compared to the rats fed with commercial animal diet. The rats fed with commercial animal diet had the highest bilirubin (5.6mg) total protein (9.2%), packed cell volume, white blood cell, red blood cell and hemoglobin concentration. Rats fed with back slope fermented pigeon pea had significant values of packed cell volume, white blood cell, red blood cell and hemoglobin concentration. There was no significant difference ($P<0.05$) in the monocytes, eosinophile and basophile of rats fed with the different samples, however rats fed with unfermented pigeon pea had the highest value of neutrophile and least value of lymphocytes. There was reduction in the alanine amino transferase and aspartate amino transferase (ALT) in all the animals' organs fed with fermented and commercial diets. The ALT and AST values of rats fed with back slope fermented pigeon pea were lower than values of rats fed with other diets. The rats fed with hot water fermented pigeon pea had the highest values of ALT and AST. The results revealed that back slope fermentation was the best method of fermentation that enhanced the quality of pigeon pea.

Keywords: Pigeon pea, fermentation methods, Wistar albino rats

INTRODUCTION

Pigeon pea *(Cajanus cajan)* is a legume crop grown in the tropics and widely consumed in Africa, Indian and the Carribbean. It contains about 19.6% protein (Okpala and Mamah, 2001) and therefore serves as an important source of vegetable protein. It is a perennial legume from the family *fabaceae.* Since its domestication in South Asia at least 3,500 years ago, its seed have become a common food grain in Asia, Africa and Latin America.

A common characteristic of dried legumes like the pigeon pea is their hard texture. Texture is an important quality characteristic of cereals and legumes. Textural characteristics of legumes may be dependent upon both seed microstructure and chemical processing. During soaking operations, the seed undergo important

physiochemical changes resulting in softer texture (Stanley and Aquira, 1985). Soaking and cooking are therefore indispensable in order to render them edible and to ensure acceptable sensory quality. Processing techniques such as boiling, roasting and germination are means of improving the nutritional value of food (Nergiz and Gokgoz, 2007). Fermentation is defined as bio-processing using microorganisms and their enzymes to achieve desirable quality characteristics of food products (Singhanai *et.al,* 2009). The microorganism involved in fermentation belongs to diverse groups, namely bacteria, yeast and filamentous moulds (Obizoba, 1998). The origin of fermented foods goes back many thousands of years. It is one of the oldest ways of food processing. Popular fermented products such as beer, bread, wine and sausages have been around for centuries (Campell-Platt. 2004). According to Lawal *et. al* (2009), fermentation improves food digestibility and nutritional quality. However, the effect of fermentation on pigeon pea has not been extensively studies. Fermented foods which are associated with lactic acid bacteria are referred to as probiotics. Probiotics are beneficial bacteria in that they favourable alter the intestinal micro flora balance, inhibit the growth of harmful bacteria, promote food digestion, boost immune function and increase resistance to infection. Starch and proteins are the major constituents of pigeon pea. The high digestibility (%) of protein and starch of pigeon pea makes it a staple for millions of people in the semi-arid tropics (SAT). Pigeon pea significantly contributes to meet the dietary requirements of crude fibre, ash, fat, magnesium, manganese and copper (Faris and Singh, 1990). Pigeon pea contains high amounts of vitamin B, carotene and ascorbic acid. Pigeon pea is a rich source of lysine but deficient in the sulfur-containing amino acid – methionine and cystein. The objective of this research was to determine the effects of different methods of fermentation of pigeon pea on the haematological and pathological parameters of wistar albino rats.

MATERIALS AND METHODS

Sample Collection

Pigeon pea *(Cajanus Cajan)* used for this research was purchased from Uchi market, Auchi in Edo State, Nigeria. The sample was transported to Food Technology Department, Auchi Polytechnic, Auchi, Edo State.

Sample processing prior to use

The pigeon peas were sorted to remove dirt, broken ones and extraneous materials which may serve as contaminants. The pigeon peas were washed with portable water treated with 5% sodium metabisulphite and drained. It was dried in hot oven model Tamro-Apata 90-544011 at 50^{o}C for 24 hours. The dried pigeon peas were divided into six portions (1kg each).

Fermentation of pigeon pea using different methods: The following fermentation methods and treatments were employed:

Step 1: 1 kg of pigeon pea was soaked in 10 litres of water in a plastic Container at 27^{o}C for 72 hours (CWFP)
Step 2: 1 kg of pigeon pea was soaked in 10 litres of water containing 2% sodium bicarbonate in a plastic container at 27^{o}C for 72 hours (SCFP).
Step 3: 1 kg of pigeon pea was soaked in 10 litres of water containing 2% potassium bicarbonate in a plastic container at 27^{o}C for 72 hours (PCFP).
Step 4: 1 kg of pigeon pea was soaked in 10 litres of water and put in water bath at 65^{o}C for 10 minutes and allowed to ferment in a plastic container at 27^{o}C for 72 hours (HWFP).
Step 5: 1 kg of pigeon pea was inoculated with isolated microorganisms for back slope fermentation in a plastic container at 27^{o}C for 72 hours (BSFP).
Step 6: 1 kg of pigeon pea was used for low moisture solid state fermentation in a plastic tray at 27^{o}C for 72 hours (SSFP).

All samples were dried and made into powder meal.

In-Vivo feeding experiment

Animal and diet

Twenty four (24) albino rats male and female were obtained from Federal University of Technology Akure (FUTA), Ondo State, Nigeria.

The rats were weighed and randomly allocated to metabolic cages. The age ranged from three to four weeks old. The rats were housed in metabolic cage fixed with a cup and a small plastic bottle to supply food and water.

The animals were acclimatized to the new environment by feeding on animal diets for seven days. The animals were reweighed and grouped into eight in such a way that the weights were close.

The rats in groups were placed on experimental food for 28 days. They were given a weighed quantity of each experimental diets in a feeding cup and water was supplied via a plastic bottle attached to the cage.

Changed in weight of the experimental animal were taken every three days. At the end of 28 days, the experimental animals were sacrificed and analysis were carried out which include haematological and pathological analysis (Fashakin *et al.,* 1986).

Haematological test

Complete blood count was performed according to Cheesbrough (2014).

Biochemical assay of blood serum

Reflotron Mo6-02<06.00 was used for the analysis of some major serum. Biochemical markers that can reveal the effects of the above treatments on the rats. The biomarkers assayed for are: Total bilirubin, Total Serum Cholesterol, Aspartate-aminotransferase (AST) and the Alkaline phosphatase (ALP) level of the serum. The general procedure involved pipetting standardized amount of the samples automatically. These were then applied on the test zone of the appropriate test strip. The strips were inserted into the test chambers and the flap closed. The results were displayed after some seconds on the computer monitor. Tests were carried out at 25^{o}C.

Total bilirubin determination

Total bilirubin determination is based on the reaction of bilirubin with the diazorium salt 2-methozy–4-nitrophenyl diazonium tetrafluoroborate to give the chromophore diazobilirubin, a coloured compound, which is measured by the releotone photometer at 567mm. The result was displayed after about 135 seconds.

Aspartate Aminotransferase (AST)

Determination

This was carried out according to the method as described by the manufacturer's manual (Randox Laboratories Ltd). In brief, 100w of test sample was mixed with 300 µL of buffer (containing 100µL phosphate buffer pH 7.4, 100 ml L-aspartate and 2ml & -oxoglutarate) and the mixture was incubated for 30 minutes at 37^{o}C. Thereafter, 500µL of 2ml 2,4-dinitropheny-iheny/drazine was added to the reaction mixture and allowed to stand for 20 min at 25^{o}C. Then, 500 w of 0.4ml NaOH was added and thoroughly mixed, the absorbance was read after 5 min at 546mn against a reagent blank and the AST activity determined. The non-fermented pigeon pea was labeled as NFP while the commercial animal diet was labeled as H.

Statistical analysis

All experiments were carried out in triplicates. Results obtained were analyzed by one way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test was used to separate means using statistical package for the social sciences (SPSS) version 16 while $P \leq 0.005$ was accepted as significant level.

RESULTS AND DISCUSSION

The result as shown in table 1, shows the haematological parameters of rats fed with non-fermented, fermented pigeon peas and commercial animal diets as control 2. The highest values of packed cell volume, white blood cells, red blood cell, haemoglobin and erythrocyte sedimentation rate were recorded in group fed with commercial animal diet with their values, 49.00 mm^3, 8.10mm^3, 5.20mm^3, 8.24% and 5.30% respectively, while rats fed with back slope fermented pigeon pea had significant values of packed cell volume, white blood cell, red blood cell, haemoglobin and erythrocyte sedimentation rate. There was no significant difference ($P<0.05$) in the monocytes, eosinophile and basophile of rats fed with the different samples. However, unfermented pigeon pea had the highest value of neutrophiles (59.6%) and least value of lymphocytes (3.4%). The backslope fermented pigeon pea had the best qualities. The safety and health status of rats fed with non-fermented pigeon pea, fermented pigeon pea and commercial animal diet was examined using some biochemical markers such as Alkaline phostate (ALP), Alanine amino transferase (ALT), Aspartate Amino transferase (AST), total protein and total bilirubin. As shown in table 2, the rats fed with commercial diet had the highest total protein (9.20%), bilirubin (5.60%), while the group fed with back slope fermented pigeon pea had the least value of Aspartate amino transferase (128.20) and Alanine amino transferase (41.10). There was significant difference ($P<0.05$) in the serum ALP activity of the rat fed with fermented diets compared to commercial diet. Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status of an organism (Luiz *et.al.* 1998). Aboderin and Oyetayo (2006) had earlier reported that a high level of hemoglobin, packed cell volume and red blood cells is an indication that the rats were not anaemia while a lower level is a sign of anaemia. The red blood cell, hemoglobin and packed cell volume were higher in rats fed with commercial animal diet and solid state fermented pigeon pea diet. White blood cell is important in defending our body against infection. White blood cell improves immune function and stimulates immunomodulatory cells. There was no significant difference ($P<0.05$) in the monocytes, eosinophile and basophile of rats fed with the different samples; however, rats fed with non-fermented pigeon pea diet had the

Table 1: Haematological parameters of rat fed with pigeon pea and commercial animal diets.

Parameter	NFP2	SSFP2	HWFP2	SCFP2	PCFP2	BSFP2	CWFP2	H
WBC (mm3)	6.10±0.05^{a}	7.90±0.01^{ab}	6.80 ±0.02^{ac}	6.700.14^{ac}	6.90±0.12^{ac}	7.80±0.01^{ab}	7.80±0.02^{ab}	8.10±0.14^{ab}
RBC (mm3)	4.31±0.03^{ad}	4.40±0.03^{ad}	4.60±0.05^{ad}	4.41±0.12^{ad}	4.90±0.01^{ad}	5.01±0.03^{ad}	4.90± 0.3^{ad}	5.20±0.12^{bc}
ESR (%)	4.16±0.01^{ae}	4.20±0.01^{ae}	4.90±0.06^{ae}	4.70±0.16^{ae}	5.00±0.02^{b}	5.10±0.12^{b}	4.90±0.03^{ae}	5.30±0.12^{bc}
PCV (mm3)	43.10±0.01^{bc}	30±0.12^{bc}	43.20±0.03^{bc}	42.20±0.13^{bc}	42.10±0.05^{bc}	47.00±0.13^{bd}	45.00±0.01^{bd}	49.00±0.14^{bd}
HGB (g/d)	14.300.01^{bd}	17.41±0.14^{cd}	15.82±0.02^{df}	16.31±0.05^{ef}	17.63±0.03^{cd}	17.92±0.05^{cd}	17.21±12.1^{cd}	8.24±0.01^{cd}
LYM (%)	32.10±0.02^{c}	41.80±0.06^{dc}	42.00±0.03^{dc}	45.60±0.03^{dc}	40.30±0.14$_{dc}$	40.20±0.01^{dc}	39.30±0.05^{dc}	39.70±0.02^{dc}
BSP (%)	2.60±0.03^{d}	2.50±0.13^{d}	2.00±0.05^{d}	2.10±0.01^{d}	2.30±0.16^{d}	2.60±0.02^{d}	2.30±0.03^{d}	2.00±0.03^{d}
MON (%)	4.20±0.01^{de}	4.01±0.02^{de}	4.70±0.05^{de}	4.01±0.01^{d}	4.00±0.13^{de}	4.20±0.03^{de}	4.10±0.01^{de}	4.50±0.05^{de}
NTP (%)	59.10±0.03^{f}	52.80±0.01^{df}	52.90±0.03^{df}	48.6006^{fd}	53.60±0.05^{df}	55.40±0.14^{dh}	57.30±0.05^{hd}	53.20±0.01^{df}
ESP (%)	3.11±0.01^{e}	3.21±0.02^{e}	3.11±0.0^{e}	3.42±0.05^{e}	3.31±0.12^{e}	3.24±0.01^{e}	3.30±0.05^{e}	3.21±0.02^{e}

Means with the same superscript along the row are not significantly different (p>0.05)

Key:

WBC = White blood cell, **RBC =** Red bood cell, **ESR =** Erlythrocyte Sedimentation Rate **PCV =** Packed cell Volume, **HGB =** Haemoglobin, **LYM =**Lymphocytes, **BSP =** Basophile, **MON =** Monocytes **NTP =** Neutriphiles, **ESP =** Eosinophile, **NFP2 =** Unfermented pigeon pea, **SSFP2 =** Solid state fermented pigeon pea , **HWFP2 =** Hot water fermented pigeon pea , **SCFP2 =** Sodium bicarbonate fermented pigeon pea, **PCFP2 =** Potassium bicarbonate fermented pigeon pea **KBSFP2 =** Back slope fermented pigeon pea, **CWFP2 =** Cold water fermented pigeon pea and **H** = commercial animal diet.

Table 2: Pathological evaluation of rats fed with pigeon pea and commercial animal diet.

Parameter	NFP2	SSFP2	HWFP2	SCFP2	PCFP2	BSFP2	CWFP2	H
AST	199.50±0.10^{a}	212.50±0.64^{a}	156.40±0.06^{ab}	208.10±0.06^{a}	134.80±0.06^{ab}	128.20±0.06^{ab}	232.80±0.06^{b}	252.1±0.00^{b}
ALT	99.33±0.58^{bc}	86.10±0.06^{bc}	74.30±0.06^{bc}	58.70±0.58^{bd}	42.70±0.06^{bd}	41.10±0.06^{bd}	48.70±0.06^{bd}	68.40±0.06^{bc}
Total Protein (g%)	8.20±0.01^{c}	8.50±0.06^{c}	6.40±0.06^{cd}	9.10±0.06^{c}	8.50±0.06^{c}	7.60±0.06^{cd}	7.70±0.06^{cd}	9.20±0.06^{c}
Biliru-Bin (mg%)	10±0.01^{a}	2.70±0.06^{de}	1.70±0.06^{de}	4.70±0.01^{ac}	3.70±0.06^{ad}	2.50±0.06^{de}	2.40±0.06^{de}	5.60±0.06^{ac}
Alkaline Phosphate (m/1)	1239.00±1.00^{ca}	1339.70±0.58^{ca}	1499.70±0.58^{ca}	1609.70±0.58^{cb}	1780.30±0.58^{cb}	1509.70±0.58^{ca}	888.70±0.58^{ca}	890.3±0.58^{ca}

Means with the same superscript along the row are not significantly different (p>0.05).

ALT=Alanine amino transferase
AST=Aspatate amino transferase
NFP2=Unfermented pigeon pea diet
SSFP2=Solid state fermented pigeon pea diet
HWFP2=Hot water fermented pigeon pea diet
SCFP2=Sodium bicarbonate fermented pigeon pea diet
PCFP2=Potassium bicarbonate fermented pigeon pea diet
BSFP2=Back slope fermented pigeon pea diet
CWFP2=Cold water fermented pigeon pea diet
H=commercial animal feed (Control)

highest value of neutrophiles and least value of lymphocytes. There was reduction in the alanine amino transferase and aspartate amino transferase in rats fed with fermented samples. However, there was significant difference (P<0.05) in the level of alanine amino transferase (ALT) and aspartate amino trasnsferase (AST) in all the animals' organs fed with fermented pigeon pea and commercial diet. The ALT and AST values of rats fed with back slope fermented pigeon pea were lower than values of rats fed with other fermented pigeon pea and commercial diet. It was reported that most liver diseases are characterized by greater ALT elevations than AST elevations with normal values ranging from 0 to 40 IU/L for AST and to 45 IU/L for AST in human. However, the obtained values of AST and LT were within the acceptable range. In this research, the obtained values were under the accepted standard values.

Conclusion

Considering all the parameters in the heamatological and pathological evaluation, backslope fermentation is the best method that enhanced the nutritional composition of pigeon pea.

Recommendation

Fermented pigeon pea with increased protein content can be developed into weaning food to solve the problem of malnutrition in children. Production of pigeon pea in a large scale should be encouraged by individuals, corporate bodies, local, state and federal governments. This can reduce the high level of unemployment, food shortage and source of income.

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