Microbiology Research Journal International

23(1): 1-6, 2018; Article no.MRJI.33604
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Effect of Different Fermentation Methods on the Microbial and Proximate Composition of Pigeon Pea (*Cajanus cajan*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AOO and VOO managed the analyses of the study. Author VOO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/33604
Editor(s):
(1) Giuseppe Blaiotta, Professor, Department of Agriculture, Division of "Grape and Wine Sciences", University of Naples Federico II, Via Universita' 100 – Palazzo Mascabruno 80055 Portici, Italy.
Reviewers:
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Complete Peer review History: http://www.sciencedomain.org/review-history/23558

Original Research Article

Received 22nd April 2017
Accepted 26th June 2017
Published 9th March 2018

ABSTRACT

Pigeon pea (*Cajanus cajan*) was subjected to different fermentation methods and treatments for 72 hr. The raw (control) and fermented samples were analyzed for microbial and proximate composition. The pH ranged from 6.8 to 4.5 after 72 hr fermentation. The microbial evaluation results showed that there was increase in microbial counts from 0hr to 48hr and reduction at 72 hr. Sodium bicarbonate treated fermented pigeon pea had the highest bacterial counts at 0 hour, (3.2±0.04) and the least counts at 72hr, (3.1±0.05) Solid state fermented pigeon pea had the highest bacterial count at 72hr, (4.9±0.02) Sodium bicarbonate treated fermented pigeon pea had the lowest fungal counts at 72hr, (2.8±0.02), while hot water treated fermented pigeon pea had the highest fungal counts, (3.6±0.05). *Bacillus subtilis, Bacillus megatarium, Staphylococcus aureus, Lactobacillus plantarum, Streptococcus lactis, Saccharomyces cerevisae, Aspergillus flavus, Aspergillus niger* and *Candida albican* were found to be associated with the fermentation of pigeon pea. At the end of 72 hr of fermentation, *Bacillus subtilis, Bacillus megatarium, Lactobacillus plantarum, Streptococcus lactis* and *Saccharomyces cerevisae* were isolated. The proximate

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analysis showed that back slope fermented sample had the highest fat content of 10.12% and least carbohydrate value of 57.84%, potassium bicarbonate treated fermented pigeon pea had the least fat value of 7.23% and the highest value of carbohydrate content of 65.3%. Cold water treated fermented pigeon pea had the highest protein content of 14.21%, hot water treated fermented pigeon pea had the highest energy value of 6,762 Kcal/g, while the non fermented (control) pigeon pea had the least energy value of 4, 296 Kca/g.

Keywords: Saccharomyces cerevisae; potassium bicarbonate; sodium bicarbonate; pigeon pea.

1. INTRODUCTION

Pigeon pea *(Cajanus cajan)* is a legume crop grown in the tropics and widely consumed in Africa, India and the Caribbean. It contains about 19.6% protein [1] and therefore serves as an important source of vegetable protein. It is a perennial legume from the family *Fabaceae*. Its seeds have become a common legume in Asia, African, and Latin America since its domestication in South Asia in about 3,500 years ago.

A common characteristic of dried legumes like the pigeon peas is their hard texture. Texture is an important quality characteristic of cereals and legumes. [2] reported that textural characteristics of legumes may be dependent upon both seed microstructure and chemical and/or physical changes occurring during processing. During soaking operation, the seeds undergo important physicochemical changes resulting in softer texture [3].

Processing techniques such as boiling, roasting and germination are means of improving the nutritional value of foods [4].

Fermentation is defined as bio-processing using microorganisms and their enzymes to achieve desirable quality characteristics of food products [5].

The microorganisms involved in fermentation belong to diverse groups, namely, bacteria, yeast and filamentous moulds [6]. 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 [7].

According to [8] fermentation improves food digestibility and nutritional quality. It alters the intestinal micro flora balance and inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection [9].

The objective of this study was to determine the effect of different fermentation methods on the microbial and proximate composition of pigeon peas *(Cajanus cajan)*. Also to determine the effect of sodium bicarbonate and potassium bicarbonate as both chemicals are used locally to soften meat and legumes during cooking.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Pigeon Peas

50kg of pigeon peas *(Cajanus cajan)* was purchased in Uchi Market, Auchi, Edo State, Nigeria. The pigeon pea was cleaned by winnowing and hand sorting. 2% each of these chemicals were percentage used as it was observed that higher percentage changed the colour of the pigeon pea as the sensory attributes were considered.

2.2 Fermentation of Pigeon Peas using Different Methods and Treatments

Treatment 1: 2 kilograms of pigeon pea was soaked in 10 litres of water for 72 hr in a plastic container and labeled as sample CWFP (cold water fermented pigeon peas). It was allowed to ferment during this period under anaerobic condition.

Treatment 2: 2 kilograms of pigeon peas was soaked in 10 litres of water containing 2% sodium bicarbonate for 72hr in a plastic container and labeled as sample SCFP (Sodium bicarbonate fermented pigeon peas).

Treatment 3: 2 kilograms of pigeon peas was soaked in 10 litres of water containing 2% potassium bicarbonate (potash) for 72hr in a plastic container and labeled as sample PCFP (potassium bicarbonate fermented pigeon peas).

Treatment 4: 2 kilograms of pigeon peas was soaked in 10 litres of water and put in a water bath at 65ºC for 10 mins and allowed to ferment in a plastic container for 72 hr and labeled as sample HWFP (Hot Water Fermented Pigeon Peas).

Treatment 5: 2 kilograms of pigeon peas was inoculated with isolated microorganisms for back slope fermentation for 72 hrs and labeled as sample BSFP (Back Slope Fermented Pigeon Peas)

Treatment 6: 2 kilograms of pigeon peas was used for low moisture solid state fermentation for 72 hr and the sample was labeled SSFP (Solid State Fermented Pigeon Peas).

The non fermented pigeon peas sample labeled as NFP (Non Fermented Pigeon Peas) was used as the control.

2.3 Microbial Analysis

Microbial analysis was carried out on non fermented pigeon peas and fermented pigeon peas using pour plate method as described by [10]. Cutturing was done at 24 hr intervals.

2.4 Determination of Proximate Composition

The proximate composition was determined using standard method of [11].

2.5 Statistical Analysis

All experiments and analyses were conducted in triplicates. Data obtained from the different parameters of the study were subjected to analysis of variance ($p<0.05$). Statistical comparison were performed using SPSS version 13.

3. RESULTS AND DISCUSSION

The microorganisms isolated and identified from the raw and fermented pigeon peas using different methods of fermentation include *Bacillus subtilis, Bacillus megatarium, Streptococcus lactic, Saccharomyces cerevisae, Aspergillus niger, Aspergillus flavus* and *Candida albican.* These microorganisms have been found to be responsible for fermentation of most legumes and cereal. These microorganisms have also been isolated in various investigations of fermented products such as alcoholic beverages and fufu [12].

The study also revealed that *Bacillus subtilis* and *Bacillus megaterium* were predominant in all the fermented samples. This may be due to the fact that these microorganisms can invade and proliferate in many kinds of food materials. *Bacillus subtilis* is known to ferment most sugars, hence involved in fermentation [10]. Moreover, *Lactobacillus plantarum* isolated from most samples belong to the group of lactic acid bacteria which are responsible for fermentation process because of their unique metabolic characteristics.

The results of the microbial counts showed decrease in the microbial counts as fermentation increases. As the pH reduces due to the production of acid, the microbial counts reduces.

The result of proximate composition revealed that the non fermented pigeon pea had the highest moisture content which may encourage microbial proliferation and food spoilage [13]. However, backslope fermented pigeon peas had the least moisture content which is significant since dry or low moisture increases shelf-life of food [14]. The crude protein value of cold water fermented pigeon peas (14.21%) was higher than other fermented samples. This may be due to the fact that microorganisms responsible for fermentation could have secreted extracellular enzymes which increases the protein content [15] while potassium bicarbonate fermented pigeon peas had the least protein content (8.87%).

Back slope fermented pigeon peas had the highest crude fibre content (8.76%). High fibre content in food helps to empty bowel and reduces the risk of constipation [16]. The Back slope fermented pigeon peas had the highest fat content (10.12%) while the potassium bicarbonate fermented pigeon peas had the highest carbohydrate content (65.53%). The hot water fermented pigeon peas had the highest energy value (6762 Kcal/g), while the non fermented pigeon peas had the least energy value (4296 Kcal/g).

Fermentation as a method of food processing increased the energy value and enrichment of the samples[17].

Table 1. Changes in bacterial count (Cfu/g) during fermentation of samples Duration (hr)

Samples	0	24	48	72
NFP	2.3±0.14	2.1±0.05	2.3±0.05	2.6±0.05
SSFP	2.6±0.12	4.5±0.05	5.1±0.12	4.9±0.02
HWFP	2.7±0.05	4.3±0.04	3.9±0.14	3.5±0.03
SCFP	3.2±0.04	4.7±0.02	4.3±0.05	3.1±0.05
PCFP	2.4±0.02	4.2±0.15	3.4±0.02	3.3±0.02
BSFP	2.3±0.05	4.5±0.02	6.8±0.03	3.2±0.05
CWFP	2.7±0.05	4.4±0.05	6.3±0.05	3.2±0.04

NOTE: NFP - Non fermented pigeon pea; SSFP - Solid State fermented pigeon pea; HWFP - Hot water fermented pigeon pea; SCFP - Sodium bicarbonate fermented pigeon pea; PCFP - Potassium bicarbonate fermented pigeon pea; BSFP - Back slope fermented pigeon pea; CWFP -Cold water fermented pigeon pea

Table 2. Changes in fungal count ($x10^5$ sfu/g) during fermentation of samples Duration (hr)

Sample	0	24	48	72
NFP	1.2±0.04	1.4±0.02	1.6±0.04	1.6±0.02
SSFP	1.4±0.02	2.1±0.02	3.6±0.05	3.2±0.05
HWFP	1.3±0.04	2.2±0.05	3.7±0.02	3.6±0.05
SCFP	1.1±0.05	2.1±0.02	3.5±0.04	2.8±0.02
PCFP	1.2±0.02	2.4±0.05	3.8±0.05	3.2±0.03
BSFP	1.3±0.05	1.3±0.02	3.7±0.05	3.4±0.04
CWFP	1.2±0.04	2.5±0.04	3.6±0.04	3.2±0.05

Table 3. Microbial succession during fermentation of pigeon peas

Samples	0hr	24hr	48hr	72hr
NFP	*Bacillus subtilis, Bacillus megatarium*	*Staphylococcus aureus, Bacillus subtilis, Bacillus megatarium*	*Staphylococcus aureus, Bacillus subtilis, Bacillus megatarium*	*Staphylococcus aureus, Bacillus subtilis, Bacillus megatarium*
SSFP	*Bacillus subtilis, Bacillus megatarium,*	*Bacillus subtilis, Bacillus megatarium, Saccharomyces cerevisae, Candida albican*	*Bacillus subtilis, Bacillus megatarium, Saccharomyces cerevisae, Aspergills flavus*	*Bacillus subtilis, Bacillus megatarium, Lactobacillus plantarum, Saccharomyces cerevisae*
HWFP	*Bacillus subtilis, Bacillus megatarium*	*Bacillus subtilis, Bacillus megatarium, Staphylococcus aureus, Aspergillus flavus, Candida albican*	*Bacillus subtilis, Bacillus megatarium*	*Bacillus subtilis, Bacillus megatarum, lactobacillus plantarium, Saccharomyces cerevisae*
SCFP	*Bacillus subtilis, Bacillus megatarium*	*Bacillus subtilis, Bacillus megatarium, Staphylococcus aureus, Aspergillus flavus, Candida albican*	*Bacillus subtilis, Bacillus megatarium*	*Bacillus subtilis, Bacillus megatarum, lactobacillus plantarium, Saccharomyces cerevisae*
PCFP	*Bacillus subtilis, Bacillus megatarium*	*Bacillus subtilis, Bacillus megatarium,*	*Aspergilus flavus, Candida albican, Saccharomyces*	*Bacillus subtilis, Bacillus megatarum,*

Samples	0hr	24hr	48hr	72hr
		Staphylococcus aureus, Aspergillus flavus, Candida albican	*cerevisae*	*lactobacillus plantarium, Saccharomyces cerevisae*
BSFP	*Bacillus subtilis, Bacillus megatarium*	*Bacillus subtilis, Bacillus megatarium, Staphylococcus aureus, Aspergillus flavus, Candida albican*	*Bacillus subtilis, Bacillus megatarium,*	*Bacillus subtilis, Bacillus megatarum, lactobacillus plantarium, Saccharomyces cerevisae*
CWFP	*Bacillus subtilis, Bacillus megatarium*	*Bacillus subtilis, Bacillus megatarium, Staphylococcus aureus, Aspergillus flavus, Candida albican*	*Aspergillus flavus, Aspergillus niger,Saccharomyces cerevisae Bacillus subtilis, Bacillus megatarium,*	*Bacillus subtilis, Bacillus megatarium, Saccharomyces cerevisae, Lactobacillus plantarum, Streptococcus lactis, Bacillus subtilis, Bacillus megatarium*

Table 4. Proximate composition of fermented and non-fermented pigeon peas parameters (%)

Samples	Moisture	Ash	Fat	Fibre	Protein	CH_20	Energy (Kcal/g)
NFP	7.44^{a}	4.97^{a}	9.32^{ab}	8.23^{b}	11.76^{bc}	58.44^{de}	4296^{a}
SSFP	6.14^{b}	4.98^{a}	8.67^{abc}	6.59^{c}	10.65^{cd}	62.97^{bc}	6572^{a}
HWFP	5.45^{cd}	3.14^{c}	9.35^{ab}	7.25^{d}	12.36^{b}	62.46^{c}	6762^{a}
SCFP	6.07^{b}	3.81^{bc}	8.10^{bc}	7.64^{c}	9.47^{dc}	64.92^{ab}	6538^{a}
PCFP	5.48^{c}	4.70^{ab}	7.23^{c}	8.19^{b}	8.87^{e}	65.53^{a}	6406^{a}
BSFP	5.24^{d}	4.43^{ab}	10.12^{a}	8.76^{a}	13.61^{1}	57.84^{e}	6643^{a}
CWFP	5.35^{cd}	4.11^{ab}	7.59^{c}	8.41^{b}	14.21^{a}	60.35^{d}	6472^{a}

Means with the same superscript down the column are not significantly different (P>0.05)
**SEM (The Standard error of the mean)*

Table 5. pH of fermented pigeon pea samples sample pH

Time (hours)	SSFP	HWFP	SCFP	PCFP	BSFP	CWFP
0	6.8±0.02	6.7±0.05	6.8±0.16	6.8±0.01	6.7±0.01	6.6±0.05
24	6.1±0.05	6.2±0.04	6.2±0.05	6.3±0.05	6.2±0.02	6.3±0.14
48	5.2±0.15	5.1±0.022	5.3±0.01	5.3±0.15	5.1±0.15	5.2±0.12
72	4.5±0.14	4.6±0.01	4.7±0.02	4.7±0.02	4.5±0.14	4.6±0.05

4. CONCLUSION

This study revealed the effect of different fermentation methods on the microbial and proximate composition of pigeon peas. The results obtained from the microbial bacteria analysis revealed the presence of lactic acid bacteria and decrease of microbial counts as the pH reduced with increase in fermentation time. The results obtained from proximate analysis revealed cold water fermented pigeon peas had the highest protein content and all the fermented samples showed appreciable amount of energy level compared to the non fermented pigeon peas.

5. RECOMMENDATION

Pigeon pea is an underutilized crop due to lack of information on the nutritional potential. It can serve as composite flour which can be used for domestic and industrial purpose. The fermented pigeon pea with increased protein content can be developed into weaning food to solve the problem of malnutrition in children.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23558