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Chemical Changes during Thermal Processing of Unfermented and Fermented Red Kidney Beans (*Phaseolus vulgaris*) and effects on *In Vitro* Protein Digestibility

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Authors' contributions

This work was carried out in collaboration among all authors. Author PFW designed the study, Author MKJ performed the statistical analysis. Author MS wrote the protocol, and the first draft of the manuscript. Author CEM, managed the analyses and the literature of the study. AuthorS JIO and OAO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Legumes are outstanding sources of macronutrients, micronutrients, phytochemicals, as well as antinutritional factors. These components present a complex system enabling interactions with different components within food matrices. The interactions result in insoluble complexes with reduced bioaccessibility of nutrients. The development of appropriate preparation technologies for use at the household and village-level become so imperative to facilitate processing and dietary availability of beans.
Aim of the Study: This study aimed to evaluate the effect of thermal processing on the chemical contents of unfermented and fermented red kidney beans (*Phaseolus vulgaris*) and the effects of the resulting changes on the *in vitro* protein digestibility. This will enhance food security and

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reduction in malnutrition.
Methodology: Unfermented and fermented *P. vulgaris* were boiled using ordinary cooking pot and a pressure pot and the chemical contents were evaluated by standard methods. *In vitro* protein digestibility was carried out by pepsin digestion.
Results: Fermentation resulted in a decrease in the traditional cooking time in the ordinary cooking pot by 40.32%. The protein content of the fermented sample increased by about 7%. The *in vitro* protein digestibility value was increased by more than 30% with greater percentage evident in fermented samples. Sulphur containing amino acids, methionine and cystein were the limiting amino acids but their contents appreciated by 6.64% and 10.92% respectively after fermentation. Total ash, crude fibre, crude fat contents of *P. vulgaris* were all affected by more than 20% during the open fermentation and cooking of unfermented beans. The antinutritional factors of *P. vulgaris* decreased most in boiled fermented samples compared with the other processing methods. There was overall improvement in the *In vitro* protein digestibility, reduction of cooking time and antinutritional factors when *P. vulgaris* was fermented and cooked.
Conclusion: The outcome of the reserach justifies the fact that combining both fermentation and cooking results in the overall improvement in the nutritional value of *P. vulgaris* as against cooking without fermentation.

Keywords: Red kidney bean; chemical composition; antinutrient and digestibility.

1. INTRODUCTION

Recent problems linked with meat consumption as source of protein have led to renewed interest in vegetation diets [1]. This phenomenon is reinforced by the fact that reserachers have emphasis that high intake of animal proteins meats may results to cardiovascular disesaes and some types of cancers. It is to this end, that intensive efforts are being made to find alternative sources of protein from the underutilizerd legumes plants in nutrition and in the formulation of new food products.

Red kidney beans are so named because of their shape which is very similar to that of the human kidney. Dry beans (*Phaseolus spp. L*.) are the most important grain legumes for human consumption [2]. Dry beans have been cultivated for thousands of years and have been reported to play an important role in the traditional diets of many regions throughout the world [3]. Beans are less significant in western diets compared to most of the developing countries [4] The daily per capital consumption of all bean products is 9g in the United States compared to about 110 g in Asia [5]. *Phaseolus vulgaris* originated from Central and South America, where it was cultivated as early as 6000 BC in Peru and 5000 BC in Mexico [6]. It was introduced to the old World by the Spaniards and Portuguese. It is now widespread and cultivated as a major food crop in many tropical, subtropical and temperate areas of the Americas, Europe, Africa and Asia [7].In Nigeria, red kidney bean is widely cultivated in Plateau State.

Kidney bean is a very good source of cholesterol-lowering fibre as are most other beans [8]. In addition to lowering cholesterol, kidney beans' high fibre content prevents blood sugar levels from rising too rapidly after a meal making these beans an especially good choice for individuals with diabetes, insulin resistance or hyperglycaemia [9].

Generally, legumes have been reported to have low nutritive value due to losw amounts of sulphur-containing amino acids, low protein digestibility and presence of anti-nutritional factors. Cooking is usually done before the use of legumes in a human diet. This improves the protein quality by destruction or inactivation of the heat-labile anti-nutritional factors [10]. However, cooking causes considerable losses in soluble solids especially vitamins and minerals [11]. Increasing the time and temperature of processing has been reported to reduce the nutritive value and available lysine of legumes [12]. Legumes are important sources of dietary protein for both human and animals, but the presence of relatively high concentration of toxins such as phytate, tannins and oxalate referred to anti-nutritive factors affects the nutritional quality by interacting with intestinal tract and also reduce protein digestibility and amino acid absorption. According to [13] unless these substances are destroyed by heat or other treatments, they can exert adverse physiological effects when ingested. The following methods,

such as Soaking, cooking, germination, fermentation or irradiation treatments may be used to improve protein nutritional value. This research aimed to evaluate the effect of thermal processing on the chemical compositions of fermented red kidney beans (*Phaseolus vulgaris)* as well as the effects of these changes on *in vitro* protein digestibility.

2. MATERIALS AND METHODS

2.1 Chemical and Reagents

The chemicals used for this research are: Pepsin, Catalyst and all other chemicals and reagents used were of analytical grades and were purchased from sigma.

2.2 Collection and Preparation of Plant Sample

Matured *Phaseolus vulgaris* (red kidney bean seeds) were purchased from local farmers in Mangu Local Governmemt Area of Plateau state, Nigeria. The identity of the bean was confirmed at the herbarium of Biological Science Department, Ahmadu Bello University, Zaria, Nigeria (Voucher Number 2403). The beans were picked, cleaned of all debris and broken seeds and then stored in a plastic container at room temperature (27-30^{o}C) for subsequent analysis.

2.3 Open Fermentation

Red kidney beans sample was rinsed with distilled water and dried in an oven at 55^{o}C for 24 hours. The rinsed beans were placed into a transparent plastic container and three cups of cold water for every one cup of dried red kidney beans was added. The beans were then allowed to soak for three (3) days uncovered and was fermented by atmospheric microorganisms [14], during which the seed coat remains intact. After fermentation, the microbial growth was terminated by drying at 55^{o}C in an oven for 24 hours [15].

2.4 Thermal Processing

The unfermented and fermented bean samples were boiled using ordinary cooking pot and pressure cooker. Boiling of unfermented bean was for 143.42 and 40.32 minutes using ordinary cooking and pressure pots respectively while 84.91 and 39.27 minutes were for fermented bean samples using ordinary pot and pressure cooking pot respectively after which it was filtered and rinsed with water in each case. The boiled beans were then dried in an oven at 55^{o}C for 24h after which the bean sample was ground in a laboratory bench mill and kept in a cool dry rubber container for subsequent analysis.

2.5 Determination of Proximate Composition

2.5.1 Determination of moisture

The method described by AOAC, [16]

2.5.2 Ash content determination

Determination of ash content was done according to the method described by AOAC, [17]

2.5.3 Determination of lipid content

The lipid content of each sample was determined by the procedure described by AOAC, [18]

2.5.4 Determination of crude fibre

Crude fibre was determined by the method of AOAC, [19].

2.5.5 Determination of nitrogen content and crude protein

Proteins are major compounds containing nitrogen primarily in the form of amino acids which are their building blocks. Nitrogen is used as an index termed crude protein as distinct from true protein. The Kjedahl method of AOAC [20] was used for the crude protein determination.

2.5.6 Ddetermination of carbohydrate content

The percentage carbohydrate was obtained by difference.

NFE = 100-(%moisture+%CP+%CF+%Ash=%Fat)

Where, NFE = Nitrogen Free Extracts

%CP = Percentage Crude Protein.

%CF = Percentage Crude Fibre

2. 6 Determination of Amino Acid Profile

The amino acid profile in the sample was determined using the method described by Adeyeye and Afolabi, [21]. The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM).

2.7 Determination of Mineral Contents

Magnesium, calcium, zinc and iron were determined using the atomic absorption spectrophotometry as described by AOAC [22].

2.8 Determination of Anti Nutritional Factors

2.8.1 Determination of cyanide

The cyanide content was determined according to the method of AOAC [23]

2.8.2 Determination of phytic acid

The phytic acid was determined using the procedure described by Lucas and Markakas [24].

2.8.3 Determination of alkaloids

The gravimetric method as described by AOAC [25] was adopted.

2.8.4 Determination of oxalates

Oxalate was determined using the method of Oke [26].

2.8.5 Determination of tannins

The tannin content of each sample was determined using the method described by Krishnaiah et al., [27].

2.9 Determination of *in vitro* Protein Digestibility

The *in vitro* protein digestibility was carried out according to the method of [28]. Two hundred (200) milligramme of the powdered bean sample was dispersed in 20cm^3 of pepsin reagent (1.5mg/ml in 0.1M phosphate buffer of pH 2.0) and shaken vigorously. All the tubes were kept in a water bath at 37^{o}C for three hours with constant shaking at 15 minutes' interval. After three hours, the digestion was stopped by removing the tubes from the water bath and placing them in ice bath for 30 minutes. The samples were then filtered through whatman No.1 filter paper and the residue washed with buffer and dried at 80^{o}C for 2 hours. The dried residue was placed in a 50cm^3 mico-kjedahl flask and analysed for nitrogen by mico-kjedahl digestion. The indigestible nitrogen was subtracted from total nitrogen of the sample to obtain digestible nitrogen using the following:

Digestible N (mg) = Total N in sample (mg) – N in residue (mg).

Digestible protein = Digestible N (mg) × Conversion factor.

$$\%\ \text{in vitro digestibility} = \text{Digestible protein/ Total protein in sample} \times 100$$

2.10 Statistical Analysis

Data obtained is expressed as mean ± standard deviation (SD). Statistical analysis was done by the One Way Analysis of Variance (ANOVA) and Paired Sample T-test using SPSS (version 20). Duncan Multiple Range Test was used to determine the source of variance at $P<0.05$.

3. RESULTS

3.1 Effects of the Different Processing Methods on Cooking Time of Red Kidney Beans (*Phaseolus vulgaris*)

Fig. 1 shows the various cooking time for the different processing methods viz: Unfermented beans boiled with ordinary cooking pot and pressure pot and fermented beans boiled with ordinary cooking pot and pressure pot. Based on these, there was a significant decrease in the cooking time of fermented beans cooked with the ordinary cooking pot compared with the unfermented beans cooked with the ordinary cooking pot.

3.2 Proximate Contents of Raw and Differently Processed Whole Grains of *Phaseolus vulgaris*

Table 1, 2 and 3 show the proximate compositions of raw and differently processed whole grains of *P. Vulgaris.*

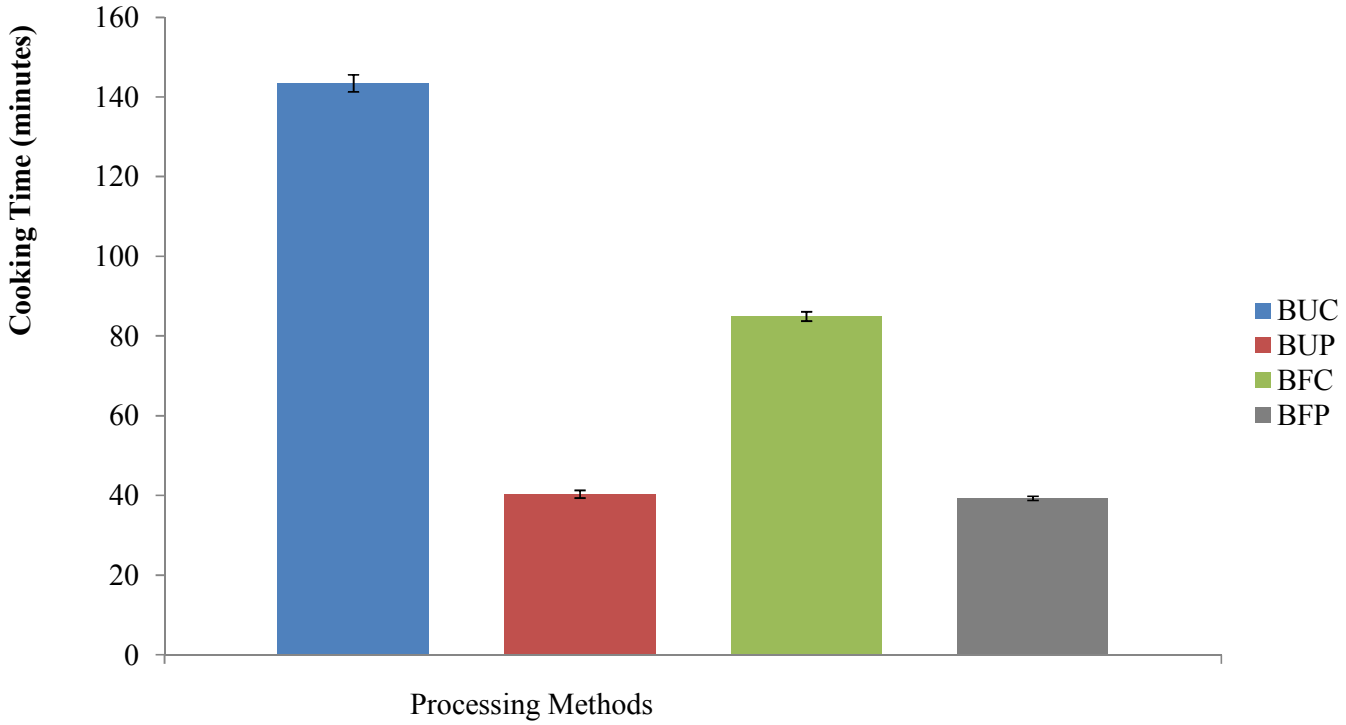

Fig. 1. Cooking time of the different processing methods

BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot. BFC - Fermented beans boiled with ordinary cooking pot. BFP – Fermented beans boiled with pressure pot.

Table 1. Effect of fermentation on the proximate contents of *Phaseolus vulgaris*

	Proximate Composition					
Processing Method	**Ash (%)**	**Crude Protein (%)**	**Fat (%)**	**Moisture (%)**	**Crude Fibre (%)**	**Carbohydrate (%)**
Raw	5.29± 0.05[a]	24.62± 0.21[a]	10.97 ±0.13[a]	2.24 ±0.05[a]	7.02± 0.47[a]	50.86 ±0.29[a]
Fermented	3.45± 0.28[b]	26.12 ±0.19[b]	8.92± 0.27[b]	2.53± 0.25[a]	3.80 ±0.25[b]	55.18± 0.65[b]

Values are Mean ± Standard Deviation. Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05)

Table 2. Effects of boiling on the proximate contents of unfermented *P. Vulgaris*

	Proximate Composition					
Processing Method	**Ash (%)**	**Crude Protein (%)**	**Fat (%)**	**Moisture (%)**	**Crude Fibre (%)**	**Carbohydrate (%)**
Raw	5.29 ±0.05[a]	24.62± 0.21[a]	8.92 ±0.27[a]	2.24± 0.05[a]	7.02 ±0.47[a]	50.86 ±0.29[a]
BUC	4.01± 0.12[b]	22.32±0.44[b]	7.32± 0.61[b]	2,54± 0.18[a]	4.45± 0.39[b]	62.83± 1.32[b]
BUP	4.17 ±0.05[b]	24.45± 0.26[c]	6.63± 0.42[b]	2.15± 0.08[a]	4.85± 0.32[b]	57.76 ±0.25[c]

Values are mean ± standard deviation. Values having the same alphabet in the same column are statistically the same (P>0.05).While values having different alphabets in the same column are statistically different (P<0.05). BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot.

Table 3. Effects of cooking on the proximate contents of fermented *P. Vulgaris*

	Proximate Composition					
Processing Method	**Ash (%)**	**Crude Protein (%)**	**Fat (%)**	**Moisture (%)**	**Crude Fibre (%)**	**Carbohydrate (%)**
Raw	5.29± 0.05[a]	24.62 ±0.21[a]	10.97± 0.13[a]	2.24± 0.05[a]	7.02± 0.47[a]	50.86 ±0.29[a]
Fermented	3.45± 0.28[b]	26.12 ±0.19[b]	8.92± 0.27[b]	2.53± 0.25[a]	3.80± 0.25[b]	55.18± 0.65[b]
BFC	3.16± 0.03[b]	26.0±1 0.10[b]	8.69± 0.41[b]	3.45± 0.04[b]	3.32± 0.33[bc]	55.29± 0.16[b]
BFP	3.21± 0.12[b]	24.80 ±0.22[a]	6.93± 0.10[c]	2.38± 0.06[a]	2.87± 0.19[c]	59.81± 0.68[c]

Values are mean ± standard deviation. Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05). BFC - Unfermented beans boiled with ordinary cooking pot. BFP - Unfermented beans boiled with pressu.

3.3 Effects of Open Fermentation of Whole Grains on the Amino Acid Profile of Red Kidney Beans (*Phaseolus vulgaris*).

The seventeen (17) amino acids determined were observed to be insignificantly (P>0.05) higher in fermented bean samples when compared to the raw bean samples. However, threonine, glycine and cysteine in fermented bean samples showed more than 10% increase when compared to the raw bean samples (Table .4)

3.4 Mineral Contents of the Different Processing Methods

Table, .5, .6 and .7 shows the mineral composition of different processed *P. vulgaris.*

3.5 Antinutritient Contents of the Different Processing Methods

Table 8, 9, and 10 shows the antinutrtient contents of of *P.vulgaris.*

3.6 Effects of the Different Processing Methods on the Protein Digestibility Profile of Red Kidney Beans (*Phaseolus vulgaris*).

The protein digestibility value was observed to be significantly {P<0.05) higher in unfermented bean samples boiled with the ordinary cooking pot (BUC) and unfermented bean samples boiled with the pressure pot (BUP) compared to the raw bean samples. However, digestibility value of unfermented bean samples boiled with the pressure pot (BUP) was observed to be insignificantly (P>0.05) higher compared to the digestibility value of unfermented bean samples boiled with the ordinary cooking pot. The digestibility value was observed to be significantly {P<0.05) higher in the fermented bean samples compared to the digestibility value of raw bean samples, unfermented bean samples boiled with the ordinary cooking pot (BUC) and unfermented bean samples boiled with the pressure pot (BUP). The digestibility value of fermented bean samples boiled with the ordinary cooking pot (BFC) and fermented bean samples boiled with the pressure pot (BFP) were observed to be significantly {P<0.05) higher compared to the raw, unfermented bean samples boiled with the ordinary cooking pot (BUC) and unfermented bean samples boiled with the pressure pot (BUP). The digestibility value in fermented bean samples samples boiled with the ordinary cooking pot (BFC) were observed to be significantly {P<0.05) higher compared to the fermented bean samples, while the digestibility value of fermented bean samples boiled with the pressure pot (BFP) were observed to be insignificantly {P>0.05) higher compared to the fermented bean samples and fermented bean samples boiled with the pressure pot (BFP) (Table 11).

Table 4. Effect of fermentation on the amino acid profile of *P. vulgaris*

Amino Acid	Concentration (g/100g of Protein)	
	Unfermented	Fermented
Lysine	6.31± 0.11[a]	6.64 ±0.14[a]
Histidine	3.41± 0.09[a]	3.59 ±0.13[a]
Arginine	7.60± 0.13[a]	8.28 ±0.08[a]
Aspartic Acid	11.20± 0.15[a]	12.31 ±0.02[a]
Threonine	3.70 ±0.14[a]	4.30 ±0.00[a]
Serine	4.01± 0.02[a]	4.20± 0.10[a]
Glutamic Acid	13.18± 0.09[a]	13.71± 0.09[a]
Proline	3.31± 0.01[a]	3.31± 0.01[a]
Glycine	4.08± 0.01[a]	4.54± 0.06[a]
Alanine	3.91± 0.03[a]	3.99± 0.05[a]
Cystein	1.19 ±0.03[a]	1.32± 0.00[a]
Valine	5.44± 0.21[a]	5.56± 0.10[a]
Methionine	1.25± 0.02[a]	1.33± 0.06[a]
Isoleucin	3.49 ±0.01[a]	3.58± 0.05[a]
Leucine	7.70 ±0.00[a]	8.00± 0.17[a]
Tyrosine	3.18 ±0.06[a]	3.49 ±0.09[a]
Phenyl Alanine	5.74 ±0.03[a]	5.99± 0.06[a]

Values are mean ± standard deviation. Values having the same alphabet in the same row are statistically the same (P>0.05). While values having different alphabets in the same row are statistically different (P<0.05).

Table 5. Effect of open fermentation on the mineral composition of *P. vulgaris*

	Mineral Contents					
Processing Method	**Potassium (%)**	**Sodium (%)**	**Calcium (ppm)**	**Magnessium (%)**	**Iron (ppm)**	**Zinc (ppm)**
Raw	1.20 ± 0.02[a]	1.45 ± 0.01[a]	926.69 ± 0.21[a]	0.19 ± 0.20[a]	744.11 ± 0.14[a]	82.46 ± 0.01[a]
Fermented	0.93 ± 0.02[b]	1.38 ± 0.02[a]	729.32 ± 0.23[b]	0.18 ± 0.44[a]	597.06 ± 0.06[b]	71.47 ±0.18[b]

Values are mean ± standard deviation.. Values having the same alphabet in the same column are statistically the same P>0.05) while values having different alphabets in the same column are statistically different P<0.05).

Table 6: Effect of boiling on the mineral contents of unfermented *P. vulgaris*

	Mineral Content					
Processing Method	**Potassium (%)**	**Sodium (%)**	**Calcium (ppm)**	**Magnessium (%)**	**Iron (ppm)**	**Zinc (ppm)**
Raw	1.2 ± 0.02[a]	0.15 ± 0.0[a]	926.69 ± 0.21[a]	0.19 ± 0.20[a]	744.11 ± 0.14[a]	82.46 ± 0.01[a]
BUC	0.88 ± 0.01[b]	0.16 ± 0.01[a]	522.56 ± 0.02[b]	0.15 ± 0.27[b]	597.06 ± 0.11[b]	58.28 ± 0,06[b]
BUP	0.95 ± 0.05[c]	0.15 ± 0.11[a]	597.24 ± 0.04[b]	0.16 ± 0.30[b]	714.71 ± 0.07[a]	38.28 ± 0.10[c]

Values are mean ± standard deviation. BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot. Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05).

Table 7. Effects of cooking on the mineral contents of unfermented *P. Vulgaris*

	Mineral Content					
Processing Method	**Potassium (%)**	**Sodium (%)**	**Calcium (ppm)**	**Magnessium (%)**	**Iron (ppm)**	**Zinc (ppm)**
Raw	1.20 ± 0.02[a]	0.15 ± 0.01[a]	926.69 ± 0.21[a]	0.19 ± 0.20[a]	744.11 ± 0.14[a]	82.46 ± 0.01[a]
Fermented	0.93 ± 0.02[b]	0.14 ± 0.02[a]	729.32 ± 0.23[b]	0.18 ± 0.44[a]	597.06 ± 0.06[b]	71.47 ± 0.18[b]
BFC	0.63 ± 0.08[c]	0.14 ± 0.05[a]	714.66 ± 0.19[b]	0.18 ± 0.02[a]	567.65 ± 0.04[b]	45.10 ± 0.26[c]
BFP	0.60 ± 0.07[c]	0.14 ± 0.02[a]	723.31 0.0022[b]	0.17 ± 0.09[a]	538.24 ± 0.04[b]	45.10 ± 0.07[c]

Values are mean ± standard deviation. Mean percentage increase. Mean percentage decrease BFC - Fermented beans boiled with ordinary cooking pot. BFP – Fermented beans boiled with pressure pot. Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05).

Table 8. Effects of open fermentation on the antinutrients contents of *P. vulgaris*

	Antinutrient Content				
Processing Method	**Phytic Acids (%)**	**Alkaloids (%)**	**Oxalates (mg/100g)**	**Cyanides (mg/100g)**	**Tannins (%)**
Raw	0.31 ± 0.01[a]	5.13 ± 0.11[a]	19.58 ± 0.06[a]	14.52 ± 0.09[a]	1.72 ± 0.05[a]
Fermented	0.26 ± 0.01[b]	2.10 ± 0.03[b]	18.23 ± 0.05[b]	10.93 ± 0.11[b]	1.20 ± 0.03[b]

Values are mean ± standard deviation. Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05).

Table 9. Effects of cooking on the antinutrient contents of unfermented *P. Vulgaris*

	Antinutrient Content				
Processing Method	**Phytic Acids (%)**	**Alkaloids (%)**	**Oxalates (mg/100g)**	**Cyanides (mg/100g)**	**Tannins (%)**
Raw	0.31 ± 0.01[a]	5.13 ± 0.11[a]	19.58 ± 0.06[a]	14.52 ± 0.09[a]	1.72 ± 0.05[a]
BUC	0.23 ± 0.00[b]	3.33 ± 0.03[b]	16.88 ± 0.03[b]	11.42 ± 0.08[b]	1.50 ± 0.14[b]
BUP	0.25 ± 0.00[c]	4.34 ± 0.06[c]	18.90 ± 0.02[c]	12.43± 0.14[c]	1.60 ± 0.02[c]

Values are mean ± standard deviation. BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot. Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05)

Table 10. Effects of cooking on the antinutrient contents of fermented *P. vulgaris*

	Antinutrient Content				
Processing Method	**Phytic Acid s(%)**	**Alkaloids (%)**	**Oxalates (mg/100g)**	**Cyanides (mg/100g)**	**Tannins (%)**
Raw	0.31 ± 0.01[a]	5.13 ± 0.11[a]	19.58 ± 0.06[a]	14.52 ± 0.09[a]	1.72 ± 0.05[a]
Fermented	0.26 ± 0.01[b]	2.10 ± 0.03[b]	18.23 ± 0.05[b]	10.93 ± 0.11[b]	1.20 ± 0.03[b]
BFC	0.12 ± 0.01[c]	1.51 ± 0.03[c]	13.88 ± 0.06[c]	8.75 ± 0.01[c]	0.94 ± 0.05[c]
BFP	0.19 ± 0.00[d]	1.80 ± 0.02[d]	19.80 ± 0.10[d]	6.74 ± 0.04[d]	1.09 ± 0.04[b]

Values are mean ± standard deviation.. BFC - Fermented beans boiled with ordinary cooking pot. BFP – Fermented beans boiled with pressure pot. Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.05)

Table 11. Digestibility profile of the differently processed red kidney beans (*P. vulgaris*)

Processing Method	%Protein in Undigested Sample	%Protein in Digested Sample	Digestibility Value (%)
Raw	24.67±0.21	8.01±0.25	32.51±0.69[a]
BUC	22.32±0.44	9.78±0.42	43.86±0.99[b]
BUP	26.45±0.26	11.94±0,18	45.39±0.99[b]
Fermented	26.12±0.19	14.36±0.22	54.87±1.29[c]
BFC	26.01±0.19	15.37±0.13	59.07±0.02[d]
BFP	24.80±0.22	13.53±0.07	57.60±0.79[c]

Values are mean ± standard deviation. BUC - Unfermented beans boiled with ordinary cooking pot. BUP - Unfermented beans boiled with pressure pot. BFC - Fermented beans boiled with ordinary cooking pot. BFP – Fermented beans boiled with pressure pot.Values having the same alphabet in the same column are statistically the same (P>0.05) while values having different alphabets in the same column are statistically different (P<0.

DISCUSSION

There was significant decrease in the cooking time of fermented beans with pressure pot and ordinary cooking pot as well as that of unfermented beans cooked with pressure pot (Fig .1). The decrease in cooking time of fermented beans cooked with both pressure pot and an ordinary cooking pot could be as a result of soaking of the beans during the fermentation period resulting in its softening and probably the shorter period of time required by the beans to absorb water during the cooking period.

The results showed that ash, fat and crude fibre contents significantly (P<0.05) decreased during the combined processing methods of fermentation and cooking. Crude protein also decreased significantly during cooking with the ordinary cooking pot but increased significantly during fermentation. Carbohydrate was observed to significantly increase during all the processing methods. The decrease in fat during fermentation agrees with the findings of [29] who worked on the effects of different processing methods on the chemical compositions of different sorghum cultivars. This decrease in fat during fermentation also agrees with the findings of Babalola and Giwa [30] who worked on the effects of fermentation on the nutritional and antinutritional properties of fermenting soybeans. The decrease in the crude fibre contents during fermentation could be as a result of the increase the crude fibre which could be due to the activities of methobolising organisms using fibre as energy source hence decreasing the fibre contents [31].

Open fermentation of whole grains of red kidney beans has no significant (P<0.05) effect on the amino acid compositions of red kidney beans (Table 4). Like other legumes sulphur containing amino acids, cystein and methionine were the limiting amino acids [32]. When combined with other protein sources, P*. vulgaris* could serve as a good source of amino acids. Red kidney bean was also found to contain high amount of Aspartic acid, glutamic acids, leucine and lysine in comparism to the other amino acids contained. This slight increase in the amino acid contents could be due to the increase in protein solubility [33].

The minerals determined in this research include: sodium, potassium, calcium, magnesium, iron and zinc. Based on the results obtained, there was a significant (P<0.05) decrease in the calcium, zinc, iron and potassium contents of the beans during the open fermentation of whole grains while there was insignificant decrease for sodium and magnesium. This decrease in minerals agrees with the finding s of ,[34] who attributed it to the leaching of these minerals in the fermenting water.

Phytic acids, alkaloids, oxalic acids, cyanides and tannins were evaluated during this research. During the open fermentation of the whole grains, phytic acids, alkaloids, cyanogenic glycosides and tannins all significantly (P<0.05) decreased. This is in agreement with the findings of [35] who attributed this decrease to degradative actions of fermenting micro organisms (Table 8).

The result of this research shows that cooking significantly (P<0,05) increased the digestibility of both unfermented and fermented red kidney beans (Table 11). This finding agrees with that of [36] that attributed to the low digestibility values to the high tannins and phytate contents which bind to the proteins and thus limiting their solubility and reducing the binding of the proteases to the proteins. The digestibility of boiled bean samples (either cooked with the

pressure pot or the ordinary cooking pot) was found to significantly increase [37]. However, reported a decrease in the digestibility values of protein in lentils and faba beans during cooking of the beans in contrast to [38] who reported an improvement in the *in vitro* protein digestibility of a 'k131 bean variety' during cooking especially when it was dehulled [39]. Attributed this increase in protein digestibility of the beans to reduction of phytate and tannin levels beyond detection as a result of dehulling.

The digestibility values of fermented bean samples (either boiled or unboiled) was found to significantly increase more than those of the unfermented boiled bean samples. Fermentation thus, improved the *in vitro* protein digestibility. This finding agrees with [40] report. This improvement in the digestibility of the fermented sample could be as a result of decrease in the phytate and tannin contents which could improve the solubility of the protein hence enhancing its digestibility. This improvement in protein digestibility could also be attributed to the degradation of complex molecules like fibre during fermentation hence increasing access of substrates to the active sites of digestive enzymes. Cooking and fermentation have been reported to result in the break down tannin-enzyme and protein-tannin complexes and released free tannins which subsequently leached out the products [41], hence, increasing the access to substrates to the active sites of digestive enzymes.

This research result showed significant ($P<0.05$) reduction in cooking time of *P. vulgaris.* This could be of economic importance as it will help reduce the cost of processing through reduction in the amount of cooking fuel used hence encouraging increase in consumption of *P. vulgaris.* Although, the fermentation period of three (3) days might be discouraging, *P. vulgaris* can be fermented in large quantity after harvest and kept for future use as this helps to disrupt the seed coats hence, softening the seed coats.although there was significant reduction in the ash, fat and crude fibre content during these processing methods, the combined method of fermentation and cooking can be employed and alternate sources of these nutrients be used to replenish these nutrients when *P. vulgaris* is processed for consumption. Despite the significant reduction in protein contents when unfermented *P. vulgaris* is cooked with the ordinary cooking pot (BUC}, there was overall improvement in protein content and protein quality when the combined processing method of fermentation and cokking was employed. The amino acid contents and the protein digestibility value increased during these processes.

CONCLUSION

There was overall improvement in the in vitro protein digestibility, reduction of cooking time and antinutritional factors when *P. vulgaris* was fermented and cooked. This justifies the fact that combining both fermentation and cooking results in the overall improvement in the nutritional value of *P. vulgaris* as against cooking without fermentation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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