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Effect of Processing on *in–vitro* Protein Digestibility and Anti–nutritional Properties of Three Underutilized Legumes Grown in Nigeria

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Authors' contributions

This work was carried out in collaboration between all the authors. Author MOA designed the study, performed the statistical analysis and approved the final manuscript. Author HI wrote the first draft of the manuscript and managed the statistical analysis while author BEE carried out the literature searches and proof read the final manuscript.

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ABSTRACT

Comparative studies are conducted on the effect of roasting and boiling methods on anti–nutritional properties and *in–vitro* protein digestibility of bambara groundnut (*Vigna subterranea* L.), scarlet runner bean (*Phaseolus coccineus* L.) and lima bean (*Phaseolus lunatus* L.) which are legume seeds grown in Nigeria. For this purpose some anti–nutritional factors and *in–vitro* protein digestibility were investigated using standard analytical techniques. Roasting method significantly reduced the anti–nutritional contents while *in–vitro* protein digestibility values were also decreased by 35.54%, 60.36% and 53.32% for *Vigna subterranean*, *Phaseolus coccineus* and *Phaseolus lunatus*, respectively. Boiling on the other hand significantly decreased the anti–nutritional properties as well as improved the *in–vitro* protein digestibility by 17.77%, 15.09% and 35.54% for *Vigna subterranean*, *Phaseolus coccineus* and *Phaseolus lunatus*, respectively. Among the two

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domestic processing methods, boiling was the most effective in reducing anti–nutritional factors and improving *in–vitro* protein digestibility.

Keywords: Protein digestibility; anti–nutrients; under–utilized legumes.

1. INTRODUCTION

Consumed regularly, legumes contribute to a healthy diet, and would help to control metabolic diseases such as diabetes mellitus [1]. Legumes such as bambara groundnut (*Vigna subterranean*), cowpea (*Vigna unguiculata*), lima bean (*Phaseolus lunatus*), groundnut (*Arachis hypogeal*), kidney beans (*Phaseolus vulgaris L*) and pigeon pea (*Cajanus cajan*) are consumed widely in Nigeria. These legumes are valuable sources of complex carbohydrates, protein and dietary fiber; contribute significant amounts of vitamins and minerals, and high energy value [2-4]. Protein contents in legume grains range from 17% to 40%, contrasting with 7–13% of cereals, and being equal to the protein contents of meats (18–25%) [5]. Poor nutritive values of the food legumes, due to the presence of some antinutritional substances, such as tannins, saponins, phytates, flavonoids, alkaloids, cyanogenic, glycosides, etc have been reported [2,6,7].

Bambara groundnut is economically important because it is an inexpensive source of high quality protein. It is highly valued among the Eastern and Northern states of Nigeria [8]. Bambara nut is processed into consumable food and taken in various forms as source of protein to Nigerians. Bambara nut (*Vigna subterranea*) is a novel legume of African origin grown mainly by subsistence female farmers intercropped with major commodities such as maize, millet, sorghum, cassava, yam, peanut and cowpea [9]. The bambara groundnut is one of the most adaptable of all plants and tolerates harsh conditions better than most crops. It is ideally suited for hot, dry regions where growing other pulses is risky. It yields on poor soils in areas of low rainfall and does not yield well in times of heavy rainfall. The cultivars had distinct colour differences ranging from cream through brown, maroon to black, with variations in the seed sizes and seed coat thicknesses [10].`The seeds can be consumed in different forms either in the immature green state or matured form. But at maturity, the seeds become very hard and therefore require boiling before any specific preparation can be carried out.

Scarlet runner bean *(Phaseolus coccineus),* a species of family Fabacceae, has been cultivated in the high parts of Mesoamerica. Its introduction into southern Columbia (Antioquia and Narino) and Europe where it is known as scarlet runner bean and haricot d'Espagne could have occurred in the seventeenth century before reaching other parts of the world such as the Ethiopian high lands [11]. Like other members of the Fabacceae, it possesses climbing stems. Its pods are gathered and left to dry in the sun before being beaten and the seeds are stored in sacks. They are cultivated by peasant farmers for home consumption in the middle belt of Nigeria. The aesthetic value of the seeds prompted their use in recreational activities such as traditional marriage in peasant communities among the Mada and Eggon tribes in Nasarawa State, Nigeria [12].

Lima bean (*Phaseolus lunatus*) is one of the most widely cultivated pulse crops both in temperate and subtropical regions. It is adapted to highly leach infertile soils of the more humid regions. Lima bean seeds are among the leguminous plants that are under–utilised in Nigeria. It is a potential supplement or even a substitute for the expensive soy meal and groundnut meal which constitute the major portion of conventional protein sources used in composite livestock feeds. The shift from this plant to other legumes such as soybean and groundnut was not unconnected with its extended cooking period resulting into the wastage of scarce and expensive fuel [13]. Also lima bean seeds contain anti–nutritional factors such as hydrogen cyanide, phytic acids, saponin, oxalate, tannin, trypsin inhibitor and haemagglutinin activity [14]. These anti–nutritional factors have been observed to inhibit absorption of nutrients and their subsequent utilisation and assimilation by animals. Besides, they cause some level of damages to some organs such as liver, kidney and spleen [15].

The presence of anti–nutritional factors such as trypsin inhibitors and chemotryosin inhibitors in legumes affects the digestibility of legume protein [16]. Other anti–nutritional factors such as tannins, phytates, anthocyannins and hemaglutinins impart bitter or unacceptable taste to the legumes, prevent protein digestibility and decrease the absorption of divalent metal ions such as F^{2+}, Zn^{2+} in the intestine. This is achieved by complexing with these metals and then making them unavailable for absorption. Many approaches have been adopted to address problems associated with legumes in food/feed.

Anti–nutritional factors are a chemical compounds synthesized in natural food and / or feedstuffs by the normal metabolism of species and by different mechanisms which exerts effect contrary to optimum nutrition [17]. Such chemical compounds, are frequently, but not exclusively associated with foods and feeding stuffs of plant origin. These anti–nutritional factors are also known as 'secondary metabolites' in plants and they have been shown to be highly biologically active. These secondary metabolites are secondary compound produced as side products of processes leading to the synthesis of primary metabolites. One major factor limiting the wider food utilization of many tropical plants is the ubiquitous occurrence in them of a diverse range of natural compounds capable of precipitating deleterious effects in man, and animals compound which act to reduce nutrient utilization and/or food intake are often referred to as anti–nutritional factors [18]. Antinutrients are chemicals which have been evolved by plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value. Some of these plant chemicals have been shown to be deleterious to health or evidently advantageous to human and animal health if consumed at appropriate amounts [19].

Some tropical seeds were reported to have high antinutrients which limit their utilization in food system [20]. Due to effect of these metabolites resulting in food poisoning, there is need to properly address this problem in developing countries like Nigeria which feed mostly on legumes and other vegetable based diets. This is because people have died of ignorance, poverty and inadequate nutrition education, especially with the African society.

To improve the nutritional quality and effective utilization of legume grains for the populace, it is essential that anti–nutritional factors be removed or reduced. So, it is necessary to establish processing technique(s) to insure its optimal utilization. Therefore, the present work was undertaken to explore the effect of two domestic processing methods (roasting and boiling) on anti–nutrients and protein quality of three under–utilized legume seeds grown in Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

About 3 kg each of fresh samples of bambara groundnut and scarlet runner bean seeds used for this study were purchased from a local market in Garaku, Nasarawa State, Nigeria while 3 kg of lima bean seeds were obtained from a Farmer in Owo local government area of Ondo State, Nigeria. All the purchased seeds were taken to the laboratory and each sample was stored in a sealed plastic container at room temperature prior treatment.

2.2 Sample Preparation

2.2.1 Raw seeds

About 500 g each of the raw seeds of bambara groundnut, scarlet runner bean and lima bean were soaked in ordinary water at room temperature and manually dehulled and dehulled seeds were dried in an oven at a temperature of 50℃. The dried dehulled seeds each were milled in attrition mill, sieved to pass through 1 mm mesh size and packaged in polyethylene container for further analyses.

2.2.2 Roasted seeds

The raw dehulled seeds (500 g) each of bambara groundnut, scarlet runner bean and lima bean were roasted on a hot cast iron pan at a temperature of 45 – 55℃. The seeds were continuously stirred until a characteristic brownish colour was obtained which indicated complete roasting. The seeds were cooled in desiccators, milled in attrition mill, sieved to pass through 1 mm mesh size and packaged in polyethylene container for further analyses.

2.2.3 Boiled seeds

The raw dehulled seeds (500 g) each of bambara groundnut, scarlet runner bean and lima bean were soaked and boiled for 45 min in distilled water at 100℃. The boiled seeds were drained using a perforated basket, after which they were dried in an oven at 50℃ until well dried. The dried seeds each were milled in attrition mill, sieved to pass through 1 mm mesh size and packaged in polyethylene container for further analyses.

2.3 Antinutritional Factors Determination

The contents of saponin, tannin, alkaloid, chemotrypsin, phytate, oxalate and cyanide were determined on each flour sample by methods described by some workers [21,22].

2.4 Determination of *In–vitro* Protein Digestibility

In–vitro digestibility was determined on each flour sample according to the method described by [23] as modified by [24]. The *in–vitro* digestibility was calculated according to the following equation [24].

$$\% \textit{ In–vitro} \text{ digestibility} = 234.84 - 22.56\,X$$

Where: X = the pH of suspension after 20 min hydrolysis of protein.

2.5 Statistical Analysis

All the data generated were analysed statistically. Parameters evaluated were grand means, standard deviation and coefficient of variation.

3. RESULTS AND DISCUSSION

3.1 Antinutritional Composition of the Seeds

Table 1 presents the antinutritional properties of bambara groundnut (*Vigna subterranea*), scarlet runner bean (*Phaseolus coccineus*) and lima bean (*Phaseolus lunatus*), respectively. The saponin content ranged from 3.2% in boiled sample to 4.2% in roasted sample. The value for the raw (4.6%) compared well with value for scarlet runner bean (4.1%) (Table 1) but has higher value than the ones reported by many authors such as Sesbania seeds (0.50–1.46%), 0.05 and 0.23 reported for mung beans and chickpeas, respectively [25]. Saponin has been shown to possess both deleterious properties and to exhibit structure dependent biological activities [26]. Saponins, in high concentrations, impart a bitter taste and stringent in dietary plants. The bitter taste of saponin is the major factor that limits its use.

In the past, saponins were recognized as antinutrient constituents, due to their adverse effects such as for growth impairment and reduce their food intake due to the bitterness and throat–irritating activity. In addition, saponins were found to reduce the bioavailability of nutrients and decrease enzyme activity and it affects protein digestibility by inhibit various digestive enzymes such as trypsin and chymotrypsin [27]. Saponins cause hypocholesterolaemia by binding cholesterol, making it unavailable for absorption. They also cause haemolysis of red blood cells and are toxic to rats [28]. Saponins from *Bulbostermma paniculatum* and *Pentapamax leschenaultii* have also been demonstrated to have anti–spermal effects on human spermatozoa [29,30]. They significantly inhibited acrosine activity of human sperms and the spermicidal effect was attributed to strong damage of the spermal plasma membrane [29]. The level of tannin in the bambara groundnut seed ranged from 0.0000103 $moldm^{-3}$ in the roasted seeds to 0.0000206 $moldm^{-3}$ in the boiled sample. The value for the raw 0.0000309 $moldm^{-3}$ is low when compared to values for scarlet runner bean (0.0000412 $moldm^{-3}$) and lima bean (0.0000567 $moldm^{-3}$) in this work (Table 1). The nutritional effects of tannins are mainly related to their interaction with protein due to the formation of complexes [31,32]. Tannin–protein complexes are insoluble and protein digestibility is decreased. Tannin acid may decrease protein quality by decreasing digestibility and palatability. Other nutritional effects which have been attributed to tannin include damage to the intestinal tract, interference with the absorption of iron and a possible carcinogenic effect [33-35].

The alkaloid content in *Vigna subterranea* ranged from 3.6% in boiled to 4.4% in roasted sample. The raw sample has a value of 6.2%. These values are very low compared to 9.6% and 8.6% obtained for *Phaseolus coccineus and Phaseolus lunatus* (Table 1), respectively but the values are higher than those obtained by [36] for black turtle bean (1.6, 1.8 and 5.0%). The values in this research are within the acceptable limit (20 mg/100 g). Alkaloids are considered to be anti–nutrients because of their action on the nervous system, disrupting or inappropriately augmenting electrochemical transmission. For instance, consumption of high tropane alkaloids will cause rapid heartbeat, paralysis and in fatal case, lead to death. Uptake of high dose of tryptamine alkaloids will lead to staggering gate and death. Indeed, the physiological effects that alkaloids have on humans are very evident. Cholinesterase is greatly inhibited by glycoalkaloids, which also cause symptoms of neurological disorder. Other toxic action includes disruption of the cell membrane in the gastrointestinal tract [37,38]. Phytate (a salt form of phytic acid) content ranged from 333.0 mg/100 g in the boiled to 346.9 mg/100 g in roasted sample (Table 1). The 353.9 mg/100 g obtained for the raw sample (bambara groundnut) is compared favourably with sesame seed (25.96 mg/100 g) [39] but lower than values reported for soybean (4050 mg/100 g), pigeon pea (1170 mg/100 g) and cowpea (2040 mg/100 g) [40], black turtle bean (11250 mg/100 g) [36] scarlet runner bean (370.01 mg/100 g) and lima bean (365.5 mg/100 g) (Table 1). Phytic acid is an important storage form of phosphorus in plant, it is insoluble and cannot be absorbed in human intestine and it has 12 replacaeble hydrogen atoms with which it could form insoluble salts with metals such as calcium, iron. zinc and magnesium. The formation of these insoluble salts renders the metals unavailable for absorption into the body. Phytate can also affect digestibility by chelating with calcium or by binding with substrate or proteolytic enzyme. Phytate is also associated with cooking time in legumes [40,36]. In areas of the world like Nigeria where cereal proteins are a major and predominant dietary factor, the associated phytate intake is a cause for concern.

Oxalate is an anti–nutrient which under normal conditions is confined to separate compartments. However, when it is processed and/or digested, it comes into contact with the nutrients in the gastrointestinal tract [41]. When released, oxalic acid binds with nutrients, rendering them inaccessible to the body. If food with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the lining of the gut [38]. Oxalate presented in this work range from 374.0 mg/100g in boiled to 418.0 mg/100 g in roasted sample for lima bean (Table 1). The raw lima bean sample has oxalate value of 475.0 mg/100 g which is higher than 254 mg/100 g for soybean and 286 mg/100 g for pigeon pea [42], but the value is lower than 225740 mg/100 g found in red kidney bean and 166890 mg/100 g found in black turtle bean [36], 495.0 mg/100 g in scarlet runner bean (Table 1) and 490.6 mg/100 g in bambara groundnut (Table 1). Oxalate is produced and accumulated in many crop plants and pasture weeds. Oxalate is a concern in legumes because high oxalate diets can increase the risk of renal calcium absorption since calcium is made unavailable to the body due to the presence of oxalate [43]. Cyanogens are glycosides of a sugar, sugars and cyanide containing aglycone. Cyanogens can be hydrolyzed by enzymes to release a volatile cyanide gas. Excess cyanide inhibits the cytochrome oxidase, the final step in electron transport, and thus blocks ATP synthesis and so tissues suffer energy deprivation and death follows rapidly. Prior to death, symptoms include faster and deeper respiration, a faster irregular and weaker pulse, salivation and frothing at the mouth, muscular spasms, dilation of the pupils, and bright red mucous membranes [44]. High level of HCN has been implicated for cerebral damage and lethargy in man and animal. The cyanide levels found in this work were between 0.0000310 – 0.0000364 moldm^{-3} for boiled and roasted bambara groundnut (Table 1), respectively. The raw sample of bambara groundnut has a value of 0.0000413 moldm^{-3} which is below the recorded value for scarlet runner bean (0.0000567 moldm^{-3}) (Table 1) and lima bean (0.0000516 moldm^{-3}) (Table 1).

Chemotrypsin and trypsin are digestive enzymes component of pancreatic juice acting in the duodenum where they perform proteolysis, the breaking down of proteins and polypeptides. Chemotrypsin inhibitors have been found in legumes to inhibit the activity of digestive enzymes hence causing poor utilization of the protein in most legumes [45]. The value chemotrypsin obtained for the raw sample of scarlet runner bean was 0.2926 g and ranged from 0.2337 g in roasted to 0.5250 g in boiled (Table 2) indicating a very significant decrease in chemotrypsin inhibitors during boiling. Researchers observed that those legumes which had the highest trypsin/chemotrypsin inhibitor activity were those in which the digestibility, as measured in *in–vivo* with rats, was most improved by cooking/boiling. The chemotrypsin inhibitors depress animal growth by interfering with the digestion and absorption of nutrients in the gastrointestinal tract [46]. Boiling and roasting were effective in lowering the levels of anti–nutrtional properties as depicted in Table 1. This agrees with the work of [47] who reported the antinutritional factors, zinc, iron and calcium in some Caribbean tuber crops and the effect of boiling and roasting on them. Boiling reduced the phytic acid to zinc molar ratio for yellow yam and cocoyam. Boiling and roasting reduced the levels of cyanoglucosides in sweet potato, yellow yam and cocoyam. Roasting greatly lowered the level of trypsin inhibitor activity compared to boiling. A similar observation was also reported for the effects of boiling and/or roasting by [48-50] on *D. lablab*, chickpeas and in other legumes, respectively.

Table 1. Anti–nutritional properties of *Vigna subterranea, Phaseolus coccineus* and *Phaseolus lunatus*

Parameter	Raw			Roasted			Boiled			Mean			SD			CV%		
	V. subterranea	***P. coccineus***	***P. lunatus***	***V. subterranea***	***P. coccineus***	***P. lunatus***	***V. subterranea***	***P. coccineus***	***P. lunatus***	***V. subterranea***	***P. coccineus***	***P. lunatus***	***V. subterranea***	***P. coccineus***	***P. lunatus***	***V. subterranea***	***P. coccineus***	***P. lunatus***
Oxalate (mg/100g)	490.6	495.0	475.0	466.2	418.0	418.0	383.0	376.2	374.0	446.6	429.7	422.3	46.1	49.2	41.3	10.32	11.45	9.78
Tannin (moldm^{-3})	3.09×10^{-5}	4.12×10^{-5}	5.67×10^{-5}	1.03×10^{-5}	2.56×10^{-5}	3.09×10^{-5}	2.06×10^{-5}	3.09×10^{-5}	4.12×10^{-5}	2.06×10^{-5}	3.26×10^{-5}	4.29×10^{-5}	6.13×10^{-6}	6.5×10^{-6}	1.06×10^{-5}	29.76	19.94	25.73
Phytate (mg/100g)	353.9	370.01	365.5	346.9	360.8	356.2	333.0	351.6	344.6	344.6	360.8	355.4	8.9	7.5	8.5	2.58	2.08	2.39
Saponin (%)	4.6	4.1	3.2	4.2	3.2	2.4	3.2	2.7	1.4	4.0	3.3	2.3	0.6	0.6	0.7	15.00	18.18	30.43
Alkaloid (%)	6.2	8.6	9.6	4.4	6.8	7.4	3.6	5.6	5.8	4.7	7.0	7.6	1.1	1.5	1.6	23.40	21.43	21.05
Cyanide (moldm^{-3})	4.13×10^{-5}	5.67×10^{-5}	5.16×10^{-5}	3.64×10^{-5}	5.15×10^{-5}	4.64×10^{-5}	3.10×10^{-5}	4.12×10^{-5}	4.12×10^{-5}	3.6×10^{-5}	4.98×10^{-5}	4.64×10^{-5}	4.21×10^{-6}	6.4×10^{-6}	4.0×10^{-6}	11.69	12.85	8.62
Chemotrypsin (g)	0.4674	0.2926	0.3506	0.4090	0.2337	0.2922	0.5843	0.525	0.4674	0.4869	0.3504	0.3701	0.1	0.13	0.07	20.54	37.10	18.91

SD = Standard deviation; CV = Coefficient of variation

Table 2. Differences of anti–nutritional properties of *Vigna subterranea, Phaseolus coccineus* and *Phaseolus lunatus*

Parameter	I – II			I – III			Mean			SD			CV%		
	Vigna subterranea	***Phaseolus coccineus***	***Phaseolus lunatus***	***V. subterranea***	***P. coccineus***	***P. lunatus***	***V. subterranea***	***P. coccineus***	***P. lunatus***	***V. subterranea***	***P. coccineus***	***P. lunatus***	***V. subterranea***	***P. coccineus***	***P. lunatus***
Oxalate (mg/100g)	24.4 (4.97%)	77 (15.56%)	57 (12.00%)	107.6 (21.93%)	118.8 (24.00%)	101 (21.26%)	6 6.00	97.90	79.00	41.6	20.90	22.00	63.03	21.35	27.85
Tannin (moldm^{-3})	2.06×10^{-5} (66.67%)	1.56×10^{-5} (37.86%)	2.58×10^{-5} (45.50%)	1.03×10^{-5} (33.33%)	1.03×10^{-5} (25.00%)	1.55×10^{-5} (27.34%)	1.55×10^{-5}	1.30×10^{-5}	2.07×10^{-5}	5.15×10^{-6}	2.65×10^{-6}	5.15×10^{-6}	33.23	20.38	24.88
Phytate (mg/100g)	7 (1.98%)	9.21 (2.49%)	9.3 (2.54%)	20.9 (5.91%)	18.41 (4.98%)	20.9 (5.72%)	13.95	13.81	15.10	6.95	4.60	5.80	49.82	33.31	38.41
Saponin (%)	0.4 (8.70%)	0.9 (21.95%)	0.8 (25.00%)	1.4 (30.43%)	1.4 (34.15%)	1.8 (56.25%)	0.90	1.15	1.30	0.50	0.25	0.50	55.56	21.74	38.46
Alkaloid (%)	1.8 (29.03%)	1.8 (20.93%)	2.2 (22.92%)	2.6 (41.94%)	3 (34.88%)	3.8 (39.58%)	2.20	2.40	3.00	0.40	0.60	0.80	18.18	25.00	26.67
Cyanide (moldm^{-3})	4.9×10^{-6} (11.86%)	5.2×10^{-6} (9.17%)	5.2×10^{-6} (10.08%)	1.03×10^{-5} (24.94%)	1.55×10^{-5} (27.34%)	1.04×10^{-5} (20.16%)	7.6×10^{-6}	1.04×10^{-5}	7.8×10^{-6}	2.70×10^{-6}	5.15×10^{-6}	2.6×10^{-6}	35.48	49.52	33.33
Chemotrypsin (g)	0.0584 (12.49%)	0.0589 (20.13%)	0.0584 (16.66%)	–0.1169 (–25.01%)	–0.2324 (–79.43%)	–0.1168 (–33.31%)	0.09	0.15	0.09	0.03	0.09	0.03	33.33	60.00	33.33

***I =** Raw sample; **II =** Roasted sample; **III =** Boiled sample; SD = Standard deviation; CV = Coefficient of variation*

Table 3. *In–vitro* protein digestibility of legume species before and after roasting and boiling

Sample	Raw	Roasted	Boiled	Mean	SD	CV%
Vigna subterranean	48.39	31.19	56.99	45.52	10.7	23.56
Phaseolus coccineus	56.99	22.59	65.59	48.39	18.6	38.44
Phaseolus lunatus	48.39	22.59	65.59	45.52	13.6	29.88

SD = Standard deviation; CV = Coefficient of variation

Table 4. Differences in *in–vitro* protein digestibility of legume species before and after roasting and boiling

Sample	I – II	I – III	Mean	SD	CV%
Vigna subterranea	17.2 (35.54%)	–8.6 (–17.77%)	12.90	4.30	33.33
Phaseolus coccineus	34.4 (60.36%)	–8.6 (–15.09%)	21.15	12.90	60.99
Phaseolus lunatus	25.8 (53.32%)	–17.2 (–35.54%)	21.50	4.30	20.00

I = Raw sample; II = Roasted sample; III = Boiled sample; SD = Standard deviation; CV= Coefficient of variation

The differences in anti–nutritional properties of *Vigna subterranea, Phaseolus coccineus* and *Phaseolus lunatus* are shown in Table 2. All the anti–nutritional factors were reduced in the three legume samples by both processing methods adopted in this study except chemotrypsin which was increased by 25.01, 79.43 and 33.31% in *Vigna subterranea, Phaseolus coccineus* and *Phaseolus lunatus,* respectively however only in boiling method. Oxalate has the highest coefficient of variation (63.03%) in *Vigna subterranea* while the least was recorded in alkanoid (18.18%) within the same sample (Table 2).

3.2 *In–vitro* Protein Digestibility (IVPD)

The results of *in–vitro* protein digestibility *(IVPD)* of bambara groundnut (*Vigna subterranea*), scarlet runner bean (*Phaseolus coccineus)* and lima bean (*Phaseolus lunatus*) legume seeds with respect to effect of roasting and boiling are shown in Table 3. Roasting caused significant decrease in protein digestibility from 48.39 to 31.19, from 56.99 to 22.59, and from 48.39 to 22.59 for bambara groundnut, scarlet runner bean and lima bean, respectively. The decrease in protein digestibility may be due to the low level of chymotrypsin a digestive enzyme which is usually inhibited by tannins. In contrast to roasting, boiling significantly improved protein digestibility in the three legume seeds. The magnitude of increase in IVPD, upon boiling ranged from 15.09% to 35.54% (Table 4). This agrees with the results of [48] and [51] who reported an increase in protein digestibility of *Dolichos lablab* bean and chickpea, respectively during boiling. The improvement of protein digestibility after boiling can be attributed to the reduction in antinutritional factors such as saponin, alkaloid, tannins and cyanide.

4. CONCLUSION

This study has revealed that, roasting and boiling processes resulted in a significant reduction in anti–nutritional properties of the three types of underutilized legume seeds. Roasting process was also found to have increased chemotrypsin inhibitors content in the three legume seeds which may affect protein digestibility. Boiling was also found to have improved *in–vitro* protein digestibility. These results clearly indicate that boiling may be useful in improving the nutritional quality of these legume seeds with respect to protein and carbohydrate utilization as well as mineral bioavailability.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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