



INVESTIGATIONS ON THE MYCOFLORA AND PROCESSING EFFECTS ON THE NUTRITIONAL QUALITY OF PEANUT (*ARACHIS HYPOGAEA* L. VAR. TS 32-1)

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ABSTRACT

The microbiological and nutritional characterization of peanut (*Arachis hypogea* L var. TS 32-1) was investigated. Bacteria and fungi were isolated from this product. The fungal isolates were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus* Speare, *Aspergillus ochraceus* Wilhelm and *Fusarium poae*. The respective mean moisture content and total acidity in samples were $8.19 \pm 0.01\%$ and $1.2 \pm 0.02\%$. Nutritional analysis showed that peanut (*Arachis hypogea* L var. TS 32-1) has interesting nutritional potential. Carbohydrate content ($7.84 \pm 0.3\%$), protein content ($33.88 \pm 0.1\%$), fat ($47.48 \pm 0.01\%$) and the presence of minerals such as calcium ($0.25 \pm 0.05\text{g/kg}$), potassium ($5.21 \pm 0.02\text{g/kg}$) and magnesium ($1.92 \pm 0.03\%$) allowed its application as supplement in infant feeding in rural areas. Anti-nutritional factors such as oxalate and phytate were also detected in samples. This nutritional potential is significantly affected by thermal processes which can reduce essentially protein and carbohydrate contents and also anti-nutritional factor levels. However, values were lower than established toxic level. Finally, more attention should be made to its microbial quality in order to preserve children's health.

Keywords: Peanut, proximate analysis, processing effects, anti-nutritional factor

INTRODUCTION

Peanut is a major annual oilseed crop and a good source of protein. Oil and protein content, fatty acid and amino acid composition, taste and flavour are important quality traits (Asibuo et al., 2008). Protein of peanut is increasingly becoming important as food and feed sources, especially in developing countries where protein from animal sources is not within the means of majority of the population. Vegetable oils are in high demand due to diseases associated with fat from animal origin. The seed has several such as peanut butter, oil, and other products. The groundnut cake has several uses in feed and infant food formulations (Asibuo et al., 2008). The literature has reported many health benefits associated with consumption of peanuts including cancer inhibition (Awad et al., 2000). This benefit is mainly attributed to micronutrients such as α -tocopherol, folate, minerals and health promoting phytochemicals, particularly resveratrol, ferulic acid and other phenolic compounds (Yu et al., 2004). From a microbiology point of view, several *in vitro* and *in vivo* investigations demonstrated the contamination of peanut by fungi (Novas and Cabral, 2002). Peanut is also very important in the vegetarian diet. It has as much or more protein than meat and contains no uric acid or cholesterol (Pamplona-Rogers, 2006). Locally, consumption of peanut is very popular among the population. It is mostly eaten in the roasted form throughout the year. A substantial part is also eaten in the cooked form, while fewer people indulge in the eating of the raw seeds (Ogunsanwo et al., 2004). However, peanuts contains some antinutritional factor such as phytates, condensed tannins, trypsin and amylase inhibitor, that may limit their usage and nutritional value. According to Njintang et al. (2001), traditional processing methods such as germination or roasting could improve the nutritional value of legume seeds. Offem et al. (1993) have reported changes on the chemical composition as a result of processing. However, few information on the effect of traditional processing on peanuts quality was reported. The aim of this study was to investigate the efficiency of processing methods on nutritional composition and the reduction or elimination of antinutritional factor in peanuts. An emphasis has been placed on identifying the most effective methods to reduce antinutritional factor in peanuts at domestic level.

MATERIAL AND METHODS

Materials

Peanuts (*Arachis hypogea* L var. TS 32-1) were obtained from Agronomical Research Center (CRA/Savè) of National Institute of Agronomical Research in Benin (INRAB). The whole seeds (raw) were divided into three (3) portions, one portion being designated as control and stored in a cold room à 4°C. The remaining seeds were processed by boiling or roasting.

Experimental procedures

About 0.5 kg of shelled peanuts seeds (harvest in 2011) were thoroughly sorted out and cleaned of stones, bad seeds, and other foreign objects and roasted in an aluminum saucepan. For the boiling process, about 0.5 kg of shelled peanuts seeds (2011 harvest) was washed and then heated with addition of water. After boiling, water was discharged and boiled peanuts were collected.

Determination of physicochemical parameters

Moisture content of samples was determined by desiccation using the method of **De Knegt and Brink (1998)**. A clean platinum dish was dried in an oven and cooled in a desiccator and weighed. From each sample, 5 g was weighed and spread on the dish, the dish containing the sample was weighed. It was then transferred into the air oven at 105°C to dry until a constant weight was obtained and the loss in mass was determined. In order to obtain the pH of the samples, 5 g of each sample was weighed, grinded and suspended in 10 ml of distilled water. The pH was determined with a digital pH-meter (HANNA HI 98129). Acidity of samples, expressed as citric acid content per unit of volume, was determined by titration with 0.01 mol/L of sodium hydroxide solution, using phenolphthalein as indicator (**AOAC, 1995**).

Nutritional analysis

The carbohydrate was determined according to phenol sulfuric acid method (**Agbo and Ronald, 1996; Ezoua et al., 1999**). A standard curve was obtained using the following concentration of sucrose in (mg/ml) 2.5, 2.0, 1.25, 1.0, 0.5 g of each sample with 9 ml of distilled water was measured into test-tube. 2 ml of phenol solution (1%) and 1 ml of concentrated H₂SO₄ solution were added. This was shaken for 15 min and boiled for 30 min. It was then allowed to cool. The absorbance was then read using a spectrophotometer (Spectrum lab 22) at 700 nm. The sugar concentration was then obtained by extrapolation from the standard curve. Protein was analyzed by the Microkjedhal nitrogen method, using a conversion factor of 6.25 and fat content was obtained by Soxhlet extraction as described by **Pearson (1976)**. Ash was determined according to the standard methods described by the Association of Official Analytical Chemists (**AOAC, 1995**). Minerals were analyzed by the method reported by **Oshodi (1992)**. Minerals were analyzed by dry-ashing 1 g of the sample at 550°C in a furnace. The ash obtained was dissolved in 10% HCl, filtered with filter paper and made up to standard volume with deionised water. Flame photometer was used to determine potassium content of the samples, while calcium and magnesium were determined using atomic absorption spectrophotometer (**Perkin Elmer, Model 403**).

Anti-nutritional factors analysis

Total oxalate was determined as described by **Day and Underwood (1986)**. 1 g of sample was weighed into 100 ml conical flask. 75 ml H₂SO₄ (3 mol/L) was added and stirred for 1 h with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. 25 ml of the filtrate was then taken and titrated while hot against 0.05 mol/L of KMnO₄ solution until a faint pink colour persisted for at least 30 s. The oxalate content was then calculated by taking 1 ml of 0.05 mol/L of KMnO₄ as equivalent to 2.2 mg oxalate (**Ihekoronye and Ngoddy, 1985; Chinma and Igyor, 2007**). Phytate was determined using the method of **Reddy and Love (1999)**. 4 g of each sample was soaked in 100 ml of 2% HCl for 5 h and filtered. To 25 ml of the filtrate, 5 ml of 0.3% ammonium thiocyanate solution was added. The mixture was then titrated with Iron (III) chloride solution until a brownish-yellow color that persisted for 5 min was obtained. A 4:6 Fe/P atomic ratio was used to calculate the phytic acid content (**Okon and Akpanyung, 2005**).

Microbiological analysis

To 25 g of each sample (shelled peanut), 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated at 30°C for 72 h. Desoxycholate was used for the total Coliforms count and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the Faecal coliforms count. In this case, plates were incubated at 44°C. Tryptone Sulfite Neomycin Agar was used for Anaerobic Sulfite-Reducer (ASR) count and tubes were incubated at 37°C for 24 h. After incubation, the number of colonies was tracked using a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, bearing in mind the factors of dilution (**Singh et al., 1991**). The isolation of fungi from samples was performed using dilution plating method. 10 g of each sample (shelled peanut) were separately added to 90 ml of sterile water containing 0.1% peptone water. This was thoroughly mixed to obtain the 10⁻¹ dilution. Further 10-fold serial dilutions up to 10⁻⁴ were made. One milliliter of each dilution was separately placed in Petri dishes, over which 10 to 15 ml of Potato Dextrose Agar with 60 µg/ml of chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2°C for 7 days (**Rampersad et al., 1999**). The identification of the bacterial isolates was based on cultural, morphological, and biochemical characteristics following standard methods (**Buchanan and Gibbons, 1974**) while that of fungi was also based on cultural and morphological characteristics using standard taxonomic schemes (**Singh et al., 1991; Bryce, 1992**).

Statistical analyses

Treatments were conducted in three repetitions. Macronutrients, micronutrients and antinutritional factors analysis were performed in triplicate for each treatment repetition. The data generated from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA) (**Alder and Roessler, 1977; Ogbeibu, 2005**).

RESULTS AND DISCUSSION

The results of physicochemical parameters and proximate composition of peanut (*Arachis hypogea* L var. TS 32-1) are shown in Tables 1 and 2. The moisture content, pH and acidity were respectively $8.19 \pm 0.01\%$, 6.3 ± 0.2 and $1.20 \pm 0.02\%$. Ash, protein, fat and carbohydrate content were $3.80 \pm 0.06\%$, $33.88 \pm 0.12\%$, $47.48 \pm 0.01\%$ and $7.84 \pm 0.30\%$, respectively. All samples analyzed were also rich in minerals such as calcium, magnesium and potassium, with a higher content of potassium ($5.21 \pm 0.02\%$) (Table 3). The analysis of anti-nutritional factors (Table 4) also revealed the presence of oxalate ($0.108 \pm 0.040\%$) and phytate ($0.51 \pm 0.09\%$). The total flora count of unprocessed peanut sample (*Arachis hypogea* L var. TS 32-1) was 2×10^1 ufc/g. The enumeration of total coliforms and fecal coliforms was less than 10 cfu/g with an absence of spores of anaerobic sulfite reducers (ASR) (Table 5). Fungal flora was high (9×10^1 cfu/g) with the presence of fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus* Speare, *Aspergillus ochraceus* Wilhelm, *Fusarium poae* (Table 5). Compared to the raw seed, results obtained from the proximate analysis of processed peanut samples depicted remarkable variation in the chemical composition of seeds ($p < 0.05$), depending on the type of process. On roasting of peanuts, ash, mineral content and fat levels did not change significantly, but there was a significant decrease in protein, total carbohydrate, moisture and antinutritional factor such as oxalate and phytate. On boiled peanuts, mineral content, protein, total carbohydrate and also antinutritional factors levels were significantly decreased. The microbiological analyses revealed that microbial contamination of all processed peanuts was very low with the absence of pathogens.

The high nutritional potential of peanut such as its proteins, fat, carbohydrates and its mineral contents (Tables 2 and 3), justified its uses as supplement in infant feeding in Benin. These findings are in agreement with those of **Asibuo et al. (2008)** who also underlined the interesting nutritional potential of peanut from Ghana.

The elevated fat content was in line with the observation of **Dwivedi et al. (1994)** who reported that oil content of peanut ranged from 44 to 56%. This result suggested that it is an interesting oleaginous crop for which the implementation of improved cropping systems should result in the economic well-being of rural people. Several studies have shown that the nutritional value of oil is mainly related to its content of fatty acids. Indeed, **Asibuo et al. (2008)** reported that the oleic and linoleic acid content of the peanut accounted for 75.30 to 81.05% of the total fatty acids. High level of oleic acid implies high oil stability and better shelf life of groundnut seeds and products because oleic acid is a monounsaturated fatty acid,

being less prone to oxidative rancidity (**Rahman et al., 2001**). According to **FAO (1994)**, during the first years of life, fat is the main source of energy needed for proper growth and physical activity. Where diets are low in fat (less than 15 % of total calories), it is difficult to ensure the energy required, which partly explains the high prevalence of malnutrition in these areas. Fats also play a structural role in providing fatty acids and cholesterol for the formation of cell membranes in all organs. Moreover, important organs such as the retina of the eye and central nervous system are mainly composed of fat. Much of fat necessary for the formation of these tissues are essential fatty acids, which cannot be synthesized by the body and must be supplied by the diet. Breast milk has fat composition very special, which explains its unique role in infant feeding. In the low-income groups the fat intake is insufficient and that this lower consumption has a negative effect on the nutritional status of children and adults in these groups.

Seed protein content was higher than cowpea which contains about 24% seed protein (**IITA, 1989**). Cowpea and peanut are the major protein sources to the poor and rural dwellers and these results also demonstrate that peanut is a valuable source of protein for improving the nutrition of humans. Indeed, from birth to age 4 months, all the nutritional needs of children are fully covered in milk. But between 4 and 6 months breast milk is not sufficient to cover the needs for energy and protein of the child. This is the period during which nutrients necessary for child growth must supplement the breast milk slurry (**Claeson et al., 2001**). Quantitative protein requirements are about 20 g per day between 6 months and 3 years. Ideally, the amino acid composition of these complementary proteins should be identical to that of breast milk that is containing the same proportion of the nine essential amino acids (**Hedley et al., 2004**). Fortunately, it is possible to reconstruct a protein mixture composition meeting the needs of the child by mixing cereal flour with legume flour.

Minerals are also important in human nutrition. It is well known that enzymatic activities as well as electrolyte balance of the blood fluid are related to adequacy of Na, K and Mg. Potassium is very important in maintaining the body fluid volume and osmotic equilibrium. Metal deficiency syndrome like rickets and calcification of bones is caused by calcium deficiency. Peanut samples analyzed have good and nutritional valuable minerals whose importance had already been emphasized (**Bowen, 1966; Bender, 1992**). However, the availability of these nutrients after ingestion depends on the antinutritional factors present in the food. The antinutrients tend to bind to mineral elements there by forming indigestible complex. Oxalate for instance binds to calcium to form complexes (calcium oxalate crystals). These oxalate crystals formed prevent the absorption and utilization of calcium. The calcium

crystals may also precipitate around the renal tubules thereby causing renal stones (**Ladeji et al., 2004**). Phytic acid (inositol hexaphosphate) is an organic acid found in plant materials (**Heldt, 1997**). Phytic acid combines with some essential elements to form insoluble salts called phytate. Phytates reduces the availability of many minerals like calcium, magnesium, iron and zinc. The ability of phytate to form complexes with these mineral can make the mineral content of a food inadequate especially for children (**Ilelaboye and Pikuda, 2009**). Phytate are also found to inhibit the protease and amylase of the intestinal tract (**Vaintraub and Bulmaga, 1991**). **Sanberg (1991)** reported that the minimum amount of phytate to cause negative effect on iron and zinc absorption were 10-50 mg per meal. Thereby, anemia and other mineral deficiency disorders are common in regions where the diet is primarily a vegetarian (**Erdman, 1979**).

The results obtained from microbial analysis, show that shelled peanuts were contaminated with microorganisms of public health concern. The most dominant flora was fungi, especially *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus* Speare, *Aspergillus ochraceus* Wilhelm and *Fusarium poae*. These fungi species are known spore formers and their growth can result in the production and accumulation of mycotoxins. The moisture content of samples would also encourage microbial growth and so deterioration. Peanut contamination by fungi does not only reduce its quality but may also lead to mycotoxin production (**Sultan and Magan, 2010**). According to **Pittet (1998)**, the mycotoxins produced by *Aspergillus spp.* of greatest significance in peanuts include aflatoxins and ochratoxin A (OTA). Several studies have reported the contamination of peanuts or peanut products by fungi (**Pildain et al., 2008; Fagbohun and Faleye, 2012**), and by mycotoxins, especially aflatoxins and ochratoxin A (**Ediage et al., 2011, Adjou et al., 2012**). The high susceptibility of peanuts contamination is mainly due to their nutritional content, useful to numerous fungi. If the hulls, which protect the seed against invasion by fungi, become damaged, the underlying cotyledons become susceptible to attack. This contamination, mainly due to the injury of the hulls, is favored by insect attack, drought occurring at the end of the vegetative cycle and poor harvesting practices.

Although some studies have been made on chemical change of peanut after germination (**Offem et al., 1993**), roasting (**Soliman et al., 1985**) or during storage (**Fagbohun and Faleye, 2012**), the present study constituted a comprehensive investigation on the influence of thermal process on the chemical composition, nutritional value and microbiological quality of peanut. Results obtained from the proximate analysis of processed

peanut samples depicted remarkable variation in the chemical composition of seeds compared to the unprocessed seeds.

The increase in moisture content of processed samples could be due to the increased imbibitions of water with boiling time. **Muller (1988)** explained that during boiling, cellulose is little affected but the middle lamella gets broken down by heat, thus making vegetables to take up water as the starch gelatinizes. The decrease in crude protein content could be attributed to leaching effect. **Gernah and Ajir (2007)** also reported a decrease in protein content with the boiling of cassava leaves. However, at the protein content of 27.02 % (roasted peanut) and 22.26 % (boiled peanut) processed peanuts are considered a very good protein source as compared to other everyday foods like eggs (12.00%), white bread (7.80%), rice (6.50%), milk (3.30%), and potatoes (2.10%) as reported by **Gamman and Sherrington (1990)**. The significant decrease of protein, carbohydrate and antinutritional factor observed after processing of peanut seeds can also resulted in the effect of heat or some migration of substances from peanuts to boiling water. According to **Ejigui et al., (2005)**, heat processing may have destroyed some of heat-labile components in food. Indeed, significant decreases were observed in antinutritional factor levels after processing. Their reduction was greater in boiling than roasted processes. **Alonso et al. (2000)** also observed a decrease of phytate in kidney beans after processing. This reduction may be due to the hydrolysis and heat destruction of some molecules of inositol hexaphosphate to penta, tetra and triphosphate (**Alonso et al., 2000**). These findings indicated that moist heat is more effective in reducing antinutritional factor levels in food and foodstuffs than dry heat. This is in accordance with the report of **Ejigui et al., (2005)**. However, the values obtained for peanut (*Arachis hypogea* L var. TS 32-1) were below the established toxic level.

Table 1 Physicochemical parameters of processed peanuts

Item	Moisture (%)	pH	Acidity (%)
Control (Unprocessed)	8.19 ± 0.01 ^a	6.3 ± 0.2 ^a	1.20 ± 0.02 ^a
Roasted peanut	7.10 ± 0.06 ^b	6.4 ± 0.1 ^a	1.40 ± 0.05 ^a
Boiled peanut	10.90 ± 0.02 ^c	6.1 ± 0.5 ^a	1.10 ± 0.02 ^a

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 2 Nutritional content of processed peanuts

Item	Carbohydrate (%)	Protein (%)	Fat (%)	Ash (%)
Control (Unprocessed)	7.84 ±0.30 ^a	33.88 ±0.10 ^a	47.48 ± 0.01 ^a	3.80 ± 0.06 ^a
Roasted peanut	5.42 ±0.50 ^b	27.02±0.30 ^b	47.33 ± 0.03 ^a	3.70 ± 0.03 ^a
Boiled peanut	4.21 ±0.10 ^c	22.26±0.50 ^c	47.39 ± 0.07 ^a	3.80 ± 0.01 ^a

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 3 Minerals content of processed peanuts

Item	Magnesium (g/kg)	Calcium (g/kg)	Potassium (g/kg)
Control (Unprocessed)	1.92±0.03 ^a	0.25 ±0.05 ^a	5.21±0.02 ^a
Roasted peanut	1.91±0.02 ^a	0.24 ±0.01 ^a	5.23±0.04 ^a
Boiled peanut	1.87±0.05 ^b	0.19 ±0.03 ^b	5.18±0.01 ^b

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 4 Antinutritional factors content of processed peanuts

Item	Percentage composition	
	Oxalate	Phytate
Control (Unprocessed)	0.108 ± 0.040 ^a	0.51 ± 0.09 ^a
Roasted peanut	0.064± 0.080 ^b	0.15 ± 0.08 ^b
Boiled peanut	0.043± 0.060 ^c	0.14 ± 0.04 ^c

Values are mean (n = 3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 5 Microbial count of processed peanuts (cfu/g)

Item	Total bacterial count	Total coliforms count	Faecal coliforms count	A.S.R spores count	Mould and yeast count
Control (Unprocessed)	1x10 ²	06	02	00	9x10 ¹
Roasted peanut	00	00	00	00	00
Boiled peanut	00	00	00	00	00
European union Criteria (2005)	-	10	10	Absence/10 g	Absence/10 g

A.S.R: Anaerobic Sulfito-Reducer

CONCLUSION

This work underlined the nutritional potentiality of peanut (*Arachis hypogea* L var. TS 32-1) and important effect of thermal processes in reducing of antinutritional factor levels and also affected the mycoflora associated with peanut. However, more attention should be paid to their secondary metabolites (mycotoxins) which are sometimes heat-resistant.

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