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Evaluation of the Yeild, Protein Content and Functional Properties of Mungbean [*Vigna radiata* (L.) Wilczek] Protein Isolates as Affected by Processing

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Abstract: The effect of processing on mungbean protein isolate yield, protein content and functional properties were evaluated. The functional properties evaluated were Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), Emulsion Capacity (EC) and nitrogen solubility. The protein isolate yield and protein content from the raw mungbean flour were 10.52 g protein/100 g flour and 87.56% respectively. Processing had significant ($p<0.05$) effect on the isolate yield, protein content and functional properties. The toasted flours yielded the highest protein isolates (10.68-8.48%), although there was progressive decrease in isolate yield as toasting time increased. Increase in sprouting time resulted to a significant ($p<0.05$) increase in isolate yield, however, no significant ($p>0.05$) decrease was observed for the protein content. Increase in boiling time markedly reduced both isolate yield (6.41-5.80%) and protein content (86.10-32.84%) respectively. The mungbean protein isolates from 60min boiled flour had the highest WAC (2.5 g/g), OAC (2.15 ml/g) and EC (22.16%) while the isolates from 90min toasted flour had the highest WAC (2.97 g/g), OAC (2.25 ml/g) and EC (18.92%). Isolates from 24 h sprouted flour gave the highest WAC (1.75 g/g), OAC (1.25 ml/g) and EC (10.96%). The functional properties of the mungbean protein isolates were significantly ($p<0.05$) improved by processing and the high solubility indicates its suitability for industrial application as protein supplements.

Key words: Mungbeans, protein isolates, functional properties, processing

INTRODUCTION

Since legume seeds are important sources of protein, complex carbohydrate and dietary fibre in the diet, there has been a worldwide interest in searching for potential utilization of unconventional legumes (Siddhuraju *et al*., 1996). Mungbean [*Vigna radiata* (L) Wilczek] is one of the lesser known legumes which have not been fully utilized to alleviate the problem of protein malnutrition common in developing countries of the world. The consumption of mung proteins can fulfil the essential amino acids requirements with the exception of the sulphur-containing amino acids (Khalil, 2006). A large section of the population of most developing countries have inadequate protein intake. However, utilization of legume protein is below their potential partly due to the deficiency of some essential amino acids in their proteins and also due to the presence of some antinutritional factors associated with their proteins (Kavas and Nehir, 1992).

In order to produce a high protein food, the protein in a legume may have to be concentrated and isolated from the legume (Udensi and Okonkwo, 2006). Protein isolation and fractionalization is aimed at separating the protein from other components in such a form that they remain (as much as possible) fully undenatured and thus retain their functionality (Zadow, 1993). Plant protein isolates are the most refined forms of proteins. Bean protein isolates are incorporated in food systems where heat treatment will be involved in order to eliminate or reduce significantly, the antinutrients contained in them. Isolated proteins often have improved appearance and taste compared with the original meal; therefore they can better be used as nutritional and functional ingredients in many food products (Mizrahi *et al*., 1967). Furthermore, they contain much higher protein content than the flour, or meal so the same supplementation can be obtained with smaller admixtures (Thompson, 1977; Soestrino, 2007).

The protein isolates from legumes have been used in various food formulations: according to Thompson (1977), mungbean protein isolates were used as protein supplement in bread flour mixtures. Yellow pea, lentil and faba bean protein isolate both from germinated and ungerminated seeds, had been reported by Hsu *et al*. (1982) to be used for replacement of 3, 5 or 8% wheat flour in bread making. Soy isolates are mainly used to improve the texture of meat products, but are also used to increase protein content, enhance flavour and as an

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emulsifier. It is also combined with other ingredients in the food industry (http://wikipedia.org, 3/5/2008), for instance, extruded rice flour fortified with soy isolates has been reported by Noguchi *et al*. (1981.
Mustafa *et al*. (1986) succeeded in producing acceptable bread and cookies with 10 and 20% cowpea isolates fortified flour. Peanut protein isolates from three different varieties (Conkerton and Ory, 1976) were used as protein supplementation in pineapple juice, as an acid type beverage at 1% level and there was no difference in flavour, texture, or aroma although the turbidity was slightly increased. Nevertheless, the use of Mungbean as a protein supplement is limited by the beany flavour and dark colour it imparts on the final product. This problem could probably be overcome by the use of Mungbean protein isolates. Mungbean protein isolate has been shown to perform many desirable functions in processed foods, such as foaming, emulsification and water absorption (El-Adawy, 2000). However, improvements in those functions would make mungbean protein isolate more desirable as a food component (El-Adawy, 2000). Therefore the aim of this study is to evaluate the effect of processing on the yield, protein content and functional properties of mungbean protein isolates.

MATERIALS AND METHODS

The mungbeans [*Vigna radiata* (L.) Wilczek] seeds were obtained from the Crop Science Department of Michael Okpara University of Agriculture, Umudike, Nigeria. All chemical reagents used for the experiment were of analytical grade.

Seeds pretreatment: The dry cleaned mungbean seeds were dehulled. The dehulled sample was further sub-divided into three sets; one was kept raw (untreated), the second set was boiled, the third set was toasted. The fourth set was germinated (sprouted) and then carefully dehulled. All these treatments were given to the mungbeans at different intervals after soaking for 12 h. The mungbean seeds were crushed to smaller fragments with the corona manual grinder after drying in the oven (65°C) and afterwards milled with a blender, using an 80 mesh sieve to sift the flour. The coarse particles were re-milled to obtain finer flour. All flour samples were stored in air-tight plastic bags until required for analysis.

Flour processing

Boiling: Three separate batches of the whole mungbeans [*Vigna radiata* (L) Wilczek] weighing 800g each were soaked in distilled water (1:3w/v) for 12 h at room temperature (~25°C) according to Mubarak (2005) and Khalil (2006) with slight modification in time of boiling. The seeds were drained and rinsed three times with 600 ml distilled water, dehulled and then boiled in tap water (100°C) in the ratio of 1:10 (w/v) on a hot plate for 30, 45, 60 and 90 min respectively. The water was drained off after each timing and the seeds dried in the oven at 65°C and cooled in a desiccator. The seeds were then dry milled, sifted with 80mesh sieve and packaged for analysis thereafter.

Dehulling: The hulls were removed manually after soaking the mungbean seeds for 12 h in distilled water (1:10w/v) according to El-Beltagy (1996).

Toasting: Three separate batches of the dehulled seeds weighing 800 g each were spread thinly in a pan and oven-dried at a fixed temperature of 120°C for time variables of 30, 45, 60 and 90 min. They were stirred intermittently to maintain uniform heating and then cooled in a desiccator after the toasting. The seeds were milled, sifted with 80mesh sieve and packaged for analysis thereafter (Emenalom and Udedibie, 2005) with slight modification in time of toasting.

Sprouting: The germination was carried out by spreading the undehulled seeds soaked in distilled water (1:3 w/v) for 12 h at room temperature (~25°C), weighing 800 g in between jute cloth and allowed to sprout in the dark for 24 and 36 h respectively. The seeds were kept wet throughout germination by spraying them with distilled water every 12 h. The sprouted mungbeans were harvested, rinsed twice in distilled water, carefully dehulled and oven dried at 65°C for 9 h and then cooled.
The dried seeds were subjected to dry milling and passed through 80 mesh sieve. The flour was cooled and packaged for analysis thereafter in air-tight plastic bags.

Preparation of mungbean protein isolates: Protein isolate was prepared using the methods described by El-Adawy (2000) with slight modification in the temperature and duration of shaking as shown in Fig. 2. Dispersions of 10% (w/v) mungbean flour in water were adjusted to pH of 9 with 0.1N NaOH at room temperature (~30°C), shaken for 1 hour and centrifuged for 15 min at 2000xg.
In order to obtain increased yields, the extraction and centrifugation procedures were repeated on the residue. The extracts were combined and the pH adjusted to 4.5 with 1N HCl to precipitate the protein. The proteins were recovered by centrifugation at 2000xg for 15 min followed by removal of the supernatant by decantation.
Protein curd was washed with distilled water and the curd was re-dispersed in distilled water (El-Adawy,

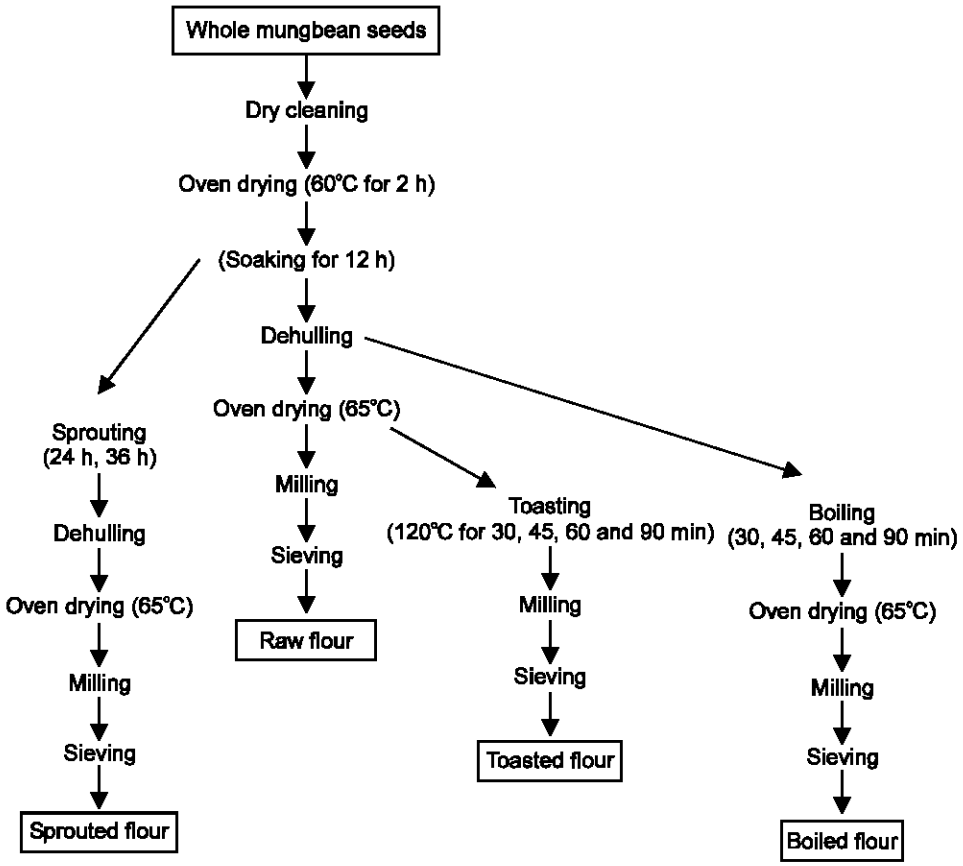

Fig. 1: Flow chart of the dehulled mungbean flour samples

2000). The resulting protein curd was separated by filtration with filter paper and washed three times using distilled water. Later the slurry was scraped out and placed in a moisture drying can and dried for 3 hours in a low temperature dryer (40°C). After drying, it was ground into fine powder using ceramic mortar and pestle (Udensi and Okonkwo, 2006). The protein isolate samples were analyzed for protein content using the micro Kjeldhal method reported by AOAC (1990). Oil and Water absorption capacities were determined using the method of Okezie and Bello (1988). Emulsion capacity was determined with the method described by Onimawo and Egbekun (1998). For nitrogen solubility studies, 1% water extract of each protein isolate sample was prepared by weighing 1g into 10ml of distilled water in a beaker, at 24°C and used for the assay. Each sample was adjusted to a given value - pH 2, 4, 6, 8 using either 1.0N NaOH or 0.1N HCl as appropriate (Fan and Sosulski, 1974). Samples were extracted at 24°C for 30 min using a magnetic stirrer and centrifuged for 15 min at 2000 rpm. Then, the nitrogen in the supernatant was estimated using the standard micro-Kjeldahl technique (AOAC, 1990). Results represent analysis of duplicate protein isolate samples.

The software package used for the statistical analysis was the version 15 of SPSS while all the analyses were carried out in three replicates. The data were evaluated for significant differences ($p = 0.05$) in their means using Analysis of Variance (ANOVA). Differences between means were separated using Duncan's Multiple Range Tests (DMRT).

RESULTS AND DISCUSSION

The effect of some processing treatments on the yield of mungbean protein isolates are presented in Table 1. The average yield of protein isolated from the raw mungbean flour was 10.52 g protein/100 g of flour. Similar result was obtained for raw mungbean protein isolate (13 g protein/100 g flour) by El-Adawy (2000). Increase in boiling time of the mungbean seed resulted to progressive decrease in the protein isolate yield from the flour. It is however, noteworthy that the toasting treatments yielded higher protein isolates than boiling and sprouting treatments. Although, increase in toasting time resulted to a progressive reduction in the protein yield, the decrease in protein yield may be mainly due to thermal degradation of the proteins (Adebowale, 2008). Sathe *et al.* (1982) also reported the formation of insoluble aggregates with sulphur-rich proteins in soybean flour when heated to 70°C and above.

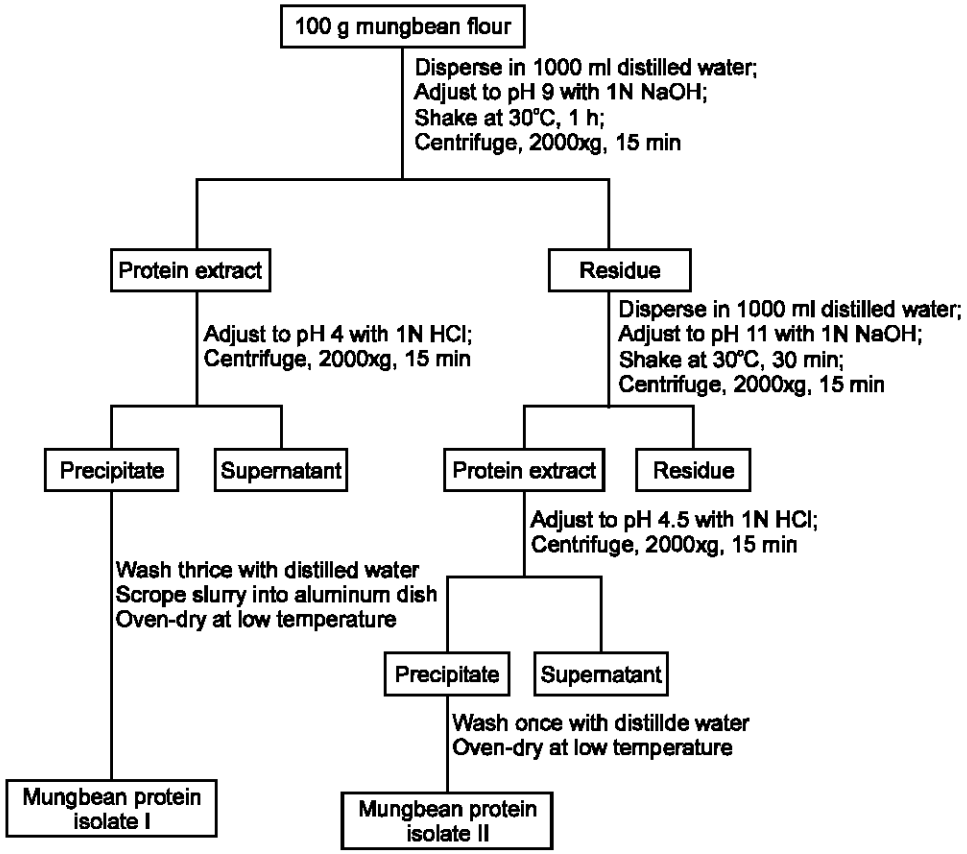

Fig. 2: Preparation of mungbean protein isolates

Table 1: Effect of some processing treatments on the protein isolation from mungbean flour at pH 9.0[a] and pH 4.0[b]

Flour sample code	Sample	Average weight of P.I. 25 g of mungbean flour	Protein isolates (P.I) yield (%)
RD	Raw dehulled	2.63^{b}±0.01	10.52^{b}±0.01
BD_{30}	Boiled for 30 min	1.61^{g}±0.02	6.41^{g}±0.01
BD_{45}	Boiled for 45 min	1.59^{g}±0.01	6.32^{g}±0.01
BD_{60}	Boiled for 60 min	1.45^{h}±0.03	5.81^{h}±0.01
BD_{90}	Boiled for 90 min	1.45^{h}±0.03	5.80^{h}±0.01
TD_{30}	Toasted for 30 min	2.67^{a}±0.02	10.68^{a}±0.01
TD_{45}	Toasted for 45 min	2.29^{c}±0.01	9.16^{c}±0.02
TD_{60}	Toasted for 60 min	2.28^{c}±0.02	9.12^{c}±0.02
TD_{90}	Toasted for 90 min	2.12^{d}±0.01	8.48^{d}±0.02
SpD_{24}	Sprouted for 24 h	1.65^{f}±0.01	6.60^{f}±0.01
SpD_{36}	Sprouted for 36 h	1.88^{e}±0.02	7.52^{e}±0.02

Means are values of triplicate determinations;
[a]Unless specified, the extractions were at 30°C for 30 min using a mungbean flour to solvent ratio of 1:15.
[b]Unless specified, isoelectric precipitation of the extracted proteins were at the pH of 4.0.
Means on the same column with different superscripts are significantly different ($p<0.05$)

Since the extraction was carried out in aqueous medium, the decrease in the extraction of protein might be due to reduced solubility of the protein with increase in temperature due to coagulation. Thompson (1977) observed a decrease in solubility at 80°C due to partial coagulation of the proteins.

However, increase in sprouting time led to a significant ($p<0.05$) increase in the protein isolate yield of the flour. This may be explained on the basis of increased solubility of the protein with increase in sprouting time. Also, increase in sprouting time was observed to increase extraction of more proteins. The protein

extraction obeys the Well mechanism for solid-liquid mass transfer which assumes that solid extraction from a solid-liquid ratio increases until a position of equilibrium is attained, after which the extraction of solute remains relatively constant (Prabhudesal, 1988). Many factors affect the extractability of protein, including particle size and quality of flour, solvent-to-flour ratio, pH and temperature during extraction, ionic strength or addition of salts into extractant (Kinsella, 1979). However, it is important to recover as much protein as possible during extraction in order to get maximum protein content in the concentrate or isolate products.

Table 2 shows the effect of some processing treatments on the protein content of the mungbean protein isolates. The protein content of the raw mungbean protein isolate was 87.56% which was similar to the result (88%) obtained by Fan and Sosulski (1974) and lower than the 92% protein content observed by Thompson (1977) after a repeated extraction. Increase in boiling treatments resulted to a progressive reduction in the protein content of the protein isolates (86.10-32.48%). This might be due to protein denaturation and leaching during the boiling of the mungbean seeds. The toasted mungbean protein isolates had lower protein contents with further reduction (74.43-22.62%) as the toasting time was increased. Lower protein solubilities as a result of thermal denaturation and polymerization of amino acids (Kato *et al*., 1985) may be accountable for the lower protein content of the toasted isolates. The sprouting treatments (24 h and 36 h) increased the protein content of the isolates (85.37% and 83.18% respectively). The sprouted flours had high protein contents and solubilities hence the high protein content of the isolates. This study has proved mungbean protein isolates to have good effect in food applications.

In Table 3, the effect of some processing treatments on the functional properties of mungbean [*Vigna radiata* (L) Wilczek] protein isolates was presented.

Table 2: Effect of some processing treatments on the protein composition of the mungbean protein isolates

Protein isolates (P.I) from mungbean flour	Protein composition of the protein isolates
Raw dehulled	87.56^{a}
Boiled	
30 min	86.10^{a}
45 min	81.72^{a}
60 min	44.51^{b}
90 min	32.84^{bc}
Toasted (120°C)	
30 min	74.43^{a}
45 min	39.41^{bc}
60 min	32.84^{bc}
90 min	22.62^{c}
Sprouted	
24 h	85.37^{a}
36 h	83.18^{a}

Values are means of triplicate determinations.
Means with different superscripts on the same column are significantly different ($p<0.05$)

The Water Absorption Capacity (WAC) of the raw mungbean protein isolates (1.12 g/g) was observed to be significantly different ($p<0.05$) from the protein isolates of the processed flours. Mesallam and Hamza (1987) reported a higher value of 2.26 g/g WAC for green gram (*Phaseolus aureus*) protein isolates. Much higher WAC values have been reported for some legumes protein isolates; winged bean isolate (5.00 g/g) and soy isolate (4.10 g/g) (Okezie and Bello, 1988). Mucuna bean protein isolates (6.00 g/g) (Udensi and Okonkwo, 2006). However, water absorption capacity, lower than the value 1.0 g/g, reported for succinlylated and acetylated mungbean protein isolates has been observed for untreated mungbean protein isolate by El-Adawy (2000). The boiling and toasting treatments were observed to significantly ($p<0.05$) increase the water absorption capacity of the protein isolates with higher values obtained in the toasted protein isolates. This could be

Table 3: Effect of some processing treatments on the functional properties of mungbean [*Vigna radiata* (L) Wilczek] protein isolates

Samples	Water absorption capacity (WAC), g/g	Oil absorption capacity (OAC), ml/g	Emulsification capacity (EC), %
Raw dehulled	1.12^{e}±0.25	1.05^{d}±0.05	19.15^{c}±0.03
Boiled			
30 min	1.55^{d}±0.05	1.75^{c}±0.25	21.45^{b}±0.62
45 min	1.33^{de}±0.29	2.15^{ab}±0.05	18.76^{d}±0.07
60 min	2.50^{b}±0.17	1.88^{bc}±0.08	22.16^{a}±0.04
90 min	1.95^{c}±0.05	1.12^{d}±0.03	16.73^{g}±0.07
Toasted (120°C)			
30 min	2.45^{b}±0.05	1.20^{d}±0.00	9.48^{i}±0.62
45 min	2.45^{b}±0.05	2.25^{a}±0.25	18.24^{e}±0.08
60 min	1.93^{c}±0.29	1.82^{c}±0.10	17.87^{f}±0.03
90 min	2.97^{a}±0.29	1.63^{c}±0.11	18.92$^{c\,d}$±0.02
Sprouted			
24 h	1.75^{c}±0.05	1.25^{d}±0.25	10.96^{h}±0.04
36 h	1.65^{c}±0.05	0.75^{e}±0.25	7.30^{j}±0.04

Values are the means ± standard deviation of triplicate determinations.
Means with different superscripts on the same column are significantly different ($p>0.05$)

explained on the basis of the fact that when a protein is heated, the bonds that maintain its secondary and tertiary structures are weakened and at some temperatures, broken. This breaking of non-covalent bonds with its resulting alteration of protein structure is termed denaturation. The early stages of thermal denaturation cause most protein molecules to begin to unfold which often lead to slight increase in the amount of water to interact with the charged groups. At some temperature, the attractive forces will have been weakened enough to allow extensive water-ion interactions. This causes an unfolding of the molecule and an increase in water binding (Feeney *et al*., 1982; El-Adawy, 2000)

The sprouting treatments were also observed to significantly ($p<0.05$) increase the WAC of the protein isolates although there was no significant difference ($p>0.05$) between the WAC of the 24 h and 36 h sprouted protein isolates (1.75 g/g and 1.65 g/g respectively). This increase in WAC might have resulted from the hydration of the mungbean seeds during soaking and sprouting which in turn unfolds the protein, thereby increasing its hydrophilic binding sites and exposing them to the aqueous phase. Udensi and Okonkwo (2006) reported a higher value (7.00 g/g) for 24 h germinated Mucuna bean protein isolates.

The Oil Absorption Capacity (OAC) of the raw mungbean protein isolates (1.05 ml/g) was observed to significantly ($p<0.05$) differ from the protein isolates of the processed flours. A higher value of oil absorption capacity (1.24 g/g) was reported for raw green gram (*Phaseolus aureus*) protein isolate by Mesallam and Hamza (1987). The values obtained for winged bean isolate (9.65 g/g); soy isolate (4.88 g/g) (Okezie and Bello, 1988) and Mucuna bean isolate (2.20 g/g) (Udensi and Okonkwo, 2006) were much higher than the OAC of mungbean protein isolates.

Oil absorption by the mungbean protein isolates were found to be significantly ($p<0.05$) increased by the boiling and toasting treatments. The difference in oil absorption could be attributable to temperature-protein interaction. The highest values of oil absorption capacity were observed in 45 min toasted protein isolates (2.25 ml/g) and 45 min boiled protein isolates (2.15 ml/g) (Table 3).

The hydrophobic portions of protein can interact with lipids during protein unfolding causing increased absorption of lipids. The oil absorption capacity is affected by several factors, such as the protein content, the surface area, the hydrophobicity, the charge and topography, the liquidity of the oil and the method used (El-Adawy, 2000). Also, oil absorption capacity of protein may depend on its capacity to entrap the oil (Kinsella, 1976).

The 24 h sprouting treatment significantly ($p<0.05$) increased the oil absorption capacity (1.25 ml/g) of the mungbean protein isolate whereas a slight reduction was observed in the 36h sprouted protein isolate (0.75 ml/g). Udensi and Okonkwo (2006) reported a significant ($p<0.05$) reduction in the OAC of the 24 h germinated protein isolate.

Significant differences ($p<0.05$) existed among the emulsifying capacity of the raw (19.15%) and processed mungbean protein isolates. Mesallam and Hamza (1987) reported an emulsion capacity of 31.4g/g for raw green gram protein isolates. The emulsion capacity of the mungbean protein isolates was markedly increased by 30 min and 60 min boiling but slightly reduced by 45 min and 90 min boiling treatment (Table 3).

However, the toasting treatments significantly ($p<0.05$) reduced the emulsion capacity of the protein isolates. Sprouting treatments also significantly reduced the emulsion capacity of the mungbean protein isolates.

A contrary observation was made by Hsu *et al*. (1982) on the emulsion capacity of the protein isolates from germinated yellow peas, lentils, and faba beans. They reported that protein isolates from those germinated legumes had higher emulsion capacity although the protein isolate from germinated pea or lentils gave severe syneresis. This could be adduced to the increases in small subunit proteins after germination (Soestrino, 2007).

Lower value (12.5 mg/g) obtained for Mucuna bean protein isolate (Udensi and Okoronkwo, 2006) was similar to that (12.9%) reported for winged bean protein isolate by Okezie and Bello (1988) but higher than that obtained for soy isolate (8.00%).

The observed increase in the emulsifying properties of the boiled protein isolates could be due to the unfolding of protein chains, thereby exposing hydrophilic residues of peptides (Feeney *et al*., 1982) which causes an improvement in emulsifying capacity. The value of some treated protein isolates could be as a result of insufficient exposure of the hydrophilic protein to the aqueous phase or due to insufficient unfolding for the hydrophobic groups of the protein to contact the lipid phase. Once a protein molecule reaches the surface (fat/water interface), it must be able to unfold enough to expose hydrophobic groups if it is to function as an emulsifier. In theory, a measure of relative hydrophobicity of a protein should be related to its ability to function as an emulsifying agent (http://www.yahoo.com–protein functionality-11/06/2009).

The industrial application of proteins such as in the production of fibers, adhesives, ingredients of coating, emulsifiers, food additives and different food products depend upon bringing proteinous materials into solution. Hence, the knowledge of protein solubility will be an important factor in selecting particular vegetable proteins for possible industrial application (Adebowale, 2008).

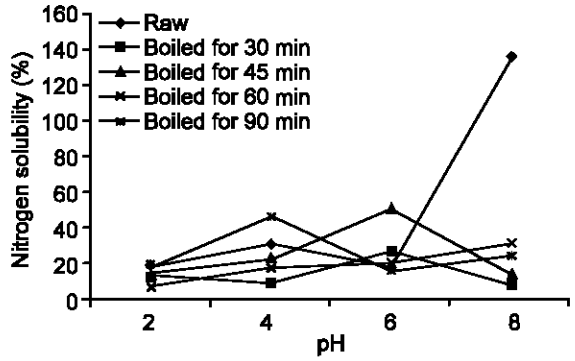

Fig. 3: Effect of boiling at different intervals on the nitrogen solubility of mungbean protein isolates

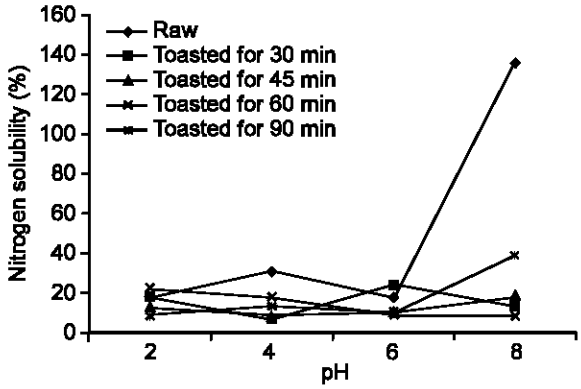

Fig. 4: Effect of Toasting at different intervals on the nitrogen solubility of mungbean protein isolates

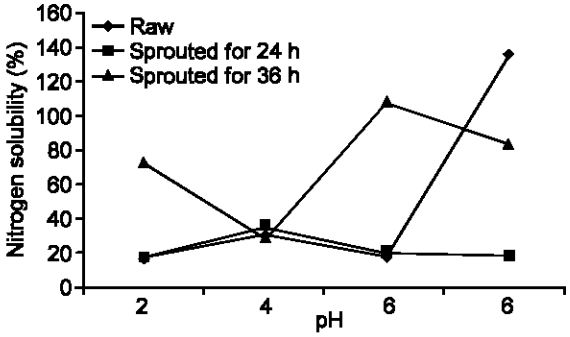

Fig. 5: Effect of Sprouting at different intervals on the nitrogen solubility of mungbean protein isolates

The pH-dependent protein solubility profile of the mungbeans protein isolate samples is presented in Fig. 3-5. Apparently, the isoelectric point of the proteins was found to be at pH 4-4.5. For the raw protein isolates, the pattern showed highest nitrogen solubility at the alkaline than acid pH with minimum solubility at the pH 2. Earlier reports of Hang *et al.* (1970) and Thompson (1977) had indicated similar results in the nitrogen solubility of mungbean protein isolates. The boiling treatments had varying effects on the nitrogen solubility of the protein isolates (Fig. 3). They had higher solubility at alkaline pH except the protein isolate from 90 min boiled flour which had higher solubility at pH 4 and least solubility at pH 6. However, it was observed that protein isolates from 30 and 45 min boiled flours had their least nitrogen solubility at pH 8 where as the protein isolate from 60 min boiled flour has its least solubility at acidic pH of 2. The effect of toasting treatment at varying times on the nitrogen solubility of protein isolates was shown in Fig. 4. For protein isolates from 30 minutes and 45 min toasted flours, the solubility reduced as pH increased and approached the isoelectric point; this was followed by progressive increase in solubility with further increase in pH.

Similar observation was reported for wing bean, chickpea and Mucuna bean protein isolates (Sathe *et al.*, 1982; Sanchez-Vioque *et al.*, 1999; Adebowale *et al.*, 2005). The protein isolates from 60 min toasted flour had its highest solubility at an acid pH 2 and the least at alkaline pH 8 while the protein isolate from 90 min toasted flour had its highest solubility at alkaline pH 8 and the least at acid pH 2. As shown in Fig. 5, the 24h sprouted flour gave protein isolates with highest nitrogen solubility at pH 4 whereas the 36 h sprouted flour yielded protein isolates with its highest solubility at alkaline pH 6. The solubility profile of a protein provides some insight into the extent of denaturation or irreversible aggregation and precipitation which might have occurred during the isolation process. It also gives an indication of how the protein could be incorporated. According to Kinsella (1976), since protein solubility affects other functionalities like emulsification, foaming and gelation, the high solubility of the mungbean protein isolates indicates that they have good functionality and could have promising food application in Nigeria for instance as protein supplements.

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REFERENCES

Adebowale, Y.A., I.A. Adeyemi and A.A. Oshodi, 2005. Variability in the physicochemical, nutritional and antinutritional attributes of six mucuna species. Chemistry, 89: 37-48.

Adebowale, Y.A., 2008. A study of the control variables during the preparation of protein isolate from Mucuna bean (*Mucuna pruriens*). EJEAFChe, 7: 3223-3238.

AOAC, 1990. Official Methods of Analysis. 15th Edn., Washington, DC., Association of Analytical Chemist 1546.

Conkerton, E.J. and R.L. Ory, 1976. Peanut proteins as food supplements: A Compositional study of selected Virginia and Spanish peanuts. J. Am. Oil Chem. Soc., 53: 754-756.

El-Adawy, T.A., 2000. Functional properties and nutritional quality of acetylated and succinylated mungbean protein isolates, pp: 1-15.

El-Beltagy, A., 1996. Effect of home traditional cooking on quality aspected of some legumes. MS Thesis, Faculty of Agriculture, Menofiya University, Shibin El-Kom Egypt. In: Nutritional composition and antinutritional factors of mungbean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Mubarak, A.E. (Ed.) 2005. J. Food Chem., 89: 489-495.

Emenalom, O.O. and A.B. Udedibie, 2005. Effect of raw, cooked and toasted *Mucuna pruriens* seeds in the performance of finisher broilers. Nig. J. Anim. Prod., 25: 115-119.

Fan, T.Y. and F.W. Sosulski, 1974. Dispersibility and isolation of proteins from legume flours. Can. Institute. Food Sci. Tech. J., 7: 256.

Feeney, R.E., R.B. Yamasaki and K.F. Geoghegen, 1982. Chemical modification of proteins: An overview. In R.E. Feeney and J.R. Whitaker, Modification of proteins: Food, nutritional and pharmacological aspects. Adv. Chem. Series 198: 3-5, Washington, DC: American Chem., 59: 520-524.

Hang, Y.D., K.H. Steinkraus and L.R. Hackler, 1970. Comparative studies on the nitrogen solubility of mungbeans, pea beans and red kidney beans. J. Food Sci., 35: 318. In: Preparation and Evaluation of Mungbean Protein Isolates. Ed. Thompson, L.U. (1977) J. Food Sci., 42: 206.

Hsu, D.L., H.K. Leung, M.M. Morad, P.L. Finney and C.T. Leung, 1982. Effect of germination on electrophoretic functional properties of yellow pea, lentil, and faba bean protein isolates. Cereal Chem., 59: 344-350. http//:wikipedia.org (2008). http://www.yahoo.com-proteinfunctionality-11/6/2009.

Kato, A., T. Fujishige, N. Matsudomi and K. Kobayashi, 1985. Determination of emulsifying properties of some proteins by conductivity measurements. J. Food Sci., 50: 56-58.

Kavas, A. and S. Nehir, 1992. Changes in nutritive value of lentils and mungbeans during germination. Chem. Microbiol. Technol. Lebesm, 14: 3-9.

Khalil, A.A., 2006. Nutritional improvement of Egyptian breed of mungbean by probiotic Lactobacilli. Afr. J. Biotech., 5: 206-212.

Kinsella, J.E., 1976. Functional properties of proteins in foods: A survey. Cr. Rev. Food Sci. Nutr., 7: 219-280.

Kinsella, J.E., 1979. Functional properties of soy proteins. J. Am. Oil Chem. Soc., 56: 242-258.

Mesallam, A.S. and M.A. Hamza, 1987. Studies on green gram (*Phaseolus aureus*) protein concentrate and flour. J. Plant Foods Human Nutr. (Springer Netherlands), 37: 17-27.

Mizrahi, S., G. Zimmerman, Z. Berk and Cogan, 1967. The use of isolated proteins in bread. Cereal Chem., 44: 193.

Mustafa, A.I., M.S. Al-Wesseli, O.M. Al-Basha and R.H. Al-Amir, 1986. Utilization of cowpea flour and protein isolate in baking products. Cereal Food World, 31: 756-759.

Noguchi, A., W. Kugimiya, Z. Haque and K. Saio, 1981. Physical and Functional Properties of Extruded rice flour fortified with soybean protein isolate. J. Food Sci., 47: 240-245.

Okezie, B.O. and A.B. Bello, 1988. Physicochemical and functional properties of winged bean flour and isolate compared with soy isolate. J. Food Sci., 53: 450-454.

Onimawo, I.A. and M.K. Egbekun, 1998. Comprehensive Food Science and Nutrition. Nigeria, pp: 206.

Prabhudesal, R.K., 1988. Leaching In: Handbook of separation techniques for chemical Engineers (ed. Schwectzer P.A) McGraw-Hill Book. New York, pp: 3-5.

Sanchez-Vioque, R., A. Clement, J. Vioque, J. Bautista and F. Millan, 1999. Protein isolates from chickpea (*Cicer arietinum*) Chemical composition, functional properties and characterization. Food Chem., 64: 243-270.

Sathe, S.K., S.S. Desphande and D.K. Salunke, 1982. Functional properties of winged bean (*Psophocarpus tetragonolobus*) L. (DC) proteins. J. Food Sci., 47: 503.

Siddhuraju, P., K. Vijayakumari and K. Janardhanan, 1996. Chemical composition and protein quality of the little known legume, Velvet bean (*Mucuna pruriens*). J. Agric. Food Chem., 44: 2636-2641.

Soestrino, U.S.S., 2007. Characterization of yellow pea (*Pisum sativum* L. Miranda) protein and the proteinate functional properties. Ph.D. Thesis. Food resource, Nutrition and Food Management department, Oregon state Univ. Corvallis, pp: 1-9. http://food.oregonstate.edu-4/2/2008.

Thompson, L.U., 1977. Preparation and evaluation of mungbean protein isolates. J. Food Sci., 42: 202-206.

Udensi, E.A. and A. Okonkwo, 2006. Effects of fermentation and germination on the physicochemical properties of *Mucuna cochinchinensis* protein isolate. Afr. J. Biotechnol., 5: 896-900.

Zadow, J.C., 1993. Why and Why Powder? In: Encyclopedia of Food Science, Food Tech. and Nutrition, Macrea Robinson, R.K. and M.J. Sadle, pp: 4888-4892.

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Sensory Evaluation of Multiple Fortified Stock Powder

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Abstract: The aim of the study was to determine consumer sensory acceptability of multiple fortified stock powder in beef stew as compared to unfortified stock powder, which was used as a control. The panelists included 10 students from the Vaal University of Technology in the Republic of South Africa aged between 18 and 23 years consisting of nine females and one male, who have some knowledge of food evaluation and were also consumers of beef. The multiple fortified stock powder was classified as a functional food in the light of the addition of a range of nutrients. Ten semi-trained panelists, evaluated three differently prepared beef stew samples based on colour, flavour, off-flavour and after flavour acceptability by using a 5-point hedonic scale. Two samples were cooked for 10 min and 30 min respectively with fortified stock powder and the other sample with unfortified stock powder. The hedonic scale ranged from "unacceptable" (which was assigned the value of one), "moderately unacceptable" (value of 2), "acceptable" (value of 3)," moderately acceptable" (value of 4) and "highly acceptable" (value of 5) respectively. The results showed that multiple fortified stock powder cooked in beef stew was appreciably accepted by the semi- trained panel. Multiple fortified stock powder is comparable to ordinary stock powder in acceptability and would be accepted when introduced into the market with accompanying education. In conclusion, sensory evaluation should precede all future micronutrient food fortification programmes and multiple fortified stock powder should be promoted as a potential functional food.

Key words: Sensory, acceptability, hedonic scale, functional food, fortification, stock powder

INTRODUCTION

Micronutrient deficiencies: Over two billion people are at risk of micronutrient deficiencies and more than one billion are affected by it (World Health Organization and United Nations International Children's Fund, 2008). The three deficiencies of greatest public health significance are those of vitamin A, iron and iodine: these could lead to serious health problems, including blindness, anaemia, mental retardation, vulnerability to infectious diseases and in extreme cases, death (Unnevehr *et al*., 2007).

According to the report of the International Vitamin A Consultative Group (2007) entitled 'Micronutrient status of the population', micronutrient intakes are inadequate in most population groups and rural and peri-urban dwellers are at a higher risk. Vorster *et al*. (2005) and Eckhardt (2007) further add that the groups with lower intakes of iron in South Africa include young children, adolescent girls and women. Standing Committee on Nutrition (2007) confirms that women especially in their reproductive age are among the most affected.

Causes of micronutrient malnutrition: The adequacy of food supplies at national level in most developing countries does not ensure that adequate food is available at the households or at the individual level. Factors that can influence the ability of an individual to acquire and utilize nutrients include:

- Food and water availability
- Food prices
- Income and purchasing power
- Women's workload
- Education level
- Local customs
- Sanitary conditions and
- Health status

The cost of ill health due to micronutrient malnutrition is difficult to quantify and it is defined as "Hidden Hunger" (World Health Organization, World Food Programme and United Nations International Children's Fund, 2007).

Prevalence of micronutrient malnutrition in South Africa: A national survey conducted by Yamanchi *et al*. (2006) on micronutrient status of South African children, revealed that xerophthalmia (clinical eye lesion leading to nutritional blindness) rates among approximately 11,000 children. Among this figure, the six to seven months of age groups were found to be surprisingly high, since clinical VAD was not considered to be a problem previously. Night blindness was prevalent in 12% of the children. Bitot's spots in 0.4-0.8%, corneal

Table 1: Micronutrients: Dietary intakes of South Africans (Bourne *et al*., 2007)

Micronutrient	Groups with lower intake
Iron	Black rural and urban settings
	Coloured Indian population Groups
	Young children, adolescent girls and women
Thiamin (B1)	Black and Indian population groups
Riboflavin (B2)	Black rural and urban settings
Niacin (B3)	Black and Indian population groups
Vitamin (B6)	All population groups of all ages except white males
Folate	Indians
	Rural black women of childbearing age
Vitamin A	Black children younger than ten years of age
	Urban black women
	Black and Indian men

xerosis in 0.2-0.7% and keratomalacia or corneal scarring in 0.1%. Prevalence of low serum retinol was higher in the rural areas thus 23% than in the urban areas 25%. Serum retinol levels were found to be low in pre-school children living in poor settlements near Durban, as seen in Table 1. This suggests that these groups should be targeted for vitamin A interventions (United Nations International Children's Fund South Africa, 2008). In addition VAD is also common among women from the poorest communities and stunting is also common (Adamson, 2006). Iron deficiency anaemia is also common among pregnant women, especially among women of Indian origin who are living in South Africa. A number of small studies reports the prevalence of anaemia in pregnancy, with 37% in the Johannesburg and 33% in the Gazankhulu area. Hatting *et al*. (2008) add that in terms of micronutrients (as shown in Table 1), riboflavin intake was found to be low in black rural and urban settings, as well as in the coloured and Indian population groups. The intake of iron was also very low in those very same population groups known to be vulnerable for iron deficiency. These include young children, adolescent girls and women.

The effects of correcting these micronutrient deficiencies are:

- Preventing up to four out of ten childhood deaths
- Lowering the maternal deaths by more than one-third
- Increasing work capacity by 40%
- Improving the population Intelligent Quotient (IQ) by 10-15 points and
- Raising the Gross Domestic Product (GDP) by 5% (De Romana *et al*., 2005).

Multiple food fortification: Multiple fortification refers to the fortification of food vehicles with two or more micronutrients. Multiple fortification addresses two or more micronutrient deficiencies in a more cost-effective manner. In Thailand for instance, vitamin A and iron are used to fortify rice. Countries like Brazil, Japan, Philippines and the United States of America are practicing double fortification (United States Agency for International Development, 2008). Multiple fortification of cereal and weaning foods/formulas has already been done successfully. For instance, micronutrient multimixes for cereals (primarily wheat), in addition to iron and/or vitamin A often include thiamine, riboflavin and niacin (World Health Organization, 2005). Efforts are still being made by the World Food Programme to develop and popularize commercial low-cost, multiple fortified weaning foods in developing countries, This is because the use of multiple fortified weaning products in developing countries has hardly met with success and the use is only restricted to the urban population with a higher purchasing power. The failure is due to existence taboos, lack of current knowledge, shortage of fuel and clean water for cooking, poor hygiene practices, short storage time and the low social prestige value for homemade products (Whiting and Calvo, 2006).

Stock powder is a staple condiment used throughout South Africa and thus fortification of stock powder with vitamin A, B1 (thiamine), B2 (riboflavin), B3 (niacin), B6 (cynocobalamin), B12 (corrinoids), folic acid and iron (cocktail of micronutrient), will provide a powerful means of delivering substantial amounts of micronutrients to many population groups. This cannot be achieved until a successful sensory evaluation of the multiple fortified stock powder has been conducted.

Sensory evaluation, a major determinant of acceptability, is a scientific discipline which encompasses all of the senses and used to evoke measure, analyze and interpret reactions to the characteristics of foods and materials as they are perceived by the senses. It is also a major determinant in the subsequent adoption and use of the product (Otoo and Asiedu, 2009). Food Industry Foundation (2005) also confirms that knowing consumers' preferences and perception of the sensory characteristics of food and drink product is very important to food manufacturers and retailers alike. Today's consumers are discerning, demanding and more knowledgeable about food and expects products which are safe, good value and of high sensory quality (Duxbury, 2005). Bourne (2002) concluded with a look at the future: with the advent of more diverse styles of foods such as functional foods, the use of sensory analysis becomes even more important.

MATERIALS AND METHODS

Determination of stock powder as a suitable vehicle for fortification: This was also a pilot study of a larger project in which the suitability of stock cube and stock powder as possible vehicle for fortification was determined.

To determine the suitability of stock powder as a vehicle for fortification, a questionnaire was developed, validated and sent out to 802 respondents (n-802). The data in the questionnaire include the amount of stock cube and stock powder consumed in a week, when and how the

stock powder is used and the choice of flavours of stock cubes and stock powders that are frequently used. The sample was randomly selected in hypermarkets, townships, towns and institutions in the Vaal Triangle area in South Africa. The survey population comprised males and females of 15 years or older. Questionnaires were completed each week for four weeks and the answers compared. Based on the results the questionnaire was accepted to be reliable and valid as in both tests correlation of 90% was found.

Methods used to develop the multiple fortified stock powder: The development of multiple fortified stock powder is as follows:

Twelve and a half kilogram of stock powder were mixed with 125 g of fortificant as instructed by Roche. The fortificants used as a cocktail of micronutrient is shown in Table 2.

Table 2: Vitamin and mineral concentration in fortified stock powder

Vitamin/mineral	Concentration per 5 g serving of stock powder
Vitamin A	500,000 RE
Vitamin B1 (thiamine)	0.420 mg
Vitamin B2 (riboflavin)	0.480 mg
Vitamin B3 (niacin)	5.400 mg
Vitamin B6	0.600 mg
Folic acid	0.134 mg
Vitamin B12	0.300 mcg
Vitamin C	18.000 mg
Iron	4.620 mg
Zinc	3.000mg

Every 5 g of multiple fortified stock powder used contained the following micronutrients: 500,000 RE of vitamin A, 0.420 mg of vitamin B1 (thiamine), 0.480 mg of vitamin B2 (riboflavin), 5.400 mg of vitamin B3 (niacin), 0.600 mg of vitamin B6, 0.134 mg of folic acid, 0.300 mg of vitamin B12, 18.000 mg of vitamin C, 4.620 mg of iron and 3.000 mg of zinc.

The mixing was done in a large stainless steel dough mixer and was covered with aluminum foil in order to prevent exposure to light and the consequent biochemical and biological deterioration of the micronutrients. The mixer was also covered so as to prevent contamination and to prevent the destruction of micronutrients through oxidation. A mixing period of thirty minutes was adhered to so as to ensure an even distribution of fortificants in the stock powder. The mixing process was well controlled so as to ensure a homogenous mixture. The powder was later subjected to a nutrient-concentration analysis in a laboratory.

The multiple fortified stock powder, as well as the unfortified stock powder, was packed in rigid 400 ml styrene curry tube containers of eight cm high and 12 cm in diametre. The containers were also provided with lids to prevent dust and dirt from contaminating the powder. The containers were coloured dark-grey to prevent the destruction of light-sensitive micronutrients such as vitamin A.

Code numbers representing fortified (Experimental) and unfortified (Control) powder, were written on the lids of the containers. This was to assist in easy identification when using them in cooking the beef stew.

Recruitment of the panelists: The sensory panelists, consisting of nine female and one male, aged 18-23 years, from Vaal University of Technology who have some knowledge of food evaluation and were also consumers of beef were recruited to evaluate the acceptability of the three beef stew samples as shown in Fig. 1. Acceptability tasting occurred in the food science laboratory of the Department of Hospitality and Food Consumer Science at the Vaal University of Technology. Panelists were recruited by polite solicitation.

The study design: The study design for the sensory panel is explained in Fig. 1. To determine the sensory acceptability of the multiple fortified stock powder, ten semi-trained panelists evaluated three differently prepared beef stew samples based on colour, flavour, off-flavour and after-flavour acceptability by using a 5-point hedonic scale. Two samples were cooked for 10 min and 30 min respectively with fortified stock powder and the other sample with unfortified stock powder. The hedonic scale ranged from “unacceptable” (which was assigned the value of 1), “moderately unacceptable” (value of 2), “acceptable” (value of 3), “moderately acceptable” (value of 4) and “highly acceptable” (value of 5) respectively. Each sample of the beef stew was presented in identical white styrene curry tubes of the same size and shape, coded randomly with a three-digit number and plastic spoons were provided to panelists for tasting. Beef stew cooked for thirty minutes with multiple fortified stock powder was coded ”570”, beef stew cooked for ten minutes with multiple fortified stock powder was coded “349” and beef stew cooked with ordinary stock powder which was used as control was coded ”298”. The containers were free from odours and contamination, which might interfere with the results. All three samples were simultaneously presented in random order. Re-tasting of the samples was also allowed.

Sample preparation: Preparation for the beef stew samples involved collection of ingredients as seen in Table 3, utensils and equipment and cooking method in Fig. 2.

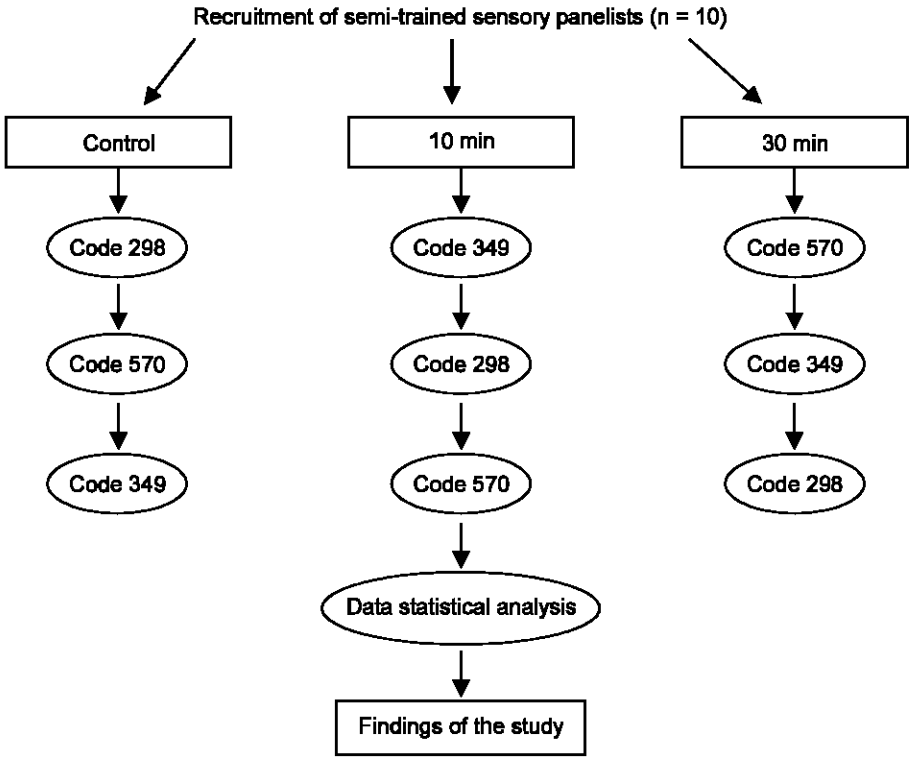

Fig. 1: Study design for sensory evaluation

Table 3: Ingredients used in the preparation of beef stew and quantities

Ingredients	Quantities in control group 60 min cooking	Quantities in experiment group 30 min cooking	Quantities in experiment group 10 min cooking
Chuck steak	800 g	800 g	800 g
Multiple fortified stock powder	-----	5 g	5 g
Unfortified stock powder	5 g	------	------
Onion	100 g	100 g	100 g
Salt	5 g	5 g	5 g
Vegetable cooking oil	15 ml	15 ml	15 ml
Cold water	250 ml	250 ml	250 ml
Boiling water	250 ml	250 ml	250 ml
End weight	1,000 g	1,150 g	1,150 g

Utensils and equipment used for the preparation of beef stew with fortified and unfortified stock powder: Utensils used for the preparation of beef stew are as follows:

- Three Identical aluminum saucepans with tight-fitting lids
- Frying pan
- Small saucepan for boiling water
- Cook's knife
- Measuring jug
- Wooden spoon and
- Chopping board

The Equipment used were:

- Electric stove and
- Electronic weighing scale

RESULTS

The results of the questionnaire on the pilot study indicated that 97% of respondents used stock cubes and 21% preferred stock powder. Respondents used various flavours of stock powder and stock cubes, with chicken being the most popular. This indicate that stock powder and stock cube is consumed by a sizeable proportion of the population and it is the right vehicle to select for fortification as shown in Fig. 3. Again the results on the daily consumption of stock cube and stock powder revealed that 79% of respondents used stock cubes and stock powder daily and 21% weekly.

Results of the sensory evaluation: The data obtained from the semi trained sensory panel were statistically analyzed using SPSS® Version 10.1. Analysis of Variance (ANOVA) for comparing mean (SD) between samples was used.

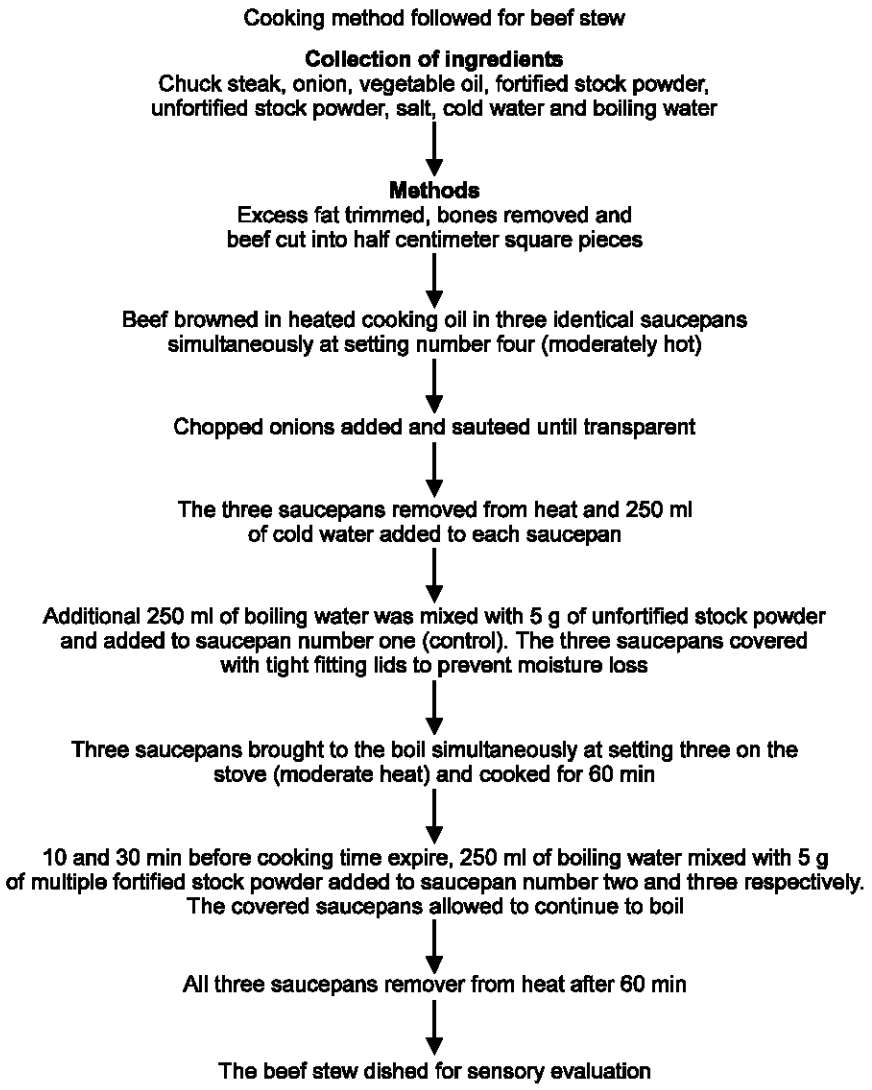

Fig. 2: Procedure for the preparation of multiple fortified beef stew

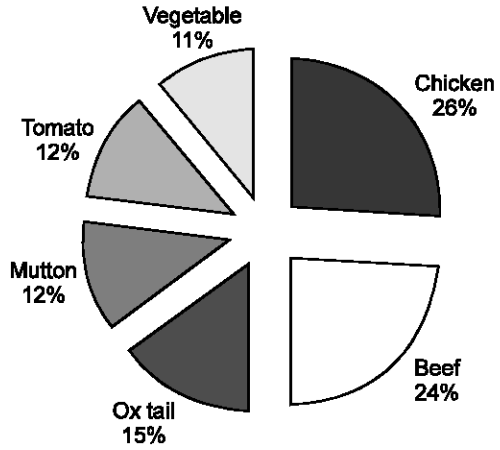

Fig. 3: Flavour popularity of stock cube or stock powder

Mean sensory scores for colour, flavour, after-flavour and off-flavour of beef stew cooked with multiple fortified stock powder for 10 and 30 min respectively and beef stew cooked with unfortified stock powder (control)

Colour sensory acceptability of beef stew: In terms of colour acceptability in Table 4, the panel rated the beef stew cooked with unfortified stock powder (control) as acceptable (3.24) on the 5-point hedonic scale. Colour of beef stew cooked with multiple fortified stock powder cooked for 10 min was acceptable (3.44) by the panel. Beef stew cooked with multiple fortified stock powder for 30 min was also rated acceptable (3.16) for colour. These indicate that there was no difference in colour between the control group and the experimental group. The 10 and 30 min can be an acceptable cooking time for the multiple fortified stock powder to obtain the desirable beef stew colour.

Table 4: Mean sensory scores for beef stew

Variable	Control group	10 min group	30 min group
Colour	3.24±0.71	3.44±0.71	3.16±0.38
Flavour	2.91±0.32	3.58±0.55	3.18±0.47
After-flavor	3.04±0.46	3.72±0.53	2.99±0.40
Off-flavour	2.77±0.72	3.08±0.90	2.94±0.56

Flavour sensory acceptability of beef stew: Data in Table 4 show that the flavour of beef stew cooked with unfortified stock powder was acceptable (2.91) by the panel. Flavour of beef stew cooked with multiple fortified stock powder for 10 min was rated moderately acceptable (3.58) by the panel. Beef stew cooked with multiple fortified stock powder for 30 min was also acceptable on the hedonic scale (3.18) in terms of flavour. These indicate that no objectionable flavor was detected in the beef stew cooked with multiple fortified stock powder for 10 min and 30 min. The 10 and 30 min cooking time should be recommended to consumers in future stock powder fortification.

After-flavour acceptability of beef stew: After-flavour taste of beef stew cooked with unfortified stock powder in Table 4 was rated acceptable (3.04) by the sensory panel. The after-flavour taste of beef stew cooked with multiple fortified stock powder for 10 min was rated moderately acceptable (3.72) and the after-flavour taste of beef stew cooked with multiple fortified stock powder for 30 min was also rated acceptable (2.99). These indicate that after the panel swallowed the stew no objectionable taste was detected in the mouth.

Off-flavour acceptability of beef stew: Data in Table 4 indicate that there was no off-flavour taste detected in the beef stew cooked with unfortified stock powder, and was rated as acceptable (2.77). Beef stew cooked with multiple fortified stock powder for 10 min was rated acceptable by the panel in terms of off-flavour (3.08). The panel detected no off-flavour taste in the beef stew cooked with multiple fortified stock powder for 30 min and was rated as acceptable (2.94).

Mean change between the control, 10 and 30 min group

Mean change of colour between the control, 10 and 30 min group: Comparing the mean change of colour between groups in Table 5, there was no statistical significant difference in colour between group one (control) and the beef stew cooked with multiple fortified stock powder for 10 min. Again, there was no statistical significant difference in colour between group one (control) and the beef stew cooked with multiple fortified stock powder for 30 min. Mean change of colour between beef stew cooked with multiple fortified stock powder for 10 min and beef stew cooked with multiple fortified stock powder for 30 min found no statistical significant difference.

Mean change of flavour between the control, 10 and 30 min group: Comparing the mean change for flavour in Table 5 between beef stew cooked with unfortified stock powder and beef stew cooked with multiple fortified stock powder for 10 min, there was no statistical difference. Again, when the flavours of beef stew cooked with unfortified stock powder and the beef stew cooked with multiple fortified stock powder for thirty minutes compared, there was no statistical significant difference shown (-0.27 where p = 0.197). There was no statistical difference in flavour between beef stew cooked with multiple fortified stock powder for 10 min and beef stew cooked with multiple fortified stock powder for 30 min (0.40, where p = 0.060).

Mean change of after-flavour between the control, 10 and 30 min group: Data in Table 5 indicate that there was no statistical significant difference by comparison in after-flavour taste between beef stew cooked with unfortified stock powder and beef stew cooked with multiple fortified stock powder for 10 min (0.68, where p = 0.03). There was no statistical significant difference in after flavour taste between beef stew cooked with unfortified stock powder for 10 min and beef stew cooked with multiple fortified stock powder for 30 min (0.05, where p = 0.812). The 10 and 30 minute cooking time should be recommended to consumers in future fortification. Also, there was no statistical difference in after-flavour taste between beef stew cooked with multiple fortified stock powder for 10 min and beef stew cooked with multiple fortified stock powder for 30 min (0.73, where p = 0.002). The acceptability indicates that high population coverage can be achieved when introduced into the market.

Mean change of off-flavour between the control, 10 and 30 min group: By comparison in Table 5, there was no statistical significant difference in off-flavour taste between beef stew cooked with unfortified stock powder and beef stew cooked with multiple fortified stock powder for 10 min (-0.31, where p = 0.356). There was

Table 5: Mean change between groups

Variable	Control group vs 10 min	Control group vs 30 min group	10 min vs 30 min
Colour	-0.2, p = 0.477	0.008, p = 0.775	0.28, p = 0.321
Flavour	-0.67*, p = 0.003	-0.27, p = 0.197	0.40, p = 0.060
After-flavor	-0.68*, p = 0.03	0.05, p = 0.812	0.73*, p = 0.002
Off-flavour	-0.31, p = 0.356	-0.17, p = 0.611	0.14, p = 0.675

no statistical difference in off-flavour taste between beef stew cooked with unfortified stock powder and beef stew cooked with multiple fortified stock powder for 30 min (-0/17, where p = 0.611). No statistical significant difference in off-flavour was detected between beef stew cooked with multiple fortified stock powder for 10 min and beef stew cooked with multiple fortified stock powder for 30 min (0.14, where p = 0.675).

DISCUSSION

No research studies could be found in the literature on sensory evaluation and acceptability to multiple fortified stock powder. The colour, flavour, after-flavour and off-flavour of beef stew cooked with multiple fortified stock powder were evaluated to assess consumer sensory acceptability. People do not eat what was not appealing and colour serves as a signal of the quality of accepted foods (Koster, 2009). Literature studied showed that visual attributes play an important role in consumer's acceptance of food and that, colour is certainly the most salient aspect of the visual aspect of food. The result again implies that, surface texture can also give important clues to the sensory properties of food and in spite of numerous ways by which the appearance attributes of food can affect consumers acceptance, the majority of research on the role of appearance in food acceptance, has focused on the influence of colour (Keith *et al*., 2007). The flavour acceptability of the multiple fortified stock powder may be attributed to the stock powder itself being a flavour improver and this again is in support of literature which indicate that most of the successful food fortification programmes in the developing world are those that use flavour and taste improvers (Eddy *et al*., 2007). Furthermore, the panelist positive attitude towards the flavour of the beef stew indicate that there was no organoleptic change to the fortified stock powder and this quality accounts for the flavour acceptability. The findings also indicate that the panelists exposure to and their prior knowledge of stock powder as well as beef might have contributed to the acceptability of the product (Ismail, 2006). This is so because panelists in the experimental group and all the panelists in the control group were already consuming stock powder and beef at home. This is confirmed in literature which stated that a commonly used vehicle is needed in order to increase the acceptability of a new product (Ismail, 2006). Faber (2005) also confirmed that acceptability behavior depends on beliefs and attitude. Previous studies done on respondents flavour and after-flavour acceptability of fortified cassava flour, which was their staple food, with groups of pregnant and lactating mothers found that cassava flour was acceptable (Faber, 2005). Finally, the findings suggest that foods prepared with multiple fortified stock powder are generally comparable in acceptability to foods prepared with ordinary stock powder. There is no significant difference. The reason for the acceptability may be that the characteristics of the stew prepared did not appear significantly changed by the fortified stock powder (Dean *et al*., 2008). It cannot be said that the fortificant used remained stable after cooking because the panel only used their senses of sight, smell and taste to evaluate the fortified stock powder and would be impossible to test the stability of the fortificant with these senses. Literature indicate that vitamin A, (fat soluble) vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_6 and vitamin B_{12} which are water soluble, are also stable to heat however, vitamin B_1 (thiamine) and folic acid, although water-solouble, are destroyed by heat (Fukuwatari and Shibatata, 2008). Accessing the stability of the multiple fortified stock powder can be served as future research.

Conclusion:

- Micronutrient food fortification is effective when it is acceptable to the respondents and they comply with the use thereof.
- The findings proposes that a common vehicle to carry the nutrients must be identified The pilot study shows that stock cube and stock powder were popular condiments that are consumed in constant quantities by a large proportion of families without segregation of the socio-economic status in the Vaal Triangle area.
- Sensory evaluation and acceptability should precede all future micronutrient fortification programmes. Micronutrient food fortification is very important and must be sustained. Multiple fortified stock powder is recommended as a potential functional food at medium and large industry because the panelists accepted the stock powder.
- Multiple fortified stock powder was accepted in terms of colour, taste and overall acceptability showing the impact of fortification of foods that are commonly consumed.
- The panelists could not differentiate between the fortified and the unfortified products.
- Micronutrient food fortification is very important and must be sustained.
- The food industries in South Africa in conjunction with all relevant role players can expand micronutrient food fortification towards attaining a sustainable and long-term solution to micronutrient deficiency.

REFERENCES

Adamson, P., 2006. Vitamin and mineral deficiency: A Global Progress Report. The Micronutrient Initiative and UNICEF.

Bourne, L.T., M.K. Hendricks, D. Marrais and B. Eley, 2007. Addressing malnutrition in young children in South Africa. Setting the context for paediatric food-based dietary guidelines. Maternal Child Nutr., 3: 230-238.

Bourne, M.C., 2002. Food Texture and Viscosity: Concept and Management. 2nd Edn., Academic Press New York and London, pp: 257-290.

Dean, M., A. Arvola, M. Vassallo, L. Lahteenmaki, M.M. Raats, A. Saaba and R. Shepherd, 2008. Predicting intensions to purchase organic food: The role of affective and moral attitudes in the Theory of Planned Behaviour. Appetite, 50: 443-454.

Duxbury, D., 2005. Sensory evaluation provides value. Food Technol., 59: 68.

De Romana, D.L., M. Salazar, K.M. Hambridge, M.E. Penny and J.M. Peerson, 2005. Longitudinal Measurements of zinc absorption in Peruvian children consuming wheat products fortified with iron only or iron and 1 of 2 amounts of zinc. Am. J. Clin. Nutr., 81: 637-47.

Eckhardt, C.L., 2007. Micronutrient malnutrition, Obesity and Chronic Disease in Countries Undergoing the Nutrition Transition Potential Links and Program/Policy Implications IFPRI, Washington, United States. International Food Policy Research Institute (IFPRI), Discussion Papers on Food Consumption and Nutrition. Series 689, Washington, United States.

Eddy, N.O., P.G. Udofia and D. Eyo, 2007. Sensory evaluation of wheat/cassava composite bread and effect of label information on acceptance and preference. Afr. J. Biotechnol., 6: 2415-2418, 18th October 2007.

Faber, M., 2005. Complementary foods consumed by 6-12-month-old rural infants in South Africa are inadequate in micronutrient. Public Health Nutr., 8: 373-381.

FIF (Food industry foundation), 2005. www.qmuc.ac.uk/fif.

Fukuwatari, T. and K. Shibatata, 2008. Urinary water soluble vitamins and their metabolite contents as nutritional markers for evaluating vitamin intakes in young Japanese women. J. Nutr. Sci. Vitaminol., 54: 223-229.

Hatting, Z., C.M. Walsh, C.J. Bester and O.O. Oguntibeju, 2008. An analysis of dietary micronutrient intakes in two age groups od black South African women. West In. Med. J., Vol. 57.

Ismail, A., 2006. India: The Land of Opportunity. Functional Foods and Nutraceuticals. January.

IVACG, (International Vitamin a Consultative Group), 2007. The cost of micronutrient interventions. Micronutrient forum. Istanbul, Turkey, 16-18 April.

Keith, T., S. Lateef, O. Olusola, P. Adewale, A. Idris, A. Kolawoye and W. Andrew, 2007. Consumer acceptability and sensory evaluation of a fermented cassava product. J. Sci. Food Agric., 87: 1449-1456.

Kõster, E.P., 2009. Diversity in the determinants of food choice: A psychological perspective. Food Qual. Preference, 20: 70-82.

Otoo, E. and E. Asiedu, 2009. Sensory evaluation: The last hurdle in varietal development of yams (Dioscorea rotundata, poir) in Ghana. Afr. J. Biotechnol., 8: 5747-5754.

SCN (Standing Committee on Nutrition), 2007. Assessing Micronutrient Deficiencies in emergencies: Current practice and future directions. Geneva, Switzerland, October.

UNICEF (United Nations Imternational Childrens Fund) South Africa, 2008. South Africa launches its first vitamin A supplementation campaign. Department of Health South Africa, Pretoria.

Unnevehr, L., C. Pray and R. PAARIBERG, 2007. Addressing micronutrient deficiencies: Alternative interventions and technologies. Agro. Biotechnol. Forum, 10: 124-134.

USAID (United States Agency for International Development), 2008. Rice Fortification in Developing Countries: A Critical Review of the Technical and Economic Feasibility April 2008. Nutrition report on micronutrient and health. Newsletter. Basel, Switzerland.

Vorster, H.H., C.S. Ventor and M.P. Wissing, 2005. The Nutrition and Health Transition in the North-West Province of South Africa. A review of the THUSA (Transition and Health during Urbanisation of South Africa) study. Public Health Nutr., 8: 480-90.

Whiting, S.J. and M.S. Calvo, 2006. Barriers to Effective Food Fortification and Supplementation. J. Nutr., 136: 1114-1116.

WHO (World Health Organisation), 2005. Guidelines on food fortification with micronutrients for the control of micronutrient malnutrition, Symposium: Food Fortification in Developing countries. Geneva.

WHO (World Health Organisation) and UNICEF (United Nations International Childrens Fund), 2008. Preventing and controlling micronutrient deficiencies in populations affected by an emergency. Geneva, Swizerland.

WHO (World Health Organisation), WFP (World Food Programme) and UNICEF (United Nations International Childrens Fund), 2007. Preventing and controlling micronutrient deficiencies in populations affected by an emergency. Geneva, Switzerland.

Yamanchi, F., T. Buthelezi and M. Velia, 2006. Gender, Labour and Prime-Age Mortality: Evidence from South Africa. FCND Discussion Papers 213.

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Compliance to Vitamin and Mineral Supplementation among Pregnant Women in Urban and Rural Areas in Malaysia

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Abstract: Low compliance has been linked to the ineffectiveness of supplementation programme among pregnant women. This cross-sectional study was carried out to determine the compliance of vitamin and mineral supplementation among pregnant women attending public antenatal clinics in urban and rural areas. A total of 118 pregnant women aged 28±4 years (urban areas = 62; rural areas = 56) were recruited. Socio demographic data and compliance to supplementation were obtained through self-reported questionnaire. Haemoglobin concentration (Hb) was obtained from the antenatal records. More than two-thirds of the subjects (79.7%) were in third trimester and less than a third (20.3%) was in second trimester. Overall, 49.2% of subjects complied with vitamin and mineral supplementation. The mean of weight and haemoglobin concentration were 63.7±15.1 kg and 11.3±1.1 g d/L, respectively. The overall percentage of subjects who complied with vitamin and mineral supplementation was 49.2%. There was no significant difference in percentage of subjects who complied with the supplements in urban (46.8%) and rural areas (51.8%) ($p = 0.587$). The prevalence of anaemia among the subjects (Hb <11 g d/L) was 42.3% ($n = 50$). Prevalence of anaemia was lower in the compliant group compared with the non-compliant group (34.5% vs 50%, $p = 0.01$). Pregnant mothers who did not comply to the supplementation had significantly lower haemoglobin concentration (11.0±1.0 g d/L) compared with those who complied (11.5±1.2 g d/L) ($p = 0.01$). Percentage of compliance in anaemic and non-anaemic subjects were 34.5 and 65.5%, respectively ($p = 0.088$). The main reasons for non-compliance reported by subjects in both areas were forgetfulness (33.9%), side effects (nausea and vomiting) (11.9%) and worry regarding big size of babies (5.1%). In summary, the incidence of anemia is still high while compliance to supplementation is still low. Comprehensive nutrition education and health promotion programme should be carried out targeting pregnant women in urban and rural to educate the importance of compliance with the vitamin and mineral supplementation during pregnancy.

Key words: Anaemia, compliance, haemoglobin, pregnancy, supplementation

INTRODUCTION

Anaemia in pregnancy is defined as reduction in the oxygen carrying capacity of the blood as a result of fewer circulating erythrocytes than normal or a decrease in the concentration of Haemoglobin (Hb) (Hoque *et al*., 2009). During pregnancy, there is an increase in amount of iron required to increase red cell mass, expand plasma volume and to allow growth of fetal-placental unit (Scholl, 2005). The deficiency occurs through reduced production or an increased loss of red blood cells. Anaemia in pregnant women in developing countries is generally presumed to be the result of nutritional deficiency. In Malaysia, the incidence of anaemia among pregnant mothers attending public antenatal clinics was reported to be 35% (Jamaiyah *et al*., 2007). Hadipour *et al*. (2010) reported a higher incidence of anaemia among Iranian pregnant women i.e. 51.4%. Iron deficiency anaemia is the most prevalent nutritional deficiency problem affecting pregnant women. Pregnant women are considered to be the most vulnerable group, since the additional demands that are made on maternal stores during this period exposes them to various latent deficiencies that manifest themselves as anaemia (Hoque *et al*., 2009). Increased iron requirements, low pre-pregnancy iron stores and continued inadequate dietary intakes of iron exacerbate this physiologic anaemia during pregnancy in many regions of this world (Christian *et al*., 2003). Brabin *et al*. (2001) reported a strong association between severe anaemia (OR 3.51, 95% CI: 2.05-6.00) and maternal mortality.

Anaemia may result from both nutritional and non-nutritional factors, specifically besides iron, deficiency of micronutrients such as vitamins A, C and B-12 and folic acid may contribute to the development of anaemia. These nutrients may affect haemoglobin synthesis either directly or indirectly by affecting absorption and/or mobilization (Ramakrishnan *et al*., 2004). Systemic

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evaluation of the efficacy of antenatal iron supplementation is known to raise haemoglobin concentration, although it effects are influenced by dose and compliance level (Christian *et al*., 2003). Inability to meet the required level for iron and other vitamins either as a result of dietary or supplementary gives rise to anaemia (Idowu *et al*., 2007). According to the World Health Organization (WHO, 1998), anaemia should be considered when the hemoglobin level is below 11 g/dL. Anaemia ranges from mild, moderate to severe and WHO classifies the hemoglobin level for each of these types of anemia in pregnancy at 10.0-10.9 g/dL (mild anemia), 7-9 g/dL (moderate anemia) and <7 g/dL (severe anemia).

Anaemia during pregnancy remains a problem in different settings despite the fact that routine provision of iron supplements has been recommended for pregnant women. The failure of iron supplementation programs to reduce anaemia in pregnant women has been attributed to various factors that influence the program delivery. Many pregnant women have little or no iron reserves and that is the reason why proper iron supplementation on a daily basis is recommended in antenatal clinics. Oral iron supplementation has been clinically shown to prevent iron deficiency (Jasti *et al*., 2005). Most Ministries of Health in developing countries including Malaysia have policies to give pregnant women either iron by itself or combined with folic acid in tablet form or prenatal vitamins. For example, national protocols in Malaysia require the provision of tablets of ferrous fumarate, folic acid, vitamin B complex and vitamin C. All pregnant women are prescribed prophylactic oral iron and folic acid supplements together with vitamin B complex and vitamin C and if the haemoglobin level ranges between 10-11 g/dL, oral treatment is indicated (Ministry of Health Malaysia, 2006). Despite these policies, prevalence of anaemia during pregnancy has not declined significantly. Many nutrition experts believe that one of the main reasons national iron supplementation program has failed is women's "non-compliance" with taking iron supplements daily (Galloway *et al*., 2002).

In Malaysia, vitamin and mineral supplementation programs have been a major strategy to reduce iron deficiency in pregnancy and the dynamic nature of nutrition problems meaning that strategies need regular review to maintain and improve their effectiveness. Despite efforts to reduce anaemia during pregnancy, information on compliance with vitamin and mineral supplementation is still considered limited. Since managing anaemia in late pregnancy poses a big challenge as it takes as long as two weeks to increase the haemoglobin level by 1 g/dL, detailed knowledge on supplements compliance might contribute to targeting efforts to those subgroups that are at higher risk. To the best of our knowledge, there is an absence of reliable epidemiological data in relation to compliance to supplementation locally. Therefore, it is difficult to evaluate intervention programme. In view of this limitation, this study was conducted with the aim of identifying compliance level with vitamin and mineral supplementation among pregnant women in urban and rural areas, factors associated with non-compliance and a comparison between blood haemoglobin and compliance level. It is hypothesized that percentage of pregnant women who complied with the supplementation is higher in urban area and haemoglobin concentration is higher in pregnant women who complied with the supplementation.

MATERIALS AND METHODS

Study locations and subjects: A cross-sectional study was conducted in three maternal and child health clinics in Selangor, central Malaysia representing urban area and five maternal and child health clinics in Johor, southern Malaysia representing rural areas. A total of 195 pregnant women were recruited by convenience sampling method. However, only 118 subjects were eligible and met the criteria of the study. The inclusion criteria in this study were Malay pregnant women of 21 to 40 years old and free from any health or obstetrics complications. Women with multiple pregnancies, gestational diabetes mellitus pregnant women or had other medical problems such as thalassemia were excluded from this study. Inform consent was obtained from each participant and ethical approval for the study was obtained from the Medical Research Ethics Committee of Universiti Kebangsaan Malaysia.

Questionnaire: A structured questionnaire was used to obtain information on demography profile, pregnancy history, eating habits during pregnancy, supplements intake, self-reported compliance level and knowledge about anaemia. This questionnaire was modified from Kalimbira *et al*. (2009). The questionnaire had been pilot-tested and tested on reliability (Cronbach-alpha = 0.825) in pregnant women attending antenatal care at maternal and child health clinic in Penang, northern Malaysia. Visits to clinics were paid to discuss about the protocol with the clinic's staffs. Subjects of the study were asked to fill in the questionnaire during their routine antenatal care at their respective clinics. Their antenatal data such as gestational week, weight and haemoglobin level were obtained from their antenatal record in the clinic. Haemoglobin level was measured using haematology analyzer available at the respective clinics. The haemoglobin values were classified according to the WHO (1972) anaemia categories [10.0 -10.9 g/dL (mild anaemia), 7-9 g/dL (moderate anaemia) and <7 g/dL (severe anaemia)].

According to Cramer *et al*. (2007), medication compliance refers to the act of conforming to the recommendations made by the provider with respect to

timing, dosage and frequency of medication taking. Therefore, medicated compliance may be defined as the extent to which a patient acts in accordance with the prescribed interval and dose of a dosing regimen. Compliance is measured over a period of time and reported as a percentage. Compliance level in this study were assessed based on the subjects' self-report questionnaire on their supplements ingestion for the past 30 days. Supplements ingestion as reported by the subjects were then compared to the prescription by doctors at the health clinic. Ingestion lower than the actual prescription was categorized as low compliance.

Statistical analysis: SPSS programme version 17.0 (Statistical Package for Social Science) was used to analyze the data. Data were summarized by using means and standard deviations and percentages. Categorical variables were expressed as counts and percentages. Data normality was checked using Kolmogrov-Smirnov test before statistical test was carried out. Group comparisons with respect to categorical data were performed by using Chi-square test. Multinomial Logistic Regression test were performed to assess the factors that contributes significantly to the compliance level. One way ANOVA was used to compare blood haemoglobin level and compliance level. The significance level was preset at 0.05.

RESULTS

Demographic profile: Of the 118 subjects, 62 (52.5%) were from urban areas and 56 (47.5%) were from rural areas (Table 1). Majority of the subjects were in the age group of 26 to 30 years old (44.9%). A large number of the subjects (67.8%) were working and having an education level up to secondary education (55.9%). More than half of the subjects' family (53.4%) had a household income between RM 1500 to RM 3500 (USD 458 to USD 1068) per month.

Antenatal profile: More than three quarter of the subjects (79.7%) were in the third trimester of pregnancy compared to 20.3% were in the second trimester. Majority of them were multiparous (58.5%) while the rest were primiparous (41.5%). For all subjects, the mean±SD of body weight was 63.7±15.1 kg and their haemoglobin concentration was 11.3±1.1 g/dL.

Compliance level among subjects: Overall, a half of the subjects did not comply with supplementation (50.8%) while 49.2% were compliant (Fig. 1). More subjects in urban areas were found to be non-compliant (53.2%)

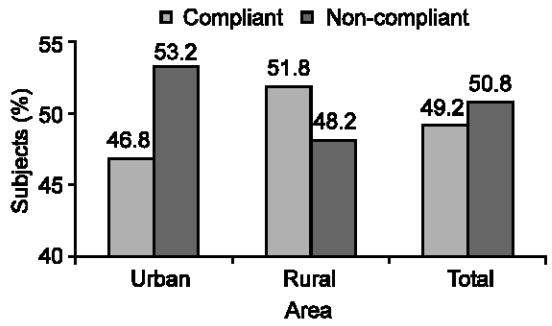

Fig. 1: Self-reported compliance with the supplementation according to areas

Table 1: Socio-demographic and antenatal characteristics of subjects

	Urban n = 62		Rural n = 56		Total n = 118	
Data	n	%	n	%	n	%
Age (years)						
21-25	14	22.6	15	26.8	29	24.6
26-30	32	51.6	21	37.5	53	44.9
31-35	12	19.4	13	23.2	25	21.2
36-40	4	6.5	7	12.5	11	9.3
Occupation						
Working	41	66.1	39	69.6	80	67.8
Housewives	21	33.9	17	30.4	38	32.2
Household income						
< RM 1500 (USD 458)	14	22.6	23	41.1	37	31.4
RM 1500-RM 3500 (USD 458-USD1068)	38	61.3	25	44.6	63	53.4
> RM 3500 (USD 1068)	10	16.1	8	14.3	18	15.3
Education						
Primary school	-	-	4	7.1	4	3.4
Secondary school	37	59.7	29	51.8	66	55.9
Tertiary level	25	40.3	23	41.1	48	40.7
Parity						
Primiparous	31	50	18	32.1	49	41.5
Multiparous	31	50	38	67.9	69	58.5
Trimester						
Second	8	12.9	16	28.6	24	20.3
Third	53	87.1	40	71.4	94	79.7

with supplements compared to their counterparts in the rural area (48.2%). The percentage of subjects in the urban areas who complied with the supplementation was slightly lower (46.8%) than their counterparts (51.8%) in the rural areas. However, this difference was not statistically significant (p = 0.305).

Incidence of anaemia during pregnancy: A total of 42.3% of the subjects in this study had anaemia (Fig. 2). There was no significant difference in the incidence of anaemia between subjects in the urban areas (46.8%) and rural areas (37.5%), p = 0.169. Only 1.6% subjects from the urban area had moderate anaemia while the rest 45.2% had mild anaemia. In the rural area, 37.5% had mild anaemia. Percentage of subjects without anaemia was 53.2 and 62.5% in urban and rural areas respectively.

Incidence of anaemia during pregnancy according to compliance level: Most of the subjects who complied with the supplementation had a normal haemoglobin concentration (65.5%) compared to the non-compliant (50.0%) (p = 0.088). Incidence of anaemia among subjects who were compliant was 34.5% and 50.0% among non-compliant. Majority of subjects who were anaemic in both compliant (37.9%) and non-compliant group (54.5%) were from urban area. On the other hand, majority of the subjects who had normal haemoglobin level in both compliant (69.0%) and non-compliant (55.6%) groups were from rural area.

Factors influencing compliance with the supplementation: The major factors reported as barriers for not complying with the supplementation were forgetfulness (33.9%), side effects (11.9%), worry to have big baby (5.1%), taking supplements other than prescribed in antenatal clinics (5.1%) and lack of knowledge (3.4%) (Table 2). Side effects of the supplements reported by the subjects were nausea (9.3%), constipation (9.3%), unpleasant taste/smell of tablets (7.6%) and vomiting (5.1%). It was found that only side effects, worry to have big baby, nausea and vomiting were significant contributing factors to poor compliance level among all subjects in this study. Nevertheless, forgetfulness was a significant contributing factor for both urban and rural subjects. Among the subjects from rural areas, working status was a significant contributing factor to non compliance (p = 0.025).

Comparison of blood haemoglobin concentration and compliance level: This study found that non-compliant pregnant women had a significantly lower blood haemoglobin concentration F (1,116) = 6.79, p<0.05), than compliant pregnant women. The mean±SD of haemoglobin concentration for non compliant pregnant women was 11.0±1.0 g/dL while for compliant pregnant women was at 11.5±1.2 g/dL.

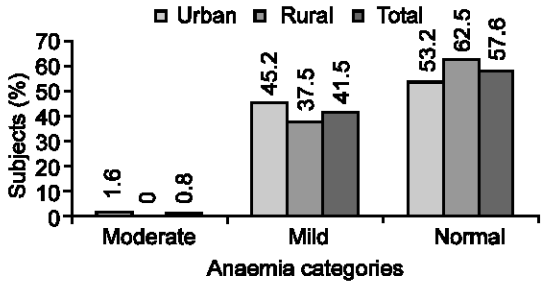

Fig. 2: Incidence of anaemia during pregnancy according to areas

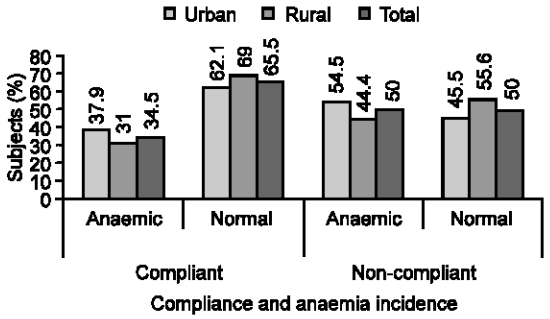

Fig. 3: Incidence of anaemia according with compliance to supplementation

Table 2: Factors influencing compliance level according to area

	Urban n = 62		Rural n = 56		Total n = 118	
Factors	n	%	n	%	n	%
Lack of knowledge on supplement intake	3	4.8	1	1.8	4	3.4
Side effects like nausea, vomiting, constipation etc.	9	14.5	5	8.9	14	11.9
Health problems	1	1.6	1	1.8	2	1.7
Unwanted pregnancy	-	-	1	1.8	1	0.8
Worry to have big baby	4	6.5	2	3.6	6	5.1
Supplements given insufficient	-	-	1	1.8	1	0.8
Taking other supplements	1	1.6	5	8.9	6	5.1
Forgetfulness	29	46.8	11	19.6	40	33.9

Table 3: Socio-demography and antenatal factors influencing compliance level according to areas

	Chi square, p value		
Factors	Urban n = 62	Rural n = 56	Total n = 118
Age	-	-	0.422
Occupation	0.025	0.640	0.190
Income	-	-	0.140
Education	0.498	-	-
Trimester	-	0.447	0.201
Parity	0.445	0.698	0.685

Table 4: Factors influencing compliance level with supplementation according to areas

	Chi square, p value		
Factors	Urban n = 62	Rural n = 56	Total n = 118
Lack of knowledge on supplements intake	-	-	0.045
Side effects	-	-	0.005
Nausea	-	-	0.031
Vomiting	-	-	0.013
Constipation	-	-	0.373
Worry regarding the size of baby	-	-	0.027
Forgetfulness	0.020	0.002	<0.001

DISCUSSION

This study aims to identify the compliance level with vitamin and mineral supplementation among pregnant women. This study has found that about 50% pregnant women did not comply with the supplementations. The result of the present study is higher than the earlier report by Saerah (1998) who reported that 26% of pregnant women did not comply with the supplements prescribed in Perak, northern Malaysia. This can be due to the higher percentage of working pregnant women in this study and working status was a significant contributor to the compliance level in urban areas.

The overall incidence of anaemia in this study was 42.3% and this value is higher compared to previous studies conducted in Malaysia (Jamaiyah *et al*., 2007; Rosline *et al*., 2005). Jamaiyah *et al*. (2007) conducted a study on maternal anaemia in Kelantan, east coast of Malaysia and reported that prevalence was 35.0%. Earlier, Rosline *et al*. (2005) reported 34.6% of anaemia incidence at the same area. The incidence of maternal anaemia among the subjects in this study is comparable with the finding published in an earlier study by Zulkifli *et al*. (1997). They reported that maternal anaemia was 47.5% among pregnant women attending health clinics in rural Kelantan.

The result of this study shows that there was no significant difference in incident of anaemia in urban and rural areas. This may be because the socioeconomic and educational characteristics of the subjects were almost similar despite the subjects lived in different areas. Previous study showed that low educational status and socioeconomic status were associated with anaemia during pregnancy (Okwu and Ukoha, 2008). Munasinghe and Van Den Broek (2006), also reported a higher prevalence of anaemia among pregnant women attending antenatal care in the rural area (72%) compared to in the urban area (57.1%).

The present study also shows that there was no significant difference between the percentage of compliance between subjects in urban (46.8%) and rural (51.8%) areas. This could partly explain why the incidence of anaemia between the two areas was not statistically significant. To our knowledge, no other studies compared the compliance with supplementations among pregnant women in urban and rural areas.

This study shows that the incidence of anaemia among subjects who did not comply with the supplementation was significantly higher compared to the compliant group and 50% of pregnant women who did not comply with the supplementation were having anaemia. This finding provides further support that compliance with supplementation do increase haemoglobin concentration during pregnancy. Zulkifli *et al*. (1997) found that only 50% of those mothers who were anaemic during their first visit to antenatal clinics, had improved haemoglobin levels by their last visit in spite of routine prophylactic oral iron supplementation for all pregnant women. The authors speculated that poor improvement in haemoglobin levels could be due to poor compliance among the subjects.

Overall, the subjects of this study reported that side effects including nausea and vomiting, worry to have big size baby and forgetfulness were the reasons for poor compliance. Other studies have produced similar results. A study by Kalimbira *et al*. (2009) in Malawi also found that 20.7% of their respondents have problems with their supplements with 43.6% of them reporting had experienced nausea. Another study in Vietnam, the respondents reported that they wrap the iron tablets in

pumpkin leaves or eat them with other food to disguise their bad smell and taste (Aikawa *et al*., 2006). It was reported that experiencing side effects was a risk factor for taking iron tablets. Different forms of iron have reduced side effects and newer forms of iron such as sprinkle, candies and beverages have the potential to reduce side effects and thus increase the compliance level accordingly (Aikawa *et al*., 2006). However, Malaysia has not embarked on the strategy of providing these newer forms of iron. According to WHO (2001) side-effects of iron tablets generally increase with higher dosages. These side-effects can be reduced if supplements are taken with meals, however the absorption is reduced by about 40%. If the supplement prescribed in the form of a single tablet, it is best ingested at bedtime. Compliance usually diminishes due to intolerance when more than one iron tablet is required. In such cases, prescribing one daily tablet instead of two is better for subjects who experience intolerance. One tablet taken consistently is preferable to the risk of total rejection or non-acceptance of supplements.

This study found that compliance level with the supplementation was not influenced by any socio-demography characteristics except for working status of urban pregnant women. Perhaps they forgot to take their supplements during working hours and some may have left their supplements at home. Saerah (1998) also reported that working mothers were more likely to be the non-compliant group. A study on iron tablets intake among adult women in Vietnam showed that majority of the respondents made efforts not to forget to take iron tablets by placing their pill within easy access or by asking their spouse to remind them (Aikawa *et al*., 2006).

A few limitations of this study should be addressed. First, the subjects of this study were recruited only from two states, Selangor and Johor. Thus, the findings may not represent the compliance level with vitamin and mineral supplementation among all pregnant women in Malaysia. Clearly, there is a need to carry out further research with a larger sample size and covering more areas throughout the nation. Second, information on compliance level with vitamin and mineral supplementation was obtained from a self-report questionnaire. Therefore, we could not reject the possibility of some subjects who did not report the actual ingestion. Methods of pill counts or microchip system to predict compliance level could be used in future studies.

Conclusion: This study has highlighted a number of important findings. First, the compliance level with vitamin and mineral supplementation among pregnant women in both urban and rural areas are found to be low and majority of them addressing side effects and forgetfulness as the main contributing factor towards non-compliance. With low compliance level, the prevalence of anaemia during pregnancy is still high among pregnant women in both urban and rural areas. The haemoglobin concentration of pregnant women who complied with the supplementation is significantly higher than the non-compliant pregnant women. The findings from this study highlight that antenatal health and nutrition intervention programmes for pregnant women is needed. A comprehensive nutrition education and health promotion program should highlight the importance of compliance with vitamin and mineral supplementation for both urban and rural pregnant women. Compliance level can be increased by focusing on disseminating the knowledge of anaemia among pregnant women and the importance of taking vitamin and mineral supplementation during pregnancy. Focus can be given to working women and to counter forgetfulness.

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REFERENCES

Aikawa, R., M. Jimba, K.C. Nguen, Y. Zhao, C.W. Binns and M.K. Lee, 2006. Why do Adult Women in Vietnam Take Iron Tablets? BMC Public Health, 6: 144.

Brabin, B.J., M. Hakimi and D. Pelletier, 2001. An analysis of anemia and pregnancy-related maternal mortality. J. Nutr., 131: 604S.

Christian, P., J. Shrestha, S.C. LeClerq, S.K. Khatry, T. Jiang, T. Wagner, J. Katz and K.P. West Jr., 2003. Supplementation with micronutrients in addition to iron and folic acid does not further improve the hematologic status of pregnant women in rural Nepal. J. Nutr., 133: 3492.

Cramer, J.A., A. Roy, A. Burrell, C.J. Fairchild, M.J. Fuldeore, D.A. Ollendorf and P.K. Wong, 2007. Medication compliance and persistence: Terminology and definitions. Value Health, 11: 44-47.

Galloway, R., E. Dusch, L. Elder, E. Achadi, R. Grajeda, E. Hurtado, M. Favin, S. Kanani, J. Marsaban and N. Meda, 2002. Women's perceptions of iron deficiency and anemia prevention and control in eight developing countries. Social Sci. Med., 55: 529-544.

Hadipour Reihaneh, A.K. Norimah, B.K. Poh, F. Firoozehchian, R. Hadipour and A. Akaberi, 2010. Haemoglobin and serum ferritin levels in newborn babies born to anaemic Iranian women: A cross-sectional study in an Iranian hospital. Pak. J. Nutr., 9: 562-566.

Hoque, M., E. Hoque and S.B. Kader, 2009. Risk factors for anaemia in pregnancy in rural KwaZulu-Natal, South Africa: Implication for health education and health promotion. S.A. Fam. Pract., 51: 68-72.

Idowu, O.A., C.F. Mafiana and S. Dopu, 2007. Anaemia in pregnancy: A survey of pregnant women in Abeokuta, Nigeria. Afr. Health Sci., 5: 295.

Jamaiyah, H., A. Das, L.T. Onn, C.W. Sun, N.M. Nordin, S. Rampal, S. Bahrin, M. Ganeslingam, K.I. Kularatnam and Z.M. Zaher, 2007. Anemia in pregnancy in Malaysia: A cross-sectional survey. A. P. J. Clin. Nutr., 16: 527.

Jasti, S., A.M. Siega-Riz, M.E. Cogswell, A.G. Hartzema, and M.E. Bentley, 2005. Pill count adherence to prenatal multivitamin/mineral supplement use among low-income women. J. Nutr., 135: 1093.

Kalimbira, A.A., B.M. Mtimuni and D.M. Chilima, 2009. Maternal knowledge and practices related to anaemia and iron supplementation in rural Malawi: A cross-sectional study. Afr. J. Food Agric. Nutr. Dev., 9: 550.

Ministry of Health, 2006. Guidelines on Nutrition Management for Anemic Pregnant Women. Nutrition Unit, Family Health and Development Unit, Ministry of Health, Malaysia.

Munasinghe, S. and N. Van Den Broek, 2006. Anemia in Pregnancy in Malawi- A Review. Malawi Med. J., 18: 160-174.

Okwu, R.N. and A.L. Ukoha, 2008. Studies on the predisposing factors of iron deficiency anaemia among pregnant women in a Nigerian community. Pak. J. Nutr., 7: 151-156.

Ramakrishnan, U., L.M. Neufeld, T. Gonzalez-Cossio, S. Villalpando, A. Garcia-Guerra, J. Rivera and R. Martorell, 2004. Multiple micronutrient supplements during pregnancy do not reduce anemia or improve iron status compared to iron-only supplements in Semirural Mexico. J. Nutr., 134: 898.

Rosline, H., W.Z. Abdullah and N.H.N. Hussain, 2005. Anemia and iron status of malay women attending an antenatal clinic in Kubang Kerian, Kelantan, Malaysia. Southeast Asian J. Trop. Med. Public Health, 36: 1304.

Saerah, S., 1998. Compliance to iron supplementation among pregnant women at public antenatal clinics in kinta district, perak. Masters Thesis, Department of Public Health, Universiti Kebangsaan Malaysia.

Scholl, T.O., 2005. Iron status during pregnancy: Setting the stage for mother and infant. Am. J. Clin. Nutr., 81: 1218S.

World Health Organization (WHO), 1972. Nutritional Anemias: Technical Report Series no. 503. Geneva: World Health Organization.

World Health Organization, United Nations Children's Fund, United Nation University (WHO), 2001. Iron Deficiency Anemia: An Assessment, Prevention and Control. A Guide for Programme Managers WHO/NHD/01.3. Geneva: World Health Organization.

Zulkifli, A., R. Jaafar, M.H.M. Hassan, M.S. Othman and A. Hashim, 1997. Anaemia during pregnancy in rural Kelantan. Mal. J. Nutr., 3: 83-90.

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Effect of Heating on the Chemical Composition and Physico - Chemical Properties of *Arachis hypogea* (Groundnut) Seed Flour and Oil

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Abstract: Groundnut (*Arachis hypogea*) seeds (raw, sun-dried and roasted) were analyzed for proximate composition and some nutritionally valuable minerals. The oil extracted from the samples was subjected to physico-chemical analysis. The results showed that the raw, sun-dried and roasted seeds contained 46.10%, 43.80%, 40.60% fat, 24.70%, 21.80%, 18.40% crude protein, 17.41%, 27.19%, 36.11% carbohydrate, 7.48%, 3.40%, 1.07% Moisture, 2.83%, 2.43%, 2.41% crude fibre and 1.48%, 1.38%, 1.41% ash respectively. There was a general decrease in the proximate composition after exposure to different heating methods but there was variation in the mineral contents of the seeds after heating. Minerals included; sodium (0.71%, 0.69%, 0.57%), phosphorus (0.68%, 0.65%, 0.69%). potassium (0.47%, 0.51%, 0.55%), zinc (0.44%, 0.42%, 0.50%), Iron (0.40%, 0.47%, 0.43%). The physico-chemical properties showed; saponification values of the raw, sun-dried and roasted groundnut oil, 201,195 and 170mg/kOH/g respectively. iodine value, 110.7, 108.5, 100.7 wijs, free fatty acid 1.180, 0.891, 1.260g/100g, acid value 2.35, 1.79, 2.52mg/kOH/g, peroxide value 0.740, 0.603, 0.470 meq/ kOH, refractive index 0.247, 0.256, 0.147. The roasted groundnut can be considered as a good source of valuable minerals, while the raw groundnut is a good source of protein with high nutrition value.

Key words: *Arachis hypogea*l, seed flour, heating effect, chemical composition, therapeutic properties

INTRODUCTION

Groundnuts (*Arachis hypogea*) or peanut is a legume which is widely grown as a food crop. It is an annual crop principally for its edible oil and protein rich kernel seeds, borne in pods which develop and mature below the soil surface. The groundnut is an herbaceous plant of which there are many varieties, some of which are Boro light, Boro red, Ela , Mokwa, Guta and campala. The fatty acids and physico-chemical analysis of the oils of the varieties had been investigated (Anyasor *et al.,* 2009).

Edible oils from plant sources are of interest in various food and application industries. They provide characteristic flavours and textures to foods as integral diet components (Odoemelam, 2005) and can also serve as a source of oleo chemicals (Morrison *et al.,* 1995). Oleo chemicals are completely biodegradable and so could replace a number of petrochemicals. In Nigeria, 1917 tons of peanuts are being produced annually (Ergul, 1988). Vegetable oils had made an important contribution to the diet in many countries, serving as a good source of protein, lipid and fatty acids for human nutrition including the repair of worn-out tissues, new cells formation as well as a useful source of energy (Gaydon *et al*., 1983; Grosso and Guzman, 1995; Grosso *et al*., 1997; 1999).

Beside income for farmers, groundnut provides an inexpensive source of high quality dietary protein and oil. The vast food preparations incorporating groundnut to improve the protein level have helped in no small way in reducing malnutrition in the developing countries. The special taste and flavour of foods containing groundnut is important in the acceptance of these food preparations (Asibuo *et al*., 2008). The quality of the oil and groundnut products depends to a large extend on the oil fraction. The oil content of groundnut differs in quantity, the relative proportion of fatty acids, geographical location , seasons and growing conditions (Adeyeye and Ajewole, 1992). Groundnut seed contains 44 to 56% oil and 22 - 30 % protein on a dry seed basis and is a rich source of minerals (phosphorus, calcium, magnesium and potassium) and vitamins E, K, and B group (Savage and Keenan, 1994).

Groundnut protein is increasingly becoming important as food and feed sources, especially in developing countries where protein from animal sources are not within the means of the majority of the populace. The seed has several uses as whole seed or processed to make peanut butter, oil soups, stews and other products. Groundnut provides considerable amount s of mineral elements to supplement the dietary requirements of humans and farm animals (Asibuo *et al*., 2008). Groundnut seeds are reported to contain

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9.5-19.0% total carbohydrates as both soluble and insoluble carbohydrate (Croker and Barton, 1957; Oke 1967; Woodroof, 1983).

The chemical composition of groundnut seeds has been evaluated in relation to protein level (Young and Hammons, 1973), amino acid composition (Young and Hammons, 1973) and fatty acid composition (Grosso and Guznam, 1993) in several countries. Groundnut is an example of vegetable protein (David, 1987) which is used in natural health care as source of proteins in balance diet. With the increase of fake drugs in many developing countries people are encouraged to eat natural products such as fruits and vegetables. Therefore, there is need to investigate the form in which most of these products should be consumed in order to conserve the proximate, mineral and fatty acid composition for the different purposes they are meant for, such as therapeutic, prophylactic and commercial (Elizabeth, 1994). It is common knowledge that in Nigeria roasted plantain, and groundnut are good for men. The roasted groundnut is particularly good. Health is the individual responsibility. We should ensure that we eat so much of the natural food items and eat them in their natural form where possible. If cooked, they should not be over-cooked as the sensitive minerals and vitamins are easily destroyed by heat. (Elizabeth, 1994). This study seeks to investigate the effect of heat on the proximate, mineral and fatty acid compositions of groundnut.

MATERIALS AND METHODS

Sample collection and preparation: Groundnuts (*Arachis hypogea*) were purchased from Araada market, Ogbomoso, Oyo State, Nigeria and was transported in a polythene bag to the laboratory. It was divided into three: one part was sun-dried for seven days, second part was roasted and the third part was air-dried for seven days. Portion (1kg) of each sample was oven dried at 100-105°C, the red skins were removed, squeezed with hand, cracked into small pieces, placed in an air tight bottle and stored in the desiccators for analysis.

Proximate analysis: Moisture content was determined by drying to constant weight at 100 -107°C in an Oven, ash content by Ignition at 550°C in a muffle furnace for 4hr, oil content by Soxhlet extraction with hexane as solvent , protein by the kjeldahl method, and crude fibre by the acid and alkaline digestive methods all described by Lees (1975). The carbohydrate content was estimated by difference, subtracting the sum of water, protein fat, crude fibre and ash percentages from one hundred.

Oil analysis: Peroxide value was evaluated according to AOAC (1990). The saponification value was determined according to the titre metric method of Pearson (1981). Iodine value was determined according to wij"s method of Pearson (1970). Acid value was determined by titre metric method of Pearson (1970).

Minerals: The mineral contents were determined by digesting the ash with 3M hydrochloric acid and using the atomic absorption spectrophotometer for magnesium, zinc, iron and the flame photometer for potassium, sodium, calcium and phosphorus (Pearson, 1981).

RESULTS AND DISCUSSION

Based on the results of the proximate composition of the groundnut as shown in Table 1, the moisture content ranged from 1.07-7.48%, ash content 1.38-1.48%, crude fibre 2.41-2.83%, crude protein 18.40 -24.70%, dried matter 92.52-98.93%, oil content 40.60- 46.10% and carbohydrate (by difference) 17.41%, 27.19%, 36.11% respectively.

Table 1: Proximate composition and mineral contents of the groundnut samples

	Groundnut		
Compositions	Raw % dry weight	Sun-dried % dry weight	Roasted % dry weight
Moisture content	7.48	3.40	1.07
Ash content	1.48	1.38	1.41
Crude fibre	2.83	2.43	2.41
Oil content	46.10	43.80	40.60
Crude protein	24.70	21.80	18.40
Carbohydrate	17.41	27.19	36.11

Results are means of three determinations

Table 2: Mineral composition of the groundnut on dry weight basis

Mineral	Raw groundnut	Sun-dried groundnut	Roasted groundnut
	(%)		
Sodium (Na)	0.71	0.69	0.57
Potassium (k)	0.47	0.51	0.55
Calcium (Ca)	1.18	1.24	1.35
Magnesium (Mg)	0.18	0.21	0.24
Iron (Fe)	0.40	0.47	0.47
Zinc (Zn)	0.44	0.42	0.50
Phosphorus (P)	0.68	0.65	0.69

Results are means of three determinations

The protein, ash and crude fibre contents were similar to the reports of Nelson and Carlos (1980). The average crude fibre contents in this result indicate the ability of groundnut to maintain internal distension for a normal peristaltic movement of the intestinal tract: a physiological role which crude fibre plays. Diet low in crude fibre is undesirable and may cause constipation and that such diet have been associated with diseases of colon like piles appendicitis and cancer. This study shows that raw groundnut is more advantageous in nutritional value than roasted groundnut, while the

Table 3: Chemical analysis of the raw and the heat treated groundnut oil

Parameter	Unit	Raw groundnut	Sun-dried groundnut oil	Roasted groundnut oil
Saponification value	Mg/KOH/g	201	195	170
Acid value	Mg/KOH/g	2.35	1.79	2.52
Iodine value	Wij's	110.7	108.5	100.7
Peroxide value	Meq/KOH	0.740	0.603	0.470
Refractive Index		0.247	0.256	0.147
Free fatty acid	g/100 g FFA	1.180	0.891	1.260

roasted one, is also advantageous in mineral contents than the raw groundnut.

Raw groundnut showed a good source of sodium, the sun-dried groundnut is a good source of magnesium and iron while the roasted groundnut is a good source of potassium, calcium, zinc and phosphorus.

The good availability of calcium, magnesium, phosphorus is a good indication that the groundnut is so rich in the minerals for bone formation. Calcium is very essential in blood clotting, muscles contraction and in certain enzymes in metabolic processes.

The moisture content of the raw groundnut was higher than the sun-dried and roasted because the raw groundnut was not previously exposed to any heat. The ash content of both the raw and roasted groundnuts were higher than the sun-dried because the sun-heat which normally contain UV-ray can easily react with some of the volatile compounds of the groundnut. The crude fibre of the raw groundnut was higher since no chemical reaction which can alter the composition was initiated. The oil was higher in raw groundnut while in both the sun-dried and roasted groundnut, the heat, depending on the intensity would have affected the quantity of oil.

The proximate compositions of the groundnuts were affected by heat and this explained why the moisture content, ash , crude fibre, extracted oil and crude protein of the raw sample were higher than those of the groundnut subjected to heat treatment. The carbohydrate content was higher in the roasted and the sun-dried than in the raw groundnut. Carbohydrate content ranging from 6.0 - 24.9% was reported by Duke (1981) in groundnut. Thus, in comparison with raw and sun-dried samples, roasted groundnut showed a general increase in mineral contents, especially in potassium, calcium, magnesium, zinc and phosphorus, and agrees with the observation of Derise *et al.* (1974) that roasted groundnut does not lead to reduction in the levels of mineral elements but rather increases the levels since volatile compounds are lost through heating (Table 2).

Table 3 shows the physico-chemical properties of groundnut oil. The saponification value ranged from 170 to 201 agreed with Pearson's (1981), 187 to 196mgkOH/g. Denniston *et al.* (2004) reported that high saponification value indicated the presence of greater number of ester bonds, suggesting that the fat molecules were intact. These properties make it useful in soap making industry, it is not attractive as a raw material because of its economic and nutritive implications Similarly, acid value of the roasted groundnut was relatively higher than the other two samples. According to Demian (1990), acid values are used to measure the extent which glyceride in the oil has been decomposed by lipase and other actions such as light and heat. The determination is often used as a general indication of the condition and edibility of oil. The iodine value reduced from raw groundnut (110.7) to the roasted groundnut oil (100.7) this indicates low degree of unsaturation and classified the oil as non-drying is recorded for most edible oil, Pearson (1981).

The peroxide values of all the oil samples were less than the standard peroxide value ($10mEqKg^{-1}$) for vegetable oil deterioration. Fresh oils have value less than $10mEq\ Kg^{-1}$ and value between 20 and $40mEqKg^{-1}$ results in rancid taste (Akubugwo and Ugbogu, 2007). The low peroxide value indicated slow oxidation of these oils according to Demian (1990), the peroxide formation is slow at first during an induction period that may vary from a few weeks to several months according to the particular oil and temperature(Pearson, 1981).There was no rancidity of oil samples in the course of this study the low peroxide value (0.44-0.74) Meq/KOH, indicate that the oil, especially the roasted groundnut oil can resist lipolytic hydrolysis and oxidative deterioration. The refractive index (0.147-0.247) shows that the oils contained some double bonds in its fatty acid (Eromosele and Pascal 2003), and that refractive index increases as the double bond increases. The free fatty acid ranged from 1.24 to 1.26g/100g, it is an indication of the percentage of fatty acid present in the oil and that the oil may likely undergo oxidation. This study indicated that groundnut should be treated differently for different purposes, all the groundnut oils may have a shelf- life, nutritional value, medicinal value and industrial applications.

Conclusion: Groundnut seed flour and oil can be use for different purposes such as nutritional, medicinal and industrial, only, if correctly treated and selected. For nutritional and industrial uses raw groundnuts are the best. For medicinal use either therapeutic (treatment of diseases) or prophylactic (prevention of diseases) roasted groundnuts are advisable, especially, for fertility in man, since roasting does not lead to reduction in the levels of the mineral elements but rather increases the levels, therefore, groundnut is a good source of oil,

protein and minerals which can be used in diets to prevent against some mineral deficiencies. This will aid to fight against malnutrition, leading to better nutrition and health in Nigeria and Africa as a whole.

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REFERENCES

Adeyeye, A. and K. Ajewole, 1992. Chemical composition and fatty acid profiles of cereals in Nigeria. Food Chem., 44: 41-44.

Akubugwo, I.E. and A.E. Ugbogu, 2007. Physicochemical studies on oils from five selected Nigerian plant seeds. Pak, J. Nutr., 6: 75-78.

Anyasor, G.N., K.O. Ogunwenmo, O.A. Oyelana, D. Ajayi and J. Dangana, 2009. Chemical analyses of Groundnut (Arachis hypogaea) oil. Pak. J. Nutr., 8: 269-272.

AOAC, 1990. Official Methods of Analysis, 15th edn. Association of official Analytical Chemists, Washington D.C.

Asibuo, J.Y., R. Akromah, Safo- Kantanka, O. Osei Adu-Dapaah, O.S. Hans Kofi and A. Agyeman, 2008. chemical composition of groundnut, *Arachis hypogaea* (L) land races. Afr. J. Biotech., 7: 2203-2208. website http://www.academic journals. Org/ AJB.

Croker, W. and L.V. Barton, 1957. Physiology of seed, chronic Botanica, Waltham, Massacusetts, P. 267.

David Werner, 1987. Where there is no Doctor a village health care handbook for Africa. Revised Ed. Macmillan publishers Ltd London, P: 122.

Demian, M.J., 1990. Principles of food chemistry. 2nd Ed. Van Nostrond Reinhold international Company Ltd, London England, pp : 37-38.

Denniston, K.J., J.J. Topping and R.L. Cariet, 2004. General Organic and Biochemistry. 4th Ed. Mc draw Hill Companies New York, pp: 432-433.

Derise, N.L., H.A. Lau and S.J. Ritchie, 1974. Proximate Composition and mineral element content of three cultivars of raw and roasted peanut. J. Food Sci., 39: 264-255.

Duke, J.A., 1981. Hand book of legumes of world economic importance. Plenum Press. New York.

Elizabeth, K., 1994. Immense help from nature workshop. 1st Ed. Elikaf Health Services Ltd, Ikeja, Lagos, P-145.

Ergul, N., 1988. Peanut production. Mediterranean Agriculture Research Institute, Ankara-Turkey, publ. Nu., 308.

Eromosele, C.O. and N.H. Pascal, 2003. Characterization and viscosity parameters of seed oils from wild plants. J. Bio Resources Tech. 86: 203-205.

Gaydon, E.M., J.P. Bianchini and J. Ratovogery, 1983. Triter pene alcohols, methyl sterols, and fatty acid five Malagasy legume seed oils. J. Agric Food Chem., 31: 833-836.

Grosso, N.R. and C.A. Guznam, 1993. Lipid protein and ash contents and fatty acid And sterol composition of peanut (*Arachis hypogaea* L.).

Grosso, N.R., E.I. Lucini, A.G. Lopez and C.A. Guzman, 1999. Chemical composition of aboriginal peanut (*Arachis hypogaea* L.) seeds from Uruguay. Grasasy Aceites, 50: 203-207.

Grosso, N.R., J.A. Zygadlo, A.L. Lamarque, D.M. Maestri and C.A. Guzman, 1997. Proximate, fatty acid and sterol compositions of aboriginal peanut (*Arachis hypogaea* L.) seeds from Bolivia. J. Sci. Food Agri., 73: 249-356.

Grosso, N.R. and C.A. Guzman, 1995. Chemical composition of Aboriginal Peanut (*Arachis hypogaea* L.) seeds from Peru. J. Agri. Food Chem., 43: 102-105.

Lees, R., 1975. Food Analysis: Analytical and Quality Control Methods for the Food Manufacturer and Buyer. (3rd edn). Leonard Hill Books, London.

Morrison, W.H., R.J. Hamilton and C. Kalu, 1995. Sun flower seed oil. In; R.J.Hamilton,(ed.) Developments in oils and fats. Blackie Academic and Professional, Glasgow, pp: 132-152.

Nelson, R.G. and A.G. Carlos, 1980. Chemical Composition of Aboriginal peanut (*Arachis hypogea* L). Seeds from Peru. J. Agric. Food Chem., 43: 102-105.

Odoemelam, S.A., 2005. Proximate Composition and selected physicochemical properties of the seeds of African oil bean (pentaclethra marcrophylla). Pak. J. Nutr. 4: 382-383.

Oke, O.L., 1967. Chemical studies on some Nigerian pulses. W. Afr. J. Biol. Appl. Chem., 9: 52-55.

Pearson, D., 1970. The chemical Analysis of Food (6th ed.) J. A. Church hill, London, pp: 510-515.

Pearson, 1981. The chemical Analysis of food (8th edn) London Church Hill Livingstone, 10: 9-12.

Savage, G.P. and J.I. Keenan, 1994. The composition and nutritive value of groundnut kernels, In: smart J (ed). The Groundnut crop: scientific basis for improvement. London : Chapman and Hall, pp: 173-213.

Woodroof, J.G., 1983. Peanuts production, Processing products 3rd edition Avi plublishing Company Inc. Westport, Connecticut.

Young, C.T. and R.O. Hammons, 1973. Variations in the protein levels of a wide range of peanut genotypes (*Arachis hypogaea* L.) Oleagineux, 28: 293-297.

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Trace Elements and Major Minerals Evaluation of *Spondias mombin*, *Vernonia amygdalina* and *Momordica charantia* Leaves

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Abstract: Samples of the plants were collected in Oyo state at Igbo-agbonin in the Ogbomoso North Local Government Area, Sabo road, Ogbomoso and were analyzed for the presence of trace elements such as; Fe, Zn, Mn, Cr, Cu, Cd, and Pb using Atomic Absorption Spectrophotometry. The results showed that trace element concentrations in *Spondias mombin* (Hog plum) were as follow: Fe, 574.00mg/kg, Zn, 59.60mg/kg, Mn, 23.00mg/kg, Cr, 66.00mg/kg Cu, 13.00mg/kg, Cd 50.00mg/kg. The mineral composition results showed that the leaves contained K 1.20%, Ca 1.05% and P, 0.32% Na 1.80%. Results of trace elements concentration in *Vernonia amygdalina* leaves were as follow: Fe, 277.30mg/kg, Zn, 74.50 mg/kg, Mn, 227.00mg/kg, Cr, 89.00mg/kg Cu, 11.00mg/kg and Cd, 4.30mg/kg. The mineral analysis revealed in the plant leaves, K 0.51%, Na 0.57%, Ca 0.45%, P 0.23%. Result of trace elements concentration in *Momordica charantia* were as follow: Fe, 8.125mg/kg, Zn, 354.8mg/kg, Mn, 37.00mg/kg, Cr, 162.00mg/kg, Cu, 21.00mg/kg, Cd, 51.40mg/kg and Pb 48.00mg/kg. the mineral analysis revealed in the plant, K 0.81%, Na 0.93%, Ca 0.90%, P 0.81%. The results obtained from the study show that the three plants are medicinal, *Spondias mombin* and *Vernonia amygdalina* are good antianaemic and antidiabetics agents, because of the high contents of Iron and chromium present in them.

Key words: *Spondias mombin, Vernonia amygdalina, Momordica charantia*, Therapeutic, Prophylactic

INTRODUCTION

The three plants have been traditionally noted for their medicinal and food values. Therefore they are referred to as medicinal plants, these are plants in which one or more of their parts contain substances that can be used for therapeutic purposes (Ogunrinola *et al*., 2004). Medicinal plants, because of their physiological effect on the structure and function of living organisms, are widely used for the prevention, diagnosis, treatment of disease and for the relief of symptoms. Thus they are referred to as therapeutic drugs (Abayomi Sofowora, 1986). Medicinal plants posses some important elements in small doses which have both therapeutics and prophylactic properties. The element are referred to as trace elements (Peter Bratter and Peter Schramel, 1980). Trace elements are required in plant mainly for the formation of pigments and enzymes in animals. They function mainly to facilitate certain vital metabolic processes. Many of these elements pair-up with vitamins in the metabolism of carbohydrates, fat, and protein. Metabolic disease will arise in the absence of trace elements.

Preliminary result of *Spondias mombin* analysis gave a wide range of antibacterial and antifungal properties (Okwu, 2001; Urugulaga and Laghton 2000). The chemistry of *Spondias mombin* has been reported by Shultes and Raffauf, 1990.

Kramer *et al*. (2006) recommended its use for pregnant woman but only after five months of pregnancy. Njoku and Akumefula, 2007 reported the mineral analysis results of the *Spondias mombin* as follow, 2.55% Potassium (K), 0.10% Sodium (Na), 0.304% Magnesium (Mg), 1.31% Calcium (Ca) and 0.20% phosphorous (P). He also reported that the *Spondias mombin* leaves contained alkaloids, flavonoids, tannins, saponins and phenolic compounds. The *Spondias mombin* leaves can be used in herbal medicine for the treatment of common cold and other diseases like prostrate cancer (Okwu and Okwu, 2004; Okwu and Ekeke, 2003).

Vernonia amygdalina, which according to Hamowa (1994) could be used in traditional medicine to treat kidney disorder and hic cups, was also recommended as stomach purgatives, antihelmintics, antiasthmatics, carminatives and anti tumor agents.

The plant as reported contained carbohydrates and glucosides, flavonoid, glycosides, sterols, lactones and volatile substances. A bitter glycoside, vernomin, has been isolated from the root (Klein 1932).

A decoction of the leaves is taken as an antipyretic and laxative and for cough (expectorant). The bark of the root, and stems are astringent and find use as a febrifuge and in diarrhoea (David, 1983).

Locally the twigs are used as tooth cleaners and are chewed as a stomachic, tonic and appetizer. The root

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after removal of the bark by scorching is also taken as a tonic and appetizer and has been described as substitute for ipecacuanha. The leaves are used in soup and are applied in local medicine for itching and parasitic skin disease (Oliver, 1960).

Momordica charantia (family: Cucurbitaceae) with the common name "bitter Cucumber" is known as "Ejirin" in the Southwest of Nigeria (Gbile, 1984). In local medicine the juice of the leaves and fruit are given as an antihelmintic and the pulverized part is applied externally against malignant ulcers. (Oliver, 1960). The presence of two acidic resins and a bitter substance called momordicine in the leaves has been reported along with vitamin C. The presence of aminobutyric acid in the leaf has been indicated. The root has been estimated to contain about 12.84% ash. The fruit given about 7% ash the major elements present are silicon, Phosphorus, Iron Sodium, Zinc, Copper, Calcium and Strontium. The dry plant was reported to contain 0.038% of an unnamed alkaloid. The results of a screening on the entire plant of Congolese Origin showed the presence of trace amount of alkaloids and saponins but the absence of flavoids, tannins, quinines, steroids and terpenes.

Bitter Cucumber has a relatively high nutritional value compared with other Cucurbits, due mainly to the Iron and ascorbic acid content (Oliver, 1960).

Momordica charantia is used in the treatment of female problems such as in the darkened and scanty period, heat in the lower abdomen, gripping or pin-like pains, when it is not easy for a woman to conceive and also good during the period of pregnancy. It is also good for the treatment of diabetes, constipation, convulsion in children and as bacteriostatic (Elizabeth, 1994).

The objective of this study is to evaluate the trace elements and major minerals present in them so as to give recommendation on the amount to consume by an individual for medicinal purposes, since less information is available on the trace elements of these three plant leaves in South-West, Nigeria.

MATERIALS AND METHODS

Samples: Fresh leaf samples of study plants were collected From Igbo-agbonin along Sabo road, Ogbomoso, Oyo State, Nigeria.

Traffic: The roads adjoining the sample sites carry a lot of commuter traffic and are major feeder routes.

Instrumentation: The determinations of the trace elements were performed with the use of a buck 210 VGP model atomic absorption spectrophotometer. The instrument's setting and operational conditions were done in accordance with the manufacturer's specification. The instrument was calibrated with analytical grade standard metal solution ($1mgdm^{-3}$) in replicate. For the determination of minerals of the test samples (Na, Ca, K and P) flame photometer Jenway, U K model was used (Institute of Agricultural Research and Training, Obafemi Awolowo University, moor plantation,Ibadan, Nigeria).

The leaf samples were washed and oven dried at 15°C for three days. There after, the samples were ground into fine powder with a porcelain mortal and pestle and stored in air tight bottles prior to use for analysis.

Physico-chemical analysis: The trace and mineral elements were determined by wet acid digestion method as described by AOAC, 1980.

0.5g of each sample in 10ml Conc. HNO_3 in a covered flask placed in a fume cupboard for three days. Thereafter, the three covered flasks with the contents were heated on a hot plate for twenty-four hours, conc. HNO_3 was added intermittently as the content reduced until the sample solutions turned colorless.

They were cooled and transfered into 50ml volumetric flask and made up to mark with distilled water. The solutions were filtered and transferred into an analytical bottle, corked, and labeled, kept for AAS and flame photometric analysis.

RESULTS AND DISCUSSION

Table 1 shows the trace elements composition of the three medicinal plants. From the table it can be seen that *Spondias mombin* contained the highest concentration of iron. Iron is important for the building up of red corpuscles, essential for formation of haemoglobin, the oxygen - carrying pigment in red blood cells. Iron is used against anaemia, tuberculosis and disorder of growth (Claude and Paule, 1979). Iron is an energizer but excess can cause fatigue but we hardly have excess if taken from natural Source (Gbolahan, 2001).

The Zinc content was found to be 59.60mg/kg in *Spondia mombin*, 74.50mg/kg in *Vernonia amygdalina* and 364.8mg/kg in *Momordica charantia* indicating that *Momordica charantia* contained large quantity of zinc. Zinc is very important for nerve function and male fertility. It is important for normal sexual development especially for the development of testes and ovaries, it is also essential for reproduction. Zinc stimulates the activity of vitamins, formation of red and white corpuscles (Claude and Paule, 1979), healthy functioning of the heart and normal growth (Elizabeth, 1994).

The manganese content was found to be 23.00mg/kg in *Spondias mombin*, 27.00mg/kg in *Vernonia amygdalina* and 37.00mg/kg in *Mormodica charantia*, since these plants contain this element in different concentration, the quantity to be taken will depend on the therapeutic need of the individual. The activity of this element is noticed in the metabolism of food which is incorporated into the bone. According to Claude and Paule (1979), manganese is necessary for the functioning of the

Table 1: Trace elements composition of *Spondias mombin* leaves on dry weight basis expressed in mg/kg

Elements	Concentration (mg/Kg)
Fe	574.00
Zn	59.00
Mn	23.00
Cr	66.00
Cu	13.00
Cd	50.00
Pb	Nd

Results are mean of five determinations

Table 2: Mineral Composition of the leaves of *Spondias mombin* on dry weight basis expressed in %

Mineral	%
K	1.20
Na	1.80
Ca	1.05
P	0.32

Results are mean of five determinations

Table 3: Trace elements composition of the leaves of *Vernonia Amygdalina* on dry weight basis expressed in mg/kg

Elements	Concentration (mg/kg)
Fe	277.30
Zn	74.50
Mn	27.00
Cr	89.00
Cu	11.00
Cd	4.30
Pb	Nd

Results are mean of five determinations

Table 4: Mineral composition of the leaves of *Vernonia amygdalina* on dry weight basis expressed in %

Mineral	%
K	0.51
Na	0.57
Ca	0.45
P	0.23

Results are mean of five determinations

Table 5: Trace elements composition of *Momordica charantia* on dry weight expressed in mg/kg

Elements	Concentration (mg/kg)
Fe	8.125
Zn	354.8
Mn	37.00
Cr	162.00
Cu	21.00
Cd	51.40
Pb	48.00

Results are mean of five determinations

Table 6: Mineral composition of *Momordica charantia* dry weight basis expressed in %

Mineral	%
K	0.81
Na	0.93
Ca	0.90
P	0.81

Results are mean of five determinations.
Nd: Not detected

pituitary gland, the pineal gland and the brain, it promotes hepatorenal function, combat anemia and also essential for growth.

The chromium content was found to be 66.00mg/kg in *Spondias mombin*, 89.00mg/kg in *Vernonia amygdalina* and 162.00mg/kg in *Mormodica charantia*. The presence of chromium even at low concentration is an indication that the plants are useful, therefore, with high concentration of chromium in *Mormodica charantia* shows that this will be more effective in the management of diabetes. It is a co-factor with insulin in carbohydrate metabolism, therefore, if chromium is deficient, insulin will not be effective (Gbolahan, 2001).

The copper content were low with 13.00mg/kg in *Spondias mombin*, 11.00mg/kg in *Vernonia amygdalina* and 21.00mg/kg in *Momordica charantia*. Copper helps in the absorption of Iron, it is therefore often seen with Iron naturally. Copper is important for cellular defense and protection of the mucous membranes, anti anemic and essential for the formation of Iron and haemoglobin (Claude and Paule, 1979).

The result of the mineral composition clearly shows that *Spondias mombin* leaves contains rich source of mineral elements (Table 1). This result become so important when the usefulness of such minerals like Ca, K, Na and P in the body are considered. Calcium is necessary for the coagulation of blood, the proper functioning of the heart and nervous system and the normal contraction of muscles. Its most important function is to aid in the formation of bones and teeth. Most of these plants containing some percentage of calcium exhibit these properties.

Sodium and Potassium are closely related in the body fluids, they regulate the acid-base balance. Sodium remains one of the major electrolytes in the blood. Without sodium the body can not be hydrated, it would dry off. At the point where some vital processes are taking place sodium is not needed, too much will cause the cell to breakdown (Gbolahan, 2001).

This explains the lower percentage or content of the element in most medicinal plants. According to Njoku and Akumefula (2007) the lower Na content (0.100g of *Spondias mombin* is an added advantage because of the direct relationship of sodium intake with hypertension in human. Another mineral useful to the bone is phosphorus. It is mineral pair of calcium, the two of them go hand in hand, they are bound together in the bone, teeth and ligament of the body. It is very important for nerves, it will produce the same bone diseases like calcium will produce (Gbolahan, 2001). It is required in small quantity. All these explain why the minerals are present in different quantity in most medicinal plants.

Table 3 shows the result of trace elements composition of *Vernonia amygdalina* (bitterleaf) and the concentration of each of the element is indicated. Iron content was shown to be 277.30mg/kg, followed in high

content by chromium with 89.00mg/kg and zinc with 74.50mg/kg with these values obtained the plant would be antidiabetic, antianaemic and antihelmintics.

The presence of manganese, 27.00mg/kg, Cu, 11.00mg/kg are useful in the areas of treatment and prevention of diseases.

Table 4 shows the percentage composition of some of the minerals obtained, this indicates that potassium (K), 0.51%, sodium (Na), 0.57, Calcium (Ca) 0.45%, Phosphorus (P) 0.23%. their functions are in line with Table 2.

The results showed the concentration of trace elements in *Momordica charantia*, element with the highest value is zinc with 354.8mg/kg this is an indication that the plant would be good for anything concerning reproductive organs, fertility and healthy functioning of the heart (Table 5). The second element is chromium with value of 162.00mg/kg which also indicates that the plant *(Momordica charantia)* would be good in the management of diabetes.

The presence of other elements also shows that *Momordica charantia* is embodiment of medicinal properties.

Table 6 show the mineral composition of *Momordica charantia*. The result of the minerals composition clearly shows that the plant contains potassium (K) 0.81%, sodium (Na) 0.93%, Calcium (Ca) 0.90% phosphorus (P) 0.81%. The importance of all the minerals had earlier been explained under Table 1.

The presence of Cd and Pb in *Vernonia amygdalina* and *Momordica, charantia* call for concern because they are not required, even in small amounts, by living organisms (Adeniyi, 1996).

Conclusion: This present study shows the trace elements and major minerals composition of three different medicinal plants, these are, *Spondias mombin*, *Vernonia amygdalina* and *Momordica charantia*. This partly shows the uses of these plants in herbal medicine. As a rich source of minerals and beneficial trace elements, these plants can be seen as potential source of useful food and drugs.

Spondias mombin and *Vernonia amygdalina* are good antianaemic agents and antidiabetics agents because of the high contents of Iron and chromium present in them.

Further studies have to be carried out to isolate, characterize and elucidate the structure of the bioactive compounds from the plants for industrial drug formulation.

REFERENCES

Abayomi Sofowora, 1986. The State of Medicinal Plants Research in Nigeria. Faculty of Pharmacy, University of Ife, Ile-Ife Nigeria, pp: 213.

Adeniyi, A.A., 1996. Determination of Cd, Cu Fe, Pb, Mn, and Zn in water leaf. (Talinium Triangulare). J. Env. Int., 22: 259-262.

AOAC, 1980. Official methods of analysis 13th Ed. Washington Dc.

Claude Barreau and Paule Solomon, 1979. The manual of Natural living. 1st Ed. Biddles Ltd, Guildford, Surrey, pp: 98-101.

David Hoffmann, 1983. The New Holistic Herbal. 3rd Ed. Findhorn press, U.S.A., pp: 241.

Elizabeth Kafaru, 1994. Immense help from nature's workshop. 1st Ed. Elikaf Health Services Ltd., pp: 207-209.

Gbile, Z.O., 1984. Verncular Names of Nigerian plants, Yoruba, Forestry Research Institution of Nigeria, Ibadan.

Gbolahan, D., 2001. Lesson Note on medical importance of trace elements. Centre for Natural Health Studies.

Hamowa, A., 1994. Pharma cological studies on vernonia amygdalins, Vertinary Medical J., 42119-97.

Klein, 1932. Flanze Analyser. Handbook. Vol: II part 2, Springer.

Kramer, A., E. Mosquera, J. Riuz and E. Rodriguez, 2006. Ethanobotany and Biological Activity of Plants utilized during pregnancy and childbirth in the Peruvian Amazon. Emanation, 4: 31-35.

Njoku, P.C. and M.I. Akumefula, 2007. Phytochemical and Nutrient Evaluation of spondias mombin leaves pak. J. Nutr., 6: 613- 615.

Ogunrinola, O.O, J.E. Odulate and B.O. Elemo, 2004. J. Res. Rev. Sci., Lagos State University, 3: 190-193.

Okwu, D.E., 2001. Evaluation of chemical composition of indigenous spices and flavouring agents. Global J. Pure Appl. Sci., 458-459.

Okwu, D.E. and M.E. Okwu, 2004. Chemical Composition of Spondia Mombin plants. J. Sustain. Agric. Environ., 6: 140-147.

Okwu, D.E. and O. Ekeke, 2003. Phytochemical Screening and mineral composition of chewing sticks in South Easter Nigeria. Global J. Pure Appl. Sci., 9: 235-238.

Oliver, A.E.P., 1960. Medicinal plants in Nigeria. College of Arts and Science Technology, River State, Nigeria, pp: 72-73.

Peter Brather and Peter Schramel, 1980. Trace elements Analytical Chemistry in Medicine and Biology, pp: 678.

Shultes, R.E. and R.F. Raffauf, 1990. The healing forest. Medicinal and toxic plant of the Northwest Amazonia, Portland, Oregon Diosconides Press.

Urugulaga, I. and F. Laghton, 2000. Plant polyphenol antioxidants and oxidative stress. Biol. Res., 33: 159-165.

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The Study of the Characteristics and Rancidity of Three Species of *Elaeis guineensis* in South East of Nigeria

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Abstract: Palm Oil (*Elaeis guineensis*) samples were extracted by two methods of extraction, cold (Eketeke) and normal. The palm oil samples extracted by cold method (Eketeke) have significantly low mean values of peroxide 2.30 (meg/kg), free fatty acid 0.12 mg/KOH/g, acid 0.19 mgKOH/g and with high iodine value 47.2 mg/KOH/g. Whereas normal method have considerable high mean values of peroxide 2.5 (meg/kg), free fatty acid, 0.23 mg/KOH/g, acid 0.16 mg/g and with low iodine value of 45.7 mg/KOH/g. The acid values of all the oil samples are not higher than 0.6 mg/g recommended for most vegetable oils in Nigeria. Steady increase of peroxide values of oils leads to rancidity. The GLC result showed that oil sample A_1 (Eketeke) at 100°C was a mixture of unsaturated (47.56%) and saturated (43.85%) fatty acid. Whereas oil sample B at 100°C was a mixture of unsaturated (49.82%) and saturated (50.17%) fatty acid. Also sample C at 100°C was a mixture of unsaturated (45.2%) and saturated (54.80%) fatty acid.

Key words: *Elaeis guineensis*, characteristics, rancidity

INTRODUCTION

Palm oil, like other oil seeds, is a fatty acid ester of glycerol commonly called triglycerides. It has a high proportion of saturated palmitic acid (C_{16}) content to which may be attributed its value in soap making. It also contains high quantity of unsaturated fats, principally those derived from oleic acid. In its natural state, palm oil contains carotenoids (0.05-0.2%) which give it the red colour (Bagepalli and Narasinga, 2003).

Other minor constituents of palm oil are sterols (0.03%), phosphatides (0.1%) and tocopherols (0.05%). Tocopherols (vitamin E active) are important and naturally occurring and widely distributed antioxidants (Carl *et al*., 2003). Antioxidants tend to protect fats by inhibiting auto-oxidation and subsequent rancidity. The amount of tocopherol present is thus a good criterion for the quality of Natural palm oil since any pro-oxidant conditions will reduce the tocopherol content of the oil (Kirk and Sawyer, 1991). RMRDC (Raw materials research and development council. Ekpa *et al*. (2001) have carried out a comparative study of triglyceride and fatty acid composition of palm oils. Osagie *et al*. (1986) studied the chemical quality and parameters and fatty acid compositions of oils of some under exploited tropical seeds.

It is therefore, the aim of this work to extract palm oil from three species of *Elaeis guineensis*, dura (Ojukwu), tenera (osukwu) and Okpuruka, using two methods namely; cold (Eketeke) and normal. Analyses of their chemical properties in its raw form were carried out. Chemical properties such as the peroxide value, free fatty acid value, iodine value, acid value, saponification value were analyzed.

MATERIALS AND METHODS

Fresh palm fruits were collected from three different communities at Umuneke-Ugiri, namely, Umuehie, Umuezealameri and Umudike in Isiala Mbano, Imo State, Nigeria.

About 700 g of each of the samples were collected. Two methods of extraction were used; cold (Eketeke) and normal.

Cold method (Eketeke) is a process where freshly harvested ripe oil palm fruits are separated from the bunch and pounded in the mortar or pit without boiling. The whole mass was fried in a clay pot at about 100°C and the fibre was pressed or squeezed for the natural oil to be collected in a container. Then the oil was filtered from impurities (Oyelola, 1979).

The normal method involves boiling freshly harvested palm fruits with about 2 litres of water for 2 h at temperature range of (100-160°C). The mass pulp was produced by pounding the boiled fruits in a mortar/pit. The whole mass was immersed in water (5 litres), stirred and the natural oil, which rose to the surface was skimmed off into another pot. The fibres where then sifted out of the water manually and finally the nuts were collected and separated from the remaining fibres. The natural oil thus obtained was boiled in smaller vessels where any fibre still present sank to the bottom. The oil was again skimmed to further remove traces of water (Ihekoronye and Ngoddy, 1985). Methylations of the extracted oil fraction prior to gas chromatography were as described by International Union of Pure and Applied Chemistry (IUPAC). HP 6890 (USA) gas chromatography with a flame ionization detector was used. The iodine, acid, peroxide, free fatty acid values were determined according to AOAC (1984).

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RESULTS AND DISCUSSION

PV - Peroxide value
AV - Acid value
IV - Iodine value
SV - Saponification value
FFA - Free fatty acid
A_1 - Oil sample (by cold method-Eketeke)
B_1 - Oil sample (by cold method-Eketeke)
C_1 - Oil sample (by cold method-Eketeke)
A_2 - Oil sample (by normal method)
B_2 - Oil sample (by normal method)
C_2 - Oil sample (by normal method)

The acid values in Table 1, showed that samples A_1, B_2 and C_1 (Eketeke) have 0.1, 0.14 and 0.13 mg/KOH/g, whereas the acid values of these samples A_2, B_2 and C_2 (Normal) increased at weekly intervals thus; 0.15, 0.16 and 0.16 mg/KOH/g. The steady weekly increase in acid values of samples A_2, B_2 and C_2 (Normal) showed that hydrolytic oxidation was gradually taken place. Also natural palm oil with high acid values are considered to be inferior to palm oils with low acid values.

The results in Table 2, showed that sample C_2 (Normal) has the highest Free Fatty Acid (FFA) value which increased at weekly intervals (0.75-1.68 mg/KOH/g) while samples A_1 (Eketeke) has the lowest FFA value which ranged between 0.5-1.35 mg/KOH/g at weekly intervals. Deterioration of a fat leads to the liberation of free fatty acid from triglycerides. The amount of Free Fatty Acid (FFA) in a fat or oil is an indicative of its level of spoilage. Palm oil samples extracted by (Eketeke) are better oils than palm oil samples processed by normal.

The iodine value of sample A_1 (Eketeke) in the first week of this research work recorded the highest value of 58.01 mg/KOH/g which gradually decreased to 52.35 mg/KOH/g, while sample C_2 (Normal) in the first week has the lowest IV value which gradually decreased to 49.93. According to the results in Table 3, samples extracted by Eketeke method have the highest iodine values but decreased at weekly intervals. The iodine value is a measure of the degree of unsaturation. High IV assures of better quality but it retards with time, more especially when environmental factors begin to reduce the potency of natural antioxidants present in natural palm oil.

The low peroxide value in the first week of carrying out this research indicated that there was no primary oxidation at the time of this analysis. The results in Table 4 showed that sample C_2 (Normal) has the highest PV of 2.68 (meg/kg) which increased weekly from 2.15-2.68 (meg/kg) while sample C_1 (Eketeke) has the lowest PV of 2.32 (meg/kg) which also increased at weekly intervals from 2.12-2.32 (meg/kg). The steady increase of the PV leads to rancidity of oil samples. The results of these research showed that palm oil samples extracted by the normal method will spoil or go rancid fast than palm oil samples extracted by Eketeke method.

Table 1: Acid Value (AV) of samples at weekly intervals

Samples	WKI	WKII	WKIII	WKIV
	(mg/KOH/g)			
A_1	0.11	0.13	0.13	0.14
B_1	0.1	0.12	0.13	0.14
C_1	0.1	0.11	0.11	0.13
A_2	0.11	0.12	0.14	0.15
B_2	0.1	0.13	0.15	0.16
C_2	0.11	0.14	0.14	0.16

Table 2: Free Fatty Acid (FFA) of samples at weekly intervals

Samples	WKI	WKII	WKIII	WKIV
	(mg/KOH/g)			
A_1	0.5	0.75	1.2	1.35
B_1	0.5	0.45	0.95	1.25
C_1	0.7	0.65	1.1	1.4
A_2	0.45	0.55	1.25	1.52
B_2	0.8	0.95	1.35	1.65
C_2	0.75	0.9	1.45	1.68

Table 3: Iodine Values (AV) of samples at weekly intervals

Samples	WKI	WKII	WKIII	WKIV
	(mg/KOH/g)			
A_1	58.01	56.7	55.25	52.35
B_1	57.87	55.67	53.65	51.35
C_1	56.65	55.43	53.45	52.45
A_2	55.63	53.45	51.61	49.45
B_2	53.12	51.45	50.13	48.15
C_2	52.14	50.15	49.75	49.95

Table 4: Peroxide Value (PV) of samples at weekly intervals

Samples	WKI	WKII	WKIII	WKIV
	(meg/Kg)			
A_1	2.1	2.15	2.25	2.35
B_1	2.1	2.13	2.14	2.4
C_1	2.12	2.22	2.18	2.32
A_2	2.13	2.14	2.2	2.38
B_2	2.14	2.15	2.25	2.48
C_2	2.15	2.16	2.31	2.68

Table 5: Saponification Value (SV) of samples at weekly intervals

Samples	WKI	WKII	WKIII	WKIV
	(mg/KOH/g)			
A_1	181.6	184.7	185.2	185.9
B_1	182.7	184.8	186.3	187.2
C_1	184.3	185.7	188.2	186.6
A_2	185.5	187.8	188.6	189.6
B_2	185.4	186.8	188.7	189.9
C_2	185.7	187.5	189.4	190.7

The results in Table 5 showed that sample C_2 (Normal) has the highest saponification value which increased in the first week of this research. The SV of sample A_1 (Eketeke) has the lowest value of 181.6 mg/KOH/g. The saponification value of oil gives an idea of the fatty acid constituents of the lipid since with long chain fatty acid constituents in a fat, lower saponification values are obtained per gram.

GLC results (Table 6) showed that palm oil sample A_1 at 100°C has almost equal concentration level of saturated (43.85621%) and unsaturated (47.56078%).

Palmitic acid has the highest concentration with a value of 38.94428%, followed by Oleic acid 37.35074%, linoleic acid 10.21004%, stearic acid 3.88235% and myristic acid respectively. The GLC spectra also shows that the saturated fatty acid to unsaturated fatty acid ratio of palm oil is close to unity and it contains a high amount of antioxidants, beta-carotene and vitamin E.

Oleic acid peaked higher with % concentration value of 38.30161% and was followed by palmitic acid 37.28138%, linoleic acid 11.52734, stearic acid 4.79217% and myristic acid 1.09285%. Caprylic, linolenic and myristoleic were not observable at this level. GLC results show that the oil sample at 100°C has almost equal concentration level of saturated 50.17105% and unsaturated 49.82895% fatty acid.

Palmitic acid (C_{16}) has the highest concentration level at 100°C with a value of 39.90422% followed by Oleic acid ($C_{18:1}$). 33.09497%, linoleic acid ($C_{18:2}$) 12.10825%, stearic acid C_{18} 5.44203% and myristoleic acid 1.07924%. The result of the oil sample has almost equal concentration level of saturated and unsaturated fatty acids.

Table 6: GLC spectra results of palm oil sample A_1 at 100°C

Peak No.	Area %	Name	
1	5.84128	-	-
2	1.80034	-	-
3	0.67605	-	-
4	1.02958	Myristic acid	C_{14}
5	0.26534	-	-
6	38.94428	Pamitic acid	C_{16}
7	3.88235	Stearic acid	C_{18}
8	37.35074	Oleic acid	$C_{18:1}$
9	10.21004	Linoleic acid	$C_{18:2}$

Saturated (43.85621%); Unsaturated (47.56078%)

Table 7: GLC spectra results of palm oil sample B_1 at 100°C

Peak No.	Area %	Name	
1	4.27925	-	-
2	1.93808	-	-
3	0.78732	-	-
4	1.09285	Myristic acid	C_{14}
5	37.38138	Palmitic acid	C_{16}
6	4.79217	Stearic acid	C_{18}
7	38.30161	Oleic acid	$C_{18:1}$
8	11.52734	Linoleic acid	$C_{18:2}$

Saturated (50.17105%); Unsaturated (49.82895%)

Table 8: GLC Spectra results of palm oil sample C_1 at 100°C

Peak No.	Area %	Name	
1	5.25882	-	-
2	2.04445	-	-
3	0.76463	-	-
4	1.07924	Myristoleic acid	$C_{14:1}$
5	0.30338	-	-
6	39.90422	Palmitic acid	C_{16}
7	5.44203	Stearic acid	C_{18}
8	33.09497	Oleic acid	$C_{18:1}$
9	12.10825	Linoleic acid	$C_{18:2}$

Saturated (54.79678%); Unsaturated (45.20322%)

Conclusion: From the foregoing investigation carried out on palm oil samples of three species of *Elaeis guineensis* oil carried out at weekly intervals, using two methods of extraction i.e Eketeke and normal palm oil. The following observations were made.

All the palm oil samples were prepared under high degree of consumable levels and quality.

The palm oils prepared by Eketeke method proved to be of high quality with regards to high iodine value, low peroxide values, low acid values low FFA% and moderate saponification value. Whereas palm oil extracted by the normal have high values of peroxide, acid, FFA, saponification values than palm oil samples extracted by Eketeke method. The results showed that oil processed by normal method are more prune/susceptible to both hydrolytic and oxidative rancidity than palm oils extracted by Eketeke method. Oxidative rancidity is a serious flavor defect and highly objectionable. It starts with the formation of hydroperoxides which then decompose to form aldehydes which have a pungent, disagreeable flavor and odor. Retardation of oxidation is brought about by using opaque, airtight containers, or nitrogen blanketing if clear glass bottles are used. For better storage, palm oils are preserved in airtight plastic containers and not with metal containers. Metal containers made of iron, copper and zinc are not good storage facilities for natural palm oil, because, these metals are pro-oxidants.

REFERENCES

AOAC, 1984. Official Method of Analysis. 15th Edn., Washington D.C. Association of Official Analytical Chemist.

Bagepalli, S. and A.N. Narasinga, 2003. Palm oil used and compatibility in industry. Am. J. Clin. Nutr., 53 (Suppl): 101555-10205.

Carl, L.A., E. Alonzo and Taylor, 2003. Chemicals Technology and Analysis of Oils, Fats and Waxes. 3 Vols, 6th Edn., London Macmillan.

Ekpa, O.D., M.I. Akpanatiatu, M. Amelio and R. Rizzo, 2001. Research work: A comparative study of triglyceride and fatty and composition of palm oils. Global J. Pure Applied Sci., pp: 61-66.

Ihekoronye, A.L. and P.O. Ngoddy, 1985. Integrated food science and technology for tropics.

Kirk and Sawyer, 1991. Oils and Fats in Composition and Analysis of Food. Label Macmillan, L. (Ed.). Publishers Ltd. London.

Ojiako, O.A. and E.I. Akubugwo, 1997. An introductory Approach to practical biochemistry CRC Publications, 25 Mbaise Rd. Owerri, Imo State.

Osagie, A.U., W.F. Okoye, B.O. Oluwayose and O.A. Dawudu, 1986. Chemical quality parameters and fatty acid composition of oil of some under exploited tropical seeds. Nig. J. Applied Sci.

Oyelola, D.O., 1979. Palm oil processing, Symposuim on Vegetable Fats and Oils. Nigerian Institution of Food Science Technology.

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The Effect of Steeping with Chemicals (Alum and Trona) on the Proximate and Functional Properties of Pigeon Pea (*Cajanus cajan*) Flour

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Abstract: The effectiveness of steeping in different chemicals (Alum and Trona) on the proximate and functional properties of Pigeon pea seed was studied. The seeds were steeped in different chemicals (Alum and Trona) with different concentrations and time intervals of 24 h and 48 h. The above treated seeds were then dehulled, dried and milled. Properties of the resulting flours were determined using standard methods and the result obtained were analyzed statistically. Results obtained showed that steeping improved the ease of dehulling pigeon pea seed with optimum at 48 h without chemical. Increase in concentration of chemicals (Alum and Trona) increased fibre content being more effective with trona (3.89-4.92%). Increase in concentration decreased protein (22.1-19.76%), fat (1.48-1.23%) and moisture content (12.27-11.33%). This happened in all cases while it increased ash (3.16-4.12%), fibre (3.89-4.92%) and carbohydrates (61.86-63.03) in proximate analyses. Increase in chemical concentration increased wettability (198sec-411sec), water absorption (1.27-4.16 ml/g) and decreased oil absorption (2.55-1.28 ml/g) except water absorption for 48h (1.65-2.25 ml/g) which had a change in trend. Increase in Trona concentration decreased gelling point (83-66°C) as a result, this product could be used in food formulation such as ice-cream because of its appetizing aroma and foaming stability.

Key words: Pigeon pea seed, food formulation, flour

INTRODUCTION

The pigeon pea (*Cajanus cajan*) belongs to the family Fabacea. It is an important grain legume crop of rain forest agriculture in the semi-arid tropics; this means that it has nodules on its roots which contain bacteria. These bacteria take nitrogen from the air, which is known as nitrogen fixation (Wikipedia, 2005). Pigeon peas are both a food crop (dried peas, flour, or green vegetable peas) and a forage/cover crop. The dried peas may be sprouted briefly and then cooked, for a flavour different from the green or dried peas. Pigeon pea originated from India, Asia and was brought millennia ago to Africa where different strains developed and by means of the slave trade to the American continent. The places/countries pigeon peas are found in the world are India, Senegal, Ghana, Togo, Nigeria, Puerto Rico Dominican Republic Hawaii, in North Australia (Wikipedia, 2005), in East Africa and in the Caribbean areas, etc. The early scientist gave it different names. It was named *Cytisus cajan* by Crawford in 1852 and *Cajanus indicus* by Valder in 1895. But different regions which they are found have different names for it. It is known as "*Arthar*" (Bengali), "*Fio-fio*" (South-East Nigeria), "*Togari*" (Canada), "*Kandi*" (Telugu), and also known as "*Gandul*", "*Guandul*", "*Gunga*" pea, Congo pea, "*Gungo*" pea and "*No-eye*" pea in different countries.

Pigeon peas are nutritionally important, as they contain high levels of protein and the important amino acids, methionine, lysine and trypotophan. In combination with cereals, pigeon peas make a well-balanced human food (Wikipedia, 2005). Pigeon peas are popular food in developing tropical countries. Nutritious and wholesome, the green seeds (and pods) serve as vegetable. Ripe seeds are source of flour, used in soups or eaten with rice. Dhal contains as much as 22% protein, depending on the location. Tender leaves are rarely used as a potherb. Ripe seeds may be germinated and eaten as sprouts. Plants produce forage quickly and can be used as a perennial forage crop or used for green manure. They are also grown as a shade crop for tree crops or vanilla, a cover crop, or occasionally as a windbreak hedge. In Thailand and N. Bengal, pigeon peas serve as host for the scale insect which produces lac or sticklac. In Malagasy, the leaves are used as food for the silkworm. Dried stalks serve for fuel, thatch and basketry. They are also used as feed for poultry (Wikipedia, 2005). The problem associated with pigeon pea, is that it is hard-to-cook and storage may leave direct and indirect effects on the nutritive value of foods and diets. Poor storage conditions with high temperature and relative humidity will result in staple foods with high moisture levels and a decrease in quality because of the millard reaction. Such conditions will also result in the growth of fungi that produce toxic compounds that have adverse effects on animals consuming such foods. Poor storage conditions can also affect nutritive value by favouring insect infestation which also results in losses of dry matter. Finally, the improper use of chemicals to protect grains can also decrease the nutritive value of the staple food. Various

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examples will be given for situations that are common in tropical developing countries (Wikipedia, 2005).

The aim of this study therefore is to investigate the effect of steeping with chemicals (Trona and Alum) on the proximate and functional properties of the flour made from pigeon pea (*Cajanus cajan*) seeds to see if it would retain some of its nutritional properties. It is hoped that the nutritional potential of pigeon pea will be exposed and its usage enhanced with this study to help in its utilization.

MATERIALS AND METHODS

Pigeon pea seed used was obtained from a local market in Enugu state. The chemicals equipment/ facilities were obtained from Ekeonunwa market in Owerri Imo State, processing laboratory of the Department of Food Science and Technology and the Department of Crop Science and Technology, Federal University of Technology, Owerri and they are high grade standard.

Production of flour: Dry seeds of pigeon pea (150 g) each were sorted, washed and steeped in required solution for 24 h and 48 h. After half of each sample was collected for further processing the steep solution being changed at 6 h intervals. The steep solutions were prepared using alum and trona powder on dry matter basis (dmb), with the solution variation based on concentration difference, ranged from 0.00%, 0.5%, 1.00% and so on. At the end of each steeping time, the samples were dehulled manually, oven dried (50-60°C) and milled separately into flour. After which they were stored in air tight containers at room temperature, ready for use in analysis.

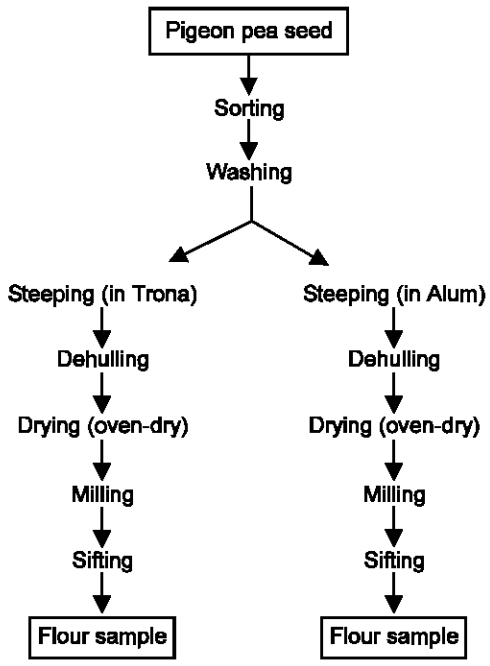

Fig. 1: Flow diagram for the production of pigeon pea flour

Analysis on flour sample: All samples were subjected to analysis to determine the proximate composition and functional properties as affected by processing conditions.

Proximate analysis: The standard AOAC (1990) methods were used to determine proximate composition of flour sample.

Analysis of the functional properties

Determination of bulk density: Method as described by Onwuka (2005) was adopted. A graduated cylinder 10 ml was weighed dry and gently filled with the flour sample. The bottom of the cylinder them gently tapped on a laboratory bench several times. This continues until no further diminution of the test flour in the cylinder after filling to mark, was observed. Weight of cylinder plus flour was measured and recorded.

$$\text{Bulk density (g/ml)} = \frac{\text{Weight of sample (g)}}{\text{Volume of sample (ml)}}$$

Determination of pH: About 10% (m/v, dmb) of flour suspension for each sample was prepared and allowed to settled at room temperature (30±2°C) for 15 min. The pH meter was switched on and allowed for 15min to stabilize. The electrodes were standardized chemically, using buffer solution of pH 7, 4 and 9 respectively, the electrode was then inserted into the test suspension and the pH value read and recorded as described by Onwuka (2005).

Determination of water absorption capacity: The method of Sosulski (1962) as described by Abbey and Ibeh (1988) was adopted. The test flour (1 g) of each treatment was weighed out into a dry, clean centrifugal tube and both weight noted. 10 ml of distilled water was poured into the tube and properly mixed with the flour to make a suspension. It was then centrifuged at speed of 3500 rpm for 15 min. after which, supernatant was discarded, then the tube and its content re-weighed and noted. The gain in weight is the water absorption capacity of the test sample.

Determination of oil absorption capacity: The method of Sosulski (1962) as described by Abbey and Ibeh (1988) was adopted. One gram (dmd) of each flour sample was weighed into a dry, clean centrifugal tube and both weight noted. 10 ml of Devon King's vegetable oil was poured into the tube and properly mixed with the flour. The suspension was centrifuged at 3500 rpm speed for 15 min then, the supernatant was discarded and the tube content re-weighed. The gain in mass is the oil absorption capacity of the sample.

Determination of swelling index: A portion (3 g) of each flour sample was weighed into a clean, dry, graduated (50 ml) cylinder. The flour sample gently leveled in the cylinder and the volume noted. 30 ml of distilled water was added to each sample. The swirled cylinder was allowed to stand for 60 min, while the change in volume recorded every 15 min. The swelling power index of each flour sample was calculated as multiple of the original volume as done by Ukpabi and Ndimele (1990).

Determination of wettability: This as described by Onwuka (2005) was adopted. One gram each flour sample was placed in a clean, dry measuring cylinder (10 ml). Placing a finger over the open end, the cylinder was inverted and clamped at a height of 10 cm from surface of a 600 ml beaker containing 500 ml of distilled water. The flour in the cylinder was gradually spread on the surface of the water on moderate speed. The time taken for the sample to be completely wet is noted as wettability.

Determination of gelling and boiling points: The method of Narayana and Rao (1982) was adopted. The flour sample (10 g) was dispersed in distilled water, in a 250 ml beaker and made up to 100 ml. A thermometer was clamped on a retort stand with its bulb submerged in the suspension. With a magnetic stirrer, the suspension was continuously stirred and heated. This continued until the suspension began to gel and the corresponding temperature recorded. The temperature as soon as boiling commenced was noted and recorded.

Determination of foam capacity: The method as described by Onwuka (2005) was adopted in the determination of foam capacity. Test flour 2 g each was mixed in 100 ml distilled water and its volume noted. The suspension was blended with a warming blender at 1600 rpm for 15 min. It was poured into a 250 ml measuring cylinder, its volume noted and recorded. Using Abbey and Ibeh (1988) formula, foam capacity expressed percentage increase in volume is as follows:

$$\text{Foam capacity} = \frac{\text{Volume of whipping - Volume before whipping}}{\text{Volume before whipping}} \times \frac{100}{1}$$

Determination of emulsion capacity: The procedure of Eke (2002) was adopted. Flour sample 2 g was mixed with 10ml of oil for 30 sec in a mixer and magnetically stirred. After complete dispersion deodorized vegetable oil (Devon Kings oil) was added continuously through a burette until emulsion breakpoint, separation into 2 layers was reached. The emulsion capacity as ml of oil emulsified per g of flour was recorded.

RESULTS AND DISCUSSION

Effect of chemical treatment, steeping time and steeping concentration on the proximate composition of pigeon pea flour: Table 1 showed the proximate composition of pigeon pea flour as affected by chemical treatment, steeping time and steeping concentration. From the results, the protein content of the bean flour decreased as the steeping time and concentration increased in all cases. This might have resulted from the breakdown of protein molecules causing it to be easily lost by leaching. Samples steeped for 24 h in

Table 1: Mean values on the proximate composition of pigeon pea flour as affected by chemical treatment, steeping time and steeping concentration

		Proximate composition (%)											
		Trona						Alum					
Steep. time (h)	Steep. Conc.	Protein	Fat	Ash	Fibre	M.C	CHO	Protein	Fat	Ash	Fibre	M.C	CHO
		(%)											
24	0.00	22.1^{a}	1.48^{ab}	3.16^{d}	3.89^{g}	12.96^{a}	61.86^{a}	22.1^{a}	1.46^{a}	3.16^{a}	3.86^{d}	12.27^{a}	61.86^{f}
	0.50	20.88^{b}	1.45^{ab}	3.94^{c}	4.67^{f}	12.52^{a}	62.02^{b}	22.02^{b}	1.38^{ab}	3.02^{b}	3.92^{cd}	12.20^{b}	61.97^{f}
	1.00	20.67^{b}	1.39^{b}	3.95^{bc}	4.73^{e}	12.35^{a}	62.18^{c}	21.95^{c}	1.32^{ab}	3.04^{b}	3.97^{c}	12.08^{c}	62.05^{e}
	1.50	20.23^{c}	1.34^{bc}	3.98^{bc}	4.78^{d}	12.04^{a}	62.37^{d}	21.57^{d}	1.27^{b}	3.07^{ab}	4.06^{b}	11.96^{d}	62.25^{d}
	2.00	20.11^{cd}	1.30^{c}	4.03^{b}	4.83^{c}	11.67^{a}	62.64^{e}	21.23^{e}	1.21^{b}	3.09^{ab}	4.14^{b}	11.72^{e}	62.5^{c}
	2.50	19.89^{d}	1.26^{c}	4.08^{ab}	4.87^{b}	11.43^{a}	62.89^{f}	21.09^{f}	1.16^{b}	3.12^{ab}	4.2^{ab}	11.64^{f}	62.8^{b}
	3.00	19.76^{d}	1.23^{c}	4.12^{a}	4.92^{a}	11.12^{a}	63.03^{g}	20.94^{g}	1.11^{b}	3.16^{a}	4.28^{a}	11.33^{g}	63.06^{a}
	LSD	0.234	0.071	0.077	0.032	ND	0.067	0.028	0.15	0.09	0.085	0.035	0.072
48	0.00	22.04^{a}	1.46^{a}	3.14^{d}	7.77^{a}	12.27^{a}	61.36^{a}	22.07^{a}	1.46^{a}	3.14^{c}	7.77^{f}	12.96^{a}	61.36^{e}
	0.50	21.74^{b}	1.38^{ab}	3.89^{c}	7.78^{a}	12.22^{ab}	61.53^{b}	21.96^{b}	1.43^{ab}	3.96^{b}	7.78^{f}	12.52^{b}	61.50^{e}
	1.00	21.58^{c}	1.33^{b}	3.95^{bc}	7.94^{b}	12.15^{b}	61.70^{c}	21.35^{c}	1.35^{b}	3.98^{b}	7.94^{e}	12.35^{c}	61.62^{d}
	1.50	21.36^{d}	1.27^{bc}	3.96^{b}	8.12^{c}	12.09^{b}	61.85^{d}	21.26^{d}	1.30^{bc}	4.00^{b}	8.12^{e}	12.04^{d}	61.75^{d}
	2.00	21.17^{e}	1.21^{c}	4.03^{ab}	8.22^{d}	11.93^{c}	62.12^{e}	21.19^{e}	1.24^{c}	4.01^{ab}	8.22^{c}	11.67^{e}	62.0^{c}
	2.50	20.98^{f}	1.16^{cd}	4.07^{a}	8.40^{e}	11.80^{d}	62.40^{f}	21.12^{f}	1.21^{c}	4.03^{ab}	8.40^{b}	11.43^{f}	62.26^{b}
	3.00	20.75^{g}	1.11^{d}	4.11^{a}	8.48^{f}	11.7^{e}	62.60^{g}	21.04^{g}	1.18^{c}	4.05^{a}	8.48^{a}	11.12^{g}	63.5^{a}
	LSD	0.04	0.09	0.08	0.07	0.09	0.089	0.063	0.08	0.04	0.07	0.089	0.179

Mean followed by same superscript are not significantly different at $p \leq 0.05$. CHO = Carbohydrate Content; LSD = Least Significant Difference; ND = Not Determined; MC = Moisture Content; Steep. = Steeping; Conc. = Concentration

trona showed a significant difference ($p \leq 0.05$) from 0.50-2.00%, while the sample steeped in alum both at 24 h and 48 h caused a significant decrease in the protein content of the flour. This decrease remained insignificant up to 3.00% concentration. Therefore, for economic reasons and better retention of protein value, it is best to steep in water for 24 h, as this had the highest protein value; since beans has become the major source of protein in the country for an average Nigerian.

With regards to the fat content, as the concentration increased, the fat content of the flour samples decreased in all cases. This is likely due to the breakdown of the molecules, thereby enhancing its leaching into the solution. Samples steeped in alum showed no significant difference ($p \leq 0.05$) from 1.00-3.00%, while trona from 24 h showed no significant difference ($p \leq 0.05$) from 2.00-3.00%, After 48 h, samples steeped in trona from 1.50-2.50% concentration showed no significant difference, while samples steeped in alum for 48 h, showed no significant difference from 1.50% and decrease in fat content was noticed. Therefore for a better keeping quality it is desirable to steep pigeon pea seed in 2.5% or 3.0% of trona solution for 48 h. This had the lowest fat content, hence reducing losses due to rancidity on storage. The ash content of the samples had a similar relationship with concentration in all cases. This owing to the possibility of attached mineral of aluminum and sodium bond over time (Michell and Robert, 1981).

In all cases, increase in concentration increased fibre content with significant difference ($p \leq 0.05$) in each of them and after 48 h steeping, there was no significant difference ($p \geq 0.05$) between the samples steeped in trona and alum. Also it was observed that samples not steeped in chemical rapidly increased the moisture content but increase in steeping concentration decreased the moisture content of the flour samples. This probably might have resulted from the replacement of water molecules by the solutes, causing loose water bonds to be lost easily during drying. For samples steeped for 24 h the decreases in moisture content for samples steeped in trona was small as not to cause significant difference among their mean. In alum for 24 h, variation occurred from 0.0-3.00% with a significant decrease in moisture content. After 48 h there was significant variation (for alum and trona as the concentration increased). This might have resulted from increased chemical reactions as time increased. Therefore for storage reasons, it is preferable to steep pigeon pea seed in 3.0% alum solution concentration for 24 h, as this had the lowest moisture content and could be time saving and possibly retard biological and chemical reactions that would take place in the bean flour on storage. The carbohydrate increased with concentration after 24 h for both alum and trona. At the end of 48 h, coherent increase or decrease was not obtained. This may have resulted from the increased chemical reaction, deterioration/leaching over time of the other proximate components which are not steady. Since carbohydrate value was dependent on them as it was determined by difference not chemically.

Effect of chemical treatment, steeping time and steeping concentration on the functional properties of pigeon pea flour: Table 2 and 3 showed the effect of chemical treatment, steeping time and steeping concentration on the functional properties of pigeon pea flour. The results obtained showed that there were significant differences ($p \leq 0.05$) among the samples steeped for 24 h and 48 h in trona and alum respectively. For the 24 h steeping samples steeped at 2.00% trona had the highest bulk density (0.73 g/ml) while the 48 h samples steeped at 2.50% had the highest bulk density (0.74 g/ml). However, with respect to alum treated samples, the highest bulk density (0.74 g/ml) was obtained with sample steeped for 48 h at 0.50% concentration. For the swelling index samples steeped for 24 h slightly decreased in 0.5% trona and as the concentration increased, the swelling index increases. Conversely, for the alum treated samples, the swelling index decreases as the concentration increased for samples steeped for 24 h and 48 h respectively. These results suggest that trona has the ability to convert starch to soluble form which will lead to increase in volume of flour whereas alum is an effective swelling inhibitor (Enwere, 1985).

However, steeping in water for 24 h is more economical to steeping in alum at different concentrations and should be utilized in foods like moin-moin or akara, where increased swelling ability is needed during processing. Table 2 also showed that samples steeped for 24 h in trona caused a decreasing effect in gelling point temperature as concentration of steep solution increased. Samples steeped in 0.50% of trona showed no significant difference ($p \geq 0.05$) from the control sample. This probably means that the quantity of trona in the solution was too small to cause a significant change; while other steeped samples in trona showed significant difference from each other, because of significant decrease in the gelling point temperature when compared to control. After 48 h of steeping in trona, increase in concentration decreased slightly the gelling point temperature, with significant difference ob served. This means that the steep solution concentration had little effect on the gelling point with increased times, as the values were closely related to those steeped for 24 h. But with alum (Table 3); the trend was different, in that, increase in concentration increased gelling point temperature. It was noticed that alum steeped for 24 h caused a significant difference ($p \leq 0.05$) in the gelling point temperature in almost all the

Table 2: Mean values on the functional properties of pigeon pea flour treated with trona

Steeping Time (h)	Steeping Conc. (%)	Bulk density (g/ml)	Swelling index (cm^3/cm^3)	Gelling point (°C)	Foam capacity (%)	Wett-ability (s)	Boiling point (°C)	pH	WAC	OAC	Emulsion capacity (ml/g)
24	0.00	0.71^{a}	1.41^{dc}	83^{a}	23.01^{d}	198^{a}	92^{a}	6.69^{e}	1.27^{d}	2.55^{a}	3.44^{a}
	0.50	0.68^{ab}	34^{f}	81^{ab}	21.19^{e}	360^{a}	88^{b}	6.74^{d}	2.38^{c}	2.22^{b}	3.51^{e}
	1.00	0.69^{a}	1.38^{e}	78^{b}	25.70^{c}	393^{a}	85^{b}	6.98^{d}	2.43^{c}	1.92^{c}	3.53^{e}
	1.50	0.71^{b}	1.43^{d}	75^{c}	26.52^{b}	399^{a}	80^{c}	7.15^{c}	3.83^{b}	1.70^{d}	3.69^{d}
	2.00	0.73^{b}	1.48^{c}	72^{d}	28.23^{a}	402^{a}	77^{c}	7.46^{b}	3.94^{b}	1.62^{e}	3.75^{c}
	2.50	0.70^{c}	1.53^{b}	69^{d}	29.94^{a}	405^{a}	72^{d}	7.77^{a}	4.05^{a}	1.48^{f}	3.81^{b}
	3.00	0.71^{a}	1.58^{a}	66^{e}	31.65^{d}	411^{a}	67^{e}	8.08^{f}	4.16^{a}	1.28^{g}	3.87^{a}
	LSD	0.03	0.031	2.86	0.21	ND	3.1	0.065	0.16	0.059	0.038
48	0.00	0.69^{ab}	1.39^{a}	82^{a}	24.61^{a}	140^{a}	93^{a}	6.63^{f}	1.50^{a}	1.65^{a}	3.42^{c}
	0.50	0.60^{a}	1.42^{a}	80^{b}	26.57^{f}	178^{b}	92^{ab}	6.81^{e}	2.98^{b}	1.49^{e}	3.51^{f}
	1.00	0.65^{ab}	1.47^{b}	77^{c}	28.53^{e}	304^{c}	89^{b}	7.12^{d}	3.34^{c}	1.57^{de}	3.59^{e}
	1.50	0.66^{b}	1.52^{c}	76^{cd}	30.49^{d}	364^{d}	88^{b}	7.19^{d}	3.46^{d}	1.62^{d}	3.74^{d}
	2.00	0.72^{b}	1.57^{d}	74^{d}	30.80^{c}	397^{e}	86^{c}	7.31^{c}	3.65^{e}	1.83^{c}	3.84^{c}
	2.50	0.74^{bc}	1.62^{e}	71^{e}	31.14^{b}	430^{f}	84^{c}	7.43^{b}	3.89^{f}	2.04^{b}	3.94^{b}
	3.00	0.72^{c}	1.67^{f}	70^{e}	31.37^{a}	463^{g}	81^{d}	7.55^{a}	4.03^{g}	2.25^{a}	4.04^{a}
	LSD	0.03	0.03	2.365	0.063	9.95	2.45	0.10	0.051	0.09	0.051

Mean followed by same superscript are not significantly different at $p \leq 0.05$. LSD = Least Significant Difference; ND = Not Determined; WAC = Water Absorption Capacity; OAC = Oil Absorption Capacity

Table 3: Mean values on the functional properties of pigeon pea flour treated with alum

Steeping Time (h)	Steeping Conc. (%)	Bulk density (g/ml)	Swelling index (cm^3/cm^3)	Gelling point (°C)	Foam capacity (%)	Wett-ability (s)a	Boiling point (°C)	pH	WAC	OAC	Emulsion capacity (ml/g)
24	0.00	0.71^{a}	1.41^{b}	83^{cd}	23.01^{b}	198^{a}	92^{b}	6.69^{a}	1.27^{d}	2.55^{a}	3.44^{a}
	0.50	0.68^{ab}	1.46^{a}	77^{e}	23.82^{a}	125^{f}	85^{d}	6.00^{b}	2.01^{c}	2.05^{b}	3.43^{a}
	1.00	0.67^{ab}	1.41^{b}	80^{e}	22.14^{c}	127^{f}	88^{c}	5.93^{c}	3.11^{b}	1.82^{c}	3.32^{a}
	1.50	0.65^{c}	1.35^{c}	81^{d}	19.99^{d}	173^{e}	92^{b}	5.64^{d}	3.19^{b}	1.67^{d}	2.91^{b}
	2.00	0.61^{cd}	1.32^{cd}	86^{c}	16.09^{e}	178^{d}	93^{ab}	5.57^{e}	3.22^{a}	1.41^{e}	3.75^{c}
	2.50	0.57^{d}	1.29^{d}	91^{b}	12.19^{f}	183^{c}	94^{a}	5.50^{f}	3.25^{a}	1.73^{d}	2.87^{b}
	3.00	0.71^{c}	1.27^{d}	96^{a}	14.90^{g}	188^{b}	95^{a}	5.43^{g}	3.28^{a}	1.99^{b}	2.85^{b}
	LSD	0.09	0.03	3.1	0.09	2.90	2.365	0.024	0.09	0.09	0.13
48	0.00	0.69^{b}	1.39^{a}	82^{a}	24.61^{a}	140^{e}	93^{ab}	6.63^{a}	1.50^{a}	1.65^{a}	3.42^{a}
	0.50	0.60^{d}	1.37^{a}	72^{b}	26.57^{f}	124^{f}	79^{e}	6.00^{b}	2.33^{b}	1.57^{b}	3.38^{a}
	1.00	0.72^{c}	1.31^{b}	76^{b}	28.53^{e}	127^{f}	82^{e}	5.42^{c}	2.42^{c}	1.53^{b}	3.01^{b}
	1.50	0.67^{bc}	1.20^{c}	80^{ab}	30.49^{d}	169^{d}	85^{d}	5.06^{d}	2.61^{d}	1.39^{c}	2.88^{c}
	2.00	0.64^{ab}	1.16^{cd}	81^{a}	30.80^{c}	193^{c}	90^{c}	4.80^{e}	2.70^{e}	1.31^{d}	2.82^{cd}
	2.50	0.6^{a}	1.12^{d}	82^{a}	31.14^{b}	197^{b}	95^{b}	4.54^{f}	2.79^{f}	1.22^{e}	2.76^{d}
	3.00	0.57^{ab}	1.08^{d}	83^{a}	31.37^{a}	201^{a}	100^{a}	4.24^{g}	2.88^{g}	1.14^{f}	2.70^{d}
	LSD	0.05	0.04	4.38	0.13	3.79	3.79	0.155	0.04	0.04	0.09

Mean followed by same superscript are not significantly different at $p \leq 0.05$. LSD = Least Significant Difference; ND = Not Determined; WAC = Water Absorption Capacity; OAC = Oil Absorption Capacity

samples when compared to the control sample. This was also similar for samples steeped for 48h in alum, though the figures were low generally (Hickson *et al*., 1982). Therefore for economic reasons, it is best to steep in 3.00% concentration of trona for 24 h, as this had the lowest gelling point temperature, hence saving time and energy cost, and should be utilized in foods where gelling property is required e.g. thickeners in soups and sauces.

It was also observed from Table 2 that, samples steeped in trona caused an increase in foam capacity as concentration increased. For 24 h samples, increase in concentration increased foam capacity until 2.50% concentration of the solution, which showed a significant decrease in foam capacity. After 48 h, increase in concentration increased foam capacity in all cases, because trona with its thickening ability enhances the trapping of air in the bean flour slurry during whipping. In contrast from Table 3, samples steeped in alum decreased in foaming capacity as concentration increased, but there was significant variation ($p \leq 0.05$) among the samples. This decrease could be desirable in food processes where excessive foaming is not required as it reduces loss due to foam spillage or the need for including an extra steep or antifoaming agent to check foaming. Therefore steeping in alum at 3.0% for 24 h is advisable.

Table 2, also showed that wettability increased with concentration in all cases, the samples steeped in trona for 24 h shows no significant difference ($p \geq 0.05$) on their mean. This probably means that time and concentration difference was too small to cause a significant

difference in wettability of pigeon pea flour. Samples steeped in alum appeared to have marked reduced difference in their wettability values when compared to those steeped in trona. This is because alum causes a reduction in density of the flour (Freeman, 2001). This is desirable as it reduces processing time and cost in food where wettability is of interest. Therefore, if wettability is a critical characteristic for choosing the sample then it should be steeped in 0.50% of alum solution for 48 h as this had the lowest value. The boiling point temperature of pigeon pea decreased as concentration increased for samples with trona and steeped for 24 h and 48 h respectively. This decrease generally might have resulted from the fact that trona being a tenderizing agent in food must have caused a softening effect on the molecular network of the flour. This made them easily attacked by heat and is desirable as it reduces energy cost and destruction of heat liable nutrients during processing.

From Table 3 increase in boiling point temperature was directly related to alum concentration, for both 24 h and 48 h samples. Concentrations as low as 0.50% caused a significant decrease in boiling point temperature compared to the control and others. Though samples steeped for 48 h generally had lower values with a step further in variation. Therefore it is techno-economically better to either steep in 0.50% solution of trona for 24 h or 0.50% of alum for 48 h as they had the lowest boiling point temperature (Fox and Cameron, 1980). It was observed that samples steeped in trona for 24 h had a slight increase in pH as concentration increased (Table 2) with significant difference ($p \leq 0.05$) among the samples. A similar result was obtained with steeping for 48 h. This general increase is because trona is slightly alkaline in nature.

However the pH of the samples steeped is alum (Table 3) were significantly different ($p \leq 0.05$) though the pH decreased as the concentration increased. Therefore steeping in water is best as it maintains the almost neutral pH of pigeon pea flour needed for the processing of certain foods (Prinyawiwatkul *et al*., 1992).

The water absorption capacity has a direct relationship with concentration in all cases (Table 2 and 3). This probably could have resulted from the loss of moisture during drying, therefore causing a high water affinity of the flour. Concentration of 0.50% caused a significant increase in the water absorption capacity of the flour when compared to control after 24 h of steeping. The increase was slight until at 1.50% of the trona solution, where a further significant increase was observed in the water absorption capacity compared to the control samples. However in all cases samples steeped at 3.00% concentration for both trona and alum had the highest water absorption capacities. Therefore it is better to steep pigeon pea in 3.00% of trona for 24 h as this had the highest water absorption capacity (4.16 cm^3/cm^3) and is desirable as this can cause an increase in volume e.g. bread (Narayana and Raon, 1982).

For oil absorption capacity, samples steeped in trona for 24 h decreased slightly as concentration increased with significant variations in their mean (Table 2). After 48 h a reverse trend was observed as slight decrease on 0.50% concentration. Where a further significant decrease was observed was sample steeped in alum as shown in Table 3, the decreasing trend was observed both in the 24 h and 48 h interval, with a little variation occurred between them. After 48 h the concentration decreased significantly from control until 3.00% concentration except for 0.50-1.00% where no further significant decrease was observed. This decrease in oil absorption capacity with respect to concentration over time, which assumed the same trend with fat was found to have resulted from increased breakdown in fat molecules, hence increasing their leaching effect (Gaman and Sherrigton, 1977). This low oil absorption capacity is desirable in frying of akara balls as less oil is absorbed by the balls, hence reducing losses due to rancidity in the akara ball on storage. It is therefore best to steep in 3.00% of alum for 48h as this had the lowest oil absorption capacity.

For the emulsion capacity as shown in Table 2 there was significant difference ($p \leq 0.05$) among the samples though as the concentration increased, the emulsion capacity increased too. This increase in emulsion capacity is desirable as it can be utilized in foods such as sausage. For samples steeped in alum as shown in Table 3, increase in concentration decreased emulsion capacity for samples steeped for 24 h and 48 h respectively. This decrease might have resulted from alum being a coagulant (cleanser) (Freeman, 2001) thereby resulting in the separation of oil and water.

Conclusion: At the end of the experiment the result obtained from this study have shown that steeping pigeon pea seed in alum and trona separately at different concentration (0.0%, 0.50%) for 24 h and 48 h caused significant variation in the proximate and functional properties of its flour. Based on this, pigeon pea can suit for various products due to its diverse utilization of combining any or all the above conditions. For better retention of nutrients, it should not be steeped for more than 24 h.

REFERENCES

Abbey, B.W. and C.O. Ibeh, 1988. Functional properties of raw and heat processed cowpea (*Vigna unguicultlata* walp) flour in Food Science.

AOAC, 1990. Official methods of Analysis. 15th Edn., Association of official Analytical chemists Washington DC.

Eke, S.O., 2002. The effect of malting on the dehulling characteristics of African yam bean and the functional properties of the flour in Food Technology.

Enwere, N.J., 1985. Effect of temperature and drying on selected functional properties and performance of cowpea flours during akara and moi-moi preparation. M.Sc. Thesis Dept. of Food Sc. and Tech. University of Nsukka.

Fox, B.A. and A.G. Cameron, 1980. Food science and chemical approach. Hodder and Stoughton, London.

Freeman, W.H., 2001. Chemistry in the community. 4th Edn., American chemical society.

Gaman, P.M. and K.B. Sherrigton, 1977. The science and food. Pergamon press Ltd.

Hickson, D.W., C.W. Diu, R.G. Morgan, V.E. Sweet, D.W. Suter and Z.C. Carpenter, 1982. Rheological properties to two heat induced protein gels. Food Sci.

Michell, J.S. and A.P. Robert, 1981. Chemistry. 5th Edn., McGraw-Hill Book Company, Japan.

Narayana, K. and M.N.S. Rao, 1982. Functional properties of raw and heat processed winged bean (*Psophocarpus tetragonolobus*) flour.

Onwuka, G.I., 2005. Food Analysis. Napthali print Lagos, Nigeria.

Prinyawiwatkul, W., L.R. Beuchat and L. Wicker, 1992. Separation protein fractions from partially defatted peanut flour and estimation of iso-electric point and molecular weights. Paper presented at 52nd annual meeting of Inst. of Food, Sci. and Tech. New Oreland June.

Sosulski, F.W., 1962. The centrifuge methods for determining flour absorption in hard red spring wheat cereal chem.

Ukpabi, U.J. and C. Ndimele, 1990. Evalaution of quality of garri produced in Imo State, Nig. Food J., 8:59-64.

Wikipedia, 2005. The encyclopedia http://en.wikipedia.og/Wiki/yard1longbean. Accessed Feb. 17 2007.

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Comparative Microbial Quality of Jedi Drinks Sold in Two Major Cities in Nigeria

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Abstract: The microbial quality of ten samples of Jedi herbal preparations sold for the treatment of anal fistula in major motor parks of Sagamu and Ibadan, both in Nigeria was studied and compared. Phytochemical analysis of the samples revealed that all the samples contained Saponins and Tannins. Alkaloids and cardenolides were also present to varying extent. These secondary metabolites are known to be responsible for the prevention of anal fistula. 100% contained *Pseudomonas aeruginosa*, 80% from Sagamu contained *Klebsiella* spp; 60% from Ibadan contained *Bacillus* spp, 30% of the samples contained *Staphylococcus aureus* and 70% contained fungi. Antibiotic sensitivity patterns revealed that the Gram-negative isolates were most sensitive to ofloxacin, a quinolone antibiotic while the Gram-positive isolates were most sensitive to Streptomycin, an aminoglycoside.

Key words: Jedi, anal fistula, phytochemical, cardenolides, antibiotic

INTRODUCTION

WHO estimates that 80% of the world's population presently uses herbal medicine for some aspect of primary health care (WHO, 2001). Africa and indeed Nigeria is not an exception. This may not be unconnected with the fact hat Africa is reported for the extraordinary richness of its flora, which totals tens of thousands of species. Asides, alternative medicines, such as herbal medicines are gaining in popularity because of typically low side effect profiles (Wilt *et al*., 2000), low cost (Vanderhoof, 2001) and high level of acceptance by patients and the majority of the population. Some managed care organizations now offer these therapies as an expanded benefit (Langyan and Ahuja, 2005).

In Nigeria, there appears o be an overwhelming increase in the public awareness and usage of herbal medicinal products in the treatment and/or prevention of diseases as a result of active mass media advertisement embarked upon by the producers and marketers of the herbal medicinal products premised upon high cost of the conventional pharmaceutical dosage forms, inaccessibility of the orthodox medical services to a vast majority of people particularly in the rural areas and the reservations by the public due to a vast majority of fake, substandard or counterfeit drugs in the market.

However, this high profile patronage is despite all the identifiable problems associated with the use of herbal medicines among which are lack of precise dose and unhygienic method of preparation; to mention but a few. One of the diseases for which the use of herbal medicine has enjoyed unprecedented patronage in south-western Nigeria is anal fistula popularly called "Jedijedi" or "Jedi" by the inhabitants of the region. This disease is characterized with pain, discharge-either bloody or purulent, pruritus ani-itching, systemic symptoms if abscess becomes infected apart from the belief of reduced libido in males having the disease.

Jedi drinks are made of medicinal plant parts macerated in water inside either glass bottle or plastic containers for about 3 days before being sold to interested consumers of which commercial motor drivers are majority. It is dispensed using plastic or glass cup depending on the quantity required by the consumers.

This study aims at comparative phytochemical analysis of Jedi preparations sold in 5 major motor parks of Sagamu (Ogun State) and 5 major motor parks of Ibadan (Oyo State) with a view to ascertaining the plants' contents similarities since the sellers are reluctant to disclose the identities of the plants often used in the preparation as well as determining the microbial load cum contents of the preparations, which is of public health concern and the antibiotic sensitivity patterns of the isolates to commonly prescribed antibiotics.

MATERIALS AND METHODS

Mueller-Hilton agar medium, Cetrimide agar medium, MacConkey agar medium, Mannitol agar medium and Sabouraud Dextrose agar medium, all ATER products by Topley House 52, Bury, Lancashire, BL96AS, UK.

Collection of samples: Samples were purchased from a major seller each from 5 major motor parks in Sagamu (Ogun State) and 5 major motor parks in Ibadan (Oyo State) into sterile bottle containers. A major seller here is defined as one who has shop where customers often assemble to buy and drink Jedi preparations.

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Phytochemical study: The following phytochemical tests were carried out on the samples. Test for alkaloids was performed using Wagner and Dragendoff reagents (Sofowora, 1994). 0.5 g of the extract was added to 5 ml of 1% aqueous Hydrochloric acid on a steam bath. This was filtered and 1 ml portion treated with a few drops of Draggendoff reagent and another 1 ml portion similarly treated with Wagner's reagent. The formation of precipitates was an indication of the presence of alkaloids.

The blood haemolysis test was used to test for Saponnins (Sofowora, 1994).

The test for anthraquinones was done by shaking 0.5 g of the extract with 5 ml chloroform for 5 min. The mixture was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink, violet or red color in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones. Test for tannins was done using Ferric chloride test. A deep green coloration showed the presence of tannins (Trease and Evans, 1989).

The Keller-Kiliani test was used to test for the presence of cardenolides. 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing one drop of Ferric chloride solution. This was then underplayed with 1 ml of concentrated H_2SO_4. A brown ring obtained at the interphase indicated the presence of a deoxy sugar typical of cardenolides.

About 1 g of the extract was dissolved in 5 ml of 2% potassium hydroxide and filtered. Formation of precipitate on addition of 10% hydrochloric acid to the filtrate confirms the presence of flavonoids.

Isolation and characterization of isolates: 0.1 ml of each sample was individually seeded in MacConkey, Cetrimide, Mannitol salt and Sabauroud Dextrose Agar media respectively and incubated appropriately with all media except Sabauroud Dextrose agar medium incubated at 37°C for 24 h with the latter at 25°C for 5 days. After incubation, each plate was examined for growth with colony counted and characterized by conventional biochemical tests.

Sensitivity test: 0.1 ml of overnight grown culture of characterized bacterial isolates was seeded in 20 ml Mueller-Hilton agar medium and the plates allowed to set on bench. Antibiotic disc was aseptically placed on the plate using sterile forceps and the plates incubated at 37°C for 24 h. The diameter of the zone of inhibition around each antibiotic was measured and the result interpreted as <12mm-resistant; 12-18 mm-moderately sensitive and >18mm-sensitive.

RESULTS

The result of phytochemical analysis reveals the samples as component of varying phytochemicals as shown in Table 1.

Microbial loads vary from park to park within city and between cities under study as shown in Table 2.

DISCUSSION

The result of phytochemical analysis as exemplified in Table 1 reveals that all Jedi drinks studied contained saponins and tannins while 70% contained alkaloids with 60% from sagamu parks and 80% from Ibadan parks. None of the samples contained anthraquinones while 30% made up of 20% and 40% from Sagamu and Ibadan parks respectively contained cardenolides.

Table 1: Results of phytochemical screening of different samples of jedi drinks from sagamu and ibadan

City	Sample Codes	Alkaloid	Anthraquinone	Saponin	Tannin	Cardenolides
Sagamu	A	-	-	+	+	-
	B	-	-	+	+	-
	C	+	-	+	+	-
	D	+	-	+	+	+
	E	+	-	+	+	-
Ibadan	F	+	-	+	+	-
	G	+	-	+	+	+
	H	-	-	+	+	+
	I	+	-	+	+	-
	J	+	-	+	+	-

Table 2: Microbial counts (cfu/ml) and types found in jedi drinks

Sample code	*Pseudomonas aeruginosa*	*Klebsiella spp*	*Bacillus spp*	*Staphylococcus aureus*	*Fungi*
A	5.0×10^3	3.1×10^3	-	-	4.1×10^3
B	4.1×10^3	-	-	5.0×10^3	-
C	4.3×10^3	5.7×10^3	-	-	5.0×10^3
D	4.0×10^3	2.3×10^3	-	-	5.1×10^3
E	5.0×10^3	4.0×10^3	-	-	6.0×10^3
F	6.2×10^3	-	-	6.0×10^3	-
G	4.1×10^3	-	2.0×10^3	-	5.1×10^3
H	5.1×10^3	-	4.2×10^3	-	-
I	5.0×10^3	-	6.1×10^3	-	6.0×10^3
J	6.1×10^3	-	-	5.5×10^3	3.5×10^3

Table 3: Antibiotic sensitivity profiles of isolated bacteria from jedi drink samples

Antibiotics	*Pseudomonas aeruginosa* (n = 10)	*Klebsiella spp* (n = 4)	Antibiotics	*Bacillus spp* (n = 3)	*Staphylococcus aureus* (n = 3)
Amoxycillin	100	50	Ampicillin	100	100
Cotrimoxazole	100	100	Cotrimoxazole	100	100
Nitrofurantoin	100	0	Nitrofurantoin	100	100
Gentamicin	100	100	Gentamicin	100	100
Nalidixic Acid	100	25	Nalidixic Acid	100	100
Ofloxacin	100	100	Colistin	100	33.3
Augmentin	100	100	Streptomycin	100	100
Tetracycline	70	0	Tetracycline	100	100

Microbial study reveals that all the samples studied had *Pseudomonas aeruginosa* which ranges between 4.1 x 10^3 and 5.0 x 10^3 for samples collected in Sagamu parks and between 4.1 x 10^3 and 6.2 x 10^3 for samples collected in Ibadan. However, none of the samples collected from Ibadan parks has *Klebsiella* while 80% of samples from Sagamu had *Klebsiella* which ranges between 2.3 x 10^3 and 5.7 x 10^3. In the same vein, none of the samples from Sagamu possesses *Bacillus spp* while 60% of the samples from Ibadan parks have *Bacillus spp* ranging between 2.0 x 10^3 and 6.1 x 10^3. *Staphylococcus aureus* was isolated from 20% of the samples collected from Sagamu parks and 40% from samples collected in Ibadan while fungus of *Penicillium* species was isolated from 80% and 60% of samples collected in Sagamu and Ibadan respectively.

Isolation of *Pseudomonas aeruginosa* in all samples studied suggests that root is a major part of the plants used in the preparation of Jedi drinks. This is because *Pseudomonas* is primarily a soil bacterium and for it to be present in all the samples is an indication that the roots were not properly washed/treated before being used. This corroborates unhygienic method of preparation usually associated with herbal products.

The presence of *Klebsiella* and *Bacillus spp* is a function of water used for the preparation and this underscore the fact that the majority of the producers of Jedi drink have no assess to potable water.

Staphylococcus aureus may have been associated with touch contamination (Joyson *et al*., 1975). Isolation of fungus, of *penicillium* species may be as a result of continuous opening to air during dispensing to consumers.

Suffice it to say that bacterial isolates from Jedi drinks are a function of the plant parts as well as water used for the preparation.

Nonetheless, all the bacterial isolates recovered from Jedi drinks have been of public health concern. For instance, *Pseudomonas spp* was implicated in infantile gastroenteritis transmitted through water and foods by Thom *et al*., 1970 while Back *et al*. (1980) and Jiva *et al*. (1988) implicated *Klebsiella* in infantile gastroenteritis. Granum and Lund (1997), McKillip (2000) and Phelps and McKillip (2002) implicated *Bacillus spp* in gastrointestinal infection characterized by diarrhea. Moreover, *Staphylococcus spp* was implicated in gastrointestinal illness by Sears and Kaper (1996) and Brooks *et al*. (1998).

Antibiotic sensitivity patterns of each bacterial isolate revealed that all *Pseudomonas* were highly sensitive to ofloxacin, Augmentin and gentamicin while least sensitive to tetracycline. *Klebsiella spp* followed similar pattern of sensitivity.

Bacillus spp was highly sensitive to gentamicin, streptomycin, cotrimoxazole, Nitrofurantoin and ampicillin while least sensitive to colistin. *Staphylococcus aureus* followed similar pattern of sensitivity with no cotrimoxazole and Nitrofurantoin susceptibility.

Conclusion: It is therefore evident that consumers of Jedi drinks are prone to gastroenteritis. This calls for constant monitoring and quality control of herbal medicinal products manufactured, advertised, sold and used in Nigeria of which Jedi drink is part.

Also, consumers of Jedi drink having gastroenteritis as a result of the drink will benefit mostly from ofloxacin, a quinolone; streptomycin and gentamicin, both aminoglycoside.

While the manufacturers of Jedi drink should be more hygienic while government should make provision and accessibility of potable water a point of duty since major isolates are corollary to producers and water used.

REFERENCES

Back, E., R. Molby and B. Kaijser, 1980. Enterotoxigenic Escherichia coli and other Gram-negative bacteria of infantile diarrhea surface antigens, haemagglutinis, colonization factor antigen and loss of enterotoxigenicity. J. Infect. and Dis., 142: 318-327.

Brooks, G.F., J.S. Butel and S.A. Moore, 1998. Medical Microbiology. 21st Edn. Appleton and Lange, Norwalk, CT.

Granum, P.E. and T. Lund, 1997. *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Lett., 157: 223-228.

Jiva, S.F.H., K. Krovacek and T. Wadstom, 1988. Enterotoxigenic bacteria in food and water from an Ethopian community. Appl. and Environ. Microbiol., 41: 1010-1019.

Joyson, D.M., C.C. Howells, R. Liddington and S.A. Williams, 1975. Contamination of fluids from a hospital pharmacy. J. Hyg. Camb., 75: 87.

Langyan, N.K. and M. Ahuja, 2005. Estimation of nickel and cobalt in herbal products, pp: 220. In: 141st British Pharmaceutical Conference Science Proceedings.

McKillip, J.L., 2000. Prevalence and expression of enterotoxins in *Bacillus cereus* and other *Bacillus spp.*, a literature review. Antonie Leeuwenhoek, 77: 393-399.

Phelps, K.J. and J.L. McKillip, 2002. Enterotoxin production in natural isolates of Bacillaceae outside the *Bacillus cereus* as a cause of abortion in a mare. Curr. Sci., 50: 458.

Sears, C.L. and J.B. Kaper, 1996. Enteric bacterial toxins: Mechanisms of action and linkage to intestinal secretion. Microbiol. Rev., 60: 167-215.

Sofowora, E.A., 1994. Medicinal plants and Traditional medicine in Africa. John Wiley and Sons Ltd.

Thom, A.R., A.P. Cole and K. Watrasiewiez, 1970. *Pseudomonas aeruginosa* infection in a neonatal nursery, possibly transmitted by a breast-milk pump. Lancet, 1: 560-561.

Trease, G.E. and W.C. Evans, 1989. A Textbook of Pharmacognosy. 13th Edn. Bacilliere Tinal Ltd., London.

Vanderhoof, J.A., 2001. Probiotics: future directions. Ann. J. Clin. Nutr., 73: 1152S-1155S.

WHO, 2001. Legal status of Traditional Medicines and Complementary/Alternative Medicine: A worldwide review. WHO publishing 1.

Wilt, T.J., A. Ishani, I. Rutks and R. MacDonald, 2000. Phytotherapy for benign prostatic hyperplasia. Public Health Nutr., 3: 459-472.

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Bacteriological and Antibiotic Sensitivity Patterns of Bacterial Isolates from Creams and Lotions Hawked in Sagamu, Ogun State

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Abstract: Fifteen cosmetic products, consisting of 10 creams and 5 lotions were randomly purchased from a local market in sagamu and their microbial qualities studied in addition to the antibiotic sensitivity patterns of different isolates obtained from the selected creams and lotions. While only one of the creams was devoid of any microorganism including fungi, organisms isolated from others include *Staphylococcus aureus* 38%; *Klebsiella*, 28%; *Pseudomonas aeruginosa*, 21%; *Bacillus* spp, 7% and Penicillium, 28%. Antibiotic sensitivity study reveals that these isolates displayed different sensitivity patterns to the antibiotics used but cotrimoxazole, tetracycline and nalidixic acid will be of assistance in case of infection from these cosmetic products. However, majority of the creams and lotions evaluated did not meet the official monograph's requirements and as such may be a potential health hazard to unsuspecting consumers moreso that all the isolates display some degree of resistance to various antibiotics used.

Key words: Cosmetic products, creams, lotions, antibiotics, resistance

INTRODUCTION

Creams and lotions are external preparations with different rheological properties. Despite this difference, they are both liable to microbial contaminations either in the course of their preparation, transportation and/or use by the consumers which may lead to their spoilage. This spoilage may lead to alteration in organoleptic properties of creams and lotions which may manifest in terms of changes in color, odour and/or taste; as well as biodegradation of active constituent of such creams and lotions.

However, spoilage may result in loss in term of cost on the part of manufacturer and infection on the part of consumers of such spoilt products.

For instance, Orth *et al.* (1996) reported an outbreak of invasive mycoses caused by *Paecilomyces lilacinus* from contaminated skin lotion in Switzerland; cutaneous manifestation of which was reported by Itin *et al.* (1998). Becks and Lorenzoni (1995) reported a link between outbreak of *Pseudomonas aeruginosa* in neonatal intensive care unit in U.S.A. and contaminated hand-lotion just as Kallings *et al.* reported in (1966) that hydrocortisone ointment containing *Pseudomonas spp* used in the treatment of ophthalmic diseases resulted in sever eye infections.

Nonetheless, reports of microbial quality evaluations of cosmetics and toiletries have mainly been from temperate countries (Malcom, 1976; Baird, 1977; Brannan and Dille, 1990) and often in response to outbreaks of infectious disease (Becks and Lorenzoni, 1995; Itin *et al.*, 1998). Few studies have been carried out in Nigeria, such as Okore (1992), Okeke and Lamikanra (2001) and Hugbo *et al.* (2003).

While some of the studies cited above dealt with just isolation of contaminating organisms and preservative efficacy studies, none of them attempted to study antimicrobial susceptibility patterns of the contaminating organisms.

In this study, however, attempt was made to study bacteriological quality of 15 selected creams and lotions hawked in Sagamu in addition to antimicrobial susceptibility patterns of the contaminating organisms.

MATERIALS AND METHODS

MacConkey agar, Cetrimide agar, Mannitol salt agar, Mueller-Hinton agar, Sabauroud Dextrose agar; all Oxoid products.

1 g of cream to be studied was accurately and aseptically weighed into sterile tube containing 4ml Ringer's solution to which 0.25% Tween 80 has been added and made up to 10 ml using the same vehicle. 1 ml aliquot was pipetted into 9 ml sterile water and serial dilution made to 10^3. From the final dilution was pipetted 1 ml and plated on the surface of sterile agar media prepared. The agar plates were incubated at 37°C for 48 h and 25°C for 7 days for bacteria and fungi respectively. Total count was done by counting the number of colonies on Mueller-Hilton agar while counts on different agar media were also made.

The isolated organism on each medium was further identified using conventional biochemical methods.

Antimicrobial susceptibility pattern of each isolate was done using conventional disc diffusion method according to NCCLS standard.

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Table 1: Container label disclosures on selected creams and lotions

Samples	Date of Production	Expiry Date	Nafdac Number	Lot Number	Manufacturer Address
Sample 1	+	+	+	-	+
Sample 2	-	-	+	-	+
Sample 3	-	+	-	+	-
Sample 4	+	+	-	-	+
Sample 5	+	+	+	-	-
Sample 6	+	+	+	-	-
Sample 7	+	+	+	+	+
Sample 8	+	+	+	-	+
Sample 9	+	+	+	-	-
Sample 10	+	+	+	-	-
Sample 11	+	+	-	-	+
Sample 12	+	+	-	+	-
Sample 13	+	+	+	-	+
Sample 14	+	+	+	-	+
Sample 15	+	+	+	+	+

Table 2: Microbial counts (cfu/ml) and types found in cream and lotion

Samples	Bacterial Count	Types	Fungal Count	Types
Sample 1	NIL	-	NIL	-
Sample 2	2.4 x10^3	*Staph. aureus*	NIL	-
Sample 3	0.8 x 10^3	*Bacillus spp*	NL	
Sample 4	0.32 x 10^3	*Staph aureus*	1.23 x 10^3	*Penicillum spp*
Sample 5	1.2 x 10^3	*Klebsiella*	NIL	
Sample 6	0.64 x 10^3	*Pseudomonas aeruginosa*	NIL	
Sample 7	2.24 x 10^3	*Klebsiella*	0.97 x 10^3	*Penicillum spp*
Sample 8	1.36 x 10^3	*Staph. aureus*	NIL	
Sample 9	0.48 x 10^3	*Pseudomonas aeruginosa*	NIL	
Sample 10	0.24 x 10^3	*Klebsiella*	NIL	
Sample 11	1.26 x 10^3	*Staph aureus*	NIL	
Sample 12	1.84 x 10^3	*Pseudomonas aeruginosa*	NIL	
Sample 13	2.4 x 10^3	*Staph. aureus*	1.54 x 10^3	*Penicillum spp*
Sample 14	2.56 x 10^3	*Klebsiella*	NIL	
Sample 15	NIL	-	1.44 x 10^3	*Penicillum spp*

RESULTS AND DISCUSSION

From the creams and lotions evaluated, only 13 of 15 had date of manufacture indicated; 14 had expiry date; 11 with NAFDAC number while only 4 had lot/ batch number. 9 had address of the manufacturer indicated as shown in Table 1. This implies that there are container label disclosure deformities on the majority of the creams and lotions evaluated. Lack of batch/lot number should be viewed with seriousness as post-marketing surveillance, hence recall, of the product would be difficult to carry out in case of untoward effect (s) development. The fact that many of these products did not disclose the address of the manufacturer in addition to lack of batch number and yet claimed to have NAFDAC number call for suspicion.

Microbiologically, organisms isolated include *Staphylococcus aureus, Bacillus spp, Klebsiella spp, Pseudomonas aeruginosa* as well as fungus, *Penicillum spp*. This result differs from that of Okeke and Lamikanra (2001) in that fungus was not isolated by them and *Escherichia coli* was not isolated from our own study. Also, *Pseudomonas aeruginosa* and *Klebsiella spp* were not isolated by (Hugbo *et al*., 2003).

Staphylococcus aureus was the predominant organism isolated i.e. 33%, *Klebsiella* accounts for 26%, *Pseudomonas aeruginosa*, 20% and *Bacillus,* 6%, least isolated as shown in Table 2.

However, counts in general ranged between 0.24 x 10^3 and 2.56 x 10^3 cfu/ml for bacteria; 0.97 x 10^3 and 1.54 x 10^3 for fungus. Moreover, the initial bacterial loads per gram of material in 8 of 15 samples exceeded 1 x 10^3 cfu which is the acceptable limit for bacteria in non-sterile topical products.

Isolation of *Pseudomonas aeruginosa* and *Bacillus*, both free-living is an indictment of the raw materials used as well as the conditions prevalent in the environment in which the products are manufactured and packaged.

Water employed in manufacture has been described as the most likely source of *Klebsiella* in cosmetics (Crowshaw, 1977) and is a likely source of *Klebsiella i*solated in this study.

Nonetheless, isolation of *Staphylococcus aureus* from creams and lotions studied is a function of personal hygiene on the part of the personnel producing the products since skin is the natural habitat of the organism. Generally, those products from where *Bacillus, Klebsiella* and *Pseudomonas* were isolated

Table 3: Antibiogram profiles of bacterial isolates from creams and lotions

Antibiotic	SAM 2	SAM 3	SAM 4	SAM 5	SAM 6	SAM 7	SAM 8	SAM 9	SAM 10	SAM 11	SAM 12	SAM 13	SAM 14
Cotrimoxazol	R	R	S	S	S	R	R	S	R	S	S	S	S
Nitrofurantoin	R	R	R	R	R	R	R	R	R	R	R	R	R
Ciprofloxacin	R	R	R	R	S	R	R	S	R	R	S	S	S
Gentamycin	S	S	S	R	R	R	R	R	R	S	R	R	R
Ofloxacin	R	S	R	R	R	R	R	S	R	R	R	S	S
Tetracycline	S	R	R	S	S	R	R	S	S	R	R	R	R
Amoxycillin	S	R	R	R	R	R	S	R	R	S	R	R	R
Nalidixin	R	R	R	S	S	S	R	R	S	R	R	R	S
Augmentin	R	S	R	R	R	R	R	R	R	R	R	R	R
Colistin	R	R	R	R	R	R	R	R	R	R	R	R	R
Erythromycin	R	R	R	R	S	R	R	R	R	R	R	R	R

R = < 12 mm, S = >18 mm

should not have been released for use considering the potential health hazard these organisms pose to consumers.

All the isolates vary in their antibiotic sensitivity patterns to all the antibiotics used for the study as shown in Table 3.

All the *Staphylococcus aureus* isolates were sensitive to at least 3 antibiotics with the exception of that isolated from sample 8 which showed sensitivity to only one antibiotic; amoxicillin.

All *Pseudomonas* isolates were sensitive to cotrimoxazole and ciprofloxacin while all *Klebsiella* isolates were sensitive to nalidixin.

The result of antibiotic susceptibility study clearly showed that resistant bacteria strains have permeated cosmetic products.

Conclusion: As shown from the study, of the 9 cosmetics with the name and address of their manufacturer, 5 were imported from the neighboring African countries such as Cote 'd ivoire with all showing a high level of contamination. This suggests that more stringent means of testing and analyzing imported cosmetics should be adopted by the regulatory agencies such as NAFDAC.

It is disheartening to see some cosmetic products with no lot number, no address of the manufacturer still carrying NAFDAC number. This gives room for suspicion as some of the products may be fake.

Moreover, manufacturers of cosmetic products should adhere strictly to the principle of good manufacturing practice

REFERENCES

Baird, R., 1977. Microbial contamination of cosmetic products. J. Soc. Cosmetic Chem., 28: 17-20.

Becks, V. and N. Lorenzoni, 1995. *Pseudomonas aeruginosa* outbreak in neonatal intensive care unit: a possible link to contaminated hand lotion. Am. J. Infection Control, 23: 396-398.

Brannan, D. and J. Dille, 1990. Type of closure prevents microbial contamination of cosmetics during consumer use. Appl. and Environ. Microbiol., 56: 1476-1479.

Crowshaw, B., 1977. Preservatives for cosmetics and toiletries. J. Soc.Cosmetics Chem., 28: 3-16.

Hugbo, P.G., A.O. Onyekweli and I. Igwe, 2003. Microbial contamination and preservative capacity of some brands of cosmetic creams. Trop. J. Pharmaceutical Res., 2: 229-234.

Itin, P., R. Frei, S. Lautenschlager, S. Buechner, C. Surber, A. Gratwohl and A. Widmer, 1998. Cutaineous manifestation of *Paecilomyces lilacinus* infection induced by contaminated skin lotion in patients who are severely immunosuppressed. J. Am. Academy of Dermatol., 39: 401-409.

Kallings, L.O., O. Ringertz, P.E. Silverton and F. Emenfeldt, 1966. Microbiological contamination of medical preparations. Acta. Pharm. Sci., 3: 219-228.

Malcom, S., 1976. The survival of bacteria in toiletries. Inhibition and Inactivation of Vegetative Microbes Vol 5 Ed. Skinner, F. and Hugo, W. pp: 305-314. London: Academic Press.

Okeke, I.N. and A. Lamikanra, 2001. Bacteriological quality of skin-moisturizing creams and lotions distributed in a tropical developing country. J. Appl. Microbiol., 91: 922-928.

Okore, V., 1992. A study of the microbiological purity of some body creams and lotions marketed in Nigeria. Afr. J. Pharmacy and Pharmaceutical Sci., 22: 166-171.

Orth, B., R. Frei, P.H. Itin, M.G. Rinaldi, B. Speck, A. Gratwohl and A.F. Widmer, 1996. Outbreak of invasive mycoses caused by *Paecilomyces lilacinus* from contaminated skin lotion. Annual J. Internal Med., 125: 799-06.

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Investigating the Metabolic Effects of the Cyclic Nucleotide Phosphodiesterase Inhibitors on Immature *balb/c Mice*

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Abstract: We have investigated the metabolic effects of IBMX (3-isobutyl-1-methyl xanthine, a non-selective PDE inhibitor), amrinone, MC7 (6-(4-(tetrahydro-2H-pyran-4-yl)-4-oxobutoxy)-4-methylquinolin-2(1H)-one) and MC9 (6-(4-(1-ethylpiperidin-4-yl)-4-oxobutoxy)-4-methylquinolin-2(1H)-one) (selective PDE3 inhibitors) on immature *balb/c mice*. Controls included groups of mice that received no intervention (control 1) and a group injected with 1mg/kg of drug carrier (control 2). The four experimental groups were weighed and injected separately with IBMX, amrinone, MC7 and MC9 (1mg/kg) daily for seven days after which they were killed and 59 blood and tissue samples were collected. The administration of drug carrier into the male mice, decreased the growth pattern during the second ($P<0.05$) and third ($P<0.001$) day. After the injection of drug carrier, there wasn't any significant difference between the growth pattern, and the concentrations of biochemical factors in the male, comparing with female mice. Therefore, a group of mice, including both male and female, was used. MC7 decreased the growth pattern on the day two ($P<0.01$). IBMX increased glucose ($P<0.05$), triglyceride ($P<0.05$) and glycogen ($P<0.01$) concentrations, whereas amrinone decreased the glucose ($P<0.05$) comparing with control 2. The ability of stress tolerating in immature female mice was more than that in the male mice. In spite of the similar inhibition influence, the experimental drugs used in this study, had different metabolic effects.

Key words: Cyclic nucleotide phosphodiesterases, growth pattern, cAMP, cGMP

INTRODUCTION

cAMP is an important second messenger, known to control many cellular processes that include: triglyceride hydrolysis, glycogenolysis, gluconeogenesis and insulin secretion (Collins *et al*., 2001). Phosphodiesterase enzymes (PDEs) play an important role in the regulation of intracellular levels of cAMP and cGMP. They have been divided into 11 families. These enzymes are often referred to as class I cyclic nucleotide PDEs (Bender *et al*., 2006).

PDE3 is often referred to as the cGMP-inhibited PDE. Two PDE3 genes, PDE3A and PDE3B, have been identified (Bender *et al*., 2006). PDE3A is relatively more abundant in the cardiovascular system and PDE3B in cells involved in energy metabolism, including adipocytes, hepatocytes and pancreatic ß-cells (Shakur *et al*., 2001; Zhao *et al*., 1997). A general characteristic of PDE3s includes their phosphorylation and short-term activation in response to insulin as well as to agents that increase cAMP (Degerman *et al*., 1996). Activation of PDE3B plays a major role in the antilipolytic action of insulin in adipose tissue (Hagstrom-Toft *et al*., 1995) and may be important in the inhibition of cAMP-induced glycogenolysis in hepatocytes (Zhao *et al*., 2000). PDE3B may also be involved in the regulation of insulin-induced glucose uptake, glucose transporter-4 (GLUT-4) translocation, and lipogenesis (Zmuda-Trzebiatowska *et al*., 2006, Eriksson *et al*., 1994).

PDE3 selective inhibitors induce lipolysis and may have effects on glucose homeostasis because they stimulate glucose-induced insulin release (Snyder, 1999; Cheung *et al*., 2003). These compounds have been used in the treatment of congestive heart failure (CHF). They produced an improvement in haemodynamic parameters (Arnold, 1993). However, long-term, oral therapy with PDE3 inhibitors increases mortality in CHF patients (Cruickshank, 1993).

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PDE3 inhibitors may also be useful for treating obese patients because they promote adipocyte lipolysis, thus mobilizing stored fat. Although, their effect on the cardiovascular system may be a major limitation to their use. So, development of isoform-specific agents that inhibit PDE3B but not PDE3A may provide a way around here this obstacle (Snyder, 1999). Since, PDE3 isoform selective inhibitors do not have the long-term side effects of non-specific PDE3 inhibitors (Movsesian, 2003), they may be useful for obese individuals (Snyder, 1999) and developing such drugs is currently being investigated.

Many obese individuals also suffer from non-insulin-dependent diabetes (NIDDM). These patients show peripheral insulin resistance and their glucose stimulated insulin release is attenuated. Since PDE3 inhibitors should elevate ß-cell cAMP, they may augment glucose-stimulated insulin release and thus have benefit for type II diabetics also (Snyder, 1999). The aim of this current study was to investigate the metabolic effects of a non-selective PDE inhibitor, such as IBMX (3-isobutyl-1-methyl xanthine), PDE3 selective inhibitors, such as amrinone (5-amino- [3, 4- bipyridin]- 6 [1H]-one), MC7 (6-(4-(tetrahydro-2H-pyran-4-yl)-4-oxobutoxy)-4-methylquinolin-2(1H)-one) and MC9 (6-(4-(1-ethylpiperidin-4-yl)-4-oxobutoxy)-4-methylquinolin-2(1H)-one), on immature male and female *balb/c mice*. To do this, the effect of these drugs on growth, serum glucose; triglycerides, and cholesterol concentrations and the hepatic glycogen content were measured.

MATERIALS AND METHODS

Male and female immature *balb/c mice* (10-12 g) were selected randomly from the animal house of the Pharmacology Department at Mashhad University of Medical Sciences. The sample size with regard to reference 11 was considered to be at least 4, with the power calculation of 90%.

The mice were provided with standard laboratory food and water. All mice, during the experiment, were maintained at 55% relative humidity and 22°C in a 12-h day/night rhythm. They had free access to water and food and weighed individually every day for a period of 1 week. This project was approved by the Ethical Committee of Alzahra University, Tehran, Iran.

The mice were divided into control (n = 25) and experimental groups (n = 34). The control 1 group (C1) was left with no treatment (male control 1, n = 7; female control 1, n = 6). The control two group (C2) (male control 2, n = 6; female control 2, n = 6) was injected with 1 mg/kg of drug carrier (normal saline solution containing dimethyl sulfoxide) using the subcutaneous procedure. The four experimental groups were weighed using Sartorius scale and injected separately, every day for 1 week with IBMX (FLUKA), amrinone (SIGMA), MC7 or MC9. Administration of drugs was carried out using the subcutaneous procedure. MC7 and MC9 were synthesized in the chemistry department, faculty of basic sciences, Ferdosi University in Mashhad. Dimethyl sulfoxide (DMSO, FLUKA) used as drug solvent. Then on day 7, after 1.5-2 hrs of injection of the drug, the mice were anesthetized using the thiopental sodium, (80 mg/kg, ip.), followed by taking a sample of blood from their heart. The serum of each sample was separated and frozen at -18°C for the subsequent measurement of glucose, cholesterol and triglyceride concentrations. Automatic enzymatic spectrophotometery (Alcyon Autoanalyser instrument) using the Iranian Man Company kits. In order to measure the glycogen concentration, the liver of each mouse was separated, weighed and frozen at -18°C. At the time of measuring, each liver sample was divided into small pieces and homogenized. Then, liver glycogen was hydrolyzed to glucose by hydrochloric acid (HCL, MERCK). Finally the glucose concentration was measured using a glucose kit (Zahedi-Asl *et al*., 2000).

Data were expressed as mean ± SEM. Both C1 male with female, C2 male with female, C1 male with C2 male and finally C1 female along with C2 female groups were analyzed using the Student t-test. C2 group and four experimental groups were evaluated using one-way analysis of variance with Dunnett's multiple comparison test. P-values less than 0.05 were considered to be significant.

RESULTS

The effect of PDE inhibitors on the growth: In C1 mice, the rate of growth in male mice was more than female mice, particularly on the 3rd ($P<0.01$) and 6th ($P<0.05$) days (Fig. 1). Total growth percentage of male mice (68%±2.6) was more than female mice (57%±1.6), over the 1 week period ($P<0.01$). However, in C2 immature mice, there was no difference between the growth pattern of male and female mice that were injected with drug carrier. Moreover, there was no significant difference between the total growth percentage of the C2 male (44%±3.9) and the female (47%±2.8) mice. Therefore, to study the effect of drugs on growth, a group of mice, including both male and female was used. In C2 male mice as compared with C1 male mice, daily administration of drug carrier decreased the growth pattern during the second ($P<0.05$) and the third ($P<0.001$) day, whereas it did not produce any significant effect on C2 female compared to C1 female mice (Fig. 1).

In the presence of amrinone, the growth pattern was at first similar to C2 and then increased by day 7 ($P<0.01$). Although, low increasing effect of the amrinone on the growth rate, during the day 2 was not significant ($P>0.05$). On the 2nd day, MC7 decreased the growth pattern sharply ($P<0.01$), and then after an initial increase, returned back to the C2 level (Fig. 2). The effect

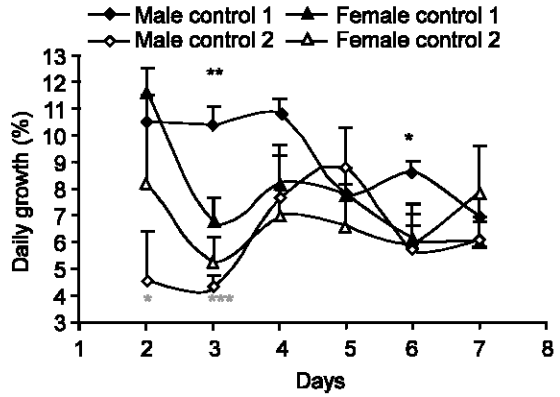

Fig. 1: The effect of drug carrier on growth pattern. Male control 1, n = 7; female control 1, n = 6; male control 2, n = 6; female control 2, n = 6. (*) is comparison of the male and female control 1 groups (C1). (*) is comparison of the male C1 group with the male control 2 group (C2). * P< 0.05, ** P<0.01, *** P<0.001. Both C1 male with female, C2 male with female, C1 male with C2 male and finally C1 female along with C2 female groups were analyzed using the Student t-test.

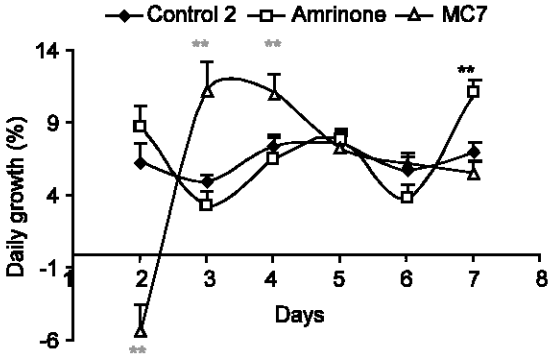

Fig. 2: The effect of PDE3 inhibitors (amrinone and MC7) on growth pattern. Control 2, n = 12; MC7, n = 8; amrinone, n = 9. (*) is comparison of amrinone group with the C2 group. (*) is comparison of the MC7 group with the C2 group, ** P< 0.01.

of MC9 and IBMX on growth pattern was similar to the effect of drug carrier in C2 group.

There was no significant effect on the total growth percentage, over the period of 1 week, and all groups grew up to 41-51% during this time.

The effect of PDE inhibitors on the serum biochemistry: There was no significant difference between the concentration of biochemical factors in the serum and liver glycogen of male comparing with female immature mice of the control group, which were administered with the drug carrier. Therefore, a group of mice, containing both male and female was used.

Serum cholesterol, triglyceride, glucose and liver glycogen concentrations for control group were 103 ± 5.2 mg/dl, 91.25 ± 11.54 mg/dl, 193.5±8.1 mg/dl and 11.5±1 mg/g respectively. IBMX increased the serum concentration of cholesterol (118.2±6 mg/dl, P>0.05), triglyceride (132.5±9 mg/dl, P<0.05) , glucose (247.8±11 mg/dl, P<0.01) and liver glycogen concentrations (24.4±4 mg/g, P<0.01) whereas amrinone decreased these concentrations to 87.6±6 mg/dl (P>0.05), 71.4±8 mg/dl (P>0.05), 158.9±12 mg/dl (P<0.05) and 6.5±2 mg/g (P>0.05) respectively.

MC7 and MC9 produced no significant effect on the above factors (P>0.05). MC7 decreased cholesterol and glucose concentrations to 96±4 mg/dl and 169.9±10 mg/dl respectively, whereas it did not change serum triglyceride (90.6±7 mg/dl) and the liver glycogen content (14.8±3 mg/g).

MC9 decreased serum cholesterol, glucose and triglyceride concentrations to 93.6±6 mg/dl; 184±8 mg/dl and 83±6 mg/dl respectively, however there was an increase in the liver glycogen storage amount (18.8±2 mg/g), (Fig. 3-4).

DISCUSSION

The effect of drugs on growth: With regard to Fig. 1, it was shown that the stress was associated with subcutaneous injection of drug carrier diminished the rate of growth on the immature male *balb/c mice*, however it did not have any effect on the female mice. In order to investigate the effect of MC7 on the growth on the 2nd day, another group (n = 8), that was injected with drug carrier, during the first 3 days, and with MC7, on the day 4, was studied. In this group, MC7 produced no significant effect on growth. This finding suggests that MC7 may have augmented the stress effect (Fig. 2). Hence, increasing the rate of growth by amrinone on the day 2 may be due to the diminishing effect of stress.

The effect of drugs on the serum biochemical factors: It is known that PDE3 inhibitors increase cAMP levels (Degerman *et al*., 1996) and stimulate glucose-induced insulin secretion. Insulin increases HMG-COA redoctase activity that regulates the synthesis of cholesterol, however increase of cAMP levels can phosphorylates and decreases its activity (Robert *et al*., 2003). The absence of any difference between the serum cholesterol concentration of four experimental groups comparing with the control group, indicates that probably these two effects have counteracted one another.

PDE3 inhibitors elevate beta cell cAMP and activate the cAMP-dependent protein kinase (PKA), and phosphorylates hormone sensitive lipase (HSL), which hydrolyzes stored triglyceride (White *et al*., 1994). PDE3

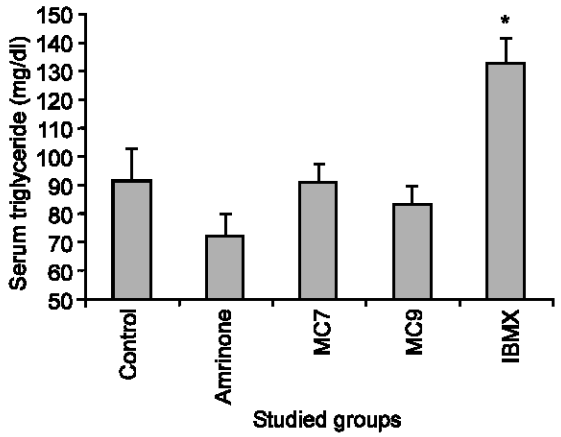

Fig. 3: The effect of PDE inhibitors on the serum triglyceride concentration. Control, n = 12; amrinone, n = 9; MC7, n = 8; MC9, n = 10; IBMX, n = 7. (*) is comparison of the four experimental groups with the control group,* P< 0.05.

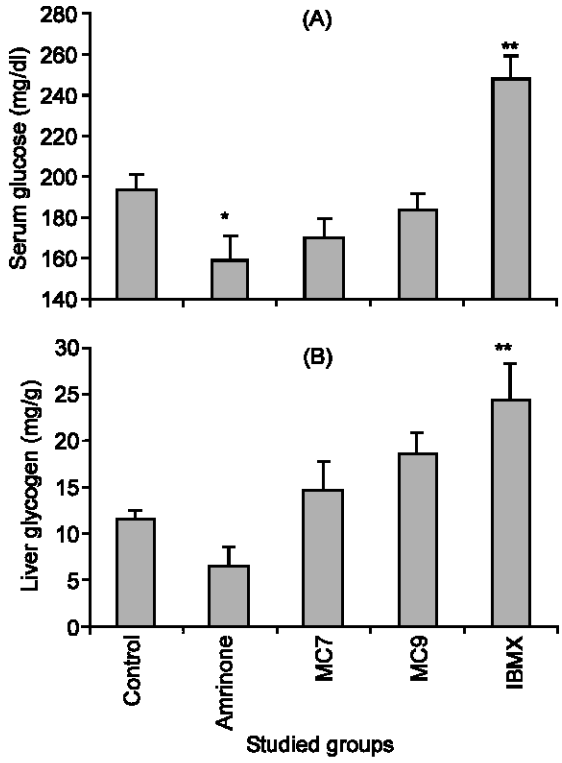

Fig. 4: The effect of PDE inhibitors on the serum glucose and liver glycogen concentrations. A, The effect of PDE inhibitors on the serum glucose concentration. Control, n =12; amrinone, n = 9; MC7, n = 8; MC9, n = 10; IBMX, n = 7. B, The effect of PDE inhibitors on the liver glycogen concentration. Control, n = 12; amrinone, n = 9; MC7, n = 8; MC9, n = 10; IBMX, n = 7. (*) is comparison of the four experimental groups with the control group, * P< 0.05, ** P< 0.01.

inhibitors promote continued breakdown of triglyceride even in the presence of an increase in the level of circulating insulin (Snyder, 1999). By the use of microdialysis technique, it has been shown that amrinone increases lipolysis in a dose-dependent manner (Arner *et al*., 1993). It has been demonstrated that iv. administration of amrinone increased blood levels of glycerol and FFA, the breakdown products of triglyceride (Wilmshurst *et al*., 1984; Ruttimann *et al*., 1994). In this study, amrinone acted as indicated above whereas IBMX showed an effect opposite to amrinone. In fact the effect of insulin on the adipose tissue, has been demonstrated by IBMX. It can be said that MC7 and MC9 have counteracted the effect of cAMP and insulin signaling on adypocytes (Fig. 3).

Present results indicated that amrinone decreased the serum glucose concentration, probably by the stimulation of insulin secretion. However, the serum glucose level was increased by IBMX (Fig. 4-A). It is necessary to mention that the effect of IBMX and amrinone on appetite was not investigated in previous studies.

One study suggest that PDE3 inhibitors stimulate hepatocyte glycogenolysis and antagonize the effect of insulin to suppress hepatocyte glycogenolysis (Snyder, 1999). In the current work, amrinone acted as above, in partially bringing down the concentration of liver glycogen. It seems that MC7 and MC9 have counteracted the effect of cAMP and insulin signaling on hepatocytes too (Fig. 4-B).

With regards to the fact that IBMX has increased both the liver glycogen content and serum glucose concentration, it could be suggested that IBMX prevented the influx of glucose into the cells of peripheral tissues. However, amrinone had an opposite action to IBMX in the immature mice.

It seems that the ability of stress toleration in immature female mice was more than that in the immature male mice. The growth effect of MC7 may be related to the augmentation of stress effect. Presumably, through an unknown mechanism, IBMX decreased the catabolism of glucose in the immature mice.

If the effect of MC7 and MC9 on the PDE activity is assessed and if these agents augment may cardiac contractility, then they might well be useful drugs for the treatment of CHF. These drugs probably are important to use, since they have less metabolic side effects than amrinone.

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REFERENCES

Arner, P., J. Hellmer, E. Hagstrom-Toft and J. Bolinder, 1993. Effect of phosphodiesterase inhibition with amrinone or theophylline on lipolysis and blood flow in human adipose tissue *in vivo* as measured with microdialysis. J. Lipid Res., 34: 1737-1743.

Arnold, J.M., 1993. The role of phosphodiesterase inhibitors in heart failure. Pharmacol. Ther., 57: 161-170.

Bender, A.T. and J.A. Beavo, 2006. Cyclic nucleotide phosphodiesterases: Molecular regulation to clinical use. Pharmacol. Rev., 58: 488-520.

Cheung, P., G. Yang, G. Boden and Milrinone, 2003. A selective phosphodiesterase 3 inhibitor, stimulates lipolysis, endogenous glucose production and insulin secretion. Metabolism, 52: 1496-500.

Collins, S. and R.S. Surwit, 2001. The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. Recent Prog. Horm Res., 56: 309-328.

Cruickshank, J.M., 1993. Phosphodiesterase III inhibitors: Long-term risks and short-term benefits. Cardiovasc Drugs Ther., 7: 655-660.

Degerman, E., M.J. Leroy, M. Taira, P. Belfrage and V.C. Manganiello, 1996. In Diabetes Mellitus, A Clinical and Fundamental Textbook. LeRoith, D. Olefsky J. and Taylor S., Lippincott Raven, Philadelphia, PA., pp: 197-204.

Eriksson, J.W., C. Wesslau and U. Smith, 1994. The cGMP-inhibitable phosphodiesterase modulates glucose transport activation by insulin. Biochem. Biophys. Acta., 1189: 163-167.

Hagstrom-Toft, E., J. Bolinder, S. Eriksson and P. Arner, 1995. Role of phosphodiesterase III in the antilipolytic effect of insulin *in vivo*. Diabetes, 44: 1170-1175.

Movsesian, M.A., 2003. Isoform-selective inhibitors and activators of PDE3 cyclic nucleotide phosphodiesterases. World Intellectual Property Organization.

Robert, K.M., K.G. Daryl, A.M. Peter and W.R. Victor, 2003. Harper's Illustrated Biochemistry, McGraw-Hill Companies, pp: 220-221.

Ruttimann, Y., R. Chiolero, J.P. Revelly, N. Jeanpretre and Y. Schutz, 1994. Thermogenic effect of amrinone in healthy men. Crit. Care Med., 22: 1235-1240.

Shakur, Y., L.S. Holst, T.R. Landstrom, M. Movsesian, E. Degerman and V. Manganiello, 2001. Regulation and function of the cyclic nucleotide phosphodiesterase (PDE3) gene family. Prog. Nucleic Acid Res. Mol. Biol., 66: 241-277.

Snyder, P.B., 1999. The adipocyte cGMP-inhibited cyclic nucleotide phosphodiesterase (PDE3B) as a target for lipolytic and thermogenic agents for the treatment of obesity. 3(4)[Expert Opinion on Therapeutic Targets], Informa Healthcare, pp: 587-599.

White, M.F. and C.R. Kahn, 1994. The insulin signaling system. J. Biol. Chem., 7: 1-4.

Wilmshurst, P.T., D.S. Thompson, S.M. Juul, B.S. Jenkins, D.J. Coltart and M.M. Webb-Peploe, 1984. Comparison of the effects of amrinone and sodium nitroprusside on haemodynamics, contractility and myocardial metabolism in patients with cardiac failure due to coronary artery disease and dilated cardiomyopathy. Br. Heart J., 52: 38-48.

Zahedi-Asl, S., H. Marahel and B. Zare, 2000. Investigating the effect of the chloroformic and water-alcoholic extracts of the Securigera Securidaca seed on the plasma glucose concentration and liver glycogen level in rats, The 14th Iranian Congress of Physiology-Pharmacology, Tehran University of Medical Sciences, pp: 16-20 (Persian).

Zhao, A.Z., M.M. Shinohara and D. Huang, 2000. Leptin induces insulin-like signaling that antagonizes cAMP elevation by glucagon in hepatocytes. J. Biol. Chem., 275: 11348-11354.

Zhao, A.Z., H. Zhao, J. Teague, W. Fujimoto and J.A. Beavo, 1997. Attenuation of insulin secretion by insulin-like growth factor 1 is mediated through activation of phosphodiesterase 3B. Proc. Natl. Acad. Sci. USA., 94: 3223-3228.

Zmuda-Trzebiatowska, E., A. Oknianska, V. Manganiello and E. Degerman, 2006. Role of PDE3B in insulin-induced glucose uptake, GLUT-4 translocation and lipogenesis in primary rat adipocytes. Cell Signal., 18: 382-390.

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The Effects of Processing on the Functional Properties of 'Oze' (*Bosqueia angolensis*) Seeds

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Abstract: 'Oze' (*Bosqueia angolensis*) found in the tropical rain forest grows in thick humid forest of undisturbed land and belongs to the family *Moracea*. Wholesome 'oze' (*Bosqueia angolensis*) seeds were given different treatments, which included blanching, cooking, roasting and malting. Malting was carried out by soaking for 24 h, germinated for 3 weeks and then dried and milled. The samples obtained from these treatments were analyzed for their functional properties. The emulsion capacity of raw 'oze' seeds was 6.6 ml while the oil and water absorption capacities were 8.9 ml and 7.8 ml respectively. The 'oze' seed flour samples had reasonable increases in the proximate composition, amino acid profile and most of the functional properties. The high emulsion, oil and water absorption capacities of the malted samples showed that 'oze' seed flour would be good as sausage extenders.

Key words: Oze seed flour, tropical rain forest, thick humid forest, sausage extender

INTRODUCTION

Oze (*Bosqueia angolensis*) referred to, as the "hospitality tree" in the cultural Igbo Community is a member of the botanical family, *Moracea*. It is a tropical rain forest tree and grows in the thick, humid forest of undisturbed land (Keay, 1989). The tree grows up to 30-40 meters high as it competes with other hard wood for sunlight (Irvine, 1961). Its green glossy leaves resemble those of 'Ogbono' (*Irvingia gabonensis*); but it is readily distinguished by the remarkably abundant latex flow observed immediately at a slash of its node. This plant called "Oze" in the Igbo speaking states of South Eastern zone of Nigeria is called "koko eran" in the Yoruba speaking states of South Western states of Nigeria (Okigbo, 1977).

In most developing tropical countries the food situation is worsening owing to increasing population; shortage of fertile land, high prices of available staples and restrictions on the importation of food (Sadik, 1991; Weaver, 1994). This has resulted in a high incidence of hunger and malnutrition, a situation in which children and women, especially pregnant and lactating women, are most vulnerable (Coulter *et al*., 1988; Pelletier, 1994). Predictions of future rates of population increase and food production emphasize the seriousness of this problem (FAO, 1990). There seems to be no immediate single solution to the problem of food sufficiency, thus interdisciplinary approach is necessary (Avery, 1991). All information on new sources of food will be of value in dealing with the food problem as suggested by Masek (1966).

While every measure is being taken to boost food production by conventional agriculture, a lot of interest is currently being focused on the possibilities of exploiting the vast numbers of less familiar food plant resources existing in the wild (RAO, 1994). Many such plants have been identified, but the lack of data on their chemical composition has limited the prospects for their broad utilization (Vijayakumari *et al*., 1994; Viano *et al*., 1995). Most reports on some lesser-known and unconventional crops indicate that they could be good sources of nutrients and many have the potentials of broadening the present narrow food base for human (Van Etten *et al*., 1967; Okigbo, 1977; Aletor and Aladetimic, 1989; Janick and Simon, 1990).

The aroma of roasted 'Oze' seed is reminiscence (resembles) that of its family member, African breadfruit; but its usual traditional dehulling process is more laborious (drudgery) than that of African breadfruit seeds. This factor has limited the traditional processing of 'Oze' to mere hot-ash roasting and a limited consequent utilization as snacking kernels, just as roasted cashew nuts. Thus 'Oze' though aromatically and morphologically more like African breadfruit is utilized mainly as indigenous snacking nuts just like cashew nuts.

Usually, the consumption of hot ash roasted Oze seed results in high gasing phenomenon, suggesting the presence of some anti-nutritional factors. Also the gas has a smell reminiscence of hydrogen sulphide suggesting the presence of sulfur containing amino acids which are among the essential amino acids needed in our daily diet.

Application of different processing methods to 'Oze' seed will give some information, which may increase the utilization of Oze seeds and enhance its potential in food

formulations. A good result will increase the awareness of the plant and may promote it as a new cash crop. This will certainly encourage its cultivation thereby saving it from being endangered. The objective of this study therefore is to investigate some of the functional properties of 'Oze' seed flour as affected by processing treatments.

MATERIALS AND METHODS

Materials collection and preparation: The 'oze' seeds with intact pulp were obtained from abandoned shrine spots at Ubomiri in Mbaitoli Local Government Area and Umuchima in Ideato South L.G.A both in Imo State. The pulp was washed off with water by rubbing with the hands. The seeds were then dried in the oven at 50-55°C for 24 h. The cleaned dry seeds were then given different treatments, which included blanching, cooking, roasting and malting after which they were dehulled. Blanching was carried out for 4, 6 and 8 min while cooking was carried out at boiling temperatures for 20, 40 and 60 min respectively. Roasting was carried out in the oven (Astell Hearson, England) at a temperature of 150°C for 45 min. Malting was done by steeping the 'oze' seeds for 24 h in water at a ratio of 1:2; (seed to water) then germinating the seeds at room temperature for 3 weeks before drying. All samples were dehulled and then milled using the manual grinder (Corona model), sieved to obtain fine powder, which were packaged in airtight plastic containers until needed for analysis.

Analysis of the functional properties of 'oze' seed flour

Determination of gelation capacity/boiling point: The gelling and boiling points were determined according to the method of Narayana and Rao (1982) with slight modification.

Three grams of each flour sample were weighed into a 50 ml beaker; each sample was dispersed in the distilled water to make 30 ml suspension using distilled water. Next a thermometer was clamped to a retort stand with its bulb submerged in the beaker. The beaker was then supported by a tripod stand, heated on a Bunsen burner and stirred gently with a stirring rod. The temperature at which the suspension began to gel was recorded. The stirring was continued until the suspension began to boil and the boiling point was also recorded.

Determination of water absorption capacity: The method described by Abbey and Ibeh (1988) was adopted with slight modification.

One (1.0 g) of each sample was put into a test tube and mixed with 10mls of distilled water. The mixture was left to stand for 30 min at room temperature being shaken every 10 min. At the end, it was blended using a magnetic stirrer for 5 min. The supernatant was carefully measured in a graduated cylinder and the volume used to calculate the volume of water absorbed and retained by the sample:

$$\text{Water absorption capacity} = \frac{(V_1 - V_2)P}{\text{Weight of sample}}$$

Where:
V_1 = is the initial volume of water used
V_2 = is the vol. remaining (not absorbed)
P = is the density of water (1.0 g/cm^3)

Determination of oil absorption capacity: For the oil absorption capacity, the Gino brand of vegetable oil was used. The method of Beuchat (1977) was followed. One gram of each flour sample was mixed with 10ml of oil for 30 sec in a mixer (Vari-whirl-mixing control set at fast speed). The sample was then allowed to stand at room temperature for 30 min. It was then centrifuged at 5000 rpm for 30 min, using a magnetic stirrer and the volume of the supernatant noted in a 10 ml graduated cylinder. The density of the oil was determined too. The volume of oil absorbed was multiplied by the density of the oil to determine the weight of oil so absorbed.

$$\text{Oil absorption capacity} = \frac{(V_1 - V_2)P}{\text{Weight of sample}}$$

Where:
V_1 = Initial volume of oil used
V_2 = Volume remaining (not absorbed)
P = density of the oil used

Determination of emulsion capacity and stability: The method of Beuchat (1977) was used. 2 g of each flour sample and 100 ml distilled water were blended at room temperature for 30 sec in Philips blender at 1600 rpm. After complete dispersion, vegetable oil (Gino) was added continuously in 5ml portions from a burette. Blending continued until the emulsion breakpoint (where a separation into two layers/phases) was observed. The emulsion capacity was expressed as ml of oil emulsified per gram of sample and was expressed as %:

$$\text{Emulsion capacity (\%)} = \frac{V_E}{V} \times \frac{100}{W}$$

Where:
W = The weight of sample
V_E = Volume of emulsion layer
V = Total volume of mixture

For the emulsion stability, the emulsion so prepared was then allowed to stand in a 250 ml graduated cylinder over time and the volume of the emulsion layer

read. The stability was measured in terms of the amount of oil that was retained in the emulsion layer and given by:

$$\text{Emulsion stability (\%)} = \frac{V_{ET}}{V} \times 100$$

V_{ET} = Emulsion volume at Time (T)
V = total volume of the mixture

Determination of bulk density: The method described by Okezie and Bello (1988) was adopted. A clean dry measuring cylinder was filled with the flour sample and the bottom of the cylinder was tapped on a table until the level could fall no further at the 100 cm^3 mark.
The weight of the flour, which occupied the 100 cm was measured and expressed as a ratio of the volume. The bulk density was given by:

$$\text{Bulk density} = \frac{W\ g/cm^3}{V}$$

Determination of the foaming capacity and stability: The method described by Coffman and Gracia (1977) was adopted. Two grams of the sample was whipped with 100 ml-distilled water in a micro blender at high speed for 5 min and quickly transferred carefully into a 250 ml graduated cylinder. The total volume of foam was noted and expressed as a ratio of the volume before blending. It was expressed as a percentage and was given by:

$$\text{Foam capacity (\%)} = \frac{Va - Vb}{Vb} \times \frac{100}{1}$$

Va = Volume of liquid and foam
Vb = Volume of mixture before whipping

The foam stability was measured in terms of how stable the formed foam lasted at room temperature. The cylinder containing the sample was left undisturbed following the foam capacity experiment. At intervals the foam volume was recorded. The foam stability was determined by measuring the foam volume at time (T). (T = life span of foam = 12 h) and expressed as the ratio of the foaming volume at the beginning.

Determination of swelling index: The swelling capacity of the samples were determined using the method of Lin *et al.* (1974), with slight modification. One gram (1 g) of the flour sample was dispersed in 10 ml of cold distilled water in a graduated centrifuge tube. The suspension was left at room temperature for 5 min to absorb water but not to swell. After 5 min the mixture was centrifuged at 2000 rpm for 30 min and the volume of the sediment recorded as initial volume.

Another 1 g of the sample was dispersed in a centrifuge tube of known weight and the suspensions heated in boiling water for 30 min. The suspension was cooled to room temperature under the tap water and then centrifuged at 2000 rpm for 30 min. using a magnetic stirrer. The volume of the heated sediment was recorded as final volume:

$$\text{Swelling index} = \frac{\text{Final Vol. after heating}}{\text{Initial Vol. before heating}}$$

Determination of wettability: The method described by Okezie and Bello (1988) was adopted. One gram (1 g) of each flour sample was measured into a 10 cm^3-measuring cylinder. The cylinder was inverted at 10cm above the water contained in 600 ml beaker. The finger was used to close the cylinder disallowing the flour sample from falling. By removing the finger and giving the cylinder a gentle tap, the flour sample was discharged into the water surface. The time taken by the sample to get completely wet was recorded as the time of wettability.

Determination of viscosity: The viscosity of each flour sample was determined by blending 10g of its flour in 90ml-distilled water using a mixer. The viscosity was measured at room temperature (28±1°C) with a Brookfied Viscometer (model LV), at 30 rpm for 5 min using spindle No 2. The viscosity readings were recorded in centipoises (cP).

RESULTS AND DISCUSSION

Functional properties of 'oze' seed flour samples: The results of the functional properties is shown in Table 1. Raw and heat-treated 'oze' seed flour samples had pH values very close to neutral (6.05-6.67) (Table 1). There was no significant difference ($p>0.05$) between the pH values of all samples irrespective of method and time of heat treatment. This implied that the seeds were neither acidic nor basic in nature. Thus its inclusion in food formulation would not influence the pH of the food product. This range of pH lies very close to the optimum (pH 7) for bacterial growth (Ejimadu, 1991) and explains why 'oze' seeds spoil rapidly when kept at room temperature. Though the raw 'oze' seeds and those cooked for 60 min had the lowest swelling index value (1.4 ml) there was no significant difference ($p>0.05$) between the swelling index values obtained for all the samples. With respective values of 4 and 5 min, the malted and raw seed flour samples had lowest wettability period as compared to all the heat treated samples. There were significant differences ($p<0.05$) between the wettability values of the blanched and all the other samples, with the blanched having values in the range of 13-16 min and the cooked having values in the

Table 1: Mean values of the functional properties of oze seed flour samples

Sample	pH	Swelling index (ml)	Wettability (min)	Bulk density	Gelation °C	Emulsion stability
Raw	6.65^{a}	1.4^{a}	5^{a}	48^{c}	70^{a}	6.6^{a}
4 min blanching	6.60^{a}	1.55^{a}	3^{b}	49^{c}	70^{a}	6.0^{ab}
6 min blanching	6.58^{a}	1.64^{a}	15^{bc}	49^{c}	70^{a}	6.0^{ab}
8 min blanching	6.54^{a}	1.90^{a}	16^{c}	50^{c}	70^{a}	6.0^{ab}
20 min cooking	6.52^{a}	1.90^{a}	21^{d}	51^{c}	70^{a}	5.8^{ab}
40 min cooking	6.50^{a}	1.73^{a}	34^{a}	52^{c}	70^{a}	5.8^{ab}
60 min cooking	6.35^{a}	1.50^{a}	40^{f}	57^{d}	80^{b}	5.8^{ab}
Roasted (45 min)	6.05^{a}	1.80^{a}	34^{e}	67^{s}	80^{b}	3.8^{c}
Malted	6.67^{a}	1.80^{a}	4^{a}	41^{c}	80^{b}	6.8^{a}
Hulls	5.80^{a}	1.80^{a}	3^{a}	25^{a}	70^{a}	1.5^{d}
LSD (±)	0.24	0.20	12.81	9.02	6.34	1.33

Sample	Emulsion capacity	Foaming stability	Foaming capacity	Oil abs. capacity	Water abs. capacity	Viscosity
Raw	3.5^{a}	5.0^{a}	4.0^{a}	8.9^{a}	7.8^{ab}	11.5^{bc}
4 min blanching	3.4^{a}	3.8^{ab}	1.8^{ab}	8.8^{a}	7.7^{ab}	12.5^{bcd}
6 min blanching	3.0^{ab}	3.5^{ab}	1.4^{b}	8.8^{a}	7.6^{ab}	13.0^{bcd}
8 min blanching	2.8^{ab}	3.0^{ab}	2.0^{ab}	8.9^{a}	7.5^{ab}	13.5^{abc}
20 min cooking	3.2^{ab}	2.4^{b}	1.4^{b}	8.3^{a}	7.4^{ab}	13.5^{ab}
40 min cooking	3.0^{ab}	1.5^{bc}	00	8.2^{a}	7.3^{ab}	14^{abc}
60 min cooking	3.0^{ab}	1.0^{bc}	00	8.4^{a}	7.2^{ab}	14.5^{b}
Roasted (45 min)	2.8^{ab}	1.0^{bc}	00	8.9^{a}	8.6^{a}	15.5^{a}
Malted	3.4^{a}	4.0^{ab}	3.0^{ab}	9.0^{a}	7.8^{ab}	11^{bc}
Hulls	1.0^{b}	0.8^{c}	00	4.3^{b}	5.4^{b}	10^{bc}
LSD (±)	0.62	1.34	0.99	1.19	0.72	1.46

Note: Means down the column with the same superscript are not significant at p>0.05

range of 21-40 min. The wettability values decreased with increased period of moist heat treatment. The ready-to-eat samples (cooked up to 40 min and roasted for 45 min) had wettability values of 34-40 min. This result indicated that the raw and malted seed flours would serve better in food formulae which require fast water absorption.

The bulk density increased with increased period of moist-heat treatment, ranging from 48 g/100 ml in the raw sample to 57 g/100 ml in 60 min cooked sample. Roasted seed flours had the highest bulk density value (67 g/100 ml) among all samples. There was significant difference ($p<0.05$) between the bulk density of the ready to eat (roasted and 40-60 min cooked) and undercooked samples. The malted samples had the lowest value (41 g/100 ml) in bulk density. Thus it would be more convenient for packaging and transportation.

The gelation temperature of samples ranged from 70-80°C with undercooked samples gelling at 70 °C while ready-to-eat samples gelled at 80°C and these values were significantly different ($p<0.05$). It is suggested therefore that this flour should not be added to formulae where gelling is required under 70°C.

Heating decreased the emulsion capacity and emulsion stability of 'oze' seed flour samples (Table 1). While raw 'oze' seed flour and malted seed flour samples had emulsion capacities of 6.6 and 6.8 ml/g with stability values of 3.5 ml and 3.4 ml respectively, heated samples had emulsion capacities ranging from 2.8 to 3.4 ml respectively. Samples blanched between 4-8 min had emulsion capacity value of 6.0 ml, while those cooked for 20-60 min had emulsion capacity value of 5.8 ml. Samples treated with dry heat (roasted) had the least value (3.8 ml) in emulsion capacity. There was significant difference ($p<0.05$) between the emulsion capacity of the roasted and the values for all other samples. The lower value (3.8 ml) for the roasted sample could be attributed to a higher degree of protein denaturation caused by dry heat as opposed to moist heat of cooking since the emulsion capacity is dependent on the nature of protein molecules and its surface properties (Sathe *et al*., 1982; Watanabe *et al*., 1984; Shamasunder and Prakash, 1994). The effect of heat processing in decreasing emulsion capacity of soy, peanut, wingbean, cowpea and brown bean flour samples McWatters and Holmes (1979), Abbey and Ibeh (1987); Abbey and Ibeh (1988). The very low emulsion capacity value (1.5 ml/g) for the hull flour samples was expected recognizing that it's protein content was 1.46% as compared to values of 6.41-11.81% in the main seed samples.

The data obtained indicated that either raw, blanched or malted 'oze' flours can be used in food formulation without much difference in emulsification power; and roasting is not desirable for flour samples intended to achieve product emulsification like in sausage; as dry heat caused higher denaturation of proteins (Sathe *et al*., 1982) desired for emulsification.

As observed for emulsion properties heating in all forms decreased the foaming properties (foam capacity and

stability) of 'oze' seed flours (Table 1). Specifically, the foaming capacity decreased from a value of 5.0 ml/g in the raw sample to a value of 1.0 ml/g in the roasted sample. The malted samples had the highest foam capacity value (4.0 mg/g) among the treated samples while the roasted and the 60 min-cooked samples had the least (1.0 ml/g) value. This low value for the roasted flour sample could also be attributed to higher degree of protein denaturation as compared to the protein nature in the raw, malted and blanched samples while the low value for the cooked samples (1.0-2.4 ml/g) could be attributed to the reduction of soluble protein by leaching (into cook-water) during cooking and the greater the period of cooking the higher the leaching effect as reported by Monteiro and Prakash (1980); Narayana and Rao (1982); Pawar and Ingle (1988); Rahama and Mastafa (1988). Interestingly, foams arising from ready-to-eat (40-60 min cooking and roasted) samples collapsed within 10 min of formation. The foams from the raw and malted samples had greater stability, 4 ml and 3 ml after 30 min. These 'oze' flour samples are not suitable for products requiring foam formations such as ice creams.

Though cooked samples had relatively lower oil absorption capacities (8.2-8.4ml/g) when compared to the values (8.8-9.0 ml/g) of all other seed flour samples. There was no significant difference ($p>0.05$) between the individual treatment samples. This implied that both raw and treated samples can be used in foods requiring oil absorption such as sausage products, with the same level of effect. The malted sample had a slightly higher level (9.0 ml/g) of oil absorption capacity than all the other samples. Though the increase was slight, but it agreed with the finding of Lund (1982) that malting increased the non-polar hydrophobic proteins resulting in superior binding of lipids. Sathe *et al.* (1982) reported that heat processing increased fat absorption capacity due to the dissociation and denaturation of the proteins but this trend was not evident in this study since the roasted sample which could have had higher oil absorption capacity value had the same value (8.9 ml/g) with the raw (unheated sample). Since oils contribute greatly in flavour perception and products with good oil absorption capacity are good flavour retainers, any of these samples could be substituted for the other in food formulation if flavour was the critical quality property (Kinsella, 1976).

Among the samples, the roasted flour had the highest value (8.6 ml/g) for water absorption capacity than all other samples whose values ranged from 7.2 ml/g (for 60 min cooked samples) to 7.8 ml/g (for raw and malted samples). There was significant difference ($p<0.05$) between the water absorption capacity value of the roasted and the values (7.2-7.8 m/g) of the other samples. Moist heat application had slight and gradual decrease effect in water absorption capacity of 'oze' seed flour samples. The hull flour sample had water absorption value of 5.4 ml/g which was lower than the values observed for all main (cotyledon) samples irrespective of treatment. It was expected that the water absorption capacity of samples would increase with increase in starch gelatinization and protein denaturation during the heating operation as in cooking and roasting (Lin *et al.*, 1974). But such increase was only slight and gradually decreased as the cooking time increased from 20-60 min cooking in this study. A high water absorption value is required for flours used in dough products and soup thickening. Thus 'oze' seed flour could be good for light thickening as in sauces but not preferable for gravies where heavy thickening is required.

With regards to the viscosity of flour samples, what was observed were relative differences between samples, with the exception of the roasted seed flour samples whose viscosity value (15.5 Cp) differed significantly ($p<0.05$) from the values (11.0-14.5 Cp) of the other samples. The viscosity values increased with increase in period of heating. Since viscosity is a measure of flow rate and an indicator of thickening ability, only the heat processed 'oze' seed flour samples should be used for instant food formulae since the viscosity values are too low compared to other seed samples used as thickeners whose viscosity values range from as high as 20-50 Cp like Okro and Ogbono (Amadi, 2004).

Conclusion: The results obtained from the project have shown that *Bosqueia angolensis* popularly known as 'oze' in Igbo speaking community yields flour which contain appreciable quantities of major nutrients like proteins and carbohydrates. The results of the studies on functional properties showed that 'oze' seed flour displayed diverse functional characteristics. From the studies it is believed that the seed has both great nutritional and functional values, which could be harnessed to meet nutritional needs and used in formulation of various foods.

REFERENCES

Abbey, B.W. and G.O. Ibeh, 1987. Functional properties of raw and heat processed brown bean (*Canavalia rosea* DC) Flour. J. Food Sci., 52: 406-408.

Abbey, B.W. and G.O. Ibeh, 1988. Functional properties of raw and heat processed cowpea (*Vigna unguiculata*) flour. J. Food Sci., 53: 1775-1777.

Aletor, V.A. and O.O. Aladetimic, 1989. Compositional evaluation of some cowpea varieties and some underutilized edible legumes in Nigeria. Nahrung, 33: 999-1007.

Amadi, C.E., 2004. Changes in the Mucilagenous and functional properties of Okro *(Abelmoschus esculentus)* as affected by different storage conditions. B.Sc. (Tech) Thesis. Dept. of FST, FUTO.

Avery, O.T., 1991. Mother earth can feed billions more. Wall Street J. Europe 20 Sept. pp: 8.

Beuchat, L.R., 1977. Functional and electrophoretic characteristics of succiylated peanut flour proteins. J. Agric. Food Chem., 25: 258-262.

Coffman, C.W. and V.V. Gracia, 1977. Functional properties of amino acid content of a protein isolate from mung bean flour. J. Food Technol., 12: 473-484.

Coulter, J.B., G.I. Suliman, M.I. Omer, S.B. MacFarlane, J.B. Moody and R.G. HendricKse, 1988. Protein-energy malnutrition in Northern Sudan: Clinical Studies. Eur. J. Clin. Nutr., 42: 787-96.

Ejimadu, A.A., 1991. Development, of processed-canned African breadfruit *(Treculia africana)* products. B.Sc. Thesis, Federal University of Technology, Owerri.

FAO, 1990. Food and Agricultural Organisation. World meat situation and outlook. Commodities and Trades Division. ME 90/1 ROME. 316.

Irvine, F.R., 1961. Woody Plants of Ghana with special reference to their uses. London, Oxford University Press.

Janick, J. and J.E. Simon, 1990 (eds). The new crop era. Timber press, Portland Oreg, USA.

Keay, R.W.J., 1989. Trees of Nigeria Oxford University Press. New York, pp: 104.

Kinsella, J.E., 1976. Functional Properties of proteins in foods. A Survey In: Critical Reviews in Food Science and Nutrition (Fluria, T.E. Ed) 7: 219-225. CRC Press Inc. Cleveland.

Lin, M.J., E.S. Humbert and F.W. Sosulski, 1974. Certain functional properties of sunflower meal products. J. Food. Sci., 39: 368-370.

Lund, D., 1982. Effect of processing on nutrient content and nutritional values of food. In: Handbook of Nutritive Value of Proc. Food. Vol. I. Food for human use : CRS Press Inc. Florida USA, pp: 3.

Masek, J., 1966. Perspectives of human nutrition. In: Problems of World Nutrition. Vol. 4. Proc. 7th Int. Congr. Nutr. Oxford, UK, pp: 780-796.

McWatters, K.H. and M.R. Holmes, 1979. Influence of moist heat on solubility and emulsion properties of soy and peanut flour. J. Food Sci., 44: 774-779.

Monteiro, P.V. and V. Prakash, 1980. Functional property of homogenous protein fraction from peanut. J. Agric. Food Chem., 25: 441-446.

Narayana, N. and N. Rao, 1982. Functional properties of raw and heat processed winged bean. *(Psophocarpus tetragonolobus)* flour. J. Food Sci., 49: 1534-1538.

Okezie, B.O. and A.E. Bello, 1988. Physicochemical and functional properties of winged bean flour and Isolate compared with soy isolate. J. Food Sci., 53: 450-455.

Okigbo, B.N., 1977. Neglected plants of horticultural and nutritional importance in traditional farming systems of Tropical Africa. 4th African Sypo. On Horticultural crops. In: Acta Horticultural, 53: 131-150.

Pawar, V.D. and U.M. Ingle, 1988. Functional properties of raw and cooked moth bean. *(Phaseolus aconitifolius)* flour. J. Food Sci. Technol., 25: 187-188.

Pelletier, D.L., 1994. The potentiating effects of malnutrition on child mortality: Epidemiologic evidence and policy implications. Nutr. Rev., 52: 409 -415.

Rahama, E.H. and M.M. Mastafa, 1988. Functional properties of peanut flour as affected by different heat treatments. J. Food Sci. Technol., 25: 11-15.

Rao, P.U., 1994. Nutrient composition of some less-familiar oil seeds. Food Chem., 50: 379-382.

Sadik, N., 1991. Population growth and the food crisis. Food, Nutrition and Agric. Alimentation. Nutr. et Agric., 1: 3-6.

Sathe, S.K., S.S. Desphande and D.K. Salunkhe, 1982. Functional properties of lupin seed. (Lupirius mutabilis). J. Food Sci., 47: 491-497.

Shamasunder, B.A. and V. Prakash, 1994. Effects of blanching on frozen storage of prawns (Physico-chem and functional properties). J. Agric. Food Chem., 42: 126-128.

Van Etten, C.H., W.F. Kwolek, J.E. Peters and A.S. Barclay, 1967. Plant seeds as protein sources for food or feed. Evaluation based on amino-acid composition of 379 species. J. Agric Food Chem., 15: 1077-1089.

Viano, J., V. Masotti, E.M. Gaydou, P.J.L. Bourreil and G.M. Ghiglione, 1995. Compositional characteristics of 10 wild plant legumes from Mediterranean French pastures. J. Agric. Food Chem., 43: 680-683.

Vijayakumari, K., P. Siddhuraju and K. Janardhanan, 1994. Nutritional assessment and chemical composition of the lesser-known tree legume. *Acacia leucophloea*. Food Chem., 50: 2858.

Watanabe, H., H. Aoki, Y. Shiras and J. Kato, 1984. Emulsion stabilizing properties of soy protein isolates mixed with Na caseinates. J. Food Sci., 49: 1080-1084.

Weaver, L.T., 1994. Feeding the weanling in the developing world. Problems and Solutions. Int. J. Food Sci. Nutr., 45: 127-134.

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Effect of Olive Leaf Extracts on the Growth and Metabolism of Two Probiotic Bacteria of Intestinal Origin

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Abstract: The increase in viable cell numbers and the production of Short-Chain Fatty Acids (SCFA) by *Bifidobacterium infantis* and *lactobacillus acidophilus*-both of human intestinal origin-were measured over 16 h at 37°C in reconstituted skim-milk (100 g L^{-1}) and skim-milk with one of three olive leaf extracts (water, ethanolic and methanolic extracts). The olive leaves were collected from a well known olive tree variety in Jordan (Nabali). All the three olive leaf extracts increased cell counts over the control with no extracts and the final values for SCFA, especially acetic acid secreted by *L. acidophilus* (1.84 gL^{-1}), were significantly higher than those observed in milk alone. The ethanolic extract of olive leaves showed highest effect on cell count and SCFA production for both bacterial species. It is proposed that the polyphenol compounds in olive leaf extracts were responsible for the stimulation of probiotic bacteria growth and metabolism and that olive leaf extracts ingested in human diet might have the same effect on desirable components of the intestinal microflora. The results of the research will be used in functional food development and food preservation purposes. This research will create a market potential for a range of new health based food to maintain optimal human health. This research is the first attempt to produce fermented milk with olive leaf extracts.

Key words: Intestinal bacteria, olive leaf extracts, growth, fatty acid production, polyphenolics

INTRODUCTION

Olive leaves are a copious by-products deriving from olive tree cultivation and olive mills. Large a mounts of leaves are principally generated during pruning of the trees and harvesting and working of the olives (De Leonardis *et al*., 2008). The industrial use of olive leaves is limited to animal feed and phytotherpy (Martin Garcia *et al*., 2003).

Olive leaves contain high quantities of phenol substances very similar to those present in olives and their derived products (De Leonardis *et al*., 2008). There is compelling scientific evidence that olive leaf polyphenols are bioactive compounds. Olive leaves or their specific organic, show antiviral (Lee-Huang *et al*., 2003), antimicrobial (Bisignano *et al*., 1999), antioxidant and anti-inflammatory (Mann *et al*., 1999; Briante *et al*., 2002) properties, atherosclerosis inhibition and hypotensive action (Khayyal *et al*., 2002; Somova *et al*., 2003; Fehri *et al*., 1994; Quiles *et al*., 2000) and anti-carcinogenic properties that lead to the prevention of some cancers (Owen *et al*., 2004) and finally, stimulation of the thyroid (Al-Qarawi *et al*., 2002).

Beneficial properties of olive leaf extracts are further enhanced by the bioavailability of their polyphenolic constituents, which are readily absorbed through the gastrointestinal tract, resulting in significant levels in the circulation system (Visioli and Galli, 2000; Vissers *et al*., 2002). In relation to human, much concern has been focused on phenolic compounds from plants and foods that may modulate microbiota in the intestine by selectively increasing that growth of *bifidobacteria* and *lactobacilli* and decreasing that harmful bacteria such as clostridia.

The olive leaf polyphenol composition is similar to that of olive oil. Oleuropein and other secoiridoids are the principle compounds, while simple phenols, enclosed hydroxytyrosol, are present but in lower amounts (Tuck and Hayball, 2002). Olive leaves contain flaronoids such as: rutin flavonol, Luteolin-7-glucoside (Pereira *et al*., 2007) The olive leave extracts was shown to have an antioxidant capacity 400% higher than vitamin C and almost double that of green tea or grape seed extract (Ryan and Robards, 1998).

In the consequence of the high incidence of civilization diseases, science and industry direct their interest to ward production of food products, which beyond the normal nutritional function, deliver health benefits. These products are called functional foods and the probiotics are an example (Fuller, 1991; Robesfroid, 2000). In recent years, the developed countries have arrived at a new food formulations, derived from the combination of nutraceutical compounds and probiotic micro-organisms and formulations are setting quite a trend (De Leonardis *et al*., 2008). The growing popularity of functional food causes increasing interest in raw materials, which can raise the prohealth value of food when supplemented (Duda-Chodak *et al*., 2008).

Different results were obtained regarding the effects of plant extracts rich in polyphenolic compounds on the growth of probiotic bacteria and other microorganisms. It was proved that plant extracts can inhibit the growth of food associated pathogens and micro - organisms

responsible for food spoiling, as well as intestinal microflora, both pathogenic and physiological (Kim *et al*., 2004; Medina *et al*., 2006; Mobe *et al*., 1999; Nagayama *et al*., 2002). Some researchers indicate that polyphenolics compounds from different plant extracts may have, also, negative effect on bacteria which are desirable for human health. Ligstroside, one of the polyphenolic compounds present in virgin olive oil, showed the strong bactericidal activity against a broad spectrum of micro-organisms both Gram-positive and Gram-negative (Duda-Chodak *et al*., 2008). Molan *et al*. (2009), found that green tea extract increased significantly the number of *Lactobacilli* and *Bifidobacterium*. However, Medina *et al*. (2006), found that olive oil was effective toward foodborne pathogens, intestinal microflora as well as positively acting micro-organisms like *L. acidophilus* and *Bif. bifidum*. The phenolic compound catechin had no influence on growth of *Clostridium* sp., but stimulated *Lactobacilli* and *Bifidobacterium* (Lee *et al*., 2006).

Only few studies refer to the effect of polyphenolic plant rich extract on probiotic bacteria. Accordingly, the knowledge of the interaction between particular micro-organisms and plant extracts rich in polyphenolic compounds is indispensable for appropriate utilization of those extracts. The doubts appear especially in the situation when food containing probiotic bacteria is supplemented with plant raw material rich in polyphenols. Duda-Chodak *et al*. (2008) reported that the probiotic yoghurt supplementation with plant materials should be proceeded by careful studies about their influence on the bacteria. Accordingly, the objective of study was to evaluate the influence of olive leaf extracts (water, ethanolic and methanolic extracts) on the growth and metabolism of *L. acidophilus* and *Bif. infantis* that had been isolated previously from infants living in Amman (Haddadin *et al*., 2004).

MATERIALS AND METHODS

In November 2009, olive leaves were randomly and directly picked from an olive tree (*Nabali* variety). The trees have 10 years old, not irrigated and no phytosanitary treatments had been applied in the last year. The leaves were collected at the operator height around the whole perimeter of each tree. The collected samples were put in plastic bags. The plant material was then dried at room temperature and powdered (20 mesh).

Olive leaf extracts preparation: Ground powdered leaves were extracted in distilled water, ethanol (70% v/v) and methanol (70% v/v) at 20% (w/v) concentration. The mixtures were mixed on rotary shaker (New Brunswick Scientific, USA) for two hours and then for 15 min in ultrasonic bath (Bandelin Electronic-RK-103 H, Germany). The mixtures were filtered through whatman no: 4 and then membrane filter (0.45 um). The obtained solid residues of the olive leaf extracts, after solvents evaporation, were redissolved in 50 ml distilled water to give 50 mg mL^{-1} expressed as (+) Catechin Equivalents (CE).

Determination of total phenolics: The concentration of phenolics in the extracts was determined by the method of Singleton *et al*. (1999) and results were expressed as (+) Catechin Equivalents (CE). Samples (0.5 ml) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich) for 5 min then 2 ml of 7.5% sodium carbonate were added. After standing for 2h at room temperature, the Absorbance was measured at 760 nm using UV/visible spectrophotometer (Jasco-V-530, Japan). The estimation of total phenolics compounds in the extract was carried out in triplicate. The concentration between 0-200 µg/ml was used as standard to produce the calibration curve.

Bacterial strains: The bacterial isolates had been identified previously as *Bifidobacterium infantis* and *Lactobacillus acidophilus* (Awaisheh *et al*., 2004) and these were stored at 4°C on slants of MRS Agar (Code: CM 361, Unipath Ltd., Basing stoke, Hants., UK) at the Nutrition and Food Technology department, University of Jordan. Prior to use in the experimental programme, 50 ml sterile MRS broth (Code: CM 359, PH 6.50±0.20) with cysteine-HCl (5 g ℓ^{-1}) in Duran bottles were inoculated with a loopful of culture and incubated at 37°C for 16 h in anaerobic jar. Once activated, each culture was maintained by subculturing weekly by adding an inoculum (0.5 ml of the previous culture) to MRS broth (50 ml) with incubation at 37°C for 16 h.

Optimum growth time for the cultures: To determine the optimal incubation time in relation to the total viable count of the two species, batches of 500 ml of reconstituted (100 g L^{-1}) skimmed milk powder (Regilait, France) were dispensed into Duran bottles and heat treated at 73°C for 30 min. After cooling to 37°C, duplicate bottles of skimmed milk were inoculated with freshly prepared cultures of *Bif. Infantis* or *L. acidophilus* (20 ml ℓ^{-1}) and the bottles incubated at 37°C. Samples were taken to determine the total viable count at the beginning of the experimental period and then after 4, 8, 12, 16 and 20 h of incubation. On each occasion, serial dilutions (down to 10^{-7}) of the fermented milk were completed in test tubes of sterile peptone (9 ml, 1.0 g ℓ^{-1}) and duplicate 0.1 ml aliquots were plated onto MRS Agar supplemented with cysteine-HCl (5 g ℓ^{-1}) and incubated at 37°C for 48 h in anaerobic jars. The results were recorded as colony-forming units (cfu) per ml of milk.

Preparation of milk with different olive leaf extracts: Skimmed milk powder (Regilait, France) was reconstituted in distilled water (100 g L^{-1}) and dispensed into sterile bottles with serew-caps. The bottles of milk were then heat treated at 73°C for 30 min in a water bath. The extracts, all with a same concentration of 50 mg mL^{-1} of (+) catechin equivalents, were sterilized by micro-filtration unit using a sterile cellulose-ester membrane (0.2 µm-Advantec MFS, Japan) fitted to a syringe that dosed the required a mount of each extract into the bottle of skim-milk. The rates of addition were 0.20, 0.40, 0.80, 1.60, 2.00, 4.00, 6.00 or 10 ml into individual bottles of skim-milk and these doses gave concentrations of olive leaf extracts (water, ethanolic and methanolic), expressed as (+) catechin equivalents, of 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 3.0 and 5.0 mg mL^{-1} of growth medium. The volume of added extract was part of the total volume of growth medium (i.e 100 mL). Control bottles of skim-milk without olive leaf extracts were prepared at the same time. A similar batches of milk and olive leaf extract were employed to monitor the release of SCFA by the selected species at a concentration of 3 mg CE mg^{-1}. This level was the optimum for the growth of both species (see later).

Estimation of growth: Duplicate bottles at each olive leaf extracts concentration were inoculated with either *Bif. infantis* or *L. acidophilus* (1 ml aliquots of an active MRS broth culture) and incubated at 37°C for 16 h in anaerobic jars; duplicate bottles of the control milk were treated similarly. After incubation, serial dilutions (down to 10^{-7}) were made as described above and the results were recorded as cfu per ml of milk.

Production of Short-Chain Fatty Acids (SCFA): Duplicate bottles of milk for each olive leaf extract at a concentration of 3 mg-CE mL^{-1} were inoculated with either *Bif. infantis* or *L. acidophilus* (1.0 ml aliquots of an active MRS broth culture) and incubated at 37°C for 16 h in anaerobic jars; duplicated bottle of the control milk were treated similarly. The short chain fatty acids in the fermented milks were measured using the method proposed by Marsili *et al.* (1981). High Performance Liquid Chromatography (HPLC) was used. The chromatographic system (Jasco System, Japan) was equipped with a manual 20 µL Loop injector, a variable wavelength ultraviolet/visible dector (Jasco Model 875, Japan) and an insulated column oven (Jasco Model 865, Japan). Column effluents were monitored at a wavelength of 210 nm and quantification was based on peak height measurements using an integrator recorder (Shimadzu-C-R6A, Japan). Analyses were performed isocratically at a flow rate of 1.0 mL min^{-1} and temperature of 25°C. The column used was a 150 x 4.6 mm Hypurity Advance (Thermo Quest, Hypersil Division, USA). The mobile phase was prepared by mixing H_3PO_4 (10g L^{-1}) with HPLC Grade methanol at a ratio of 95: 5. The mobile phase was micro-filtered using a PTFE membrane (0.2 µm) and then degassed by sonication and helium purging.

Acetic, propionic and butyric acids (Sigma, USA) were used as standards. Stock solutions of different concentrations of each acid were prepared, namely 100, 200, 300, 600 and 1000 mg L^{-1}. Each concentration was injected in duplicate to obtain its retention time and area under the curve. The coefficient of correlation (r), regression equation and standard curves for each acid were calculated using Microsoft office excel 2003. The test of significance of coefficient of correlation (r) values was carried out at 0.01 probability. The recovery percent of each acid was determined by adding a known a mount of each acid to a sample of fermented milk and, after mixing, taking 5 mL of the test mixture.

This sample was then centrifuged for 10 min. at 4000 rpm and the supernatant micro-filtered and analyzed using the HPLC. The same procedure was applied to the experimental samples.

Measurement of pH: A sub-sample (10 ml) of the each fermented milk use used to measure the pH using a digital pH meter Model HI8519 (Hanna Instruments, Germany).

Statistical analysis: The General Linear Model (GLM) produced by the Statistical Analysis System (SAS) version 7 (SAS® System for Microsoft® Windows® 2001), was used to analyze the data. Differences between the means of treatments were tested using the Least Significant Difference (LSD) test at $p<0.05$.

RESULTS AND DISCUSSION

The growth of *Bif infantis* and *L. acidophilus* in skim-milk is shown in Table 1 and the trend was for the maximum viable cell count to be achieved after 16 h incubation. It was decided that all the test cultures could be incubated for 16 h, as the secretion of SCFA was considered as potentially the most important effect of the addition of olive leaf extracts.

Table 1: Growth of *Bif. Infantis* and *L. acidophilus* in reconstituted skim milk (100 g L^{-1}) at 37°C and sampled at the times indicated; all figures as CFU ml^{-1} and means of duplicate samples from two bottles

Time	*Bif. infantis*	*L. acidophilus*
0.0	7.50×10^6	6.50×10^5
4	2.50×10^7	3.50×10^6
8	3.25×10^7	2.50×10^7
12	4.00×10^7	6.50×10^7
16	5.10×10^7	7.80×10^7
20	4.40×10^7	2.80×10^7

Table 2: Effects of different concentrations (mg catechin equivalents/ml of growth medium) of three olive leaf extracts on the growth of *Bif. infantis* over a period of 16 h; all figures as cfu ml^{-1} and means±SD of triplicate samples from three bottles of milk

Concentration of extract (mg CE ml^{-1})	Water extract ($x10^8$)	Ethanolic extract ($x10^8$)	Methanol extract ($x10^8$)
0.0	$0.48±0.015^{ab}$	$0.65±0.050^{abcl}$	$0.68±0.015^{abc}$
0.1	$0.65±0.010^{ab}$	$0.82±0.010^{abc}$	$0.75±0.050^{abc}$
0.2	$0.92±0.060^{c}$	$0.65±0.474^{abci}$	$0.81±0.011^{abc}$
0.4	$1.77±0.251^{de}$	$1.65±0.050^{d}$	$1.10±0.100^{d}$
0.8	$1.75±0.050^{de}$	$2.15±0.217^{e}$	$2.10±0.100^{ef}$
1.0	$2.07±0.104^{f}$	$2.80±0.100^{f}$	$2.30±0.173^{ef}$
2.0	$2.89±0.130^{gh}$	$4.06±0.115^{gh}$	$3.46±0.35^{g}$
3.0	$2.91±0.076^{gh}$	$4.20±0.100^{gh}$	$3.79±0.085^{ha}$
5.0	$0.15±0.010^{i}$	$0.41±0.020^{aci}$	$0.12±0.002^{i}$

• Means within a column with a different superscript letter are significantly different at ($p \leq 0.05$)

Table 3: Effects of different concentrations (mg catechin equivalents/ml of growth medium) of three olive leaf extracts on the growth of *L. acidophilus* over a period of 16 h; all figures as cfu ml^{-1} and means±SD of triplicate samples from three bottles of milk

Concentration of extract (mg CE ml^{-1})	Water extract ($x10^8$)	Ethanolic extract ($x10^8$)	Methanol extract ($x10^8$)
0.0	$0.37±0.010^{ai}$	$0.44±0.045^{a}$	$0.32±0.020^{a}$
0.1	$0.86±0.076^{b}$	$0.90±0.050^{b}$	$0.74±0.032^{bc}$
0.2	$1.10±0.100^{c}$	$0.13±0.020^{c}$	$0.93±0.025^{bc}$
0.4	$2.06±0.152^{d}$	$2.41±0.085^{d}$	$1.40±0.050^{d}$
0.8	$2.43±0.076^{e}$	$2.84±0.050^{e}$	$2.45±0.150^{e}$
1.0	$3.00±0.200^{fg}$	$3.68±0.170^{f}$	$3.06±0.096^{f}$
2.0	$3.13±0.057^{fg}$	$3.94±0.150^{g}$	$3.41±0.175^{g}$
3.0	$3.64±0.083^{h}$	$4.30±0.200^{h}$	$3.80±0.086^{h}$
5.0	$0.36±0.006^{ai}$	$5.53±0.208^{i}$	$6.56±0.493^{i}$

• Means within a column with a different superscript letter are significantly different at ($p \leq 0.05$)

The total viable counts of *Bif. infantis* and *L. acidophilus* in skimmed milk with different concentrations of the three olive leaf extracts are summarized in Table 2 and 3, respectively. Nine concentrations of each extracts were used in this research and highest counts of *Bif. infantis* and *L. acidophilus* were related to both concentration and the type of extract. The level of 3 mg of (+) Catechin Equivalents (CE) per ml of the growth medium (100 mL) for all olive leaf extracts samples had the most significant effect on the count of *Bif. infants* and *L. acidophilus*. At 5 mg (+) catechin equivalents mL^{-1} of the growth medium for all extracts, the counts of *Bif. infantis* and *L. acidophilus* showed a significant drops than the other lower concentrations. These results are in agreement with those reported by De Leonardis *et al.* (2008), in which the antimicrobial activities of olive leaf extract showed no inhibition on *streptococcars thermophilus* and *Lactobacillus delbruechii spp bulgaricus* up to concentration of 3.2 mg ml^{-1} (as hydroxytyrosil) in the growth medurm.

They concluded that olive leaf extracts can be added as an integrator or antioxidant to fermented milk to increase both the quality and the nutritional value of the final milk product, without inducing any negative effects on the viability of the lactic acid bacteria. Similar results were, also, obtained by Molan *et al.* (2009), in which the addition of green tea extract resulted in a significant increase in the number of *lactobacilli* and *bifidobacteria*. This prebiotic activity of olive leaf extracts may be related to the higher total phenolic contents of these extracts (Molan *et al.*, 2009).

This prebiotic activity of olive leaf extracts, as non-carbohydrate prebiotics, is needed to stimulate the growth of probiotic bacteria without side effects such the enhancement of fructooligosaccharides to the growth of non-probiotic bacteria such as *Clostridium perfringens* and *Eubacterium biforme* (Bello *et al.*, 2001).

The mechanism by which phenolic extracts increased the growth of probiotic bacteria is not known. Molan *et al.* (2009) presented a possible partial explanation for this enhancing effects is the ability of polyphenols, in green tea extract, to act as antioxidant and antiradical agent to modulate the oxidative stress in the medium generated by the metabolic activities and consequently provide a better environment for the growth and multiplication of these bacteria. The importance of this research, is in the production of functional dairy products with an acceptable sensory properties and containing 300 mg CE per 100 ml milk. This product will enhance gut health and decrease putrefactive products and increased organic acids by lowering pH (Hara, 1997).

The statistical analysis did reveal that ethanolic extract of olive leaves had significantly better effect on the growth of *Bif. infantis* and *L. acidophilus* than the other extracts. This effect may be due to the ability of ethanolic extract to contain certain components that support the growth of *Bif. infantis* and *L. acidophilus*.

Table 4: Production of short-chain fatty at optimal catechin equivalents (3 ml CE ml^{-1} of growth medium) of three extracts by *Bif. infantis* over a period of 16 h; all figures as g L^{-1} and means±SD of triplicate samples from three bottles of milk

Olive leaf extract	Acetic acid	Propionic acid	Butyric acid	pH
Water extract	0.95±0.045^{a}	0.65±0.045^{a}	0.50±0.050^{a}	4.60±0.100^{abc}
Ethanolic extract	1.78±0.047^{b}	0.94±0.045^{b}	0.75±0.032^{b}	4.50±0.050^{abc}
Methanolic extract	1.31±0.030^{c}	0.80±0.050^{c}	0.62±0.0493^{c}	4.50±0.050^{abc}
Control	0.010±0.000^{d}	0.010±0.000^{d}	0.010±0.000^{d}	4.80±0.100^{d}

• Means within a column with a different superscript letter are significantly different at ($p \leq 0.05$)

Table 5: Production of short-chain fatty at optimal catechin equivalents (3 ml CE ml^{-1} of growth medium) of three extracts by *L. acidophilus* over a period of 16 h; all figures as g L^{-1} and means±SD of triplicate samples from three bottles of milk

Olive leaf extract	Acetic acid	Propionic acid	Butyric acid	pH
Water extract	1.10±0.050^{a}	0.94±0.040^{a}	0.73±0.032^{abc}	4.61±0.065^{a}
Ethanolic extract	1.84±0.041^{b}	1.11±0.032^{bc}	0.96±0.040^{b}	4.30±0.100^{bc}
Methanolic extract	1.54±0.081^{c}	1.05±0.066^{bc}	0.81±0.037^{c}	4.40±0.100^{bc}
Control	0.010±0.000^{d}	0.010±0.000^{d}	0.010±0.000^{d}	4.800±0.100^{d}

• Means within a column with a different superscript letter are significantly different at ($p \leq 0.05$)

The SCFA produced during fermentation are determined by the substrates available, their fermentability and the rate of breakdown (Parrett and Edwards, 1997) and the highest amounts found in this study were acetic acid, followed by propionic acid and butyric acid. This pattern is in with agreement with the results reported by Haddadin *et al*. (2007) and Topping and Clifton (2001), in which the concentrations of acetic, propionic and butyric acids were in the order: acetate > propionate = butyrate.

Regarding the metabolism of *Bif. infantis* (Table 4), There was significant difference between the different extracts with respect to the concentration of acetic, propionic and butyric acids and the control. The ethanolic extracts of the fermented milk contained the highest amount of all SCFA, followed by the methanolic extract and water extract. The ethanolic extract, also, supporting both the highest quantity of butyric acid and cell count. In the samples with *L. acidophilus*, there were significant differences between the three extracts with respect to the concentration of acetic acid, also, there was a significant difference between the extracts and the control (Table 5). The ethanolic extract showed the highest concentration for propionic and butyric acids and cell counts. In the milk samples containing *L. acidophilus*, there was a significant difference between the extracts and the control. The values of SCFA in the control milk were below the level of detection. The results showed that the ethanolic extract of olive leaves seems to contain components that favour the growth and the production of SCFA by *Bif. infantis* and *L. acidophilus*. Cornu *et al*. (1984) suggested that phenolic compounds in any plant extract could have an activating or inhibiting effect on probiotic bacteria, according to their constitution and concentration and the bacterial strain. The growth rate stimulation by phenolic compounds and the increase in cell density during the later stage of cell incubation could be related to their ability to metabolize these phenolic compounds (Stead, 1994; Vivas *et al*., 1997; Reguant *et al*., 2000).

The release of lactic acid is an indication of the activity of the probiotic bacteria (Ustunol, 2000) and the pH of all the cultures was monitored to provide an indication of total acidity (see Table 4 and 5). The milk fermented with *Bif. infantis* in the presence of olive leaf extracts had a significant lower pH than the control, suggesting an appreciable amount of lactic acid had been produced. In the case of *L. acidophilus,* the milks with olive leaf extract had significantly lower pH than the control (Table 5) and these values may be related to the amounts of lactic acid produced. These results of lower pH values in the milk with olive leaf extracts could be attributed to the phenolic compounds of the extracts, which are known to serve as an oxygen scavengers and to reduce the redox potential of the growth media, as probiotic bacteria grow better in the absence of oxygen (Alberto *et al*., 2004).

Overall, it is clear that all the olive leaf extracts beneficially influenced the growth and the metabolism of these two organisms of intestinal origin and it might be reasonable to assume that olive leaf extracts ingested by a consumer would have a similar effect on the native population of these species in the lower intestine. As a consequence of this research, it is expected that the twin beneficial attributes of polyphenolic rich extracts and probiotics of a fermented milk will create a market potential for a group of new health based functional food. If polyphenolics were the principal activators of the test bacteria, then olive leaf extracts may well have the beneficial role in promoting probiotic bacteria and inhibiting harmful bacteria such as *Clostridium* and *Escherichia coli*, thus maintaining optimal human health.

REFERENCES

Alberto, M.R., C. Gomez-Cordoves and M.C.M. De Nadra, 2004. Metabolism of gallic acid and catechin by *Lactobacillus hilgardi* from wine. J. Agric. Food Chem., 52: 6465-6469.

Al-Qarawi, A.A., M.A. Al-Damegh and S.A. El-Mougy, 2002. Effect of freeze dried extract of *Olea europaea* on pituitary-thyroid axis in rats. Phytother. Res., 16: 286-287.

Awaisheh, S.S., M.S.Y. Haddadin and R.K. Robinson, 2004. Incorporation of nutraceuticals and probiotic bacteria into a fermented milk. Int. Dairy J., 15: 1184-1190.

Bello, F.D., I. Walter and W.P. Hammes, 2001. *In vitro* study of prebiotic properties of Levan-type exopolysacch-a rides form lactobacilli and non-digestible carbohydrates using denaturing gel electrophoresis. Syst. Applied Microbiol., 24: 232-237.

Bisignano, G., A. Tomaino, R. La Cascio, G. Crisafi, N. Uccella and A. Saija, 1999. On the *in vitro* antimicrobial activity of oleuropein and hydroxytyrosol. J. Pharm. Phrmacol., 51: 971-974.

Briante, R., M. Patumi, S. Tereziani, E. Bimuto, F. Febbraio and R. Nucci, 2002. *Oleo europaea* L. leaf extract and derivatives: Antioxidant properties. J. Agric. Food Chem., 50: 4934-4940.

Cornu, M.C., A. Marchand, E. Meurville and J.M. Belin, 1984. Incidences des composes phenoliques sur des bacteries lactiques et acetiques isolees du vin. Sci. Aliment, 4: 73-79.

De Leonardis, A., A. Acetini, G. Alfano, V. Macciola and G. Ranalli, 2008. Isolation of a hydroxytyrosol rich extract from olive leaves *(Olea Europaea L.)* and evaluation of its antioxidant properties and bioactivity. Eur. Food Res. Technol., 226: 653-659.

Duda-Chodak, A., T. Tarko and M. Statek, 2008. The effect of antioxidants on *Lactobacillus casei* cultures. Acta Sci. Pol., Technol. Aliment, 7: 39-51.

Fehri, B., J.M. Aiache, A. Memmi, S. Korbi, M.T. Yacoubi, S. Mrad and J.L. Lamaison, 1994. Hypotension, hypoglycemia and hypouricemia recorded after repeated administration of aqueous leaf extract of *Olea europaea L.* J. Pharm. Belg., 49: 101-108.

Fuller, R., 1991. Probiotics in human medicine. Gut., 32: 439-442.

Haddadin, M.S.Y., S.S. Awaisheh and R.K. Robinson, 2004. Production of yoghurt with probiotic bacteria Isolated from infants in Jordan. Pak. J. Nutr., 3: 290-293.

Haddadin, M.S.Y., I. Nazer, S.J. Abu Raddad and R.K. Robinson, 2007. Effect of honey on the growth and metabolism of two bacterial species of intestinal origin. Pak. J. Nutr., 6: 693-697.

Hara, Y., 1997. Influence of tea catechin on the digestive tract. J. Cell. Biochem., 27: 52-58.

Khayyal, M.T., M.A. El-Ghazaly, D.M. Abdallah, N.N. Nassar, S.N. Okpanyi and M.H. Kreuter, 2002. Blood pressure lowering effect of an olive leaf extract *(Oleo europaea)* in L-NAME induced hypertension in rates. Arzneimittel-Forschung/Drug Res., 52: 797-802.

Kim, S., C. Ruengwilysup and D.Y. Fung, 2004. Antimicrobial effect of water-soluble tea extracts on foodborne pathogens in laboratory medium and in a food model. J. Food Prot., 67: 2608-2612.

Lee-Huang, S., L. Zhang, P.L. Huang, Y.T. Chang and P.L. Huang, 2003. Anti-HIV Activity of Olive Leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. Biochem. Biophys. Res. Commun., 307:1029-1037.

Lee, H.C., A.M. Jenner, C.S. Low and Y.K. Lee, 2006. Effect of tea phenolics and their metabolites on intestinal microbiota. Res. Microbiol., 157: 876-884.

Mobe, K., M. Yamado, I. Oguni and T. Takahashi, 1999. *In vitro* and *in vivo* activities of tea catechin against *Helicobacter pylori*. Antimicrob. Agents. Chemother., 43: 1788-1791.

Mann, C.P., V. Galletti, G. Cucciolla, Montedoro and V. Zappin, 1999. Olive oil hydroxyltyrosol protects human erythrocytes against oxidative damages-possible role in cancer. J. Nutr. Biochem., 10: 159-165.

Marsili, R.T., H. Ostapenko, R.E. Simmons and D.E. Green, 1981. High Performance liquid Chromatographic determination of organic acids in dariy products. J. Food Sci., 46: 52-57.

Martin Garcia, A.I., A. Moumen, D.R. Yanez Ruiz and M. Al-Caide, 2003. Chemical composition and nutrients availability for goats and sheep of two-stage olive cake and olive leaves. Anim. Feed Sci. Tech., 107: 61-74.

Medina, E., A. de Castro, C. Romero and M. Brenes, 2006. Comparison of the concentrations of phenolic compounds in olive oils and other plant oils, correlation with antimicrobial activity. J. Agric. Food Chem., 54: 4954-4961.

Molan, A.L., J. Flanagan, W. Wei and P.J. Moughan, 2009. Selenium-containing green tea has higher antioxidant and prebiotic activites than regular green tea. Food Chem., 114: 820-835.

Nagayama, K., Y. Iwamura, T. Shibata, I. Hirayama and T. Nakamura, 2002. Bacterial activity of phlorotannins from the brown alga *Ecklonia kurome*. J. Antimicrob. Chemother, 50: 889-893.

Owen, R.W., A. Giacosa, W.E. Hull, R. Haubner, B. Spiegelhalder and H. Bartsch, 2004. Olives and olive oil cancer prevention. Eur. J. Cancer, 13: 319-326.

Parrett, A.M. and C.A. Edwards, 1997. *In vitro* fermentation of carbohydrate by breast fed and formula fed infants. Arch. Dis. Childhood, 79: 249-253.

Pereira, A.P., I.C.F.R. Ferreiara, F. Marcelino, P. Valentao, P.B. Andrade, R. Seabra, L. Estevinho, A. Bento and J.A. Pereira, 2007. Phenolic compounds and antimicrobial activity of olive *(Olea europaea L. Cv Cobrancosa)* Leaves. Molecules, 12: 1153-1163.

Quiles, J.L., A.J. Far Quharson, D.K. Simpson, I. Grant and K.W.J. Wahle, 2000. Olive oil phenolics: Effects on DNA Oxidation and redox enzyme mRNA in prostate cells. Br. J. Nutr., 88: 225-234.

Reguant, C., A. Bordons, L. Arola and N. Rozes, 2000. Influence of phenolic compounds on the physiology of (*Enococcus ceni* from wine). J. Appl. Microbiol., 88: 1065-1071.

Robesfroid, M., 2000. Prebiotics and probiotics are they functional foods? Am. J. Clin. Nutr., 71 (suppl.): 1682S-1687S.

Ryan, D. and K. Robards, 1998. Phenolic compounds in olives. Critical Review. The Analyst (May), 123: (31R -44R).

SAS System for Microsoft Windows®, 2001. Version 7 (TS P1). SAS Institute Inc. Cary, NC, USA.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Reventos, 1999. Analysis of total phenols and other oxidation substances and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299: 152-178.

Somova, L.I., F.O. Shode, P. Ramnanan and A. Nados, 2003. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea, subspecies Africana* leaves. J. Ethnopharmacol., 84: 299-305.

Stead, D., 1994. The effect of chlorogenic, gallic and quinic acids on the growth of spoilage strains of *Lactobacillus collinoides* and *lactobacillus brevis*. Lett. Appl. Microbiol., 18: 112-114.

Topping, D.L. and P.M. Clifton, 2001. Short-chain fatty acids and human colonic function: Roles of resistant starch and non-starch polysaccharides. Physiol. Rev., 81: 1031-1064.

Tuck, K.L. and P.J. Hayball, 2002. Major phenolic compounds in olive oil: Metabolism and health effects. J. Nutr. Biochem., 13: 636-644.

Ustunol, Z., 2000. The effect of honey on the growth of *Bifidobacteria*. Summary of a research project funded by the National honey Board and conducted at Michigan State University. www.honey.com/ pressrm/research/bifido.html.

Visioli, F. and C. Galli, 2000. Biological properties of olive oil phytochemicals. Crit. Rev. Food Sci. Nutr., 42: 209-221.

Vissers, M.N., P.L. Zock, A.J. Roodenburg, R. Leenen and M.B. Katan, 2002. Olive oil phenols are absorbed in human. J. Nutr., 132: 409-417.

Vivas, N., A. Lonvaud-Funel and Y. Glories, 1997. Effect of phenolic acids and anthocyanins on growth, viability and malolactic activity of a lactic acid bacterium. Food Microbiol., 14: 291-300.

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Nutritional and Sensory Analysis of Soya Bean and Wheat Flour Composite Cake

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Abstract: The proximate and sensory analysis of the soya bean and wheat flour composite cake has been performed. This was done to determine the nutritional content and the general acceptability of the composite cake as compared to the pure wheat flour cake. The analysis revealed that the protein and fat content of the cake increased with the addition of the soya bean. Thus the composite cake is more nutritious than the pure wheat flour cake. The sensory analysis also revealed that the cake with 70% wheat flour and 30% soya beans was more acceptable than the pure wheat flour cake. However, cake with more than 30% soya beans was not generally acceptable.

Key words: Soya bean, wheat flour, cake

INTRODUCTION

According to Clerk and Herbert (2000), cake is a form of food that is usually sweet and often baked. Cakes normally combine some kind of flour (normally wheat flour), a sweetening agent (sugar), fats and liquid. In Ghana cakes are normally eaten as a dessert of choice at ceremonial occasions such as weddings, anniversaries and birthdays.

According to Tull (2000) cakes produced from wheat flour alone lack adequate protein needed for growth, repair of tissues and building of cells. In addition, cake made from wheat flour is too expensive for the average person from a developing country to afford. A lot of efforts has been made and still being made to promote the use of composite flours in which flour from locally grown crops and high protein seeds replace a portion of wheat flour for use in bread production, thereby decreasing the demand for imported wheat and producing protein-enriched bread (Giami *et al*., 2004; Olaoye *et al*., 2006). Soy-based foods may provide additional benefits for the consumer for example due to their hypolipidemic, anticholesterolemic and counteratherogenic properties and also to their reduced allergenicity (Sipos, 1988). Consequently, soy milk based yoghurts offer a considerable appeal for a growing segment of consumers with certain dietary and health concern. In addition, soy milk yoghurt has several nutritional advantages over cow milk yoghurt such as, reduced levels of cholesterol, of saturated fat and free of lactose. Soya bean, which is possibly the richest natural food in proteins, vitamins and minerals, could therefore be a good substitute for wheat floor in the preparation of cakes.

In this work, wheat flour-soya bean composite cakes are prepared. Nutritional and sensory analysis are made and the results presented.

MATERIALS AND METHODS

Soybeans and wheat flour were both purchased from the Kotokuraba market in Cape Coast, Central region of Ghana. They were sent to the laboratory of the Food Research Institute, Council for Scientific and Industrial Research, for processing. The soybeans were processed into flour, using the method of IITA (1990) (Fig. 1). The process ensures effective removal of most anti-nutritional factors.

Six samples were prepared by mixing soybean flour with wheat flour in the proportions indicated in Table 1, using a Kenwood food mixer KN 201, England. The mixing was done to ensure a homogeneous mixture of the samples.

Table 1: Percentage composition of the soyabean flour-wheat flour composite cakes

	Percentages of wheat flour and soya bean flour	
Sample	Wheat flour	Soyabean flour
A	100%	0%
B	90%	10%
C	80%	20%
D	70%	30%
E	60%	40%
F	50%	50%

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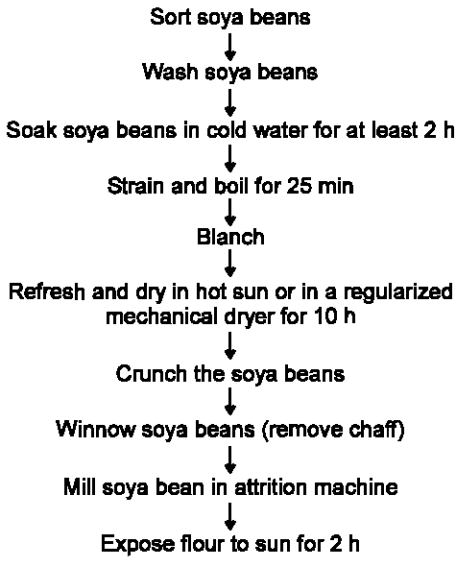

Fig. 1: Flow chart for the preparation of soya bean flour

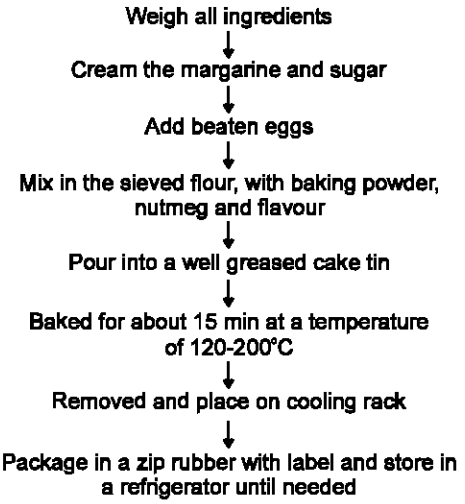

Fig. 2: Flow chart for the preparation of soyabean-wheat flour composite cakes

The flow chart in Fig. 2 demonstrates the method used in the preparation of the soya bean flour and wheat flour composite cakes.

The compositions of fat, moisture, ash, energy, protein and carbohydrate in the composite cakes were determined in the Chemistry Laboratory of the Food Research Institute using the standard AOAC methods.

Sensory analysis was performed on the cakes using a 5-point hedonic scale according to Watts *et al.* (1989) with a scaling range of 1-5 (1 = poor, 2 = fair, 3 = good, 4 = very good and 5 = excellent). A panel of 50 members was used. The Statistical Package for Social Sciences (SPSS) version II for Windows was used to analyze the data of the data of the hedonic test. Samples with significant means were separated using the Least Significant Difference (LSD) according to Ihekoronye and Ngoddy (1985).

RESULTS AND DISCUSSION

The results of the proximate analysis of the wheat flour-soya bean flour cake are indicated in Table 2.

It is observed from Table 2 that the moisture content of the cake reduces slightly as the amount of soya bean flour increases. Ash, which is the inorganic residue remaining after an inorganic matter has been burnt, is found to be almost constant for all the samples studied. The substitution of soya bean flour for the wheat flour is also found to increase the fat and the protein content of the cake while the amount of carbohydrate is reduced. The increase in the protein content agrees with the report of other workers (Olaoye *et al.*, 2006) and indicates that generally the substitution of soya bean flour for wheat flour in the production of cake increases the nutritional content of the cake. It is also observed from Table 2 that the values of the parameters investigated, such as fat and protein, peaked with the 30% soyabean cake sample.

Table 3 gives the organoleptic evaluation of the mean scores of the wheat flour-soya bean flour cakes.

The taste of the cake refers to the sweet sensation caused in the mouth by contact with the cake due to the sweetening agent. From the results of the organoleptic evaluation of the mean scores, sample D was ranked with the highest mean of 4.0400±0.1369. In relation to the response keys of taste, it implies that sample D was ranked very good. The difference between the samples is significant. The results indicate that the taste of sample D is preferred to the other samples.

The colour of the cake expresses the level of sensation the cake produces on the eye by the rays of light due to additional colouring agent such as margarine and eggs. Even though the difference between the mean is insignificant at 1% level, sample D has a little higher mean than the other samples. Thus, the colour of sample D is preferred to the colour of the other samples.

The flavour of the cake is the mingled nice sensation of smell and taste the cake produces due to the addition of flavouring substances. From the mean scores of the organoleptic evaluation, sample D ranked with 4.2800±0.1247 as the highest. The differences between the means of the samples are quite significant. It can therefore be concluded that sample D was preferred to the other samples in terms of flavour.

The texture of the cake refers to the smoothness, feel or appearance of the surface of the cake. From the mean scores of the organoleptic table of texture, sample D has the highest. The differences between the means of the samples are significantly different. Therefore, these results indicate that sample D is ranked as the best from the other samples in terms of texture by the panelists.

Table 2: Proximate composition of soyabean flour-wheat flour composite cakes

	Parameters				
Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate
A	20.7	1.8	16.4	6.8	54.3
B	20.2	1.8	18.2	7.4	50.1
C	19.4	1.9	25.5	8.9	44.3
D	18.3	1.9	26.0	9.3	43.9
E	18.3	1.9	26.2	9.3	43.9
F	18.3	1.9	26.2	9.3	43.9

Table 3: Hedonic sensory mean scores of the soyabean flour-wheat flour composite cakes

	Sensory quality				
Sample	Taste	Colour	Flavour	Texture	General acceptability
A	3.4000±0.1151	3.6800±0.1323	3.7600±0.1231	4.2800±0.1311	4.5000±0.1317
B	3.1200±0.1234	3.4000±0.1400	3.0800±0.1508	3.5600±0.1516	4.0000±0.1340
C	3.2400±0.1231	3.7200±0.1247	3.3600±0.1134	3.7400±0.1334	4.1800±0.1057
D	4.0400±0.1369	4.2400±0.1473	4.2800±0.1247	4.5600±0.1075	4.8400±0.1495
E	3.0400±0.1276	3.0400±0.1713	3.2000±0.1278	3.8800±0.1166	3.6800±0.1441
F	3.3467±0.0584	3.1600±0.2274	3.4400±0.1433	3.8000±0.1457	3.9600±0.1538

The general acceptability expresses how the consumers or panelists accept the product generally. Even though the mean scores of samples A and D are quite close (with that of D being a little higher than that of A), the impression is that consumers are more inclined to accept cake from two-composite flour of 30% soyabean and 70% wheat flour than only wheat flour, since sample D is ranked as the best in terms of all the sensory parameters. The P-value ($p<0.05$) obtained from the one-way analysis of variance (F-test) indicates that the difference between the means for the samples are insignificant at 1% level. This result agrees quite well with the work of Alabi and Anuonye (2007).

Conclusion: The proximate analysis of the wheat flour-soyabean flour cakes indicates that the nutritional content of the cake increases as the amount of soya bean increases. It is also observed that consumers prefer the two-composite cake of 30% soyabean and 70% wheat flour than only wheat flour cake.

REFERENCES

Alabi, M.O. and J.C. Anuonye, 2007. Nutritional and sensory attributes of sy-supplemented cereal meals, Nig. Food J., 25: 100-110.

Clerk, D. and E. Herbert, 2000. Food Facts. 4th Edn., Nelson and Son Ltd., pp: 55.

Ihekoronye, A.O. and P.O. Ngoddy, 1985. Integrated food science and technology for the tropics, Macmillan Publishers.

IITA, 1990. Soybeans for good health: How to grow and use soybeans in Nigeria. IITA Publications, p 23.

Giami, S.Y., T. Amasisi and G. Akiyor, 2004. Comparison of bread making properties of composite flour from kernels of roasted and boiled African breadfruit (*Treculia Africana* decne) seeds. J. Mater. Res., 1: 16-25.

Olaoye, O.A., A.A. Onilude and O.A. Idowu, 2006. Quality characteristics of bread produced from composite flours of wheat, plantain and soybeans. Afr. J. Biotechn., 5: 1102-1106.

Sipos, E.F., 1988. Edible uses of soybean protein. Presented at Soybean Utilisation Alternatives Symposium. Univ. of Minnesota, Feb. 16-18, Minneapolis, MN.

Tull, A., 2000. Food and Nutrition. 3rd Edn., University Press, pp: 32-40.

Watts, B.M., G.L. Yimaki, L.E. Jeffery and L.G. Ekics, 1989. Basic sensory methods for food evaluation. International Development Research Centre, Ottawa, Ontario, Canada, pp: 68-79.

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The Nutritional Value of "Poha Beer" (Tamarind Fruit Drink) and its Social Usage in Tamale Metropolis

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Abstract: Tamarind fruit drink popularly known as "Poha Beer" in most parts of the Northern Ghana was prepared to test the nutritional value of the fruit. There is inadequate or lack of scientific data on its nutritional value and its usage especially in Ghanaian communities. This study was therefore carried out to survey the general usage of the tamarind fruit drink in the Tamale Metropolis and also to assess the nutritional value of some nutrients in the drink so as to contribute to the literature as well as bridge the knowledge gap. The study employed the use of questionnaire and the standard Association of Official Analytical Chemists (AOAC) official method of analyzing nutritional composition of foods to determine the vitamin C, the protein and carbohydrate composition values present in the Tamarind fruit drink. The results of the study indicate that "Poha Beer" generally compares favourably with most fruit juice (beverage) on the market. Considering the vitamin C level of the "Poha beer", it is very likely that, it could promote iron absorption. Reasons for non-preference include: taste, unhygienic method of preparation, unattractive packaging and its light nature. Improving on hygienic preparation, handling and good packaging could help curb the problem of non-preference by the populace in Tamale metropolis.

Key words: Tamarind, Poha, nutritional value, Tamale

INTRODUCTION

Tamarind fruit drink is popularly known as "Poha beer" in Northern Ghana and as its name suggests it is a drink (beverage) processed from the Tamarind fruit. The whole Tamarind plant, right from the root to the leaves, is of immense use, ranging from medicinal, decorative, brewing and dye production (Alfred and Ngoddy, 1992; Sidibe and Hofmanand, 1996).

Tamarinds are slow-growing, long-lived, evergreen trees that under optimum conditions can grow 80 feet high with a spread of 20-35 ft., in its native eastern Africa and Asia. However, in Southern California it seldom reaches more than 15-25 ft. in height (National Research Council, 2008). According to Watson and Dallwitz (1992) it is a legume popular in many tropical and sub tropical areas as an ornamental and as a fruit produce. It is from the class magnoliopsida and the family fabaceae. The genus Tamarindus is monotypic (having only a single species).

Although it is one of the conspicuous trees in the three Northern regions-Upper East, Upper West and Northern region, it is considered as a wild fruit and is not cultivated in Ghana. Also it can be seen across the entire nation.

Popenoe (1974) found the pulp of the fruit to be very rich in vitamin C and sugar. Hence it is used in syrups, juice concentrates, curries, pickles and meat sauces. The sugar in it is mainly glucose and fructose. Vitamin C is very important antioxidant in the body and it is also said to have a laxative effect (Kennedy and Santa, 2005; Ronald, 1998).

Problem statement: In Ghana, there are several claims on the usefulness of the Tamarind fruit and as such has led to it being one of the most patronized beverage by the people of Northern region, most especially during the period of Ramadan, These claims range from nutritional to medicinal and hence it is highly preferred to several beverages, alcoholic and non alcoholic sold on the market especially within the Muslim communities. In spite of the high patronage of this beverage in most parts of northern Ghana and the several claims on the nutritional value of the fruit, there is inadequate or lack of scientific data on its nutritional value and it usage specially in the Ghanaian communities. The paucity of literature relating to tamarind "Poha beer" (fruit drink) raises a number of pertinent research questions stated below:

- What are some of the vitamins in the "Poha beer"?
- What is the level of knowledge concerning the health benefit/risk concerning the use of "Poha beer"?
- What is the extent of usefulness of the "Poha beer" in Tamale metropolis?

The research questions underscore the aim and objectives of the study. The overarching aim is to assess the social uses of "Poha beer" (Tamarind fruit juice) and its nutritional value. The specific objectives are as follows:

- To determine the amount of vitamin C present in the beverage;

- To determine the amount of glucose present in the beverage;
- To determine the social usage of "Poha beer in Tamale metropolis and
- To determine the factors which limits the patronage and consumption of "Poha beer".

Literature review

Origin of Tamarind tree: Tamarind is native to tropical Africa and grows wild throughout the Sudan. It was introduced into India so long ago; it has often been reported as indigenous there. It is extensively cultivated in tropical areas of the world. Sometime during the sixteenth century, it was introduced into America and today is widely grown in Mexico (California Rare Fruit Growers, 1996).

Growth habit of Tamarind tree: Tamarinds are slow-growing, long-lived, evergreen trees that under optimum conditions can grow 80 feet high with a spread of 20-35 ft., in its native eastern Africa and Asia. However, in Southern California it seldom reaches more than 15-25 ft. in height (Caribbean Food and Nutrition Institute, 1993; Tamale *et al*., 1995).

Foliage: The bright green, pinnate foliage is dense and feathery in appearance, making an attractive shade tree with an open branch structure. The leaves are normally evergreen but may be shed briefly in very dry areas during the hot season. There are usually as many as 10-20 nearly sessile ½-1 inch, pale green leaflets per leaf. The leaflets close up at night (Caribbean Food and Nutrition Institute, 1993; Tamale *et al*., 1995; Morton, 1987).

Flowers: The inconspicuous, inch-wide, five-petalled flowers are borne in small racemes and are yellow with orange or red streaks. The flower buds are pink due to the outer colour of the 4 sepals which are shed when the flower opens (Tamale *et al*., 1995).

Fruit: The 3-8 inch long, brown, irregularly curved pods are borne in abundance along the new branches. As the pods mature, they fill out somewhat and the juicy, acidious pulp turns brown or reddish-brown. When fully ripe, the shells are brittle and easily broken. The pulp dehydrates to a sticky paste enclosed by a few coarse strands of fiber. The pods may contain from 1-12 large, flat, glossy brown, obviate seeds embedded in the brown, edible pulp. The pulp has a pleasing sweet/sour flavour and is high in both acid and sugar. It is also rich in vitamin B and high in calcium. There are wide differences in fruit size and flavour in seedling trees. Indian types have longer pods with 6-12 seeds, while the West Indian types have shorter pods containing only 3-6 seeds. Most tamarinds in America are of the shorter type (California Rare Fruit Growers, 1996) (Fig. 1, 2).

Fig. 1: Tamarind fruit. Source: http://www.wikipedia.org/*Tamarindus indica*

Fig. 2: Tamarind tree branch with the fruit. Source: http://www.wikipedia.org/*Tamarindus indica*

Location: The tamarind ultimately becomes a fairly large tree, so this should be kept in mind when planting out the tree. It is planted in full sun and is highly wind-resistant with strong, supple branches. The tree generally forms a beautiful spreading crown that casts a light shade (Dassanayake *et al*., 1991; National Research Council, 2008).

Soils: Tamarinds tolerate a great diversity of soil types but do best in deep, well drained soils which are slightly acid. Trees will not tolerate cold, wet soils but are tolerant of salt spray and can be planted fairly near the seashore (Morton, 1987; National Research Council, 2008).

Irrigation: The tamarind is adapted to semiarid regions of the tropics and can withstand drought conditions quite well. Young trees require adequate soil moisture until they become established, but mature trees do quite well without supplemental irrigation (California Rare Fruit Growers, 1996).

Fertilization: Young trees require fertilization every 2-3 months with a 6-6-3 NPK or similar analysis fertilizer. The requisite amount is 1/4 lb. and gradually increased to about ½ lb. Thereafter, young trees could receive ½ lb. per application, per year of tree age, 3-4 times a year. Bearing trees can be fertilized with 8-3-9 NPK or similar analysis, at rates of about ½ lb. per application per year of tree age. Microelements, particularly iron may be required for trees in alkaline soils (California Rare Fruit Growers, 1996).

Propagation: Rootstocks are propagated from seed, which germinate within a week. Seeds retain their viability for several months if kept dry. Plant seeds ½ inch deep in containers filled with a UC soilless type potting

media. Tamarind should be selected from trees of good production and quality. Even so, seedlings will be variable in quality and slow to bear. Veneer grafting, shield (T or inverted T) budding and air layering may be used to propagate desirable selections. Such trees will usually fruit within 3-4 years if provided optimum growing conditions. Seedlings should begin to produce fruit in 6-8 years, while vegetative propagated trees will normally bear in half that time (California Rare Fruit Growers, 1996).

Young trees should be planted in holes larger than necessary to accommodate the root system. They should be planted slightly higher than existing ground level to allow for subsequent settling of the soil and a water basin should be built around each tree to assure adequate moisture for young trees. Spacing of trees is normally 20-25 ft. in commercial orchards. However, solitary trees planted in Southern California rarely exceed 15 feet in diameter (California Rare Fruit Growers, 1996).

Pests and diseases: In California tamarinds are generally free of pests and diseases, although ants will sometimes spread black and olive scales. In India there are a host of pests that attack the tree, including mealy-bugs, caterpillars, aphids, white flies, trips and a variety of scales. Various weevils and borers can also infest the ripening pods or stored fruits (California Rare Fruit Growers, 1996).

Harvest: Tamarind fruits mature in late spring to early summer. They may be left on the tree for as long as 6 months after maturity so that the moisture content will be reduced to 20% or lower. Fruits for immediate processing are often harvested by pulling the pod away from the stalk. Mature trees are capable of producing 350 lb. of fruit a year. Ripe fruit in humid climates is readily attacked by beetles and fungi, so mature fruit should be harvested and stored under refrigeration (California Rare Fruit Growers, 1996).

Tamarinds may be eaten fresh, but they area most commonly used with sugar and water in the American tropics to prepare a cooling drink. The pulp is used to flavour preserves and chutney, to make meat sauces and to pickle fish. Candy can be made by mixing the pulp with dry sugar and moulding it into desired shapes (California Rare Fruit Growers, 1996).

Nutritional value of food: Food nutrients are classified generally into two namely macro-nutrients and micro-nutrients (Hamilton and Whitney, 1993; Lavon, 2001). Macro-nutrient basically include carbohydrates, fats and proteins whose constituent substance supply energy and build tissue. Micronutrient on the other hand embodies vitamins and minerals that the body uses in much smaller amounts to regulate and control body processes (William, 1994; Corinne *et al*., 1992). The nutritional value of a food refers to the amount of both macro and micro-nutrients present in that food item, in this case the Tamarind fruit drink.

Minerals: Minerals are inorganic nutrients that are required in minute quantity in the human organism. They are widely distributed in nature and perform metabolic functions like building, activating, regulating, transmitting and controlling (William, 1994; Susan, 2000). They are important nutrients which are categorized based on the amount needed per day. On a broader note if we require 100mg (1/50 a teaspoon) or more per day of a mineral, it is considered a major mineral; otherwise it is considered a trace minerals (Mallory and Julian, 1998; Wardlaw, 2003).

The more presence or supply of minerals in our diet is not an end in itself. The ability to absorb minerals from diet depends on so many factors (William, 1994; Charlotte and Nancy, 1991). Spinach for example contains a lot of calcium but only about 5% of it can be absorbed because it contains high concentration of oxalic acid and calcium-binder. Generally the more refined a plant food is-as in the case of white flour-the lower its mineral content (Wardlaw, 2003; King and Burgess, 1998).

Vitamins: They are vital organic dietary substances that are not an energy producing carbohydrate, fat, or protein and is usually necessary in only small quantities to perform a particular metabolic function or to prevent an associated deficiency disease and it cannot be manufactured by the body and therefore must be supplied in food (Watson and Dallwitz, 1992; William, 1994). Vitamins are thus classified into fat soluble (vitamins A, D, E and K) and water soluble vitamins (vitamins B and vitamins C) (Garrow and James, 1993). Vitamins can however be obtained from both plant and animal sources. Vitamins are very essential. It is also worth noting that when they are accumulated to some levels in the body, can cause toxic effects. Theoretically toxicity of any vitamin is possible, however toxicity of the fat soluble vitamins A and D are most frequently observed vitamin E and the water soluble vitamins niacin vitamin B and vitamin C can also cause toxic effects, but only when consumed in very large amount (15-100) time human need or more (Hampli and Hall, 2002; Wardlaw, 2003).

Preservation of vitamins in food: Vitamins in food can be lost usually from the time period of harvested or gathered to consummation. Water soluble vitamins particularly thiamine vitamin and foliate can be destroyed with improper storage and excessive cooking, heat, light, exposure to open air which can destroy vitamins. The sooner food is eaten after harvest the lesser the chance of nutrient lost (Charlotte and Nancy, 1991). Freezing apparently is a good method of preservation than those

methods (blending, drying, frying etc) (Alfred and Ngoddy, 1992).

Function of vitamin C: It functions include assistance in the synthesis of protein and collagen. This protein is highly concentrated in connection tissues; epithelial tissue bone, teeth, tendons and blood vessels. The vitamin also plays an important role in wound healing (Remero and Rodriguez, 1992).
In addition, it helps the body to use calcium and other nutrients to build bones and blood vessels. It also assists iron absorption by keeping iron in its most absorbable form and destroys free radicals in the body (King and Burgess, 1998). Apart from this it helps to strengthen the immune system of man and also contribute to reducing stress, heart diseases and cancer (Wardlaw, 2003). Food sources of vitamin C include Green pepper, Potatoes, Citrus fruits, Cauliflower, Broccoli, Papayas and Strawberries etc.

MATERIALS AND METHODS

"Poha beer" production: The preparation of the "Poha beer" involves several key stages as shown in Fig. 3.

Threshing: The outmost covering is carefully threshed out manually or separated one at a time with the hands. This is done carefully to prevent soiling and contamination of the fruit to ensure safety.

Fermentation: After the outer cover has been removed, the fruits are kept in a container with water sprinkled on them and left to ferment.

Moulding: The fruits are then moulded into balls for use and storage. The moulding reduces the surface area. This prevents bacteria and fungal spore's development in the fruits. This preserves it for a longer time while ensuring its quality as well.

Soaking: The fruits are water soaked either with cold or warm water to help extract the pulp. It ensures good dissolution. All the water soluble nutrients sip out of fruit into solution. Cold or slightly warm water is used to ensure that much volatile nutrients such as vitamin C are not lost.

Mashing: The soaked fruits are mashed in enough water to extract enough pulp. It increase rate of dissolution.

Decanting: After the substance has been mashed, the seed aid suspending matter (fibre or pieces of outer cover) are removed leaving the drink where all the unwanted materials are removed with a sieve.

Spicing: Spices are added to taste. The basic spices usually include ginger, cloves and peppercorns.

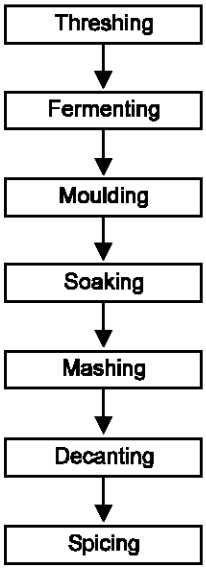

Fig. 3: Processing steps of "Poha beer"

Quantitative data on nutritional value
Sample source and preparation: A sample of Tamarind fruits was collected from fine tamarind fruit sellers in Tamale Central Market. The sample was soaked with warm water. The pulps of the fruits were extracted in the form of juice. The juice was stored in a refrigerator. After keeping the sample for a week, it was analyzed for its proximate compositions and mineral composition using standard Association of Official Analytical Chemists (AOAC) (1990) official method.

Protein/total nitrogen: The protein content in the Tamarind fruit drink was determined by measuring the total nitrogen using the Kjeldahl method (this is a food composition determinant procedure propose by Kjeldahl) of AOAC. 2.00 grams sample and half of selenium based catalyst tablet and 0.5 g of anti-bumping agent was added to the digestion flask. 25 ml of concentrated H_2SO_4 (Sulphuric acid) was added and the flask was shooked gently to make the sample wet. The mixture was then digested until a clear solution was obtained. Solution was cooled to room temperature. The digested sample was then transferred into a 100ml volumetric flask and made to the mark using distilled water. 10 ml aliquot of the digested was measured into the disposition chamber of a distillation apparatus.
A 15 ml 40% Sodium Hydroxide (NaOH) solution was added and the solution distilled. The ammonia released was trapped into 25 ml of 2% boric acid solution containing mixed indicator. A colour change from pink to green was observed as the ammonia was trapped. Distillation was contained for 5 min. The boric ammonia acid solution obtained was titrated against 0.1 ml of Hydrogen Chloride (HCl). The percentage protein was then calculated as shown in appendix 4a.

Moisture: Moisture was determined using the method of AOAC (1990). 5 g of the sample was transferred to previous weighed and dried dish. Dish was place in a hot air oven thermostatically controlled at 105°C for 5 h. Dish was then removed, placed in desiccators to cool and weighed. It was replaced in an oven and weighed. This was repeated until constant weight was obtained.

Estimation of vitamin C (Trimetric method): Vitamin C content was measured using the trimetric method.

Materials: Ascorbic acid, indophenols and sample. A burette was filled with 0.001% of indophenols solution. A solution containing 1g/dm^3 of Ascorbic acid was prepared (such that 1 cm^3 = 1 m of Vitamin C). 10 cm^3 solution of Ascorbic acid was acidified with three drops of diluted hydrochloric acid. It was run in indophenols solution until the solution was permanently pink.
For easy and accurate calculations, it was taken that if x cm^3 of sample is required; 1cm^3 of indophenols solution is equivalent to 10/x mg of vitamin C. Having standardized the indophenols solution, 10 cm^3 of the given sample was taken and treated in a similar way. The mass and volume of vitamin C was calculated as in appendix 4c.

Sugar: The total sugar was measured using the Abc tetrameter. The sample (dry) was divided corresponding to the international sugar scale, 1996. In taking the measurement a rounded glass rod was used to introduce 2-3 drops of sample into the funnel shaped opening on the right side of the measuring prism. The prism body was turned until a boundary was exactly in the intersection point of telescopes cross hair line by means of the focusing telescope. Reading were taken using the reading telescope which has a graduated circle which carries graduation for refractive indices and sugar percentage (dry substance) side by side Model: Carl Zeiss JENA SRN 291765.

Data analysis: The quantitative data were subjected to descriptive statistics using statistical tools such as percentages, tables, bar and pie charts. However the quantitative data were analyzed using nutrient specific protocols as indicated above.

Quantitative data on survey stand of the study

The survey area: The survey was carried out in Tamale central market, with the characteristics of a metropolitan setting, such as ethnicity, educational levels, religion reflected in the respondents' responses based on their diverse socio-demographic backgrounds.

Sample size determination: The sample size was determined using the statistical formulae:

$$N = z^2 pq/d^2$$

When,
n = Sample size
z = Statistical certainty chosen
p = Estimated prevalence/ level of investigation
q = 1-p
d = Precision desired
z = 99% = 1.96
p = 0.5
q = 0.5
d = 0.14
Therefore, n = $(1.96)^2$ x 0.5 x 0.5/$(0.14)^2$ = 49

Selection of participants: Participants were selected from the Tamale central market. It was mainly women dealing with "Poha beer" and a cross section of their customers. These customers included school children.

Information acquisition: It was acquired from participants via the use of semi-structured questionnaires. It was basically done in a form of conversation and written down later (shortly after the conversations). This was done to get full participation of the participants.

RESULTS

The nutritional value of "Poha beer" obtained from proximate analysis are given in Table 1.

Table 1: Nutritional value of "Poha beer"

Item	Amount
Protein	1.75%
Total solids	22.74%
Ash	1.04%
Sugar	26%
Vitamin C	30 mg/100 ml

Nutritional values of some beverages: The under listed drinks and their nutritional values were those sampled from the Tamale Central Market for the purpose of comparing them with that of "Poha Beer".

Sampling procedure: All drink with nutritional labeling in six Supermarkets in Tamale central market square were selected.

Demographic characteristics of respondents: A total of 50 people were sampled and interviewed. Table 3 gives details of the demographic characteristics of the respondents.
In terms of age distribution, most of the participants (46%) were within the age group of 26-35 years followed by 36% for those within the age group 10-25 years. The age range of 36-55 recorded the least number of participants (18%).
The participants were mainly Dagombas (64%) with Ewes being the least (2%). Majority of the participants were Muslims (62%) followed by Christians (38%).

Table 2: Nutritional value of massig fruit drinks

	Nutrient				
Drink	Carbohydrates (mg) (100 ml)	Protein (%)	pH	Ash	Acid (%)
Massig Banana	20	1.65	3.58	1.77	2.49
Massig Guava	18	3.5	3.4	1.06	1.41
Massig Melon	14	1.60	3.75	1.03	1.38

Mineral present: Fe, Na, K, Ca. The guava drink has been enriched with Vitamin C*

Table 3: Socio demographic characteristics of the participants

Sex	Number of respondents	Percentage
Males	21	42
Females	29	58
Ethnicity		
Dagomba	32	64
Gonja	8	16
Kasena	4	8
Akans	2	4
Ewes	1	2
Others	3	6
Religion		
Muslim	31	62
Christians	19	38
Education		
Primary	5	10
Middle/j.S.S	16	32
Secondary/s.S.S	10	20
Tertiary	9	18
No formal education	4	8
Arabic	6	12
Occupation		
Trader	20	40
Salary worker	4	8
Students	21	42
No employment	5	10

Table 4: Utilization of Poha beer

Items	Number respondents	Percentage
Source		
Market	18	36
Self-process	8	16
Relatives/friends (gifts)	3	6
Reasons for use		
Cheap not expensive	19	38
Readily available	4	8
Medicinal value	6	12
Amount consumed-50 ml cup	**Frequency**	**Percentage**
3 + cups daily	4	8
1-2 cups a week	14	28
3-4 cups in 2 weeks	2	4
Don't know	6	12
Occasionally	3	6
Preference		
Poha beer	13	26
Others	37	74

The detailed information on the participants socio-demographic characteristics are as illustrated in Table 3.

Table 4 shows the sources of "Poha beer" and some responses of the respondents.

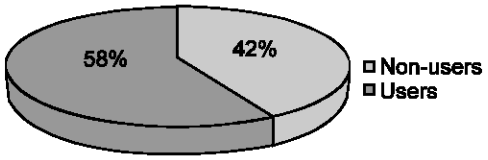

Fig. 4: A pie chart showing users and non-users

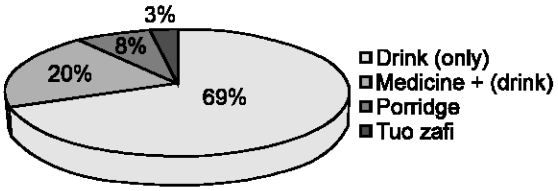

Fig. 5: A pie chart showing the utilization of "Poha Beer"

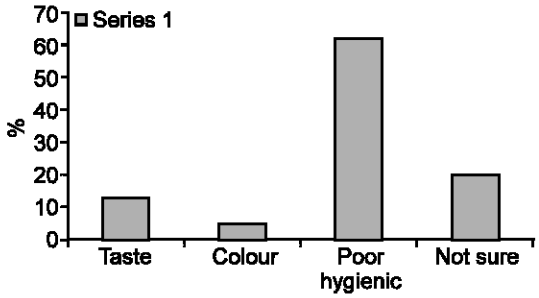

Fig. 6: A bar chart showing the reason for non-utilization

Figure 4 shows a high user's level (58%) of the beverage while 42% do not use it at all.

Figure 5 shows the utilization of "Poha beer". It could be seen that 69% of the respondents take "Poha beer" as a beverage (drink only), 20% of them took it because of its medicinal value while 8% and 3% user for porridge and T Z respectively.

Figure 6 is a bar chart showing the reasons for non-utilization, it revealed that the high level of non-utilization of "poha beer" was due to poor hygienic preparation (62%) and the taste (13%) while 5% do not use it because of its colour 20% were not sure.

Figure 7 is showing the reasons for preference of "Poha beer", 62% of the respondents prefer it to other drinks because they believe it has medicinal values, 23% of them prefer it because they believe it has a laxative effect and 15% prefer it for its taste.

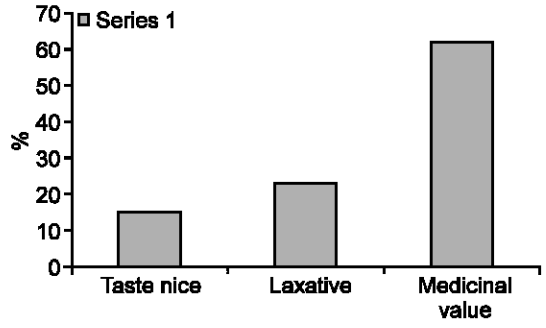

Fig. 7: A bar chart showing the reasons for preference of "Poha beer"

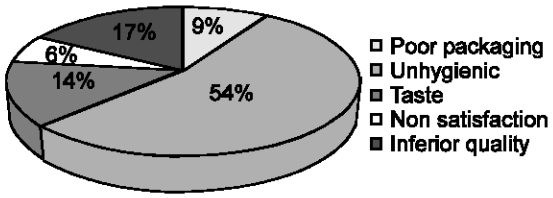

Fig. 8: A pie chart showing reasons for non-preference of "Poha beer"

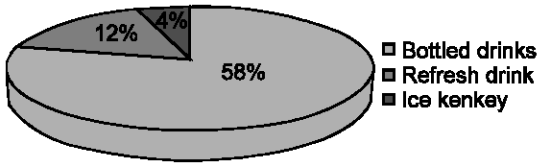

Fig. 9: Preference for other drinks

The pie chart it shows the distribution of reason for non preference "Poha beer" (Fig. 8). The result showed that 54% of the respondents do not prefer the drink because they believe it is unhygienically prepared, 14% of them do not prefer it because of its taste, 17, 9 and 6% do not prefer it because they find it to be non-satisfactory, poor packaging and an inferior drink respectively.

Figure 9 shows preference of other drinks. When "Poha beer was compared to other drinks majority of the respondents (58%) prefer bottled drinks to "Poha beer", 12% of the respondents prefer refresh and 4% prefer "ice kenkey" because they find it to be thicker than "Poha beer".

DISCUSSION

The nutritional value of a food refers to the amounts of nutrients present in the particular food. A food item is said to be nutrient rich with particular nutrients, when it has large amounts of those nutrients in it (Lavon, 2001). "Poha beer" nutritionally contains macronutrients (carbohydrates and proteins) and micronutrients (minerals and vitamins).

It contains substantial amount of carbohydrates-sugar (26%) (Table 1), which is mainly glucose and fructose. The beer could therefore be said to be a good source of carbohydrate, hence an energy giving drink. According to Wardlaw (2003) the presence of glucose in the drink could aid the absorption of calcium.

The protein content of the Tamarind fruit drink was 1.75% and hence it is relatively high compared to Massig's banana (1.60%) and melon drink (1.60%).the guava drink (3.5%).

Vitamin C is an essential vitamin, which plays a vital role in wound healing (Garrow and James 1993), reduction of the risk of cancer, heart diseases and stress (Ronald, 1998; Wardlaw, 2003). Vitamin C also aids the synthesis of collagen. A protein that is concentrated in bone, teeth, tendons, blood vessels and connective tissues (Garrow and James, 1993). "Poha beer" may be regarded as an important source of vitamin C. It contains 30 mg per 100 ml. Vitamin C (40% RDA), which compares favourable with the recommended daily allowance (75 mg). It is still a good source of vitamin C (Table 1) compared to other food drinks, for example, Pure Heaven (25 mg). The values for Massig Banana, Massig Guava, Massig Melon were however not shown indicating negligible amounts. "Poha beer" however has a lower vitamin C content as compared to Ceres (35 mg) and Farm Fresh (40 mg). Tapioca and other fruit drinks have the same amount of vitamin C as contained in "Poha beer" even though they have been enriched with vitamin C.

Based on the vitamin C content, it could be said that the beer promotes the absorption of iron. It is very likely that the consumption of adequate amount of poha beer could help reduce the prevalence of iron deficiency anaemia. This is because according to Kennedy and Santa (2005), Wardlaw and Insel (1995) vitamin C enhance bioavailability of non-haem iron. This is however possible if some amounts of non-haem iron have been consumed in a diet.

Utilization of "Poha beer" in Tamale metropolis: The survey reveals that 5% of the sampled population uses "Poha beer" (Fig. 4). Most people prefer it because of its medicinal value (Fig. 5). The beverage was readily available and cheap. This possibly explains its high patronage by the people. The medicinal values could be due to vitamin C, which is known to boost immune system in man. It is also known to be involved in the development of epithelial tissues (Wardlaw, 2003). Beside its uses as medicine and as a beverage, it is also used in the preparation of food in Northern Ghana, 8 and 3% of the respondents used it in the preparation of Tuo Zaafi and porridge respectively (Fig. 4).

Consumption of the beverage is usually high during the month of Ramadan. This could be due to the fact that majority of the people in the study area are Moslems (Table 4) and possibly use it to break their fast or as a starter meal.

Figure 7 show that most of the respondents prefer other drinks to "Poha beer". Several reasons were given for either non-preference or non-utilization. These include poor hygiene (62%) as shown in Fig. 6 and to a limited extent the taste. This could be due to the educational level of the respondents. It could be seen from Table 3 that only 12% of the respondents had no formal education.

The unhygienic nature could be due to the mode of preparation and the environment in which it is sold. Another reason for the low usage of this beverage is the poor packaging which makes it unattractive. Most people would have preferred the beverage bottled, cannedor sealed in polyethylene sachets like voltic water (Fig. 9). Information gathered from interactions with respondents revealed that the respondents did not really know the nutritional value of "Poha beer" and though its prestigious consuming other canned or bottled drinks, which are relatively more expensive.

The surveys also revealed that majority of the respondents were traders (Table 3) who could in a way afford other drinks/beverages in the market.

Most of the school children saw it as an inferior beverage of its poor packaging and unhygienic mode of preparations. Some of the respondents do not take it because they claim it has some laxative effect on them when they consume it, this could be due to the possible presence of microbes and its unhygienic nature.

On a whole, usage level and non-usage level of the "Poha beer" could also be attributed to it being in Tamale metropolises and hence have people of diverse ethnicity and socio-economic backgrounds (Table 3).

Conclusion and Recommendations: The results of the study indicate that "Poha beer" generally compares favourably with most fruit juice (beverage) in the market. The micronutrient tested of vitamin C (30 mg/100 ml).

The vitamin C content of "Poha beer" is lower than that of Ceres (35 ml/L) and farm fresh (40 mg/L) but has the same amount as in Tampico and Liquid fruit, though these were vitamin C enriched. It however has a higher vitamin C level than the Massig's fruit juice (banana, guava and melon). It is possible that there was loss of vitamin C during the processing of the juice. Considering the vitamin C level of the beverage; it is very likely that it could promote iron absorption.

Reasons for non-preference included: taste, poor hygiene and packaging, inferior quality and its light nature. Improving on hygiene, good packaging and adequate preparation could help curb the problem of non-preference. Adequate spicing would also contribute to solving this problem.

The following recommendations are made based on the study findings:

- Nutritionally accepted preservatives that will increase it shelf live should be investigated and consequently added in it preparation so as to have a sealed and hygienic package that can be display along side other drinks on the shelf.
- Awareness should be created on the nutritional values of "Poha beer" and it possible nutritional benefits so as to encourage increased production and consumption thereby creating a ready market for the beverage. This would go a long way to alleviate poverty especially in our rural settings via job creation.
- A national survey should be conducted, so as to access it marketability national.
- Further research should be conducted on the other nutrients of "Poha beer".
- Studies should be conducted on possible health effects of "Poha beer".

REFERENCES

Alfred, I.I. and P.O. Ngoddy, 1992. Integrated Food Science and Technology for the Tropics. Macmillan Press Ltd, London and Basingstoke, pp: 86-88.

AOAC, 1990. Official methods of analysis. 15th Edn., Association of Official Analysis Chemists, Washington, DC. Ohioline.osu.edu/hyg/5000/5552.html.

California Rare Fruit Growers, 1996. http://www.crfg.org/pubs/ff/tamawind.htm Retrieved August 2008.

Caribbean Food and Nutrition Institute PAHO/WHO, 1993. In collaboration with Ministry of Health, Jamaica Nutrition hand book for community workers in the tropics. 2nd Edn., Macmillan Press Ltd.

Charlotte, M.P. and J.P. Nancy, 1991. Nutrition Essentials and Diet Therapy. 6th Edn., W.B. Saunder Company, pp: 123-130, 173-190, 203-213.

Corinne, B.C., L.K. Debruyne and E.N. Whitney, 1992. Nutrition and diet therapy principles and practice. 3rd Edn., Washington DC: West Publishing Co.

Dassanayake, M.D. and F.R. Forsberg (Eds.) 1991. A Revised handbook to the flora of Ceylon. Washington, DC.

Garrow, J.S. and W.P.T. James, 1993. Contemporary Nutrition and Dietetics. 9th Edn.

Hamilton, E.M. and E.N. Whitney, 1993. Nutrition concepts and controversies. 5th Edn., Washington DC: West publishers.

Hampli, S.J. and R. Hall, 2002. Dietetic approaches to US Hunger and Food Insecurities. J. Am. Diet. Assoc., 102: 919.

Kennedy and Santa, 2005. Vitamin C. www.vitaminc foundation.org Retrieved July, 2008.

King, F.S. and A. Burgess, 1998. Nutrition and Developing Countries. 2nd Edn. Oxford: oxford University Press.

Lavon, J.D., 2001. Nutrition Almanac. 5th Edn.

Mallory Boylan, L. and Julian Spallholz, 1998.Nutrition: Chemistry and Biology CRC.

Morton Julia, F., 1987. Fruits of warm climate. Wipf and stock publishers. pp: 115-121.

National Research Council, 2008. Tamarind. Lost crops of Africa: Volume III: Fruits. Lost Crops of Africa. National Academies Press. ISBN 978-0 -309-10596-5. page - 149. Retrieve 2008-07-17.

Popenoe, W., 1974. Manual of tropical and subtropical Fruits. Hafner Press, pp: 432-436.

Remero, M.A. and Rodriguez, 1992. Determination of Vitamins C and orange acid in various fruits HPLC J. Chromatographic Sci., 30: 433-437.

Ronald, P., 1998. Antioxidant capacity and benefits of fruits and vegetables: Blue berries, the leader of the pack.

Sidibe, M.P. and M.F. Hofmanand, 1996. Baoba-home growth vitamin C for Africa.

Susan, G.D., 2000. Nutrition Essential for Nursing Practice. RDBS.

Tamale, E., N. Jones and Pswarayi-Riddihough, 1995. Technologies Related to participatory forestry in tropical and subtropical countries. World Bank Publications.

Wardlaw, G.M., 2003. Comtemporary Nutrition; Issues and Insight. 5th Edn., Churchill Livingston, Edinburgh, London.

Wardlaw, G.M. and P.M. Insel, 1995. Perspectives Nutrition. 13th Edn., Mosby, London, pp: 395, 437-467, 467-480.

Watson, L. and M.J. Dallwitz, 1992 (onwards). Families of flowering plants: Description, Illustration Identification and information retrieval. WCB/McGraw-Hill, pp: 187-200, 239-289.

William, R.S., 1994. Essential of Nutrition and Diet Therapy. 6th Edn., Mosby Westline, London.

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Improvement of the Biochemical Properties of Watermelon Rinds Subjected to *Saccharomyces cerevisae* Solid Media Fermentation

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Abstract: Improvement of the nutritional quality of watermelon rinds was attempted by *Saccharomyces cerevisae* solid media fermentation. The fermented rinds were analyzed with regard to proximate composition, antinutrient contents and phytonutrient properties and compared to unfermented watermelon rinds. Results revealed a significant increase ($p<0.05$) in the protein, lipid, ash and crude fibre contents. There were significant decreases ($p>0.05$) in the antinutrient contents of the fermented watermelon rinds. Significant increases ($p<0.05$) were observed in the phenolic and flavonoid contents of the fermented watermelon, as opposed to the significant decrease ($p>0.05$) of the alkaloid and saponin contents. These results indicate that *Saccharomyces cerevisae*, a cheap, non-pathogenic and saprophytic fungus, would efficiently improve the nutritional qualities of watermelon rinds and reduce the antinutrition levels.

Key words: Watermelon rinds, *S. cerevisae*, antinutrients and phytonutrients

INTRODUCTION

Watermelon, *Citrullus lanatus* (Thunb.) is a tropical fruit which grows in almost all part of Africa and South East Asia (Koocheki *et al*., 2007). It belongs to the family of cucumber (*Cucurbitacea*). It is large, oval, round or oblong in shape. The skin is smooth, with dark green rind or sometimes pale green stripes that turn yellowish green when ripe. Watermelon is a very rich source of vitamins and often used as an appetizer or snack, depending on how it is prepared (Kerje and Grum, 2003). It also serves as a good source of phytochemical and lycopene, a red carotenoid pigment which acts as antioxidant during normal metabolism and protects against cancer (Perkins-Veazie and Collins, 2004). Watermelon contains a significant amount of citrulline and after consumption of several kg an elevated concentration is measured in the blood plasma; this could be mistaken for citrullinaemia or other urea cycle disorder (Mandel *et al*., 2005). The rind is usually discarded, applied to feeds or fertilizer. But they are also edible and sometimes used as a vegetable. In China, they are stir-fried, stewed, or more often pickled. When stir-fried, the de-skinned and de-fruited rind is cooked with olive oil, garlic, chili peppers, scallions, sugar and rum. Pickled watermelon rind is also commonly consumed in the Southern US, Russia, Ukraine, Romania and Bulgaria (Southern U.S. Cuisine, 2010). Medicinally, watermelons are mildly diuretic. Watermelons contain large amounts of beta carotene and are a significant source of lycopene (Collins *et al*., 2005). In Nigeria, watermelon rinds are fermented, blended and consumed as juice.

Food fermentation can be described as a process of subjecting food to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food (Campbell-Platt, 1987). This study aims at investigating nutrient enrichment of watermelon rinds using cheap, non-pathogenic and saprophytic aerobic *Saccharomyces cerevisae*

MATERIALS AND METHODS

Sample preparation: Watermelon fruits were purchased from a local market at Lagos, Nigeria. The rinds were peeled-off and subjected to fermentation in plastic jars. A pure strain of *Saccharomyces cerevisae* was sub-cultured and inoculated into 500 g of the soaked rinds as the starter culture and 730 ml nutrient solution (urea (80 g), $MgSO_4$ $2H_2O$ (7 g), KH_2PO_4 (13 g) and citric acid (20 g)) and then allowed to ferment for 38 h. The micro-fungi fermented watermelon rinds were then blended. Another 1 kg of unfermented watermelon rind blend was also prepared. They were stored in laboratory freezers for further analysis.

Sample analysis

Proximate analysis: The proximate composition (protein, ash, lipid, crude fibre and carbohydrate) of the micro-fungi fermented and unfermented watermelon rinds was evaluated using the standard AOAC method (1997).

Anti-nutritional factors: Tannin was determined by the modified Vanillin - HCl method using 1.0 mg/ml of

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catechin in 1% HCl-MeOH as standard, the coloured substituted product was measured at 500 nm (Price *et al*., 1978). Phytate was determined by the anion exchange method (Harland and Oberleas, 1986) using KH_2PO_4 as standard. Oxalate was determined by titrimetrically (Falade *et al*., 2004) precipitation as calcium oxalate and titrated against standard potassium permanganate. The oxalate was calculated as sodium oxalate equivalent.

Phytonutrient analysis: The qualitative and quantitative phytonutrient properties of the fermented and unfermented samples were determined using standard methods described by Harborne (1993); Boham and Kocipai (1994); Ebrahimzadeh *et al*. (2007) and Nabavi *et al*. (2008).

Statistical analysis: Statistical significance was established using One-Way Analysis of Variance (ANOVA) and data were reported as mean ± standard deviation. Statistical analyses were carried out using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, IL.USA).

RESULTS AND DISCUSSION

The WHO food safety unit has set high priority to the research area of fermentation as a technique for food preparation/storage (Sahlin, 1999). Fermentation has been shown to improve protein quality and digestibility, vitamin B content and microbiological safety and keeping quality (Hotz and Gibson, 2007). Since watermelons are in abundance in almost all parts of Africa, methods for enhancing the nutrient content and reducing the antinutrients without adversely affecting the acceptability become very important as this fruit has potential to improve nutrition, boost food security, foster rural development and support sustainable land care. This study lays emphasis on the rinds.

The results of the proximate analysis (Table 1) revealed that the protein content of the *Saccharomyces cerevisae* fermented watermelon rinds was significantly higher ($p<0.05$) than the unfermented watermelon rinds. This high protein content could be attributed to the ability of the *Saccharomyces cerevisae* to secrete some extracellular enzymes (protein) into the watermelon rinds during their metabolic activities on the rinds during fermentation by the fungi (Oboh and Akindahunsi, 2003). The multiplication of the fungi in the watermelon rinds in the form of single cell proteins could also provide a reason for the increase in the protein content of fermented watermelon rinds (Akindahunsi *et al*., 1999). The observed increase in lipid content of the fermented watermelon rinds could be as a result of possible transformation of carbohydrate to fat (Lehninger, 1987). Certain fungi have been reported to produce microbial oil during the course of fermentation (Akindumila and Glatz, 1998). There was a significant change in the ash and crude fibre contents of the fermented watermelon rinds. The increased ash content is an indication of increased mineral contents.

Table 1: Result of proximate analysis of fermented and unfermented watermelon rinds

Parameters (%)	UFWM	FWM
Moisture	91.22±0.65	87.06±0.29
Ash	0.92±0.04	1.61±0.07
Lipid	0.69±0.06	0.81±0.04
Protein	1.52±0.05	2.80±0.05
Crude fibre	0.97±0.04	1.78±0.06
Carbohydrate	4.68±0.10	5.94±0.10

Key: UFWM = Unfermented Watermelon Rinds; FWM = Fermented Watermelon Rinds. Data = mean±SD, n = 4

Results of the antinutrients analysis are shown in Table 2. Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients. A significant reduction ($p>0.05$) in the phytic acid content was observed in the *Saccharomyces cerevisae* fermented watermelon rinds compared to the unfermented rinds. Phytic acid has been shown to form insoluble complexes with calcium, zinc, iron and copper (Cheryan, 1980), interfering with their absorption. Fermentation can induce phytic acid hydrolysis via the action of microbial enzymes, which hydrolyze phytic acid to lower inositol phosphates. It can be said that *Saccharomyces cerevisae* brought about the hydrolysis.

Such hydrolysis is important because myoinositol phosphates with <5 phosphate groups (i.e., IP-1 to IP-4) do not have a negative effect on zinc absorption (Lonnerdal *et al*., 1989) and those with <3 phosphate groups do not inhibit non-heme iron absorption (Sandberg *et al*., 1999; Hurrell, 2004). Low molecular weight organic acids (e.g., citric, malic, lactic acid) produced during fermentation generates a low pH that optimizes the activity of endogenous phytase (Teucher *et al*., 2004). The tannin content of *Saccharomyces cerevisae* fermented watermelon rinds was significantly lower ($p>0.05$) than the unfermented watermelon rinds. Tannins have been reported to affect nutritive value of food products by forming complex with protein thereby inhibiting digestion and absorption (Oboh and Akindahunsi, 2003). It also interferes with metal ion availability. Recent studies suggest that free or protein-complex condensed and hydrolysable tannins are more effective than small phenolics in antioxidant activities (Hagerman, 2002). Oxalate has a negative effect on mineral availability. Reduced oxalate content was observed in *Saccharomyces cerevisae* fermented watermelon rinds at a significant difference ($p<0.05$) compared to the unfermented rinds. Diets high in oxalate increases the risk of renal calcium absorption and has been implicated as a source of kidney stones (Chai and Liebman, 2004).

Table 2: Results of antinutrient analysis of fermented and unfermented watermelon rinds

Antinutrients	FWM (mg/100 g)	UFWM (mg/100 g)
Tannin	12.15±0.09	13.23±0.42
Phytic acid	394.88±4.43	620.50±6.57
Oxalate	90.00±3.35	158.00±1.65

FWM = Fermented Watermelon Rinds; UFWM = Unfermented Watermelon Rinds. Data = mean ± SD, n = 4

Table 3: Results of phytonutrient screening of fermented and unfermented watermelon rinds

Phytonutrients	FWM	UFWM
Saponin	+	+
Phlobatanin	-	-
Terpenoid	-	-
Flavonoid	+	+
Cardiac glycoside	+	+
Phenol	+	+
Alkaloids	+	+

Key: + = Present; - = Absent. FWM = Fermented Watermelon Rinds; UFWM= Unfermented Watermelon Rinds

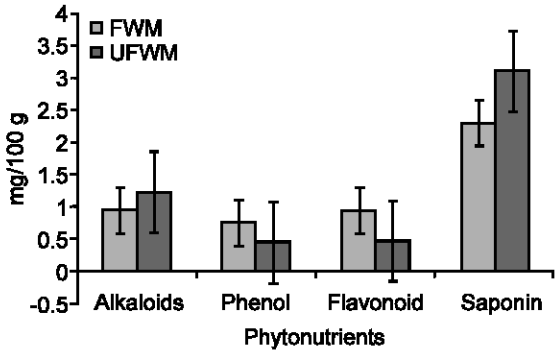

Fig. 1: Qualitative analysis of phytonutrients of fermented and unfermented watermelon rinds. Data = means ± SD. FWM = Fermented Watermelon Rinds; UFWM = Unfermented Watermelon Rinds

Phytonutrient analysis (Table 3 and Fig. 1) revealed the presence of saponin, flavonoid, cardiac-glycoside, alkaloids, phenols and saponins. Phytonutrients are natural bioactive compounds from plants with general benefits to human health. Saponin, flavonoids, phenol and alkaloids contents were quantified (Fig. 1). There was a significant decrease in the alkaloid and saponin contents of *Saccharomyces cerevisae* fermented watermelon rinds. Alkaloids, comprises of a large group of nitrogenous compounds and widely used as cancer chemotherapeutic agents. Alkaloids have also been reported to interfere with cell division (Valero and Salmeron, 2003). Saponins have been reported to possess haemolytic activity and cholesterol binding properties (Nijveldt *et al*., 2001). They serve as natural antibiotics, helping the body to fight infections and microbial invasions (Nijveldt *et al*., 2001). There was a significant ($p<0.05$) increase in the total phenolic and flavonoid contents of *Saccharomyces cerevisae* fermented watermelon rinds compared to that of the unfermented rinds. The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. The flavones and catechins seem to be the most powerful flavonoids for protecting the body against ROS (Sodipo *et al*., 2000). Studies have revealed that consumption of flavonoids can be used in the management of coronary heart disease (Knekt *et al*., 1996). The relationship between total phenol content and antioxidant activity has been widely studied in different foodstuffs (Jayaprakasha *et al*., 2008). Antioxidant activity of foodstuff significantly increases with the presence of high concentration of total phenol and flavonoid contents. Therefore the increased phenolic and flavonoid contents of the fermented watermelon rinds indicate high potential antioxidant activities.

Conclusion: Results from this study indicate that *Saccharomyces cerevisae*, a cheap, non-pathogenic and saprophytic fungus, would efficiently increase the nutritional qualities of watermelon rinds and reduce the antinutrition levels.

REFERENCES

AOAC, 1997. Official methods of analysis of the Association of Analytical Chemists, Washington DC.

Akindahunsi, A.A., G. Oboh and A.A. Oshodi, 1999. Effect of fermenting cassava with Rhizopus oryzae on the chemical composition of its flour and gari. Riv. Ital. Sostanze Grasse, 76: 437-440.

Akindumila, F. and B.A. Glatz, 1998. Growth and oil production of Apiotrichum curvatum in tomato juice. J. Food Protect., 61: 1515-1517.

Boham, B.A. and A.C. Kocipai, 1994. Flavonoids and condensed tannins from the leaves of Hawaiian *Vaccinum vaticulatum* and *V. calycinum*. Pacific Sci., 48: 458-463.

Campbell-Platt, G., 1987. Fermented foods of the world-a dictionary and guide, Butterworths, London.

Chai, W. and M. Liebman, 2004. Assessment of oxalate absorption from almonds and black beans with and without the use of an extrinsic label. J. Urol., 172: 953-957.

Cheryan, M., 1980. Phytic acid interactions in food systems. Crit. Rev. Food Sci. Nutr., 13: 297-335.

Collins, J.K., A.R. Davis, P.M. Perkins-Veazie and E. Adams, 2005. Sensory evaluation of low sugar watermelon by consumers, Hort Sci., 40: 883.

Ebrahimzadeh, M.A., S.J. Hosseinimehr, A. Hamidinia and M. Jafari, 2007. Antioxidant and free radical scavenging activity of Feijoa sallowiana fruits peel and leaves. Pharmacologyonline, 1: 7-14.

Falade, O.S., A.F. Dare, M.O. Bello, B.O. Osuntogun and S.R.A. Adewusi, 2004. Varietal changes in proximate composition and the effect of processing on the ascorbic acid content of some Nigerian vegetables. J. Food Technol., 2: 103-108.

Hagerman, A.E., 2002. Tannin Chemistry. Ann E. Hagerman: Miami.

Harborne, J.B., 1993. Phytochemical methods: A guide to modern technique in plant analysis. Chapman and Hall: New York.

Harland, B.F. and D. Oberleas, 1986. Anion exchange method for determination of phytates in food: collaborative study. J. Assoc. Off. Anal. Chem., 69: 667-670.

Hurrell, R.F., 2004. Phytic acid degradation as a means of improving iron absorption. Int. J. Vitam. Nutr. Res., 74: 445-452.

Hotz, C. and R.S. Gibson, 2007. Traditional Food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. J. Nutr., 137: 1097-1100.

Jayaprakasha, G.K., B. Girennavar and B.S. Patil, 2008. Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different *in vitro* model systems. Bioresour. Technol., 99: 4484-4494.

Kerje, T. and M. Grum, 2003. The origin of watermelon (*Cucumis melo)*: A review of the literature. www.vitacost.com.

Knekt, P., R. Jarvinen, A. Reunanen and J. Maatela, 1996. Flavonoid intake and coronary mortality in Finland: A cohort study. Biomed. J., 312: 478-481.

Koocheki, A., S.M.A. Razavi, E. Milani, T.M. Moghadam, M. Abedini, S. Alamatiyan and S. Izadkhah, 2007. Physical properties of watermelon seed as a function of moisture content and variety. Int. Agrophysics, 21: 349-359.

Lehninger, A.L., 1987. Bioenergetics and metabolism, principle of biochemistry, 2nd Preprint, CBS.

Lonnerdal, B., A.S. Sandberg, B. Sandstrom and C. Kunz, 1989. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. J. Nutr., 119: 211-214.

Mandel, H., N. Levy, S. Izkovitch and S.H. Korman, 2005. Elevated plasma citrulline and arginine due to consumption of *Citrullus vulgaris* (watermelon). Berichte der deutschen chemischen Gesellschaft, 28: 467-472.

Nabavi, S.M., M.A. Ebrahimzadeh, S.F. Nabavi, A. Hamidinia and A.R. Bekhradnia, 2008. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica Mey*. Pharmacologyonline, 2: 560-567.

Nijveldt, R.J., E. Van Nood, D.E.C. Van Hoorn, P.G. Boelens, K. Van Norren and P.A.M. Van Leeuwen, 2001. Flavonoids: A review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr., 74: 418-425.

Oboh, G. and A.A. Akindahunsi, 2003. Biochemical changes in cassava products (flour and gari) subjected to *Saccharomyces cerevisae* solid media fermentation. Food Chem., 82: 599-602.

Perkins-Veazie, P. and J.K. Collins, 2004. Flesh quality and lycopene stability of fresh-cut watermelon, Postharvest Biol. Technol., 31: 159-166.

Price, M.L., S.V. Scoyoc and L.G. Butler, 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem., 26: 1214-1218.

Sahlin, P., 1999. Fermentation as a Method of Food Processing. Lund University of Technology, Lund.

Sandberg, A.S., M. Brune, N.G. Carlsson, L. Hallberg, E. Skoglund and L. Rossander-Hulthen, 1999. Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. Am. J. Clin. Nutr., 70: 240-246.

Sodipo, O.A. Akinniyi and J.V. Ogunbameru, 2000. Studies on certain characteristics of extracts from the bark of *Pansinystalia macroceras* (K. Schem) pierve Exbeille. Global J. Pure Appl. Sci., 6: 83-87.

Southern U.S. Cuisine, 2010. Judy's Pickled Watermelon Rind. www.southernfood.about.com; Retrieved 2010-03-07.

Teucher, B., M. Olivares and H. Cori, 2004. Enhancers of iron absorption: Ascorbic acid and other organic acids. Int. J. Vitam. Nutr. Res., 74: 403-419.

Valero, M. and M.C. Salmeron, 2003. Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. Int. J. Food Microbiol., 85: 73-81.

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Production of Cocoyam, Cassava and Wheat Flour Composite Rock Cake

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Abstract: The proximate and sensory analysis of the cassava-cocoyam supplemented wheat flour rock cake has been made. This was done to investigate the nutritional value and the general acceptability of the cassava flour and cocoyam flour supplemented rock cake. The proximate analysis indicate that the moisture content, ash and the carbohydrate increase with increasing cassava and cocoyam flour concentration. Generally the ash content of composite rock cakes increases as the level of supplementation increases implying that the inorganic nutrients in the composite rock cake is richer than that of wheat rock cake. It is observed from the organoleptic analysis that generally, whole wheat rock cake and cassava and cocoyam supplemented rock cake with cassava and cocoyam flour up to 30% is preferred to rock cake with cassava and cocoyam flour beyond 30%. Thus cassava and cocoyam flour can be used to substitute for wheat flour up to about 30%.

Key words: Cocoyam flour, cassava flour, proximate analysis, sensory evaluation

INTRODUCTION

Over the years, the demand for pastry products in Ghana has been on the increase. Pastries such as rock cakes, cakes and turnovers are sold at every corner of the streets of the urban centres of Ghana as snacks. In as much as the demand for pastry products increase, the cost of the products also becomes very expensive (Dotsey, 2009). This high cost is due to the fact that, urbanization in Ghana has increased the consumption of processed food and bakery products as well as increased the demand for imported products. To reduce imports and to save foreign exchange, it has been proposed that wheat be substituted with alternative local products such as cassava, cocoyam, rice, sweet potato and maize flours in the production of cakes.

Two types of crops are known by the name *cocoyam*, and are both herbaceous plants. The most common one found on the Ghanaian market is formally known as new cocoyam (*Xanthosoma sagittifolium*) and its leaves are used as vegetables (kontomire). The second type is known as old cocoyam (*Colocasia esculenta*) or taro; it grows in marshy areas and unlike the new cocoyam, its leaves are not eaten (Dotsey, 2009).

Cocoyam (*Xanthosoma sagittifolium*) contribute significant portion of the carbohydrate content of the diet in many regions in developing countries and provide edible starchy storage corms or cormels. Although they are less important than other tropical roots such as yam, cassava and sweet potato, they are still a major staple in some parts of the tropics and sub-tropics (Opara, 2002; Ojinaka *et al*., 2009).

The high content of calcium oxalate crystals 780 mg per 100 g in some species of cocoyam, has been implicated in the acridity or irritation caused by cocoyam. Oxalates tend to precipitate calcium and make it unavailable for use by the body. The acridity of high oxalate cultivars of cocoyam can be reduced by peeling, grating, soaking and fermenting during processing (Food-info.net, 2010). Cocoyam is used essentially the same way as yam, although it is not considered as prestigious as yam. Its flour has the added advantage that, it is highly digestible and so is used for invalids and as an ingredient in baby foods.

Cassava (*Manihot esculenta*) is one of the most important crops in Ghana. In Africa, most cassava that is produced is used for human food though in recent times, the industrial utilization is on the ascendency. It is estimated that cassava provided about 40% of the calories consumed in Africa (UNICEF, 1991). In Ghana, the crop has several uses such as in:

Ampesi = Boiled cassava tubers normally eaten with stew
Akple = Prepared from a mixture of cassava and corn dough
Fufu = Boiled and pounded cassava eaten with soup
Yakayake = Steamed cassava dough
Kokonte = Dried unfermented cassava chips, milled into flour and made into a thick paste and accompanied with soup, just to mention a few (Dotsey, 2009)

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Recently, varied percentages of wheat flour to cassava flour have been used to produce rock cake and pastry products successfully (UNICEF, 1991). The use of locally produced cassava flour to replace wheat flour as a source of carbohydrate, would reduce the cost of production and save on foreign exchange. Cassava does not contain any gluten and so if used to replace wheat flour 100%, the quality of the product will be different. A suitable ratio for replacing wheat flour that will appeal to consumers will depend on the kind of food. However, the properties of cassava flour are similar to those of wheat flour and therefore can partially substitute for wheat flour in many wheat-based products.

According to Kent and Evers (1994) flours milled from other crops such as maize, millet, sorghum, cassava, potatoes and rice has been added to wheat flour to extend the use of the local crops to reduce the cost of wheat importation. This is practiced mostly in tropical countries where the soil and climate are not favourable for commercial large scale production of wheat. Satisfactory rock cake has been made from such composite flour through a blend of wheat flour with other cereals and root crops. In this work, composite rock cake made from wheat flour, cassava flour and cocoyam flour is studied for its nutritional qualities. The analysis of its acceptance by consumers is also made.

MATERIALS AND METHODS

Cocoyam, cassava and wheat flour were both purchased from the Kotokuraba market in Cape Coast, Central region of Ghana. They were sent to the laboratory of the Food Research Institute, Council for Scientific and Industrial Research, for processing. The cassava and cocoyam were processed into flour.

Preparation of cassava and cocoyam flour: The fresh roots of cassava was washed and peeled and the peeled roots were also washed. It was then cut into 7-8 cm thick discs. It was weighed, arranged randomly on the drying trays in single layers and placed in the drying machine (Hot Air Oven Dryer) at the temperature of 65°C for 9-10 h. After drying, the moisture content of the cassava should be less than 8%. The next day, the cassava was heated for 1 h to remove acquired moisture and milled using attrition milling machine into flour and sieved through an 80 mesh sieve.

The cocoyam was washed, peeled, washed again. It was then cut into 3-4 cm thick discs. They were weighed and arranged randomly on the drying trays in single layers and placed in the drying machine (Hot Air Oven Dryer) at the temperature of 65°C for 9 h. The next day, the cocoyam was heated for 1 h because it was a little moist. After that it was milled using a double disc attrition milling machine into flour and sieved.

Seven composite samples were prepared by mixing cocoyam, cassava and wheat flour in the proportions indicated in Table 1, using a Kenwood food mixer KN 201, England. The mixing was done to ensure a homogeneous mixture of the samples.

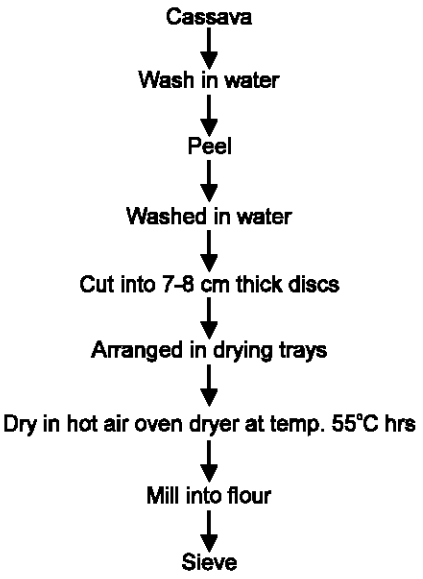

Fig. 1: Flow chart for preparation of cassava flour

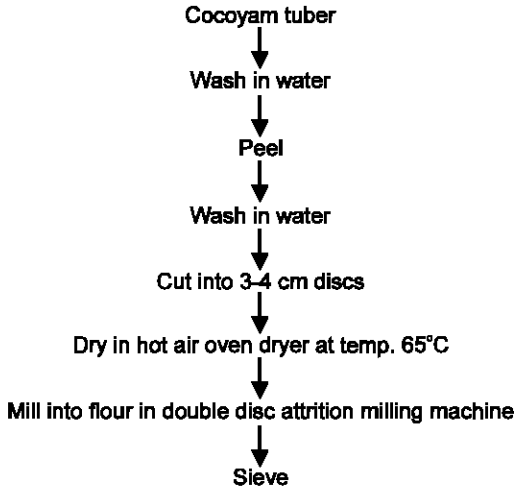

Fig. 2: Flow chart for preparation of cocoyam flour

Table 1: Sample formulation

Samples	Wheat flour (%)	Cassava flour (%)	Cocoyam flour (%)
A	100	0	0
B	90	5	5
C	80	10	10
D	70	15	15
E	60	20	20
F	50	25	25
G	40	30	30

All the seven samples were baked using the standard method (Ceserani and Kinton, 2008) (Fig. 3). All dry ingredients were mixed together in a bowl until a sandy

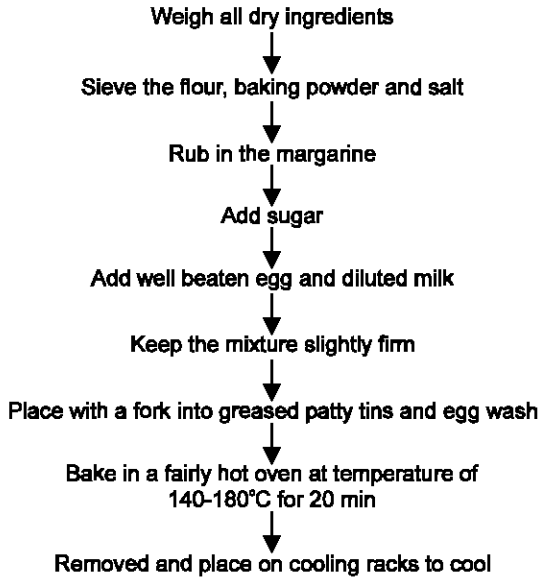

Fig. 3: Flow chart for preparation of rock cakes

texture was achieved. The diluted milk, beaten egg and flavouring were added and mixed thoroughly until a slightly firm mixture was achieved. The mixture were placed into greased patty tins with a fork to achieve a rough surface, it was egg washed and placed in a fairly hot oven at 140-180°C for 20 min. It was removed and placed on a cooling rack to cool.

The sensory attributes including colour, taste, texture, flavour, aroma, appearance and general acceptance were evaluated by untrained 30 member panel, using a 5-point Hedonic scale according to Watts *et al.* (1989) with a scale ranging from 1 to 5 with 1 representing the least score (dislike extremely) and 5 the highest score (like extremely). Analysis of Variance (ANOVA) was performed on the data gathered to determine differences, while the least significant test according to Ihekoronye and Ngoddy (1985) was used to detect differences among the means.

Proximate analysis of samples was determined according to AOAC (1990; 2000). The samples were analyzed for moisture content, ash, protein, fat, carbohydrate (by difference) and energy (Atwater Factor).

RESULTS

Table 2 shows the result of the proximate composition of the wheat-cassava-cocoyam composite rock cake samples. Table 3 gives the percentage acceptance of the sensory qualities of the rock cake samples.

DISCUSSION

From Table 1 it can be seen that the moisture content of the samples studied in this work ranged from 21.6-22.6%. Different food materials have different capacity for absorbing/retaining moisture which may exist as occluded or absorbed water. As a result, it can be deduced that some moisture will be found in the samples as observed during the study (Eddy *et al.*, 2007; Eddy, 2004; James, 1984). The slight increase in the moisture content could be due to the high moisture content of both cassava and cocoyam.

The protein content of the composite rock cake samples were 7.4, 8.0, 6.9 and 6.5% for samples A, C, D and E respectively. The protein content decreased as the amount of the cassava and cocoyam flour increased. Generally, the protein content of all samples were relatively low because, wheat, cassava and cocoyam are poor sources of protein (Oyenuga, 1992; Okaka and Isieh, 2002).

Generally, the fat content of the rock cake samples studied in this work increased in the order of supplementation.

Table 2: Proximate analysis of cassava and cocoyam flour supplemented rock cake samples

		Sample						
Parameter	Method	A	B	C	D	E	F	G
Moisture (%)	AOAC (1990)	21.6	-	20.2	22.4	22.6	-	-
Ash (%)	AOAC (2000)	1.4	-	1.0	1.9	2.0	-	-
Fat (%)	AOAC (2000)	14.2	-	20.8	22.8	23.0	-	-
Protein (%)	AOAC (1990)	7.4	-	8.0	6.9	6.5	-	-
Carbohydrate (%)	By difference	45.7	-	46.0	50.0	55.4	-	-
Energy (kcal/100 g)	Atwater factor	422.3	-	416.8	400.8	379	-	-

Table 3: Percentage score on comparative sensory evaluation of cassava and coconut flour supplemented rock cake

	Organoleptic parameter						
Sample	Colour	Taste	Texture	Appearance	Aroma	Flavour	Overall acceptance
A	93.3	100	93.3	93.3	100	100	100
B	93.3	86.7	93.4	80.0	93.3	93.3	93.3
C	86.7	80.0	86.7	86.7	93.3	96.7	93.3
D	63.3	80.0	73.4	73.3	80.0	86.7	100
E	33.3	53.3	46.7	30.0	60.0	56.7	63.0
F	26.7	26.7	50.0	26.7	33.3	40.0	53.3
G	20.0	33.4	56.3	20.0	33.3	33.3	46.7

This could be due to the presence of the cocoyam flour since cassava has been found to reduce the fat content of rock cake (Eddy *et al*., 2007).

The ash content is also found to increase as the compositions of cassava flour and cocoyam flour increased. Generally the ash content of composite rock cakes increases as the level of supplementation increases implying that the inorganic nutrients in the composite rock cake is richer than that of wheat rock cake.

The carbohydrate of the rock cake samples ranged from 45.7-55.4% with higher values obtained in composite flour rock cake compared to the 100% wheat rock cake. This observation may be attributed to the high content of carbohydrate in cassava and cocoyam. According to Enwere (1998), of all the solid nutrients in roots and tubers, carbohydrate predominates. Carbohydrate supplies quick source of metabolizable energy and assists in fat metabolism.

Table 3 shows the percentage score on comparative sensory valuation of the rock cake samples studied in this work. The panelists accept the colour of the rock cake samples A and B equally (93.3%). The acceptance of the colour is found to decrease with increasing amount of cassava flour and cocoyam flour.

The acceptance of the taste of the rock cake samples A, B, C and D were found to be 100, 86.7, 80 and 80% respectively. It indicated that even though the panelists seem to prefer the whole wheat rock cake, they found the taste of supplemented rock cake samples up to 70% wheat flour to be quite good.

The texture of the whole wheat rock cake and the 90% wheat rock cake was accepted by 93.3% of the panelists. The 80% wheat rock cake was accepted by 86.7% while the 70% wheat rock cake was accepted by 73.4%. The texture, like the taste, was found to decrease with increasing amount of cassava and cocoyam flour.

93.7% of the panelists preferred the appearance of the whole wheat rock cake to the other rock cake samples. 80, 86.7 and 73.3% preferred the appearance of samples B, C and D respectively.

The aroma of the 100% wheat flour was preferred by 100% of the panelists and 93.3% of the panelists accepted the 90% and 80% wheat flour samples. 80% of the panelists preferred the 70% wheat flour sample. The acceptance of the flavour follows a similar pattern.

It is observed from Table 3 that the 100, 93.3, 93.3 and 100% of the panelists accept the rock cake samples A, B, C and D respectively. The mean of the overall acceptance ranged from 3.43±0.680 to 4.712±0.221, with the highest mean for sample A and the lowest for sample G. The differences in the means of samples A, B, C and D are significantly indifferent ($p<0.05$). Thus generally, the addition of cassava and cocoyam up to 30% level is acceptable to consumers just as the whole wheat rock cake.

Conclusion: The proximate analysis of the cassava and cocoyam supplemented wheat rock cake shows that the protein content of the rock cake decreased slightly with increasing amount of cassava and cocoyam flour. The organoleptic analysis also indicates that generally, whole rock cake and cassava-cocoyam-supplemented rock cake with cassava-cocoyam up to 30% is preferred to rock cake with cassava-cocoyam flour beyond 30%. Thus substitution of wheat flour with cassava-cocoyam flour up to 30% could help produce rock cake which could be accepted and also affordable to many Ghanaians.

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REFERENCES

AOAC, 1990. Methods of Association of Official Chemists. Official Methods of Analysis, 15th Edn., Virginia Assoc. Official Analytical Chemist, USA., pp: 1141.

AOAC, 2000. 920.03 Official Methods of the Association of Official Chemists. Official Analytical Int, Arlington VA.

Ceserani, V. and R. Kinton, 2008. Practical Cookery. 10th Edn., John Wiley and Sons, New York.

Dotsey, P., 2009. The use of cocoyam, cassava and wheat flour composite in the production of rock cakes, HND Dissertation, Cape Coast Polytechnic, Cape Coast, Ghana, 1: 7-40.

Eddy, N.O., 2004. Effect of processing on the chemical composition of some Nigerian food crops, M.Sc. Theses, University of Uyo, Uyo, Nigeria.

Eddy, N.O., P.G. Udofia and D. Eyo, 2007. Sensory evaluation of wheat/cassava composite rock cake and effect of label information on acceptance and preference. Afr. J. Biotechnol., 6: 2415-2418.

Enwere, N.J., 1998. Foods of plant origin. Afro-Orbis publication ltd., pp: 194-1999.

Food info.net., 2010. Tropical Roots and tubers: Cocoyam. Wageningen University, The Netherlands.

Ihekoronye, A.I. and P.O. Ngoddy, 1985. Integrated food science and technol, for the tropics. 1st Edn., McMillan Publishers, pp: 261, 265, 291.

James, E.G., 1984. Analytical chemistry of foods, Blackie Academic and Professional, London.

Kent, N.L. and A.D. Evers, 1994. Kent's Technology of Cereals, 4th Edn., Pergamon press, New York.

Ojinaka, M.C., E.N.T. Akobundu and M.O. Iwe, 2009. Cocoyam starch modification effects on functional, sensory and cookies qualities. Pak. J. Nutr., 8: 558-567.

Okaka, J.C. and M.I. Isieh, 2002. Development and quantity evaluation of cowpea wheat biscuits. Nig. Food J., 8: 56-62.

Opara, L.U., 2002. Edible Aroids: Postharvest Operation in: AGST/FAO: Danilo, M. (Ed.). Massey University, New Zealand.

Oyenuga, V.A., 1992. Nigerian Food and Feeding Stuffs, Ibadan, Ibadan University Press.

UNICEF, 19991. Cassava in Tropical Africa: A reference Manual; International Institute of Tropical Agriculture, Ibadan.

Watts, B.M., G.L. Ylimaki, L.E. Jeffrey and L.G. Ekics,1989. Basic sensory methods for food evaluation. International Development Research centre, Ottawa, Ontario, Canada, pp: 68-79.

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Utilization of Soybean Flour in the Production of Bread

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Abstract: The proximate and sensory analysis of the soy-supplemented wheat flour bread has been made. This was done to investigate the nutritional value and the general acceptability of the soy-supplemented bread. The proximate analysis indicate that the moisture content, ash and the protein increase with increasing soya bean flour concentration. The increase in protein concentration indicates that supplementation of wheat flour with soyabean flour would greatly improve the protein nutritional quantity of bread. It is observed from the organoleptic analysis that generally, whole wheat bread and soy-supplemented bread with soybean flour below 30% is preferred to bread with soybean flour beyond 30%.

Key words: Soybean flour, proximate analysis, sensory evaluation

INTRODUCTION

Bread is a major flour product which is normally consumed by majority of Ghanaians at breakfast, lunch and sometimes dinner (Tsatsu, 2009). It is an important stable food, the consumption of which is steady and increasing in Nigeria. It is however, relatively expensive, being made from imported wheat that is not cultivated in the tropics for climatic reasons (Edema *et al*., 2005; Olaoye *et al*., 2006). A lot of efforts has been made and still being made to promote the use of composite flours in which flour from locally grown crops and high protein seeds replace a portion of wheat flour for use in bread production, thereby decreasing the demand for imported wheat and producing protein-enriched bread (Giami *et al*., 2004; Olaoye *et al*., 2006).

Soybean (Glycine max) belongs to the family leguminosae and sub-family papillionnideae. It is a remarkable source of protein for both animals and human consumption and is also a leading source of edible oils and fats (Singh *et al*., 1999; Alabi *et al*., 2001). As an important component crop, soybean the legume richest in nutrients and the one from which the most dietary products are made is used in various traditional farming systems of various countries (Pamplona, 2005). It contains valuable phylochemicals and has extraordinary capacity to nourish and prevent diseases. Soya has the advantages of containing virtually no sodium, a mineral that cause fluid retention in the tissues; this makes it very suitable in cases of cardiovascular disease.

Soya is also known to be a good source of the trace elements copper, zinc and manganese and can be said to contain all the nutrients needed in food (Ampofo, 2009). It has been proved that daily consumption of soybean between 30 g and 50 g substitute for an equal amount of animal-base protein produces the following results:

- 9.3% reduction in total cholesterol
- 12.9% reduction in LDL cholesterol (harmful)
- 2.4% increase in HDL cholesterol (beneficial)
- 10.5% reduction in triglycerides (Pamplona, 2005)

Apart from the higher nutritional content of soybeans, it is also very cheap compared to wheat flour. According to a survey done by Ampofo (2009) at different markets in Ghana, a cup of soybeans cost GH¢0.50 whiles a cup of wheat flour cost GH¢0.80. This and the high nutrient content of the soybean have necessitated the need to formulate a soybean composite for the production of bread to make bread cheaper for the ordinary Ghanaian. In Ghana today, soybean is used in the dried and fresh forms and is available as soy protein in concentrated, isolated or texturized form, soy milk and cooked soybeans (Ampofo, 2009). Soybean is the only source that contains all the amino acids. Its use in the production of bread as composite flour has been reported (Kure *et al*., 1998; Dhingra and Jood, 2002; Basman *et al*., 2003; Olaoye *et al*., 2006).

MATERIALS AND METHODS

Soybeans and wheat flour were both purchased from the Kotokuraba market in Cape Coast, Central region of Ghana. They were sent to the laboratory of the Food Research Institute, Council for Scientific and Industrial Research, for processing. The soybeans were processed into flour, using the method of IITA (1990) (Fig. 1). The process ensures effective removal of most anti-nutritional factors.

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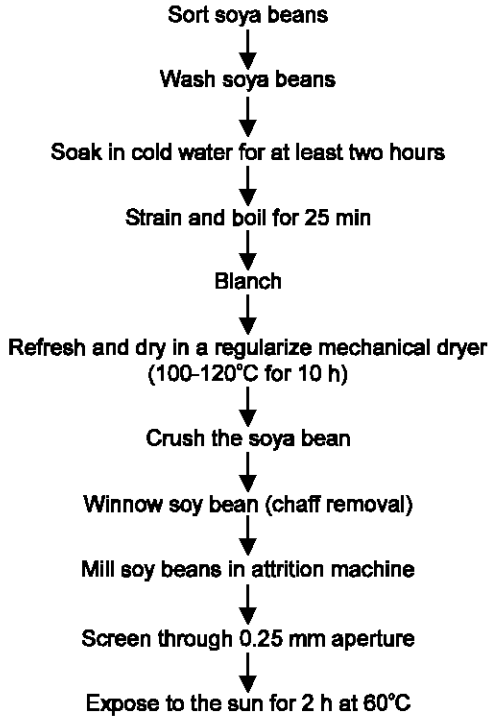

Fig. 1: Flow chart for the production of soy flour

Table 1: Composition of the samples used in this work

Sample	Soya flour (%)	Wheat flour (%)
A	0	100
B	10	90
C	20	80
D	30	70
E	40	60
F	50	50

Six samples were prepared by mixing soybean flour with wheat flour in the proportions indicated in Table 1, using a Kenwood food mixer KN 201, England. The mixing was done to ensure a homogeneous mixture of the samples.

The six sample formulations were baked using the straight dough method (Kinton and Ceserani, 2008) (Fig. 2). All ingredients were mixed in a Kenwood food mixer (Model A 907 D) for approximately 5 min. The dough were left in bowls to prove covered with damp clean muslin cloth for approximately 55 min at room temperature (29°C), the dough was then knocked back and moulded into a loaf, placed in a loaf tin and further proved in a proving cabinet for 90 min at 30°C, 85% relative humidity and baked at 250°C for 30 min (Giami *et al*., 2004). It was removed and placed on a cooling rack and packaged in zip lock polythene with label and stored in a refrigerator under temperature 32°C until needed.

The sensory attributes including crust, colour, taste, texture and general acceptance were evaluated by untrained 40 member panel, using a 5-point Hedonic

Fig. 2: Flow chart for the production of bread

scale according to Watts *et al*. (1989) with a scale ranging of 1 to 5 with 1 representing the least score (dislike extremely) and 5 the highest score (like extremely). Analysis of Variance (ANOVA) was performed on the data gathered to determine differences, while the least significant test according to Ihekoronye and Ngoddy (1985) was used to detect differences among the means.

Proximate analysis of samples was determined according to AOAC (1990, 2000). The samples were analyzed for moisture, ash, protein, fat, carbohydrate (by difference) energy (Atwater Factor).

RESULTS AND DISCUSSION

The results of the proximate analysis are presented in Table 2. The moisture content is found to increase as the soya flour content was increased. Soy flour, derived from ground soybeans, is found to bring moisture to baked goods (Soy foods Association of North America, 2010).

The ash content increased with the increase in proportion of soybean flour in the bread. This result agrees with results of other workers (Olaoye *et al*., 2006). The increase in ash content could be due to the higher ash content of the soya bean than in the wheat flour. The soybean seeds have been reported to contain an appreciable quantity of minerals and fat (Ariahu *et al*., 1999; Onyeka and Dibia, 2002; Plahar *et al*., 2003).

The protein content of the bread was observed to increase with increasing soybean flour concentration. This indicates that supplementation of wheat flour with soybean flour would greatly improve the protein nutritional quantity of bread. The increase in the protein

Table 2: Proximate analysis of the soyabean wheat flour composite bread

		Sample					
Parameter	Method	A	B	C	D	E	F
Moisture (%)	AOAC 925.10 (1990) 15th Edition	21.9	-	36.9	-	-	35.0
Ash (%)	AOAC 923.03 (2000) 17th Edition	1.1	-	1.7	-	-	1.7
Fat (%)	AOAC 920.03 (2000) 17th Edition	1.1	-	1.5	-	-	2.0
Protein (%)	AOAC 984.13 (1990) 15th Edition	7.5	-	13.2	-	-	13.2
Carbohydrate (%)	By difference	59.0	-	37.7	-	-	36.0

content of the bread could be due to the significant quantity of protein in the soybean seeds (Asiedu, 1989; Kure *et al*., 1998; Basman *et al*., 2003). The high protein content in the soybean supplemented breads studied in this work would be of nutritional importance in many developing countries like Ghana where many people are unable to afford foods with high protein because such foods are quite expensive.

The carbohydrate contents decreased with increase in the proportion of the soy flour in the soybean flour supplemented bread. This trend supports the claim of Akpapunam and co-workers (1997).

Table 3 gives the percentage score of the comparative sensory evaluation of the soya bean bread. The crust as applied here is the outside layer of the bread. It should be smooth and golden brown. 100 percent of the panellists prefer the crust of samples B and D while 95 percent prefer sample A. Thus, the panellists seem to slightly prefer the crust of the soya-supplemented bread to the whole wheat bread. However, the crusts of the samples with beyond 30% soya bean flour do not appeal to the panellists. It can be seen from Table 4 that there is insignificant difference between samples A, B and D at 5% level.

The colour of bread talks about the appearance of the bread, how it looks like, if it is appealing to the eyes, inviting and bright. 100 percent of the panellists prefer the colour of the whole wheat bread to that of the soy-supplemented bread. However, the mean of the whole wheat bread and the soy-supplemented bread up to 30% soybean flour is insignificantly different at 5% significant level. Like the crust, the colour of the bread with soyabean flour beyond 30% does not seem to appeal to the panellists.

The taste of the bread refers to the sweet sensation caused in the mouth by contact with the bread due to the sweetening agent. 100 percent of the panellists prefer the taste of samples A and B while 95% and 85% prefer the tastes of sample C and D, respectively. There seems, however, to be no significant difference between the tastes of the whole wheat bread and the soy-supplemented bread up to 30% of soybean flour.

Texture is the quality of the bread that can be decided by touch, the degree to which it is rough or smooth, hard or soft. The panellists prefer sample A and B equally, as confirmed by the organoleptic evaluation. However, the

Table 3: Percentage score on comparative sensory evaluation of soya bean bread

Sample	Crust	Colour	Taste	Texture	Overall acceptance
A	95	100	100	70	80
B	100	95	100	70	100
C	85	85	95	61	53
D	100	90	85	50	50
E	70	62	43	55	35
F	43	38	40	55	12

Table 4: Organoleptic evaluation of soya bean bread

Scale	Crust	Colour	Taste	Texture	Overall acceptance
A	2.2000	2.3250	1.8750	1.7500	2.0000
B	2.3750	2.2500	1.5000	1.7500	2.5000
C	1.1250	1.9250	0.9750	1.5250	1.3250
D	2.3000	2.1625	0.7500	1.2500	0.8750
E	1.2000	1.8500	0.7350	1.3750	0.3000
F	0.6200	1.8500	0.07350	1.3750	0.3000

mean indicates that there is insignificant difference between the colours of the samples at 5% significant level.

The overall acceptance expresses how the consumers or panellists accept the product generally. It is observed that 80% of the panellists accept the whole wheat flour while 100 of the panellists accept the bread with 10% soybean flour. The analysis of variance indicates that there is a significant difference between the samples with soybean flour below 10% and those beyond 10% at 5% significance level.

It is observed from the organoleptic analysis that generally, whole wheat bread and soy-supplemented bread with soybean flour below 30% is preferred to bread with soybean flour beyond 30%. The preference of the panellists for the sensory attributes of the wheat flour bread may be due to the familiarization of consumers to the normal whole wheat flour.

Conclusion: The proximate analysis of the soy-supplemented wheat bread shows that the nutritional content of the bread increased with the soybean content. The organoleptic analysis also indicate that generally, whole bread and soy-supplemented bread with soybean flour below 30% is preferred to bread with soybean flour beyond 30%. The preference of the wheat flour bread may be due to the familiarization of consumers to the

normal whole wheat flour. Public enlightenment on the nutritional importance of the soy-supplemented foods would help enhance the acceptability of the soy-supplemented bread.

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REFERENCES

Akpapunam, M.A., G.IO. Badifu and E.P. Etokudo, 1997. Production and quality characteristics of Nigerian Agidi supplemented with soy flour. J. Food Sci. Technol., 34: 143-145.

Alabi, M.O., J.C. Anuonye, C.F. Ndaeji and A.A. Idowu, 2001. Comparison of the growth and development of selected children in soybean and non soybean producing and utilization of villages in Niger State, Nigeria. Poly Math J., 2: 8-12.

Ampofo, V., 2009. Production and sensory analysis of soybean and wheat flour composite cake, HND Dissertation, Cape Coast Polytechnic, Cape Coast, Ghana, 1: 5-7.

AOAC, 1990. Methods of Association of Official Chemists. Official Methods of Analysis, 15th Edn., Virginia Assoc. Official Analytical Chemist, USA., pp: 1141.

AOAC, 1990. AOAC Method 925.10 Official Methods of the Association of Official Analytical Chemists, 15th Edn., 777, Arlington, VA, USA.

AOAC, 2000. 920.03 Official Methods of the Association of Official Chemists. Official Analytical Int, Arlington VA.

Ariahu, C.C., U. Ukpabi and K.O. Mbajunwa, 1999. Production of African breadfruit (Trecuilia Africana) and soybean (Glycine Max) seed based food formulations: Effects of germination and fermentation on nutritional and organoleptic quality. Foods Human Nutr., 54: 193-206.

Asiedu, J.J., 1989. Processing Tropical crops. McMillan Education Ltd. London, 1st Edn., 21.

Basman, A., H. Koksel and P.K.W. Ng, 2003. Utilization of Transgluranase use to increase the level of barley and soy flour incorporation in wheat flour breads. J. Food Sci., 68: 2453-2453-2460.

Dhingra, S. and S. Jood, 2002. Physico-chemical and nutritional properties of cereal- pulse blends for bread making. Nutr. Health, 16: 183-94.

Edema, M.O., I.O. Sanni and A.I. Sanni, 2005. Evaluation of maize-soybean flour blends for sour maize bread production in Nigeria. Fri. J. Biotechnol., 4: 911-918.

Giami, S.Y., T. Amasisi and G. Ekiyor, 2004. Comparism of bread making properties of composite flour from kernels of roasted and boiled African breadfruit (*Treculia Africana decene*) seeds. J. Mat. Res., 1: 16-25.

Ihekoronye, A.I. and P.O. Ngoddy, 1985. Integrated Food Science and technol, for the tropics. 1st Edn., McMillan publishers. pp: 261, 265, 291.

IITA, 1990. Soybeans for good health: How to grow and use soybeans in Nigeria. IITA publication, ISBN 97813101698. pp: 23.

Kinton, R. and V. Ceserani, 2008. Practical Cookery. 10th Ed. John Wiley and Sons, New York.

Kure, O.A., E.J. Bahago and E.A. Daniel, 1998. Studies on the proximate composition and effect of flour particle size of acceptability of biscuits produced from blends and plantain flours. Namoda Tech. Scope J., 3: 17-22.

Olaoye, O.A., A.A. Onilude and O.A. Idowu, 2006. Quality characteristics of bread produced from composite flours of wheat, plantain and soybeans. Afri. J. Biotechnol., 11: 1102-1106.

Onyeka, U. and I. Dibia, 2002. Malted weaning food made from maize, soybean, groundnut and cooking banana. J. Sci. Food Agric., 82: 513-516.

Pamplona, R., 2005. Encyclopedia of food and their healing power.

Plahar, W.A., B.O. Okezie and C.K. Gyato, 2003. Development of a high protein weaning food by extrusion cooking using peanuts, maize and soybeans. Plant Foods Human Nutr., 58: 1-12.

Singh, S.R., K.O. Rachie and Pashiell, 1999. Soyabeans for the tropics. New York.

Soy foods Association of North America, 2010. WWW.soyfoods.org.

Tsatsu Tsikata, C., 2009. Utilization of soybeans in the production of bread, HND Dissertation, Cape Coast Polytechnic, Cape Coast, Ghana, 1: 4-28.

Watts, B.M., G.L. Ylimaki, L.E. Jeffrey and L.G. Ekics, 1989. Basic sensory methods for food evaluation. International Development Research Cenre, Ottawa, Ontario, Canada, pp: 68-79.

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Urinary Tract Infection as a Predictor of Childhood Malnutrition in Southern Sindh, Pakistan

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Abstract: A cross-sectional study to examine the association of urinary tract infection (UTI) with protein-energy malnutrition and microcytic hypochromic anemia (Iron deficiency anemia) was conducted among children in southern sindh Pakistan. A total of 150 children aged up to 5 years were studied. The data were collected using structured questionnaires, anthropometric measurements and laboratory analysis of blood and Urine samples. The results showed that 31.6% of the children were infected with *Escherichia coli*, while 56.5, 41.3 and 15.1% had significant underweight, stunting and wasting, respectively. Urinary tract infection with *Escherichia coli* was statistically identified as a strong predictor of significant wasting in this study population.

Key words: Urinary tract infection, malnutrition, iron deficiency anaemia

INTRODUCTION

Escherichia coli (E. coli) have been recognized as the most common UTI pathogen worldwide. *E. coli* organisms form part of the normal microbial flora of intestinal tract of humans and animal. They can also be found in water, soil and vegetation. In tropical and temperate countries (Jakobsson *et al*., 1999; Coulthard *et al*., 1997), children are more frequently infected than adults, particularly those who are malnourished (Watson, 2003). In the developed countries, *Escherichia coli* has been implicated as a cause of UTI among children (Royal College of Physicians, 1991; Smellie *et al*., 1995) and In developing countries, however, *Escherichia coli* is endemic and *Escherichia coli infection* as a predictor of childhood malnutrition commonly seen in children aged up to 5 years (Goonasekera *et al*., 1996; Jacobsson *et al*., 1989). Most often the infections are asymptomatic. Usually, acute symptomatic individuals present with cystitis, pyelitis, and pyelonephritis (Alexander *et al*., 1990). *E. coli* is the commonest pathogen isolated from patients with cystitis. Chronic *E. coli* infection in children is usually associated with clinical manifestations of Anemia, Weight loss, decreased muscle mass and weakness, Dry scaly skin (Lewis *et al*., 1999). The association of *E. coli* with Proteinenergy Malnutrition (PEM) and recently with Iron Deficiency Anaemia (IDA) has been reported by some researchers. In addition, a positive association between *E. coli* and PEM has been described before (Farhat *et al*., 2004). *E. coli* causes malabsorption and can lead to IDA, although clinical IDA in patients with *E. coli* infection has not been reported (Farhat *et al*., 2004; Wolfish *et al*., 1993).

Children with *E. coli* had anaemia it may be to hemoturia and treatment with antibiotic did not improve the condition (Watson, 2004). The study also suggested that besides antibiotic agent, supplementary with iron should be given to prevent microcytic hypochromic anaemia. However, little is known regarding UTI and its association with malnutrition in southern sindh Pakistan. This study was carried out to examine the association between UTI, PEM, and IDA among children (South Bedfordshire Practitioners' Group, 1990; Pisacane *et al*., 2004).

MATERIALS AND METHODS

Study areas and study population: This is part of a cross-sectional study on the relationship between urinary tract infections and childhood malnutrition in southern sindh Pakistan. The child almost belongs to ruler area of southern sindh. Most of the residents of the area worked as laborers, farmers and some did odd jobs selling forest products. Houses were made of mud or bricks and cements with any cement plaster. Most of them do not had supplies water. The study population was aged upto 5 years. All children who agreed voluntarily through their parents to participate were included in this study. Of 150 children studied, only 136 children delivered urine specimens for examination, and analyses for the association between UTI and malnutrition were based on these children. The study included both males and females. Most of the children's mothers are not completely aware about the washing after pass stool by the children. Most of the children included in this study do not have proper toilet. In addition, their personal hygienic practices were poor. The data were collected using a structured questionnaire, anthropometry and laboratory analysis of blood and urine samples.

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Structured questionnaire: The data were collected over a period of 6 months, beginning in July 2009. During many visits, the parents were read an Informed Consent Form and permission was obtained from parents whose children participated in this study. Each of the children was given a code number accordingly and particulars were entered in the data sheet. The parents were interviewed directly on the personal particulars of the children, as well as socio-economic status, using a standard questionnaire. Date of birth and birth weight were obtained from birth certificates, while immunization status was obtained from each child's health record.

Anthropometry: All children underwent anthropometrics measurement as follows: children were weighed without shoes using Seca scales, which had intervals of 0.5 kg; height was measured to the nearest 0.1 cm using a calibrated scale consisting of a wooden platform with a scale and a sliding head piece. To reduce intra-individual error, weight and height were measured twice and the mean value was used for the analysis.

Blood examination: Approximately 6-7 ml venous blood was collected by disposable syringes through vein puncture technique from cubital vein. An aliquot (3 ml) was transferred in the EDTA tube immediately after collection for heamotological analysts, and remaining blood was transferred into a plain tube, taken to laboratory for biochemical analysis. The blood was collect in plain tube allowed to clot and the tubes were centrifuged at 3000 rpm for 10 min to obtain the serum. Total protein and albumin were determined colorimetrically using the microlab 300 analyzer. Anaemic children (low haemoglobin concentration) with low red blood cells indices (Hct, MCV, MCH, MCHC) were considered to have Anemic. Serum Total protein and albumin was recorded in g/l and children with serum Total protein levels less than 35 g/l and albumin level 2.2 g/l were considered to have hypoal-buminaemia.

Urine culture examination: Urine samples were collected into wide mouth screw-cap sterile 100 ml containers. Mothers of the female children were instructed to cleanse the area around the urethral opening with clean water, dry the area and collect the urine with the labia held apart. Label the container with the date, number of the child. Sample was delivered to the lab as soon as possible. Possible pathogens of UTI in Gram positive are *Enterococci*, *Staphylococcus saprophyticus*, *Haemolytic streptococci* and in Gram negative *E. coli*, *Proteus* species, *Pseudomonas*, *aeruginosa*, *Klebsiella* strain, *Salmonella typhi*, *Salmonella paratyphi*, *Neisseria gonorrhoeae*.

RESULTS

One hundred and thirty six children (61 males; 75 females) aged upto 5 years with a mean age of 3.076±2.02 years. The overall significant underweight was 62.3% with low values of Total protein and albumin. Table 1 shows that 31.6% were positive for *E. coli* infection. Overall, females had a higher prevalence of UTI than males. Prevalence of UTI was slightly higher in children in the age group 2-3 years children but it does not have significant difference, Urine culture analysis identified with *E. coli* infection as a predictor of significant underweight and significant wasting in this study population.

Table 1: Comparison of frequency and % of *E. coli* infection with all others in different age groups

Age (years)	No. of children examined	No. of children infected	No. of *E. coli* infection
Up to 1 years	22 (16.1%)	13 (9.5%)	7 (5.1%)
1-2 years	32 (23.5%)	18 (13.2%)	8 (5.9%)
2-3 years	30 (22.0%)	23 (16.9%)	11 (8.0%)
3-4 years	28 (20.5%)	19 (13.9%)	9 (6.6%)
4-5 years	24 (17.6%)	18 (13.2%)	8 (5.9%)
Total No.	n = 136	91 (66.9%)	43 (31.6%)

Table 2: Frequency and % of males and females children

Gender	No. of children examined	No. of children infected	No. of *E. coli* infection
Males	59 (43.5%)	36 (26.4%)	18 (13.2%)
Females	78 (57.5%)	55 (40.4%)	25 (18.4%)

DISCUSSION

The classical definition of significant bacteriuria (>105 organisms/ml [or >108/l]) is still applied in childhood with the proviso that any bacteriology reports should always be interpreted in the clinical context. The problems of obtaining urine specimens in children have already been alluded to (Watson, 2003; Lambert and Coulthard, 2003).

Anemia, Weight loss, decreased muscle mass and weakness, Dry scaly skin Edema (swelling, due to lack of protein), Hair that has lost its pigment, Brittle and malformed (spooned) nails, Chronic diarrhea, Slow wound healing, Bone and joint pain , Growth retardation (in children), Mental changes such as confusion and irritability, Goiter all are the symptoms of malnutrition (Dohil *et al*., 1994). General malnutrition often develops slowly, over months or years. As the body's store of nutrients is depleted, changes begin to happen at the cellular level, affecting biochemical processes and decreasing the body's ability to fight infections. Over time, a variety of symptoms may begin to emerge (Kontiokari *et al*., 2001).

The present study observed a high prevalence of UTI by the *E. coli* among the children who participated in this study. Alan (2004) reported that *Escherichia coli* are responsible for at least 80% of UTI but other organisms include *Proteus*, *Enterococcus*, *Pseudomonas* and *Klebsiella* species. *Staphylococcal aureus* and *Staphylococcal epidermidis* are urinary pathogens in small children and young women. Any organism may cause sepsis in this young age group with the kidney

Table 3: Biochemical findings of patients

Test	Mean±SD (n = 136)	Reference values
Total protein (g/dl)	3.9±0.9	5.5-8.5 g/dl
Albumin (g/dl)	1.9±0.7	3.5-5.0 g/dl
Blood glucose level (mg/dl)	62.04±23.78	80-180 mg/dl
Urea (mg/dl)	20.48±18.45	10-50 mg/dl
Creatine (mg %)	0.51±0.81	0-1.0 mg %

Table 4: Hematological finding of children

Test	Mean±SD	Reference values
Hb (g/dl)	8.6±2.8	12.0±2.0
RBCs (Cmm)	3.1±1.2	4.7±0.7
Hct (%)	26.7±11.9	41.0±4.0
MCV (fl)	67.8±24.2	84.0±7.0
MCH (dg)	20.8±6.6	29.5±20.5
MCHC (g/dl)	26.4±4.0	33.0±2.0

and urinary tract becoming involved by hematogenous spread from a generalized septicemia (Lambert and Coulthard, 2003; Gorelick and Shaw, 1999).

The prevalence of *E. coli* was slightly higher in children aged 2-3 years. This may indicate that high rate of transmission of the infection occurs in this age group; it spreads poor hygienic condition, within households, perhaps from person to person, as young children usually play in the house and have very close contact with other members of the household (Gorelick and Shaw, 1999).

With positive results of UTI we also observed the significantly low values of Total protein (3.9±0.9 g/dl) and albumin (1.9±0.7 g/dl), Hemoglobin and all the indices include Hct, MCV, MCH, MCHC then the reference normal values, which predicate the protein energy malnutrition and the presence of microcytic hypochromic anemia which is often iron deficiency anemia in the children with positive UTI.

Conclusion: Urinary tract infection with *Escherichia coli* was statistically identified as a strong predictor of childhood malnutrition in this study population.

REFERENCES

Alan Watson, R., 2004. Pediatric urinarytract infection. EAU Update Series 2, pp: 94-100.

Alexander, S.R., G.S. Arbus and K.M.H. Butt, 1990. The 1989 Report of the North American Pediatric Renal Transplant Cooperative Study. Pediatr Nephrol., 4: 542-553.

Coulthard, M., H. Lambert and M. Keir, 1997. Occurrence of renal scars in children after their first referral for urinary tract infection. BMJ., 315: 918-919.

Dohil, R., E. Roberts, K. Verrier Jones and H.R. Jenkins, 1994. Constipation and reversible urinary tract abnormalities. Arch. Dis. Child., 70: 56-57.

Farhat, W., G. McLorie and D. Geary, 2004. The natural history of neonatal vesicoureteric reflux associated with antenatal hydronephrosis. J. Urol., 164: 1057-1060. A.R. Watson/EAU Update Series, 94-100.

Goonasekera, C.D.A., V. Shah, A.M. Wade, T.M. Barratt and M.J. Dillon, 1996. 15-year follow-up of renin and blood pressure in reflux nephropathy. Lancet, 347: 640-643.

Gorelick, M.H. and K.V. Shaw, 1999. Screening tests for urinary tract infection in children: a meta-analysis. Pediatrics, 104: 54.

Jacobsson, S.H., O. Eklof, C.G. Eriksson, L.E. Lins, B. Tidgren and J. Winberg, 1989. Development of hypertension and uraemia after pyelonephritis in childhood, 27 year follow up. BMJ., 299: 703-706.

Jakobsson, B., E. Esbjorner and S. Hansson, 1999. Minimum incidence and diagnostic rate of first urinary tract infection. Pediatrics, 104: 222-226.

Kontiokari, T., K. Sundqvist, M. Nuutinen, T. Pokka, M. Koskela and M. Uhari, 2001. Randomised trial of cranberry-lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. BMJ., 322: 1571-1573.

Lambert, H. and M. Coulthard, 2003. The child with urinary tract infection. In: Clinical paediatric nephrology 3 oxford, Webb, N. and R. Postlethwaite (Eds.). Oxford University Press, pp: 197-225.

Lewis, M., A.R. Watson, G. Clark, W. Van't Hoff and J. Shaw, 1999. Report of the paediatric renal registry. In: The UK Renal Registry: Second Annual Report. London: Renal Association, pp: 175-187.

Pisacane, A., L. Graziano, G. Mazzarella, B. Scarpellino and G. Zona, 2004. Breastfeeding and urinary tract infection. J. Pediatr., 120: 87-89.

Royal College of Physicians, 1991. Report of a working group of the research unit Royal College of Physicians. Guidelines for the management of acute urinary tract infection in childhood. J. R. Coll. Physicians Lond., 25: 36-42.

Smellie, J.M., S.P.A. Rigden and N.P. Prescod, 1995. Urinary tract infection: a comparison of four methods of investigation. Arch. Dis. Child., 72: 247-250.

South Bedfordshire Practitioners' Group, 1990. Development of renal scars in children: Missed opportunities in management. BMJ., 301: 1082-1084.

Watson, A.R., 2004. Urinary tract infection in early childhood. J. Antimicrob. Chemother., 34(A): 53-60.

Watson, A.R., 2003. Urinary tract infections. In: Forfar and Arneil's Textbook of Pediatrics, McIntosh, N., P.J. Helms and R.L. Smyth (Eds.). Edinburgh: Churchill Livingstone, pp: 613-621.

Wolfish, N.M., N.E. Delbrouck, A. Shannon, M. Matzinger, R. Stenstrom and P.N. McLaine, 1993. Prevalence of hypertension in children with primary vesicoureteral reflux. J. Pediatr., 123: 559-563.

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Improving Carcass Quality of Indigenous Cattle of West Sumatera Fed Local Feed Resources

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Abstract: Lack of adequate nutrition all year round is one of the major causes of the low productivity of ruminants. An operational policy announced by the Indonesian government to achieve the target to be self sufficient on meat demand 2010 is to develop feed and ration for beef industry based on agricultural and industrial-wastes besides exploiting biodiversity that has not been fully implemented by the farmers. A feeding trial with 12 Pesisir cattle, indigenous cattle of West Sumatera weighed 120-150 kg aged 2-3 yrs old were carried out to investigate the effect of feeding local feed resources on their growth performance and carcass characteristics; dressing percentage, rib eye area, lean and fat percentage. Animals were divided into two equal groups of similar average body weight, assigned to Completely Randomized Design and were individually fed for 90 days. The first group was offered control feed as practiced by farmer and the second group was offered treatment diet based on ammoniated rice straw and chipped cassava. This feeding trial was followed by a slaughter experiment on one animal from each group to study of carcass traits. Cattle receiving a treatment diet performed better ($p<0.05$) in all parameters measured, than those receiving control diets. There was significant difference ($p>0.05$) observed between the two groups with regard to Average Total Body Weight Gain (ATG) and Average Daily Body Weight Gain (ADG) 0.20 kg/d to 0.68 kg/d respectively. Dressing percentage was significantly higher in treatment diet (52.59%) than in control diet (50.82%). The rib eye area, lean and fat percentage was significantly higher in treatment diet than in control diet. It was concluded that feeding improved low quality local feed resources could have a positive effects on growth performance and carcass traits of indigenous cattle.

Key words: Local feed resources, ammoniated straw, chipped cassava, indigenous cattle, carcass quality

INTRODUCTION

Population growth, increasing income, food and nutrition awareness and increased community outside the flow of tourists to Indonesia cause meat demand for domestic consumption continues to increase. The increased demand has yet to be met by increased productivity of meat-producing livestock. Increase livestock population, productivity and carcass quality should continue.

For this purpose livestock production methods should be directed to the improvement of meat quality criteria in accordance with international market requirements. These efforts may also result in foreign exchange savings if the result is used to substitute high-quality meat that had been imported. The problem is how to improve the quality of local beef, so that the local cattle farmers can receive the appropriate price. Fattening are the best effort in improving the productivity and quality of local beef, because the cattle can be fed to suit your needs, high energy and good quality feeds. Thus if the feed of cattle feed is improved by providing a high-protein and energy of the result will be better.

It is necessary to find alternative feed sources such as the utilization of agricultural and industrial waste. In this case West Sumatra has a great potential, but not optimally used yet. Among the sources of feed materials which are often found in West Sumatra is rice bran, coconut cake, corn, sago, straw and others. Rice straw is an agricultural-product whose production is high and most are available throughout the year.

Response of livestock productivity and carcass and meat quality can vary within the same breeds, between breeds, genders and among environmental factors including diet and fattening period. Many of the local cattle in West Sumatra that can be used for the fattening including Sapi Pesisir, Bali cattle and Ongole. Of the three breeds of local cows is the most common and is genetic in West Sumatra is the Sapi Pesisir. With good feeding for fattening cattle are expected to be able to have higher productivity.

Based on the above issues it is necessary to put an effort to improve productivity and quality of beef carcass by farming intensively with different fattening period. Cattle are given protein concentrate and high energy and improved ammoniated rice straw amoniasi.

The research objective is to determine the effect of feeding level and duration of fattening on growth and characteristics of sapi pesisir.

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Hypothesis in this research is the provision of different rations and long fattening period affects the rate of growth and carcass quality of Sapi Pesisir.

MATERIALS AND METHODS

Animals: Twelve male cattle aged 2-2.5 years were used in this study. Each cattle was placed in individual cages and having the adaptation period for 30 days.

Diets: Rations formulated based on the available material in the ratio of Concentrations (C) and Ammoniated Straw (AS) 50%: 50% for treatment A1 and 75% and 25% for the treatment of A2. Concentrate was composed of a combination of bran, corn, coconut oilcake, sago, minerals and salt. Composition of ration treatment and the composition can be seen in the Table 1.

Table 1: Composition of concentrate (% DM)

Ingredients	Percentage (%)
Rice bran	40
Corn	18
Coconut oil cake	30
Sago	10
Mineral	1
Salt	1
Total	100

Equipments: Equipments used in the study, including: livestock field scales, feed scales, a set of tools abatoir, carcass weight, back fat measurement and thick plastic sheets for measuring grid area of rib eye tendon.

Methods: This research was design using factorial randomized block design in 2 x 2 with 3 groups. The first factor (factor A) was the provision of 2 ration levels i.e: A1 = 50% concentrate + 50% ammoniated straw and A2 = 75% concentrate + 25% ammoniated straw. The second factor (factor B) was the long fattening period, B1 = 3 months and B2 = 4 month.

Statistical model used in accordance to Steel and Torrie (1993):

$$Y(ijk) = \mu + \alpha i + \beta j + \alpha\beta(ij) + rk + \epsilon ijk$$

Whereas:

Y (ijk) = The value of observations in treatment I and j, replications to k
μ = Value of the common
αi = Effect of treatment to the cow I
βj = Influence of the treatment to the long of fattening period
βα (ij) = Interaction of treatment effect to the I and j
rk = k influence the group
ϵijk = Experimental error

Parameters observed in this study were: Daily weight gain and carcass characteristics (carcass percentage, fat thick wide back and rib eye tendon).

Daily body weight gain: Calculated based on final body weight minus initial weight divided by the time (days) between the two weighing (kg).

Carcass weight percentage: Calculated based on the ratio between hot carcass weight (fresh) cut multiplied by 100%.

Back fat thickness: Determined by measuring the thickness of fat on length of approximately three-quarters of cross-sectional slices of tendon between the ribs, rib eye to the 12 and 13 (Soeparno, 1992).

Tendon rib eye area. Calculated by measuring tendon cross-sectional rib eye area (Longissimus dorsi) at the incision between the ribs 12 and 13 (Soeparno, 1992). Surface slices of rib eye tendon affixed with transparent plastic and then drawn with markers. Images of the tendon cross-sectional rib eye is read with a plastic grid.

RESULTS AND DISCUSSION

Animal growth: One way to see the growth of beef cattle is to measure the increase of body weight per unit of time. Increase average daily weight sapi pesisir intensively with different ration level and duration of fattening period can be seen in the following table.

Results of variety analysis show no interaction effect between the level of feeding and long fattening period to increase the daily weight Sapi Pesisir. Ration level was giving significant effect ($p<0.05$) to increase the daily body weight gain, while the long of fattening period show no significant effect ($p>0.05$).

From the table above, showed that the average daily body weight gain fed with A2 diet (75% concentrate + 25% ammoniated straw) had a higher effect (0.78 kg per cattle per day) compared with ration A (50% concentrate + 50% straw amoniasi) 0:58 kg per cattle per day. The higher increased of daily body weight gain when fed

Table 2: Chemical content of ingredients (% DM)

Ingredients	DM	CP	CF	Fat	Ash	NFE	TDN
Rice bran	85.89	12.58	14.18	5.69	6.92	60.63	62.90
Corn	89.33	10.23	3.98	3.94	3.04	78.81	80.80
Coconut oilcake	84.09	18.09	14.99	9.78	8.19	48.95	91.35
Sago	25.12	2.53	4.46	0.17	2.99	89.85	83.84
Mineral	96						
Salt	100						
Ammoniated straw	46.60	7.81	39.92	1.79	20.80	29.68	46.00

Table 3: Chemical composition of diets (%)

Components	Diets (Factor A) A1 (50% conc + 50% Ammoniated straw)	A1 (75% conc + 25% Ammoniated straw)
Dry matter	63.37	71.75
Crude protein	10.06	11.36
Crude fibre	25.63	18.47
Fat	3.86	4.90
Ash	13.43	9.75
NFE	45.89	54.00
TDN	60.74	68.12

Table 4: Average daily body weight gain of Sapi Pesisir (kg/head/day)

Factor A (Ration level)	Factor B (Fattening period) B1 (3 mth)	B2 (4 mth)	Average
A1 (50% C + 50% AS)	0.58	0.58	0.58[a]
A2 (75% C + 25% AS)	0.80	0.77	0.78[b]
Average	0.69	0.68	0.68

Notes: Different superscripts in the same row indicate significantly different effects ($p<0.05$)

rations A2 from A1 was closely related to composition of concentrates. The higher the percentage of concentrate in the ration means higher level of protein and energy rations, thus will result in the increase of body weight gain. This is in accordance with the opinion Soeparno (1992) that consumption of higher protein and energy would produce a growth rate faster. The influence of nutrients will be greater when the treatment started early period of growth. So the growth of cattle can be manipulated with different nutritional treatment. Ngadiono (1995) research results on Sumba Ongole Cattle (SO), Brahman Cross Cattle (BX) and the Australian Commersial Cross cattle (ACC), which are intensively for 4 months with feeding concentrate averaging 85% a daily body weight increase in cattle SO, BX and ACC each for 0.85, 0.78 and 0.82 kg/head/day accordingly.

The high increase of body weight Sapi Pesisir cattle fed concentrate feed (75% 25% ammoniated straw, compared with 50% concentrate + 50 ammoniated straw), possibly caused by feeding higher concentrations causes the higher protein digesting. Thus would lead to an increase of muscle meat or increased protein and fat accumulation in muscle. The rate of increase of body weight gain of Sapi Pesisir fed with different levels diets and fattening period can be seen in the following graph (Fig. 1).

An increase of body weight Sapi Pesisir fed high concentrate up to 75% due to cattle were still in the growth stage and had traditionally maintained without any concentrated diet. According to Tulloh (1978) in cattle production business, rapid growth and increase high body weight is needed, especially to achieve a certain weight with a relatively shorter time. Nitis and Lana (1984) mentioned that the supplement concentrates caused more efficient use of rations as a manifestation of higher digestion.

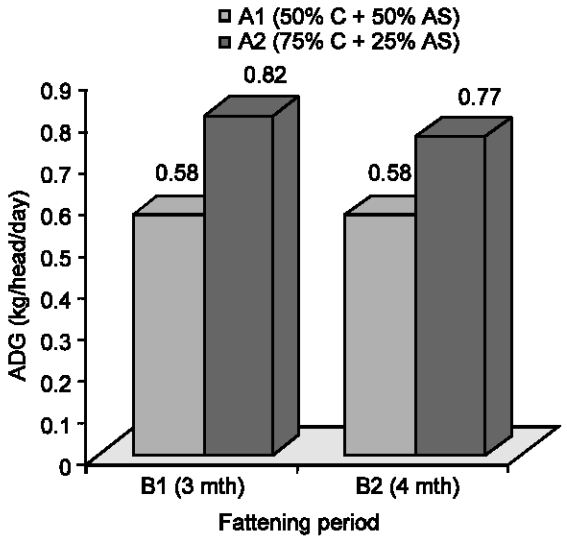

Fig. 1: Gambar 1. Histogram of average daily weight gain

Table 5: Average carcass percentage of Sapi Pesisir (%)

Factor A (Ration level)	Factor B (Fattening period) B1 (3 mth)	B2 (4 mth)	Average
A1 (50% C + 50% AS)	51.92	52.77	52.34
A2 (75% C + 25% AS)	52.37	53.66	53.00
Average	52.13[a]	53.21[b]	52.67

Notes: Different superscripts in the same row indicate significantly different effects ($p<0.05$)

Carcass percentage: Percentage of Sapi Pesisir carcass kept intensively with different fattening period can be seen in Table 5.

The results showed that there was no interaction between the level of feeding with level of fattening period to the percentage of carcass ($p>0.05$). While longer fattening period showed a significantly different effect on the percentage of carcass ($p<0.05$) while the level of ration did not.

These differences indicated that the cow was still in productive age, so the longer fattening period the higher body weight gain as a result of increased fat deposition and muscle fiber expansion. From the data obtained in this study reaffirms the theory of growth that carcass weights increased with increasing age of cattle for growth or increase the weight of the tissues that make up the carcass (meat, bones and fat). After the animals reach mature body weight, the weight gain is decreasing (Berg and Butterfield, 1976). Adult body weight of Sapi Pesisir achieved at the age of 4 years and above (Saladin, 1984). Swatland (1984) states that after the age of puberty (at around age cows 2-3 years) development of carcass tissue components is more dominated by the development of fat, while muskulus (muscle) develops slowly, whereas the bone relative to cease.

Table 6: Average back fat thickness of Sapi Pesisir (mm)

Factor A (Ration Level)	Factor B (Fattening period) B1 (3 mth)	B2 (4 mth)	Average
A1 (50% C + 50% AS)	2.167	2.500	2.334[a]
A2 (75% C + 25% AS)	2.500	3.333	2.916[b]
Average	2.334[A]	2.916[B]	2.625

Notes: Different superscripts in the same row indicate significantly different effects (p<0.05)

Table 7: Average rib eye area of Sapi Pesisir (cm^2)

Factor A (Ration Level)	Factor B (Fattening period) B1 (3 mth)	B2 (4 mth)	Average
A1 (50% C + 50% AS)	66.33	68.50	67.42[a]
A2 (75% C + 25% AS)	67.00	71.00	69.50[b]
Average	67.17[A]	69.75[B]	68.46

Notes: Different superscripts in the same row indicate significantly different effects (p<0.05)

Back fat thickness: Average back fat thickness of Sapi Pesisir kept intensively with different fattening periods, can be seen in Table 6.

The results of statistical analysis showed that there was no interaction effect between the level of feeding level, with fattening period on back fat thickness of Sapi Pesisir, while the influence of each factor shows the significant effects (p<0.05).

Feeding level on treatment A1 (50% concentrate + 50% ammoniated straw) with the average back fat thickness 2.334 mm is highly significant (p<0.05) to A2 (75% concentrate + 25% ammoniated straw) with average value of 2.916 mm. The higher average back-fat thickness due to A2 provided a higher concentrations than A1. The growing cattle fed high concentrations will deposit primarily body fat accumulation or subcutaneous fat. Back fat thickness is an indicator to determine the fatty body or carcass. The back fat thickness of Sapi Pesisir were lower than Sumba Onggole cattle. The research of Ngadiono (1995) on cows given 85% of feed concentrates for cattle 4 months results in back fat thickness of 0:09 inch. Maynard and Loosli (1969) as cited by Magdalena (1991) states that the age of cattle and food eaten by livestock affects the body fat.

Nutrition is probably the most important environmental factors affecting carcass composition, especially the proportions of fat (Soeparno, 1992). In addition that nutrients affect and change the level of fatty carcass on a particular body weight. Improvement of feed energy level and energy consumption will increase carcass fat levels, provided that the protein is not a limiting factor. Increased energy rations will increase subcutaneous fat, the proportion of carcass fat and lower proportion of meat (Arthoud *et al*., 1977).

The length of fattening period also gave a significant effect (p<0.05), where the 4-month period with average value of 2.916 mm was higher than at 3 months period with average value of 2.334 mm. This due to the accumulation of fat. According to Soeparno (1992) the growth rate occurs after the age of puberty from the fastest is fat, then muskulus and relative bone has stopped respectively. Swatland (1984) states that the deposition of fat during fattening following 3 phases that are marbling in internal organs, kidneys and mesentery, then subcutan and intermuscular network, and the last is intramuscular fat (marbling).

Rib eye area: The area of rib eye of Sapi Pesisir kept intensively under different fattening period was shown in Table 7.

Results of analysis showed that there was no interaction between factor A (ration level) and factor B (Fattening period) on the tendon rib eye area. While each factor rations level and duration of fattening showed a significant effect (p<0.05) against tendon rib eye area of Sapi Pesisir.

Rib eye area with level A1 rations (50% concentrate + 50% ammoniated straw) produced values averaging 67.42 cm^2, significantly different (p<0.05) with A2 (75% concentrate + 25% ammoniated straw) with values averaging 69.50 cm^2. The higher the level of concentrate in the rations, the rib eye area increased. The higher average tendon rib eye area given a higher concentrate (75%) led to increased accumulation of fat and protein in muscle meat. Increased tendon rib eye was also closely associated with increased carcass weight. With the increase in carcass weight means increased tendon wide rib eye. In accordance with the opinion Suwarno (1980) in Yusnayeti (1986) that there was of a positive relationship between the large tendon rib eye with the cow's carcass weights of cattle. Each increase of 1 cm2 area of rib eye tendon causes the increase of carcass weight of 2.90 kg.

Fattening period also provide a high significant effect (p<0.05) against broad tendon rib eye of Sapi pesisir, which is fattening period up for 4 months with average 69.75 cm^2 had a larger rib eye tendon than the 3-month period (67,167 cm^2). This due to the length of the fattening there will be increasing the size of muscle fibers. Besides also showed a tendency to an increase in fat in the muscle for longer fattening period. In accordance with the opinion of Romans and Ziegler (1974) that the size of the proportion of carcass tendon can be determined from the broad tendon rib eye. Arka (1984) reported the age of cattle provides a very real impact on tendon wide rib eye. With increasing age of the broad tendon rib eye also increases.

Conclusion:

- There was no interaction between the level of feeding rations to the length of fattening period on growth, carcass percentage, back fat thick and rib eye area of Sapi Pesisir (p>0.05).

- Factors of feeding level influenced significantly ($p<0.05$) on the growth, back fat thickness and rib-eye tendon area. The higher the percentage of concentrate in the ration, the faster the growth, back fat and the thick rib eye tendon expanded.
- Carcass characteristics of fattened Sapi Pesisir cattle for 4 months was better than for 3 months, where the percentage of carcass, fat backs and wide tendon rib eye area also increased ($p<0.05$).

REFERENCES

Arka, I.B., 1984. Pengaruh Penggemukan Terhadap Kualitas Daging dan Karkas pada Sapi Bali. Disertasi Doktor. Universitas Padjadjaran, Bandung.

Arthoud, V.H., R.W. Mandigo, R.M. Koch and A.W. Kotula, 1977. Carcass composition, quality and palatability attribute of bull and steers fed different energy levels and slaughtered at four ages. J. Anim. Sci., 44: 53-64.

Berg, R.T. and Dan R.M. Butterfield, 1976. New Concepts of Cattle Growth. Sidney University Press.

Magdalena, 1991. Hubungan Antara Luas Udamaru dan Tebal Lemak Punggung Terhadap Persentase Karkas Kerbau Jantan. Fakultas Peternakan, Universitas Andalas.

Maynard, L.A. and J.K. Loosli, 1969. Animal Nutrition, 6th ed. Tata McGraw-Hill Publishing Co. Ltd, New Delhi.

Ngadiono, N., 1995. Pertumbuhan serta Sifat-Sifat Karkas dan Daging Sapi Sumba Ongole, Brahman Cross dan Australian Commercial Cross yang Dipelihara Secara Intensif pada Berbagai Bobot Potong. Disertasi, Institut Pertanian Bogor, Bogor.

Nitis, I.M. and I.K. Lana, 1984. Effects of Replacing 30% of the Roughage with Concentrate on the Performance of Bali Steer. Udayana University, Denpasar.

Romans, J.R. and P.T. Ziegler, 1974. The Meat We Eat. 10th Edn., The Interstate Printers and Publisher, Inc., Danville, Illinois.

Saladin, R., 1984. Pedoman Beternak Sapi Potong. Fakultas Peternakan Universitas Andalas, Padang.

Soeparno, 1992. Ilmu dan Teknologi Daging. Gajah Mada University Press. Yogyakarta.

Steel, R.D.G. and J.T. Torrie, 1993. Principles and Procedure of Statistics. McGraw Hill Book Co. I.C. New York.

Suwarno, 1980. Hubungan antara luas urat daging mata rusuk dengan bobot karkas pada sapi Peranakan Ongole, sapi Bali dan Kerbau. Fakultas Peternakan Institut Pertanian Bogor.

Swatland, H.J., 1984. Strukture and Development of Meat Animals. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Tulloh, N.M., 1978. Growth, Development, Body Composition, Breeding and Management. In: A Course Manual in Beef Cattle Management and Economics. Bowker, W.A.T., R.G. Dumsday, J.E. Frisch, R.A. Swan and N.M. Tulloh (Eds.). Australian Vice Chancellors Committee. Academity Press. Pty. Ltd, Brishbane, pp: 59-91.

Yusnayeti, 1986. Hubungan antara luas udamaru dan tebal lemak punggung terhadap persentase karkas sapi. Fakultas Peternakan, Universitas Andalas.

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Major Antinutrients Found in Plant Protein Sources: Their Effect on Nutrition

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Abstract: Compounds or substances which act to reduce nutrient intake, digestion, absorption and utilization and may produce other adverse effects are referred to as antinutrients or antinutritional factors. Seeds of legumes and other plant sources contain in their raw state wide varieties of antinutrients which are potentially toxic. The major antinutrients includes: toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phytohaemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors. These antinutrients pose a major constraint in the use of plant protein sources in livestock feeds without adequate and effective processing. The level or concentration of these anitnutrients in plant protein sources vary with the species of plant, cultivar and post-harvest treatments (processing methods). This paper reviews the nutritional effect of major antinutrients present in plant protein sources.

Key words: Antinutrients, plant protein, legumes

INTRODUCTION

Antinutrients or antinutritional factors may be defined as those substances generated in natural feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exerts effect contrary to optimum nutrition. Being an antinutritional factor is not an intrinsic characteristic of a compound but depends upon the digestive process of the ingesting animal. Trypsin inhibitors, which are antinutritional factors for monogastric animals, do not exert adverse effects in ruminants because they are degraded in the rumen (Cheeke and Shull, 1985). Many plant components have potential to precipitate adverse effects on the productivity of farm livestock. These compounds are present in the foliage and seeds of virtually every plant that is used in practical feeding (D'Mello, 2000).

Nutritional effect of major antinutrients in plant protein sources: The major antinutrients mostly found in plant protein sources are toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phytohaemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors.

Toxic amino acids: A wide range of toxic non-protein amino acids occur in the foliage and seeds of plants. These toxic non-protein amino acids appear to play a major role in determining the nutritional value of a number of tropical legumes (D'Mello, 1982). It has been proposed that these amino acids act antagonistically towards certain nutritionally important amino acids (Liener, 1980). Fowden (1971) suggested that the metabolic pathways culminating in the synthesis of certain non-protein amino acids might reflect subtle alteration in the genome responsible for directing the formation of crucial amino acids. Bell (1971) reported that while non-protein amino acids function primarily as storage metabolites, they may also provide an adaptive advantage to the plants, for example to render the plant less susceptible to attack by various animals and lower plants. Some of these toxic amino acids includes; djenkolic acids, mimosine and canavanine.

Mimosine, a toxic non-protein amino acid structurally similar to tyrosine, is contained in the legume *Leucaena leucocephala* (D'Mello and Acamovic 1989; D'Mello, 2000). Mimosine has been proven effective in defleecing sheep and goats (Jacquemet *et al*.,1990; Luo *et al*., 2000). Mimosine a pyridoxal antagonist, which inhibits DNA replication and protein synthesis; thus, it may elicit defleecing by arresting cell division in the follicle bulb (Reis, 1979). In monogastric animals, mimosine causes poor growth, alopecia and reproductive problems. Levels of *Leucaena* meal above 5-10% of the diet for swine, poultry and rabbits generally result in poor animal performance.

The major symptoms of toxicity in ruminants are poor growth, loss of hair and wool, lameness, mouth and

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oesophageal lesions, depressed serum thyroxine level and goitre. Some of these symptoms may be due to mimosine and others to 3, 4-dihydroxypyridine, a metabolite of mimosine in the rumen (Jones and Hegarty, 1984).

Djenkol beans (*Pithecolobium lubatum*) when ingested sometimes lead to kidney failure which is accompanied by the appearance of blood and white needle-like clusters in the urine. The clusters are sulphur-containing amino acids known as djenkolic acids which are present in the bean in the free state, to the extent of 1-4%. This toxic amino acid is structurally similar to cystine, but it is not degraded in the animal body. Due to its insolubility it crystallizes out in the kidney tubules and escapes through urine (Enwere, 1998).

The toxic, non-protein amino acid, canavanine, occurs widely in unbound form in various legume plants of the sub-family *Papillonoideae* (Bell *et al*., 1978) and abundantly in jack bean (*Canavalia ensiformis* (L). DC), constituting up to 63 g/kg dry weight of the seed (Ho and Shen, 1966). Canavanine, a structural analogue of arginine, was first isolated from jackbean by Kitagawa and Tomiyama (1929).

Canavanine is believed to exert its toxic influence by virtue of its structural similarity with the nutritionally indispensable amino acid, arginine. Canavanine may antagonize arginine and interfere with Ribonucleic Acid (RNA) metabolism (Rosenthal, 1982). Canavanine has been demonstrated to reduce feed intake of non-ruminants but this was observed only at the equivalent of about 300 g/kg dietary level of raw jackbean (Tschiersch, 1962).

Saponins: Saponins are a heterogeneous group of naturally occurring foam-producing triterpene or steroidal glycosides that occur in a wide range of plants, including pulses and oil seeds such as kidney bean, chickpea, soybean, groundnut, lupin and sunflower (Liener, 1980; Price *et al*., 1987; Jenkins and Atwal, 1994). It has been reported that saponins can affect animal performance and metabolism in a number of ways as follows: erythrocyte haemolysis, reduction of blood and liver cholesterol, depression of growth rate, bloat (ruminants), inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption (Cheeke, 1971). Saponins have also been reported to alter cell wall permeability and therefore produce some toxic effects when ingested (Belmar *et al*., 1999). Saponins have been shown to bind to the cells of the small intestine thereby affecting the absorption of nutrients across the intestinal wall (Johnson *et al*., 1986).

The effect of saponins on chicks have been reported to reduce growth, feed efficiency and interfere with the absorption of dietary lipids, cholesterol, bile acids and vitamins A and E (Jenkins and Atwal, 1994).

Cyanogenic glycosides: Some legumes like linseed, lima bean, kidney bean and the red gram contain cyanogenic glycosides from which Hydrogen Cyanide (HCN) may be released by hydrolysis. Some cultivars of *Phaseolus lunatus* (lima bean) contain a cyanogenic glycoside called phaseolutanin from which HCN is liberated due to enzyme action, especially when tissues are broken down by grinding or chewing or under damp conditions (Purseglove, 1991). Hydrolysis occurs rapidly when the ground meal is cooked in water and most of the liberated HCN is lost by volatilization. HCN is very toxic at low concentration to animals. HCN can cause dysfunction of the central nervous system, respiratory failure and cardiac arrest (D'Mello, 2000).

Tannins: Tannins are water soluble phenolic compounds with a molecular weight greater than 500 daltons. They have the ability to precipitate proteins from aqueous solution. There are two different groups of tannins:- hydrolyzable tannins and condensed tannins. Condensed tannins are widely distributed in leguminous forages and seeds. Cattle and sheep are sensitive to condensed tannins, while goats are more resistant (Kumar, 1983; Kumar and Horigome, 1986; Kumar and Vaithiyanathan, 1990; D'Mello, 2000).

Tannins may form a less digestive complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes (Kumar and Singh, 1984). Tannin-protein complexes involve both hydrogen-bonding and hydrophobic interactions. The precipitation of the protein-tannin complex depends upon pH, ionic strength and molecular size of tannins. Both the protein precipitation and incorporation of tannin phenolics into the precipitate increase with increase in molecular size of tannins (Kumar and Horigome, 1986). However, when the molecular weight exceeds 5,000 daltons, the tannins become insoluble and lose their protein precipitating capacity and degree of polymerization becomes imperative to assess the role of tannins in ruminant nutrition (Kumar, 1983; Lowry, 1990). Tannins have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity. Helsper *et al*. (1993) reported that condensed tannins were responsible for the testa-bound trypsin inhibitor activity of faba beans. Tannins also have the ability to complex with vitamin B_{12} (Liener, 1980). Other adverse nutritional effects of tannins have been reported to include intestinal damage, interference with iron absorption and the possibility of tannins producing a carcinogenic effect (Butler, 1989).

Phytic acid: Phytic acid occurs naturally throughout the plant kingdom and is present in considerable quantities within many of the major legumes and oilseeds. This includes soybean, rapeseed and cotton seed. Matyka *et al*. (1993) reported that about 62-73% and 46-73% of the total phosphorus within cereal grains and legume

seeds being in form of organically bound phytin phosphorus, respectively. As phytic acid accumulates in storage sites in seeds, other minerals apparently chelates to it forming the complex salt phytate (Erdman, 1979). Studies by Martinez (1977) revealed that in oilseeds, which contain little or no endosperm, the phytates are distributed throughout the kernel found within subcellular inclusions called aleurone grains or protein bodies.

Whole soybeans have been reported to contain 1-2% phytic acids (Weingartner, 1987; Osho, 1993). The major part of the phosphorus contained within phytic acid are largely unavailable to animals due to the absence of the enzyme phytase within the digestive tract of monogastric animals. Nwokolo and Bragg (1977) reported that in the chicken there is a significant inverse relationship between phytic acid and the availability of calcium, magnesium, phosphorus and zinc in feedstuffs such as rapeseed, palm kernel seed, cotton seed and soybean meals. Phytic acid acts as a strong chelator, forming protein and mineral-phytic acid complexes; the net result being reduced protein and mineral bioavailability (Erdman, 1979; Spinelli *et al*., 1983; Khare, 2000). Phytic acid is reported to chelate metal ions such as calcium, magnesium, zinc, copper, iron and molybdenum to form insoluble complexes that are not readily absorbed from gastrointestinal tract. Phytic acid also inhibits the action of gastrointestinal tyrosinase, trypsin, pepsin, lipase and α-amylase (Liener, 1980; Hendricks and Bailey, 1989; Khare, 2000). Erdman (1979) stated that the greatest effect of phytic acid on human nutrition is its reduction of zinc bioavailability.

Gossypol: Gossypol is a naturally occurring polyphenolic compound present in the pigment glands of cotton seed (*Gossypium spp*). The average gossypol content varying from 0.4-2.4% within glanded cotton seeds to less than 0.01% free gossypol within some low-gossypol cotton seed meals (Liener, 1980; Robinson and Brent, 1989; Castaldo, 1995). Reduced lysine availability has been reported with cotton seed protein due to the ability of gossypol to bind with the reactive epsilon amino group of lysine during heat processing (Wilson *et al*., 1981; Robinson, 1991; Church, 1991). The general symptoms of gossypol toxicity are depressed appetite, loss of weight, laboured breathing and cardiac irregularity. Death is usually associated with reduced oxygen-carrying capacity of the blood, haemolytic effects on erythrocytes and circulatory failure. Dietary gossypol also causes olive-green discolouration of yolks in eggs (Church, 1991; Olomu, 1995; McDonald *et al*., 1995).

Oxalates: Oxalates affects calcium and magnesium metabolism and react with proteins to form complexes which have an inhibitory effect in peptic digestion. Ruminants, however unlike monogastric animals can ingest considerable amounts of high-oxalate plants without adverse effects, due principally to microbial decomposition in the rumen (Oke, 1969).

The hulls of sesame seeds contain oxalates and it is essential that meals should be completely decorticated in order to avoid toxicities (McDonald *et al*., 1995). Chemical analysis carried by Alabi *et al*. (2005) on locust bean seeds revealed that the testa of locust bean seeds had the highest concentration of oxalate (4.96 mg/100 g) followed by the pulp (3.40 mg/100 g) and the cotyledon (1.15 mg/100 g). Olomu (1995) reported that pigeon pea contains about 0.38% oxalic acid. Oxalic acid binds calcium and forms calcium oxalate which is insoluble. Calcium oxalate adversely affects the absorption and utilization of calcium in the animal body (Olomu, 1995).

Goitrogens: Goitrogenic substances, which cause enlargement of the thyroid gland, have been found in legumes such as soybean and groundnut. They have been reported to inhibit the synthesis and secretion of the thyroid hormones. Since thyroid hormones play an important part in the control of body metabolism their deficiency results in reduced growth and reproductive performance (Olomu, 1995). Goitrogenic effect have been effectively counteracted by iodine supplementation rather heat treatment (Liener, 1975).

Lectins (phytohaemagglutinins): Phytohaemagglutinins or lectins are glycoproteins widely distributed in legumes and some certain oil seeds (including soybean) which posses an affinity for specific sugar molecules and are characterized by their ability to combine with carbohydrate membrane receptors (Pusztai, 1989). Lectins have the capability to directly bind to the intestinal muscosa (Almeida *et al*., 1991; Santiago *et al*., 1993), interacting with the enterocytes and interfering with the absorption and transportation of nutrients (particularly carbohydrates) during digestion (Santiago *et al*., 1993) and causing epithelial lesions within the intestine (Oliveira *et al*., 1989).

Although lectins are usually reported as being heat-labile, their stability varies between plant species, many lectins being resistant to inactivation by dry heat and requiring the presence of moisture for more complete destruction (Ayyagari *et al*., 1989; Poel *et al*., 1990; Almeida *et al*., 1991).

Protease inhibitors: Protease inhibitors are widely distributed within the plant kingdom, including the seeds of most cultivated legumes. Protease inhibitors have the ability to inhibit the activity of proteolytic enzymes within the gastrointestinal tract of animals (Liener and Kakade, 1980).

Trypsin inhibitor and chymotrypsin inhibitor are protease inhibitors occurring in raw legume seeds. Protease inhibitors are the most commonly encountered class of

antinutritional factors of plant origin. These inhibitors have been reported to be partly responsible for the growth-retarding property of raw legumes. The retardation has been attributed to inhibition of protein digestion but there is evidence that pancreatic hyper-activity, resulting in increased production of trypsin and chymotrypsin with consequent loss of cystine and methionine is also involved (McDonald *et al*., 1995).
Trypsin inhibitors have been implicated in reducing protein digestibility and in pancreatic hypertrophy (Liener, 1976). Trypsin inhibitors are polypeptides that form well characterized stable complexes with trypsin on a one-to-one molar ratio, obstructing the enzymatic action (Carlini and Udedibie, 1997). Protease inhibitors are inactivated by heat especially moist heat, because of even distribution of heat (Bressani and Sosa, 1990; Liener, 1995).

Chlorogenic acid: Sunflower meal contains high levels of chlorogenic acid, a tannin like compound that inhibits activity of digestive enzymes including trypsin, chymotrypsin, amylase and lipase (Cheeke and Shull, 1985). Because chlorogenic acid is uncondensed and non-hydrolyzable, its content of 1% or more of a total of 3-3.5% phenolic compounds in sunflower meal is not reported in tannin assays. Chlorogenic acid is also a precursor of ortho-quinones that occur through the action of the plant enzyme polyphenol oxidase. These compounds then react with the polymerize lysine during processing or in the gut. Although the toxic effects of chlorogenic acid can be counteracted by dietary supplementation with methyl donors such as choline and methionine. Chlorogenic acid is reported to be readily removed from sunflower seeds using aqueous extraction methods (Dominguez *et al*., 1993).

Amylase inhibitors: Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. Starches are complex carbohydrates that cannot be absorbed unless they are first broken down by the digestive enzyme amylase and other secondary enzymes (Marshall and Lauda, 1975; Choudhury *et al*., 1996). Pigeon pea have been reported to contain amylase inhibitors. These inhibitors have been found to be active over a pH range of 4.5-9.5 and are heat labile. Amylase inhibitors inhibit bovine pancreatic amylase but fail to inhibit bacterial, fungal and endogenous amylase. Pigeon pea amylase inhibitors are synthesized during late seed development and also degraded during late germination (Giri and Kachole, 1998).

Conclusion: The presence of antinutrients in plant protein sources for livestock feeding is a major constraint that reduces their full utilization. To be able to justify the overall nutritional potential or value of any plant protein source, proper assessment of the type, nature and concentration of the antinutrients present in the protein source and also the bioavailability of nutrients to the ingesting animal is necessary. Employing appropriate and effective processing techniques or combination of techniques could help reduce or eliminate the adverse effects of these antinutritive constituents in plant protein sources and thereby improve their nutritive value. Supplementation of some minerals, animo acids and vitamins could help reduce or neutralize the negative effect of antinutritional factors in plant protein sources for livestock nutrition. The concentration or level of the antinutritive constituents in these protein sources vary with the species of plant, cultivar and post-harvest treatments (processing methods). Since antinutrients vary among plant cultivars, therefore the use of genetically improved low-antinutritive cultivars or varieties could be a possible option for livestock feeding.

REFERENCES

Alabi, D.A., O.R. Akinsulire and M.A. Sanyaolu, 2005. Qualitative determination of chemical and nutritional composition of *Parkia biglobosa* (jacq.) Benth. Afr. J. Biotechnol., 4: 812-815.

Almeida, N.G., A.M. Calderon de la Barca and M.E. Valencia, 1991. Effect of different heat treatments on the antinutrional activity of *Phaseolus vulgaris* (variety; *ojo de cabra*) lectin. J. Agric. Food Chem., 39: 1627-1630.

Ayyagari, R., B.S.N. Rae and D.N. Roy, 1989. Lectins, trypsin inhibitors, BOAA and tannins in legumes and cereals and the effects of processing. Food Chem., 34: 229-238.

Bell, E.A., 1971. Comparative biochemistry of non-protein amino acids. In: Chemotaxonomy of the leguminosae. (Editors: Harborne, J.B., B. Boulter and B.L. Turner). Academic Press, New York, pp: 179-206.

Bell, E.A., J.A. Lackey and R.M. Polhill, 1978. Systematic significance of canavanine in the *Papillonoideae* (*Faboideae*). Biochemical Systematics Ecol., 6: 201-212.

Belmar, R., R. Nava-montero, C. Sandoval-castro and J. M. McNab, 1999. Jack bean (*Canavalia ensiformis* L. DC.) in poultry diets: Antinutritional factors and detoxification studies-a review. World Poult. Sci. J., 55: 37-59.

Bressani, R. and J.L. Sosa, 1990. Effects of processing on the nutritive value of *Canavalia* Jackbeans [*Canavalia ensiformis* (L.)]. Plant Foods Human Nutr., 40: 207-214.

Butler, L.G., 1989. Effects of condensed tannins on animal nutrition. In: Chemistry and significance of condensed tannins. (Editors: Hemingway, R.W. and J.J. Karchesy). Plenum Press, New York, pp: 391-402.

Carlini, C.R. and A.B.I. Udedibie, 1997. Comparative effects of processing methods on haemagglutinating and antitryptic activities of *Canavalia ensiformis* and *Canavalia braziliensis* seeds. J. Agric. Food Chem., 45: 4372-4377.

Castaldo, D.J., 1995. Extruded cottonseed: A novel ingredient for dairy feeds. Feed Int., 16: 14-16.

Cheeke, P.R., 1971. Nutritional and physiological implications of saponins: A review. Can. J. Anim. Sci., 51: 621-623.

Cheeke, P.R. and L.R. Shull, 1985. Tannins and Polyphenolic compounds. In: Natural Toxicants in Feeds and Poisonous Plants. AVI Publishing company, USA.

Choudhury, A., K. Maeda, R. Murayama and E.P. Dimagno, 1996. Character of a wheat amylase inhibitor, preparation and effects on fasting human pancreaticobiliary secretions and hormones. Gastroenterology, 111: 1313-1320.

Church, D.C., 1991. Livestock feeds and feeding. 3rd Edn., Prentice Hall Incorporation, New Jersy, USA, pp: 546.

D'Mello, J.P.F., 1982. Toxic factors in some tropical legumes. World Rev. Anim. Prod., 18: 41-46.

D'Mello, J.P.F., 2000. Anti-nutritional factors and mycotoxins. In: Farm animal metabolism and nutrition. CAB International Wallingford, UK, pp: 383-403.

D'Mello, J.P.F. and T. Acamovic, 1989. *Leucaena leucocephala* in poultry nutrition: A review. Anim. Feed Sci. Technol., 26: 1-28.

Dominguez, H., M.J. Nunez and J.M. Lema, 1993. Chlorogenic acid removal during aqueous processing of sunflower kernels. Grasas of Aceite (Espana), 44: 235-242.

Enwere, N.J., 1998. Foods of plant origin: Processing and utilization with recipes and technology profiles. Afro-orbis publications Ltd., Nsukka, pp: 301.

Erdman, J.W., 1979. Oilseed phytates: Nutritional implications. J. Am. Oil Chemist Soc., 56: 736-741.

Fowden, L., 1971. Amino acid biosynthesis. In: Biosynthetic pathways in higher plants. (Editors: Pridham, J.B. and T. Swain). Academic Press, New York, pp: 73-99.

Giri, A.P. and M.S. Kachole, 1998. Amylase inhibitors of pigeon pea *(Cajanus cajan)* seeds. Phytochemistry, 47: 197-202.

Helsper, J.P., J.M. Hoogendijk, A. Van Norel and K. Burger-meyer, 1993. Antinutritional factors in faba beans (*Vicia faba* L.) as affected by breeding toward the absence of condensed tannins. J. Agric. Food Chem., 41: 1058-1061.

Hendricks, J.D. and G.S. Bailey, 1989. Adventitious toxins. In: Fish nutrition. 2nd Edn., Halver, J.E. (Ed.). Academic Press Inc., New York, USA, pp: 605-651.

Ho, H.K. and C. Shen, 1966. Studies on the distribution of canavanine in Taiwan plants and its metabolic change during germination. Chinese Agric. Chem. Soc., (special issue) pp: 23-31.

Jacquemet, N., J.M. Fernandez, T. Sahlu and C.D. Lu, 1990. Mohair quality and metabolic profile of Angora goats during acute mimosine toxicity. J. Anim. Sci., 1: 400(Abstract).

Jenkins, K.J. and A.S. Atwal, 1994. Effects of dietary saponins on faecal bile acids and neutral sterols and availability of vitamins A and E in the chick. J. Nutr. Biochem., 5: 134-137.

Johnson, L.T., J.M. Gee, K. Price, C. Curl and G.R. Fenwick, 1986. Influence of saponins in gut permeability and active nutrient transport *in vitro*. J. Nutr., 116: 2270-2272.

Jones, R.J. and M.P. Hegarty, 1984. The effect of different proportions of *Leucaena leucocephala* in the diet of cattle on growth, feed intake, thyroid function and urinary excretion of 3-hydroxyl-4(IH)-pyridine. Aust. J. Agric. Res., 35: 317.

Khare, S.K., 2000. Application of immobilized enzymes in soybean processing. The Third International Soybean Processing and Utilization Conference (ISPCRC III): 2000 of the Innovation Era for Soybeans. 15-20, October, 2000, Tsukuba, Ibaraka, Japan, pp: 381-382.

Kitagawa, M. and T. Tomiyama, 1929. A new amino acid compound in the Jackbean and a corresponding new ferment. J. Biochem. (Tokyo), 11: 265-271.

Kumar, R., 1983. Chemical and biochemical nature of fodder tree leaf tannins. J. Agric. Food Chem., 31: 1364-1367.

Kumar, R. and T. Horigome, 1986. Fractionation, characterization and protein precipitating capacity of the condensed tannins from *Robinia pseudoacacia* (L.) leaves. J. Agric. Food Chem., 34: 487-489.

Kumar, R. and M. Singh, 1984. Tannins: Their adverse role in ruminant nutrition. J. Agric. Food Chem., 32: 447-453.

Kumar, R. and S. Vaithiyanathan, 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol., 30: 21-38.

Liener, I.E., 1975. Antitryptic and other anti-nutritional factors in legumes. In: Nutritional improvement of food legumes by breeding. (Editor: M. Milner) Wiley Interscience publication, John Wiley and Sons, New York, pp: 239-258.

Liener, I.E., 1976. Legume toxins in relation to protein digestibility-a review. J. Food Sci., 41: 1076-1081.

Liener, I.E., 1980. Heat labile antinutritional factors. In: Advances in legume science, (Editors: R.J. Summerfield and A.H. Bunting), Kew London, Royal Botanic Gardens, pp: 157-170.

Liener, I.E., 1995. Possible adverse effects of soyabean anticarcinogens. Am. Inst. Nutr., 125: 7445-7505.

Liener, I.E. and M.L. Kakade, 1980. Protease inhibitors. In: Toxic constituents of plant food stuffs (Editor: I.E. Liener) Academic Press, New York, pp: 7-71.

Lowry, J.B., 1990. Toxic factors and problems: Methods of alleviating them in animals. In: Shrubs and tree fodders for farm animals (Editor: C. Devendra) IDRC, Canada, pp: 76-88.

Luo, J., A.J. Litherland, T. Sahlu, R. Puchala, M. Lachica and A.L. Goetsch, 2000. Effects of mimosine on fiber shedding, follicle activity and fiber regrowth in Spanish goats. J. Anim. Sci., 78: 1551-1555.

Marshall, J.J. and C.M. Lauda, 1975. Purification and properties of *Phaseolus vulgaris*. J. Biol. Chem., 250: 8030-8037.

Martinez, W.H., 1977. Evaluation of proteins for Humans. (Editor; C.E. Bodwell) Publishing Co., Inc., Westport, CT, pp: 203.

Matyka, S., G. Bogusz and W. Korol, 1993. Phytate contents in cereal grains, legume and rape seeds. Biuletyn Informacyjny Przemyslu Paszowedo (Poland), 32: 37-43.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan, 1995. Animal nutrition. 5th Edn., Longman group Ltd., UK., pp: 607.

Nwokolo, E.N. and D.B. Bragg, 1977. Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. Can. J. Anim. Sci., 57: 475-477.

Oke, O.L., 1969. Oxalic acid in plants and in nutrition. World Rev. Nutr. Diet., 10: 263-303.

Oliveira, A.C., B.C. Vidal and V.C. Sgarbieri, 1989. Lesions of intestinal epithelium by ingestion of bean lectins in rats. J. Nutr. Sci. Vitaminol. (Japan), 35: 315-322.

Olomu, J.M., 1995. Monogastric animal nutrition, principles and practice. Jachem publication, pp: 320.

Osho, S.M., 1993. Developed soybean technologies for small-scale and industrial levels. A manual workshop on small-scale and industrial level processing of soybean, July, 1993, Ibadan, Nigeria.

Poel, A.F.B., J. Van der Blonk, D.J. Zuilichem and M.G. Oort, 1990. Thermal inactivation of lectins and trypsin inhibitor activity during steam processing of dry beans (*Phaseolus vulgaris)* and effects on protein quality. J. Food Sci. Technol., 53: 215-228.

Price, K.R., I.T. Johnson and G.R. Fenwick, 1987. The chemistry and biological significance of saponins in foods and feeding-stuff. Crit. Rev. Food Sci. Nutr., 26: 27-135.

Purseglove, J.W. 1991. Tropical crops: Dicotyledons. Longman Scientific and Technical Co published in the United States with John Wiley and Sons Inc., New York.

Pusztai, A., 1989. Biological effects of dietary lectins. In: Recent advances of research in antinutritional factors in legume seeds: Animal nutrition, feed technology and analytical methods. Wageningen (Netherlands), Pudoc, pp: 17-29.

Reis, P.J., 1979. Effects of amino acids on the growth and properties of wool. In: Physiological and Environment Limitations to Wool Growth. (Editors; J.L. Black and P.J. Reis). University of New England Publishing Unit, Armidale, Australia, pp: 223-242.

Robinson, E.H., 1991. Improvement of cottonseed meal protein with supplemental lysine in feeds for channel catfish. J. Applied Aquac., 1: 1-14.

Robinson, E.H. and J.R. Brent, 1989. Use of cotton seed meal in channel catfish feeds. J. World Aquac. Soc., 20: 250-255.

Rosenthal, G.A., 1982. Plant non-protein amino and imino acids: Biological, biochemical and toxicological properties. New York and London, Academic Press, pp: 95-113.

Santiago, J.G., A. Levy-Benshimol and A. Carmona, 1993. Effect of *Phaseolus vulgaris* lectins on glucose absorption, transport and metabolism in rat everted intestinal sacs. J. Nutr. Biochem., 4: 426-430.

Spinelli, J., C.R. Houle and J.C. Wekell, 1983. The effect of phytates on the growth of rainbow trout *(Salmo gairdneri)* fed purified diets containing various quantities of calcium and magnesium. Aquaculture, 30: 71-83.

Tschiersch, B., 1962. *Uber das Vorkommen von Canavanin*. Flora, 150: 87-94.

Weingartner, K.E., 1987. Processing, nutrition and utilization of soyabeans. In: Soyabean for the tropics. (Editors: S.B. Singh, K.O. Rachie and K.E. Dashiell). John Wiley and Sons Ltd, New York, pp: 149-177.

Wilson, R.P., E.H. Robinson and W.E. Poe, 1981. Apparent and true availability of amino acids from common feed ingredients for channel catfish. J. Nutr., 111: 923-929.