PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 9 (11): 1039-1042, 2010
ISSN 1680-5194

Effects of Effective Microorganisms on Yield and Quality of Vegetable Cabbage Comparatively to Nitrogen and Phosphorus Fertilizers

Kwizera Chantal[1,2], Shao Xiaohou[1,2], Wang Weimu[1,2] and Basil T. Iro Ong'or[3]
[1]Key Laboratory of Efficient Irrigation-Drainage and Agricultural Soil-Water Environment in Southern China, Hohai University, Ministry of Education, China
[2]College of Water Conservancy and Hydropower Engineering, Hohai University, Nanjing 210098, China
[3]Department of Civil and Structural Engineering, Masinde Muliro University of Science and Technology, Kakamega, 190-50100, Kenya

Abstract: The misuse and excessive use of fertilizers resulted in the loss of soil sustainability and a declined productivity, have increased the need of use of Effective Microorganisms Technology (EM) as an alternative to such practices to meet the future nutritional requirements of the people. This study aimed at appraising the effects of EM on Leaf Area (LA) and Photosynthesis (PH) of vegetable cabbage comparatively to Nitrogen (N) and Phosphorus (P) fertilizers. Results showed an increased leaf area for treatments with EM, while others demonstrated its reduction. Significant among treatments was recorded with $p<0.05$. Likewise, EM has improved photosynthesis. This suggested that EM improve plant yield and quality, resulting in a fulfillment of a sustainable agriculture.

Key words: EM Technology, chemical fertilizers, vegetable cabbage, leaf area index, photosynthesis

INTRODUCTION

From the ancient times, the principal objective of using chemical fertilizers in agriculture has been to approach the best production in quantity and quality for the humankind (Parr *et al*., 1992). Unfortunately, their excessive use has caused many problems as soil degradation leading to the reduction of plant yield and quality. To reverse these trends, Effective Microorganisms Technology (EM) has been adopted as an alternative solution.

Developed by Professor Teruo Higa in Okinawa in the 1970's (Yamada and Xu, 2000), Effective Microorganisms has been put into application since 1980 (Teruo Higa, 2005). It is a mixture of three principal microorganisms (photosynthetic bacteria, lactic acid bacteria and Yeasts) applied to alter the use of chemical fertilizers and change the microbial diversity and interaction in soils and plants (Primavesi and Kinjo, 1997). Yamada and Xu (2000), affirmed higher crop maze productivity due to the use of EM (Yamada and Xu, 2000). Foregoing research reported an increased crops production by using EM (Teruo Higa, 2005). In this study, a comparative assessment on the responses of Cabbage to Effective Microorganisms and chemical fertilizer (NP) was done. Specifically, the:

- Influence of EM and Chemical fertilizer on leaf area of cabbage is analyzed
- Effectiveness of Effective Microorganisms on photosynthesis of cabbage is determined

MATERIALS AND METHODS

Experimental site: The study was carried out in Greenhouse of Water-Saving Park, located in Jiangning Campus of Hohai University. The climate is sub- humid, which belongs to the north subtropical climate zone with an average rainfall of 1.106 mm.

Experimental design: EM, N and P were considered with two levels for each: L1 (2% of EM regarding to the water requirement, 100 mg N/kg of dry soil and 75 mg P_2O_5/Kg of dry soil) and L2 (5% of EM regarding to the water requirement, 200 mg N/kg of dry soil and 150 mg P_2O_5/Kg of dry soil). 150 mg K_2O/Kg were supplied as a basal fertilizer. All treatments were replicated 3 times as depicted in the Table 1 below. The vegetable Cabbage was sown on 28th November 2006 and transplanted on 1st January 2007 in 39 pots with 7.5 kg of dry soil and 4 plants per pot.

Samples and data analysis

Samples: Three PH measurements were done on 7th February, 9th March and 20th March 2007 using LI-6400 portable photosynthesis system, while LA was measured after every 2 weeks until 20th March 2007.

Data analysis: Statistic comparative analysis of photosynthesis (transpiration rate (E), net photosynthetic rate (P_n), stomatal conductance (G_s), Intercellular CO_2 content (ci)) and plant leaf area between all treatments

Corresponding Author: Kwizera Chantal, Key Laboratory of Efficient Irrigation-Drainage and Agricultural Soil-Water Environment in Southern China, Hohai University, Ministry of Education, China

Table 1: Treatment design

Treat. No.	Treatments	EM	Fertilizers (g/pot) NH_4NO_3	KH_2PO_4	K_2SO_4
1	CK	0	0	0	0
2	EM1N1/EM1P1	2%	2.143	1.077	1.393
3	EM1N2	2%	4.286	1.077	1.393
4	EM2N1/EM2P1	5%	2.143	1.077	1.393
5	EM2N2	5%	4.286	1.077	1.393
6	EM1P2	2%	2.143	2.154	0.704
7	EM2P2	5%	2.143	2.154	0.704
8	N1	0	2.143	1.077	1.393
9	N2	0	4.286	1.077	1.393
10	P1	0	2.143	1.077	1.393
11	P2	0	2.143	2.154	0.704

Treat. No. = Treatment Number

was conducted, with a probability value $p<0.05$ using Excel and SPSS.

RESULTS AND DISCUSSION

Effect of EM on the leaf area: Result showed an increased LA for treatments with EM than others (Fig. 1 and 2). The highest increase was recorded by EM2N2 (167.056%, 16.014%) comparatively to CK and N2 respectively (Fig. 1). On the other hand, EM2P2 (170.702%, 27.051%) exhibited the greatest increase as compared to CK and P2. However Significant among treatments was recorded with $p<0.05$. Higher LA may be due to the effect of EM on plant root development, followed by better fostering with nutrients to the plant. This indicates an enhanced biomass production and photosynthetic capacity. Primavesi and Kinjo (1997) reported an increased LA on beans) (Primavesi and Kinjo, 1997). Higa and Parr (1994) argued a better photosynthetic capacity with an increased LA on maze (Higa and Parr, 1994).

Effect on photosynthesis: Result demonstrated an increased P_n for treatments with EM than others except EM1N2 and EM2P2 (Fig. 3, 4). The highest P_n was recorded by EM2N2 (23.188%, 18.592%) as compared to CK and N2 respectively (Fig. 3). Significant increase was observed for EM1P2 (12.229%, 6.961%) comparatively to CK and P2 successively (Fig. 4). Matthew Wood and Higa (2005) reported an increased P_n on plant maze due to the continual supply of nutrient and hormones from EM (Matthew Wood and Higa, 2005). Study of Yamada and Xu (2000), argue that EM contains phytohormones or others biologically active substances that cause the delay of senescence of plants and increase P_n (Yamada and Xu, 2000). The P_n reduction recorded by EM1N2 (4.869%, 8.418%) and EM2P2 (3.88% 3.78%) comparatively to the others was attributed to the reduction of stomata conductance. This is in support with results found by Meloni *et al.* (2003) and Dubey (2005), who reported a reduced P_n for cotton crop due to the reduction of stomata conductance (Meloni *et al.*, 2003; Dubey, 2005).

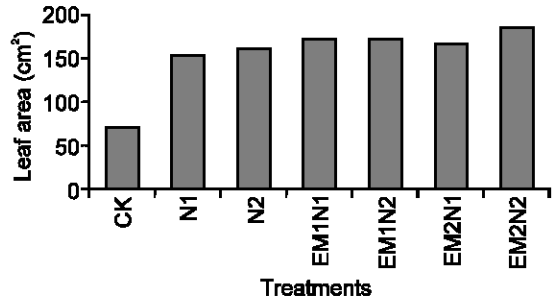

Fig. 1: Leaf area analysis with EMN

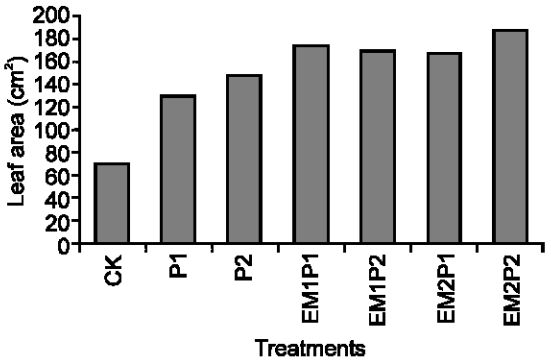

Fig. 2: Leaf area analysis with EMP

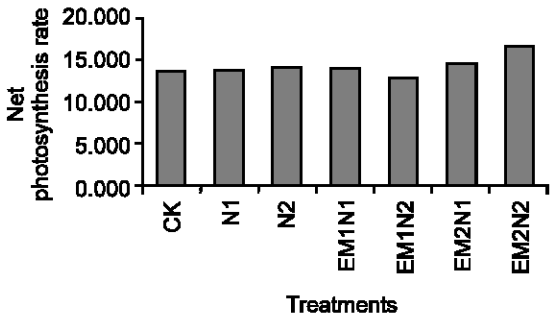

Fig. 3: Net photosynthesis rate (P_n) (µmol m^{-2} s^{-1}) analysis with EMN

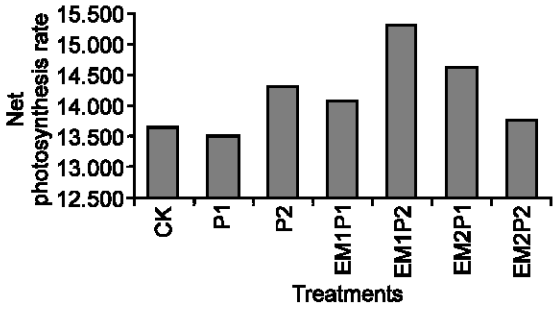

Fig. 4: Net photosynthesis rate (P_n) analysis (µmol m^{-2} s^{-1}) with EMNP

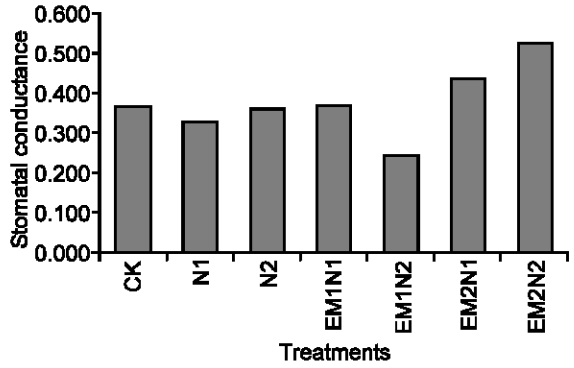

Fig. 5: Analysis of stomata conductance (G_s) (µmol m^{-2} s^{-1}) with EMN

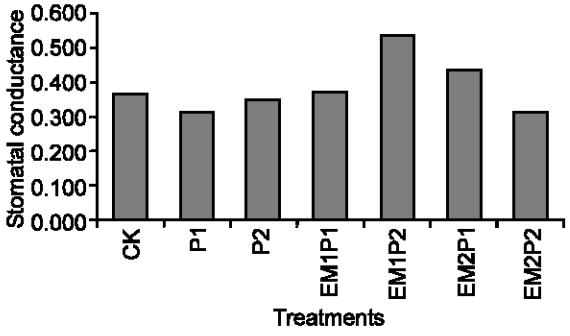

Fig. 6: Analysis of stomata conductance (G_s) (µmol m^{-2} s^{-1}) with EMP

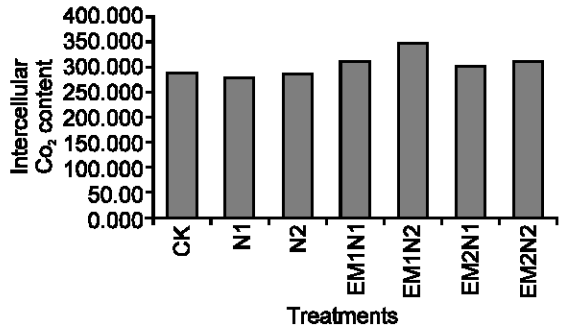

Fig. 7: Analysis of Intercellular CO_2 content (ci) (µmol mol^{-1}) with EMN

G_s was higher for all treatments applied with EM except EM1N2 and EM2P2 (Fig. 5, 6). The highest level was shown by EM2N2 (43.68%, 45.48%) comparatively to the control and N2 (Fig. 5). On the other hand, treatment EM1P2 (46.79%; 52.81%) was the most efficient in increasing G_s (Fig. 6). E differs much among the treatments (Fig. 9, 10). It was higher in control and others treatments without EM than these fertilized with EM. Little difference among treatments was observed for Ci (Fig. 7, 8). It was higher for treatments applied with

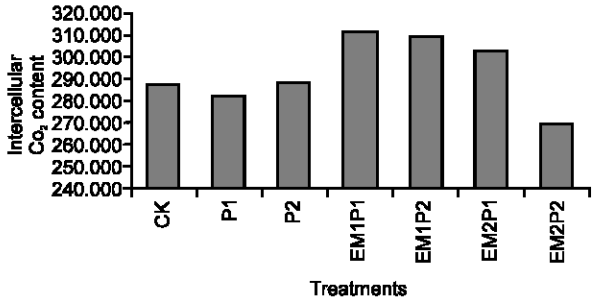

Fig. 8: Analysis of Intercellular CO_2 content (ci) (µmol mol^{-1}) with EMP

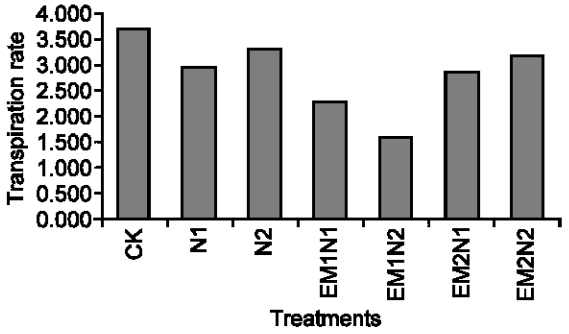

Fig. 9: Analysis of transpiration rate (E) (µmol m^{-2} s^{-1}) with EMN

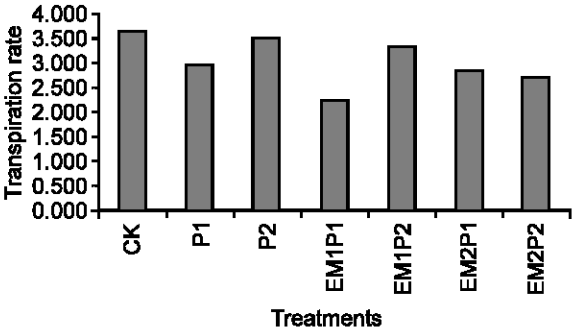

Fig. 10: Analysis of transpiration rate (E) (µmol m^{-2} s^{-1}) with EMP

EM than others and the control. The maximum was shown by treatment EM1N2 (21.58%) comparatively to both the control and N2 (Fig. 7). With Figure 8, the greatest level was recorded by EM1P1 (8.43%, 10.48%) comparatively to the control and P1 respectively. All this could conclude that the effects in improving photosynthesis could be expected from EM Technology.

Conclusion: By using these results above as basis, the following conclusions could be drawn:

1. EM effectively increases the leaf area; hence significant increase in yield, ascribed mainly to the stimulation of vegetable biomass production.

2. These data also enable a more precise assessment of the economic benefits that greenhouse growers can expect from Effective Microorganisms Technology, taking into account the specificity of the winter climatic conditions and greenhouse characteristics.
3. Compared to fertilizer applied and controlled grown vegetable-cabbage, the EM positively impacts by increasing the photosynthesis.
4. Producing safe food is the important target worldwide. However outcomes showed a little improved production due to the effect of EM. Hence it should be considered as a support fertilizer, to enhance other fertilizer's capacity to supply nutrients to plants and fulfill sustainable development of agriculture. At the same time furthers research on photosynthesis are recommended for more understanding.

REFERENCES

Dubey, R.S., 2005. Photosynthesis in plants under stressful conditions. In: M. Pessarakli, Editor, Handbook of photosynthesis (2nd Edn.), CRC Press, Florida, pp: 479-497.

Higa, T. and J.F. Parr, 1994. Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment. International Nature Farming Research Center, Atami, Japan.

Matthew Wood and T. Higa, 2005. Microorganism for sustainable community Development : A National Case Study of Cooperation and C-Prosperity in North Korea for the preservation of Environmental, Agricultural, Economic and Cultural Integrity.

Meloni, D.A., M.A. Oliva, C.A. Martinez and J. Cambraia, 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot., 49: 69-76.

Parr, J.F., R.I. Papendick, S.B. Hornick and R.E. Meyer, 1992. Soil quality attributes and relationship to alternative and sustainable agriculture.

Primavesi, A. and S. Kinjo, 1997. Comparison of the effect of EM and other bacterial and mineral product on bean yiel, Brasil.

Teruo Higa, 2005. The Technology of Effective Microorganisms in- Beneficial.

Yamada, K. and H. Xu, 2000. Properties and applications of an organic fertilizer inoculated with Effective Microorganisms. In Xu, H.; Parr, J.F.; Umemura, H. (Eds) Nature Farming and Microbial Applications, New York, pp: 255-268.

Pakistan Journal of Nutrition 9 (11): 1043-1046, 2010
ISSN 1680-5194

Nutritional and Quality Attributes of Wheat Buns Enriched with the Larvae of *Rhynchophorus phoenicis* F.

Ifie Idolo
Department of Animal Science, Faculty of Agriculture, Delta State University,
Asaba Campus, Asaba, Delta State, Nigeria

Abstract: The larva of *Rhynchophorus phoenicis* is one of delicacies consumed by the people in Southern Nigeria. Proximate analysis of the Larva found it to be nutritious containing protein, fat, ash and carbohydrate at the following ratios (9.96, 25.72, 1.91 and 1.98% on wet basis respectively). A process was developed for incorporating the larva into processed wheat buns. The process involved substitution of wheat flour with the flour of the larva at 5% 10% and 15%. Results of the nutritional analysis of the buns showed increase in the protein, energy, Ca, Mg, Zn and Vitamins content with progressive increase in the larva content. The sensory evaluation showed that no significant difference ($p<0.05$) were observed between the 0% and 5% substitution level in the sensory attributes of aroma and flavor, taste texture and general acceptability. However differences were significant in the colour ($p<0.05$). The buns with 20% larva content was the least accepted in nearly all the quality attributes evaluated. The result shows that an acceptable buns product can be processed with the inclusion of the larva at 5% level to enhance the nutritional quality of the product.

Key words: *Rhynchophorus phoenicis*, proximate analysis, wheat buns, nutritional quality, sensory evaluation

INTRODUCTION

Insects have played an important part in the history of human nutrition in Africa, Asia and Latin America (Bodenheimer, 1951). Hundreds of species have been used as human food. Some of the more important groups include grass-hoppers, caterpillars, beetle grubs and (sometimes) adults, winged termites (some of which are very large in the tropics), bee, wasp and ant brood (larvae and pupae) as well as winged ants, cicadas and a variety of aquatic insects. Ordinarily, insects are not used as emergency food during shortages, but are included as a planned part of the diet throughout the year or when seasonally available. One of such insects popularly consumed in Nigeria is the larvae of the beetle *Rhynchophorus phoenicis F*. It is commonly consumed raw, fried, smoked, roasted and it may be eaten as part of a meal or as a whole meal. Moreover, the nutritional value of this larva has been evaluated (Ekpo and Onigbinde 2005; Okaraonye and Ikewuchi 2008; Banjo *et al*., 2006) and found to be highly nutritious with high quality of macro protein and micronutrients (minerals and vitamins). Thus its addition in diet has been greatly encouraged as a means of combating the problem of malnutrition.

Consequently, the objective of this work aimed at incorporating the larvae flour into common snack-buns made from wheat flour by substituting it (wheat flour) with the larvae at different levels and evaluating the nutritional and sensory qualities of the wheat-larvae buns product.

MATERIALS AND METHODS

Live larvae of Rhynchophorus *phoenicis* were purchased from Ogbe-Ijoh waterside market at Warri South Local Government area of Delta State. The live sample along with their feed were transported to the laboratory in a well ventilated container for analysis at the Nigeria Institute for Oil Palm Research (NIFOR) Benin City, Nigeria and used within ten hours of collection.

Proximate analysis: The larvae were analyzed chemically according to the Official Methods of Analysis (AOAC, 1990). Determinations were performed for water content, crude fiber (structural carbohydrates), fats, protein and mineral salts.

Wheat-based larva buns making process: The processes involved in the manufacture of the buns are shown in Fig. 1. Four blends of wheat and larva flour for the production of wheat buns, were prepared by mixing the wheat flour with the flour from the larva at the following levels 0%, 5%, 10%, 15%. The buns were processed by deep frying in oil at a temperature of 150°C for 12 min.

Nutritional analysis of processed buns: The processed wheat buns were analyzed for the energy, protein, Ca, Mg and Zn contents. The Minerals were analyzed using an Atomic absorption spectrophotometer (AAS, Model SP9, Pychicham UK) the protein content with the Kjedahl method (AOAC, 1990) and the energy content was measured with the use of the Gallenkamp Ballistic Bomb calorimeter (AOAC, 1990). The Vitamin contents were determined using the procedure of (AOAC, 2005).

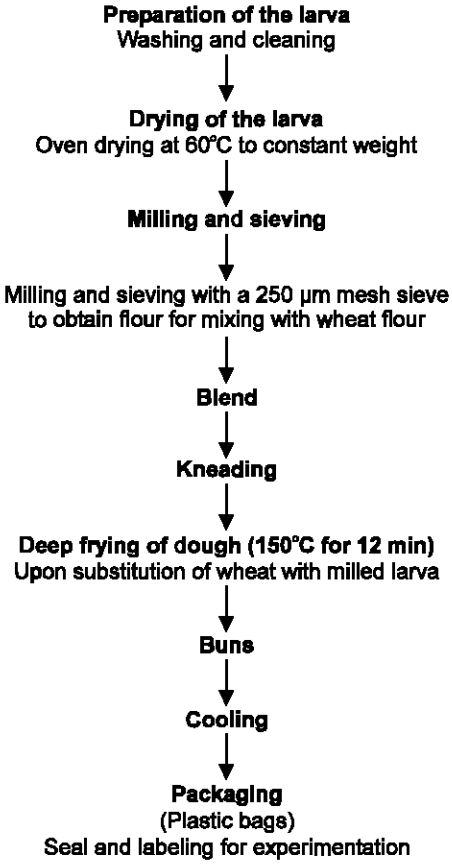

Fig. 1: Flow chart for the production of wheat-based larva enriched buns

Sensory evaluation: The sensory attributes, including colour, texture, aroma/flavour, taste and overall acceptability, were evaluated by a semi trained 20-member panel, using a 7-point Hedonic scale with 1 representing the least score (dislike extremely) and 7 the highest score (like extremely). Analysis of Variance (ANOVA) was performed on the data gathered to determine differences, while the least significant test was used to detect significant differences among the means (Ihekoronye and Ngoddy, 1985).

RESULTS AND DISCUSSION

The results of the proximate analysis are presented in Table 1.

The moisture content is quite high (60.43%), similar result was reported by (Ekpo and Onigbinde, 2005) where a moisture content of 61.5% was reported. The moisture content is an index of water activity (Olutiola *et al*., 1991) and is used as a measure of stability and susceptibility to microbial contamination (Uraih and Izuagbe, 1990). Consequently, the shelf life of the larvae can be improved upon by further processing methods such as drying, frying and roasting. The fat value of

Table 1: Proximate composition of the larvae of *Rhynchophorus phoenicis*

Nutrient	Wet weight (%)	Dry weight (%)
Moisture	60.43	-
Fat	25.72	65.00
Protein (Nx6.25)	9.96	25.16
Ash	1.91	4.82
Carbohydrate (caluted by difference)	1.98	5.02

Result represents the mean of the three determinations

(25.72%) on wet basis increased to (65.0%) on dry basis. Ekpo and Onigbinde (2005) reported a value of 66.1% on dry basis, while Banjo *et al*. (2006) reported a value of 31.4% for the larvae. Furthermore, the crude protein value of 21.56% on dry basis reported in this work shows that the larvae is a rich source of protein and this implies that its use in the diet of human can be exploited to meet the daily requirements of protein intake which is about 23-56 g (FAO/WHO/UNU, 1991; Chaney 2006). A relatively high value of 4.82% ash (dry basis) is observed when compared to other reported values of meat, meat products and egg (Watt and Merril, 1963). Similar values were obtained by Ekpo and Onigbinde (2005) and Okaraonye and Ikewuchi (2008) who reported ash contents of 5.73% and 4.71% dry basis, respectively.

Nutritional composition: the nutritional content of the wheat-larva buns and the results are presented in Table 2.

The protein content was observed to increase progressively in proportion to the percentage of larvae flour added. The larvae of *Rhychophorus Phoenicis* have been reported (Ande, 1991; Fasoranti, 1997) to be a rich source of digestible proteins able to make up for the dietary imbalance as they form real sources of food for man and other animals. Furthermore, of particular interest is the high level of leucine, lysine and threoenine reported by Ekpo and Onigbinde (2005) to be present in the insect larva. Lysine and threonine are limiting amino acids in wheat, rice, cassava and maize based diets prevalent in the developing world (Hill, 1970; Ozimek *et al*., 1985). Therefore, the inclusion of the larva into these staples would enhance the nutritional quality in these diets. The magnesium, zinc and calcium content were also noted to assume the same trend as the protein content with the highest values observed for the 15% larva flour substitution. Zinc deficiency has been known to cause poor growth and impairment of sexual development (Chaney, 1997). In addition, magnesium is needed for more than 300 biochemical reactions in the body. It helps maintain normal muscle and nerve function, keeps heart rhythm steady, supports a healthy immune blood and regulates blood sugar levels (Saris *et al*., 2000). Results of mineral content of the larvae of

Table 2: The nutritional content of the wheat-based larva buns

Rhychophorus phoenics larva content (%)	Nutrient parameter							
	Crude protein (%)	Energy (Kcal/kg)	Mg	Ca	Zn	Vit A	Vit B	Vit C
			(mg/100 g)					
0	14.7	1024	1.12	112.4	0.57	10.30	0.03	7.45
5	17.7	1130	1.48	125.6	1.07	10.63	0.04	8.75
10	19.2	1268	1.62	142.6	1.10	11.63	0.05	9.75
15	21.6	1574	2.75	152.7	1.60	12.75	0.07	10.25

Rhynchophorus Phoenicis reported by (Ekpo and Onigbinde, 2005) showed that consumption of 100 g of the larva would meet the RDA value for iron, zinc, copper, magnesium and manganese in most third world countries. Furthermore, the high content of iron and zinc in many edible insects is of particular interest. Iron deficiency is a major problem in women's diets in the developing world, particularly among pregnant women and especially in Africa (Orr, 1986). The energy content also increased in proportion to the level of larva flour added. Malnutrition in developing countries is as much or more a problem of calorie deficiency as of protein deficiency (Defoliat, 1992). Protein Energy Malnutrition (PEM) contributes to more than 50% of the deaths of children under five years all over the developing countries. The fat level presented in Table 1 implies that a 100 g sample of the larva will meet the calorific needs in most developing countries (Davidson *et al*., 1973). Similarly, there were increases recorded in the Vitamin contents with increasing larva content in the wheat-based larva buns. *Rhynchophorus phoenicis* has been reported to be a rich source of vitamins, particularly thiamin and riboflavin (DeFoliart, 1992). In each case, 100 g of these insects providing more than the minimum daily requirement.

The mean sensory scores for the wheat-based larva substituted buns and whole wheat buns are presented in Table 3.

The Analysis of Variance (ANOVA) showed that the control (0%) did not differ significantly ($p \geq 0.05$) from the 5% larva substituted buns in the sensory attributes except in colour where there was significant difference ($p \leq 0.05$) probably due to Maillard reaction which occurred during the frying process. Hence larva flour substitution at 5% in wheat-based buns was adequate for the product that would enjoy general consumer acceptance comparable to the traditional whole wheat buns. However at higher larva flour supplementation varying significant differences occurs in comparison with the control at the same probability level. The buns with 15% larva substitution had the least scores in nearly all the quality attributes evaluated and it was generally unacceptable by the consumer despite, its nutritional advantage when compared to the other buns samples. Consequently, public enlightenment is needed on the nutritional benefits of supplementation of flour with the larva of Rhynchophorus *Phoenicis* in the production of wheat based buns.

Table 3: Mean score for hedonic sensory attributes of samples

Attributes	Level of larva flour addition			
	0%	5%	10%	15%
Colour	6.1^{a}	5.3^{a}	2.9^{b}	3.0^{b}
Texture	5.15^{a}	4.9^{a}	3.4^{b}	2.7^{b}
Aroma and flavour	5.85^{a}	5.05^{ab}	4.2^{bc}	3.95^{c}
Taste	6.05^{a}	5.25^{a}	3.65^{b}	3.25^{b}
Overall acceptability	6.35^{a}	5.4^{a}	3.6^{b}	3.5^{b}

Scale: 7-point hedonic where, 1 = Dislike extremely 7 = Like extremely. Mean scores in rows with same letters are not significantly different ($p \geq 0.05$)

Conclusion: Buns produced with larva flour substitution, up to 15% were found to be nutritionally superior to that of the whole wheat buns. It has also been found that buns baked with 5% composite flour were not significantly different in most sensory attributes from the control. It is recommended that up to 5% larva flour could be adopted in buns making processes, without affecting quality adversely. The product has the potential of addressing the problems of malnutrition and food insecurity plaguing the least developed countries.

REFERENCES

Ande, A.T., 1991. Some aspects of the Biology of Insects (Lepidoptra: Saturnidae, Coleoptera: curculionidae). Nig. J. Entom., 24: 106-129.

AOAC, 1990. Methods of the Association of Official Chemists. Official Methods of Analysis, 15th Edn., Virginia Assoc. Official Analytical Chemists, USA., pp: 1141.

AOAC, 2005. Official Methods of Analysis of the Association of Official Analytical Chemists, 17th Edn., AOAC, Gaitherburg MD., USA.

Banjo, A.D., O.A. Lawal and E.A. Songonuga, 2006. The nutritional value of fourteen species of edible insects in Southwestern Nigeria. Afr. J. Biotechnol., 5: 298-301.

Bodenheimer, F.S., 1951. Insects as Human Food, W. Junk, The Hague, pp: 352.

Chaney, S.G., 1997. Principles of Nutrition II. Macronutrients. In: Textbook of Biochemistry with clinical correlations. (T.M. Devlin, Ed). Wiley-Liss Inc., New York, USA., pp: 1108-1136.

Chaney, S.G., 2006. Principles of Nutrition II Micronutrients. In: Textbook of Biochemistry with Clinical Correlation, Delvin, T.M. (Ed). John Wiley and Sons, New York, pp: 1091-1120.

Davidson, S., R. Pasmore and J.F. Brock, 1973. In: Human Nutrition and Dietetics (5th Edn.). The English language book society and Churchill Livingstone, pp: 77.

DeFoliart, G., 1992. Insects as human food. Elsevier Science (publishers), pp: 295-399

Ekpo, K.E. and A.O. Onigbinde, 2005. Nutritional Potentials of the larva of *Rhynchophorus phoenicis* (F). Pak. J. Nutr., 4: 287-290.

FAO/WHO/UNU, 1991. Energy and Protein requirements: Report of a Joint FAO/WHO/UNU Expert consultation. WHO Technical Report Series 724. http: //www.fao.org/docrep/003/aa040e/AA040 E01.htm.

Fasoranti, J.O., 1997. The value of African insects as human food supplements. J. Sci. Tech., 10: 1-5.

Hill, M.M., 1970. Food Guides-Their Development and Use. Nut. Prog. News (US Department of Agriculture). Washington DC., pp: 1-5.

Ihekoronye, A.I. and P.O. Ngoddy, 1985. Integrated food Science and Technol. for the tropics. 1st Edn., McMillan publishers, pp: 185-190.

Okaraonye, C.C. and J.C. Ikewuchi, 2008. *Rhynchophorus phoenicis* (F) larva meat: Nutritional value and health implications. J. Bio. Sci., pp: 1-5.

Olutiola, P.O., O. Famurewa and H.G. Sonntag, 1991. An Introduction to General Microbiology, A Practical Approach. 1st Edn., Heidelberger Verlagsanstalt and Druckerei GmbH Heidelberg, Germany, ISBN: 3-894-042-4.

Orr, B., 1986. Improvement of women's health linked to reducing widespread anemia. Int. Health News, 7: 3.

Ozimek, L., W.C. Sauer, V. Kozikowski, J.K. Ryan, H. Jorgensen and P. Jelen, 1985. Nutritive value of protein extracted from honey bees. J. Food Sci., 50: 1327-1332.

Saris, N.E., E. Mervaala, H. Karppanen, J.A. Khawaja and A. Lewenstam, 2000. Magnesium: An update on physiological, clinical and analytical aspects. Clinica Chimica Acta, 294: 1-26.

Uraih, N. and Y. Izuagbe, 1990. Public Health, Food and Industrial Microbiology. 1st Edn., Uniben Press: Nigeria, ISBN: 978-2027-00-6.

Watt, B.K. and A.L. Merril, 1963. Consumption of food: Raw, processed and prepared. U.S. Dept. Agric. Handbook, 8: 1-189.

Pakistan Journal of Nutrition 9 (11): 1047-1051, 2010
ISSN 1680-5194

Contribution of Nutrition Education in Dietary Habits of Overweight and Obese Females in Hathras City (U.P.)

Sushma Yadav[1] and Anita Singh[2]
[1]Department of Home Science, Sri R.D.A.K. P.G. College, Hathras, (UP) India
[2]Department of Home Science, Sri Agrasen P.G. College, Permanandpur, Varanasi, (UP) India

Abstract: The present study was carried out (1) to provide nutrition education to the overweight and obese females and (2) to evaluate impact of nutrition education on their food habit and practices of physical exercise. The data were collected with the help of "Questionnaire cum Interview Technique." The questionnaire having all relevant information was pretested and pre designed the data were collected at two phases: one at initial phase of the study before implementation of nutrition education and secondly at post phase of the nutrition education. The base line study was conducted on 300 overweight and obese females purposely selected from four areas of the city. Equal number of subjects (one hundred each) were considered from high, middle and low income groups. Due to various constraints the nutrition education was imparted to 150 subject only. The inferences were drawn with the help of suitable statistical tools. Majority of the females were vegetarian (95.33%) at the initial phase of the study, but there was no change in this habit after imparting the nutrition education ($p>0.05$NS). So for as consumptions of food items and practices of physical exercise were concerned, there were significant changes ($p<0.05$). Practicing of good food habits were developed and consumption of sweet dishes, fatty fried items and cold drinks were discouraged.

Key words: Prevalence, nutrition-education, food habit, food consumption

INTRODUCTION

Obesity senses when the energy consumption exceeds the requirement to accomplish basic metabolism and physical activities of the subject. Additional factors causing obesity are genetic disorders, hypothyroidism, medication of psychotic drugs, insufficient sleep, stress, sudden smoking caseation, sedentary life style, eating disorders and intake of high glycemic diet (Barness *et al*., 2007; Wood House, 2008; Vaharatian, 2009).

Prevalence of obesity is increasing rapidly in developed as well as in developing countries of the world. The women in the middle and menopausal age groups are becoming easy prey of the disease in comparison to male counterparts. Further the prevalence of obesity in males and females is taking the form of epidemic requiring attention of the health care provides, policy makers and administrators of the affected regions (Sidhu and Tatla, 2002; Subramanyam *et al*., 2002; Sidhu *et al*., 2005).

The principle of reduction in extra energy by the obese subjects has been proved on effective method to eliminate obesity and nutrition education is helpful in dissemination of the messages regarding various aspects of prevention of the disease. The subjects are educated to reduce energy consumption by consuming low amount of carbohydrate and sugar diet. So far as production of energy is concerned, fat is the most energy densed macro-nutrient and it provides lows satiety feeling than other nutrients. In addition its great flavour and palatability lead to heavy consumption of fatty food items causing obesity. The obese subjects are also advised to use minimum amount of fat, if not zero; use of mustered oil or unsaturated fat as alternative of saturated fat (Duvigneaud *et al*., 2007; Mohanty *et al*., 1994; Krauss *et al*., 2000; Foster *et al*., 2003; Stern *et al*., 2004; Strycher, 2006).

In view of the aforesaid descriptions, the present study was formulated to accomplish following objectives:

- To provide nutrition education to the subjects and
- To evaluate impact of nutrition education on food intake habits and practices of physical exercise.

Consequently the hypothesis "the nutrition education has significant impact on knowledge of the nutrients, food habits and physical exercises practiced by the overweight and obese females" was framed.

MATERIALS AND METHODS

The data were collected with the help of questionnaire cum interview technique. A pretested and predesigned questionnaire having all relevant information was used for this purpose. The information were collected at base line period and secondly at post phase of the nutrition education. The base line information were collected from 300 overweight and obese females aged 30-50 years. These subjects were selected from 4

Corresponding Author: Sushma Yadav, Department of Home Science, Sri R.D.A.K. P.G. College, Hathras, (UP) India

representative areas of Hathras City. Purposive sampling technique was employed for the selection of overweight and obese subjects. Due to various constraints the nutrition education was imparted to 150 overweight and obese women from high, middle and low income groups considering equal number from the each group.

Inspite of large number of nutrition education programmes going on in our country, the nutrition and health status of our population in general and particularly in overweight and obese females are unsatisfactory requiring special attention in order to achieve atmost well being of the desired subjects.

The present study highlighted that the overweight and obese females were ignored about the need of nutritious diet for themselves. Generally females did not cook special low calorie and low fat diet, but they use to take a potion of food from the meals cooked for the whole family. The females were unaware about the detrimental effects of imbalanced and insufficient food intake. It was interesting that the females knew about the fatly food but still they were not accustomed about the correct methods of preparation of zero oil and low calorie recipe which is most necessary is the preparation of recipes for the ultimate reduction of fat in the body.

Designing and implementation of the nutrition education: The nutrition education packages were designed on the basis of the information collected during baseline survey. First of all, the components of nutrition education were identified from the finding of the base line survey. In identifying these components, the positive and effective procedures were adopted in view of following facts:

- Nutrients
- Cooking demonstration
- Diet chart according to BMI on low fat, low carbohydrate and low calorie diet

Techniques of nutrition education

Diet chart according to BMI: The diet chart provided to the subjects were 1000 Kcal, 1200 Kcal; 1400 Kcal and 1600 Kcal as per suitability to the subjects in view of their BMI.

Health and fitness tips: Tips given were easiest, most effective, simplest and the most economical method to impart education from person to person particularly to the smaller groups.

Cooking demonstration of low calorie and zero oil recipes: The females were grouped in batches of 5-10 and the venue and time were decided according to convenience of the females keeping in view of their occupation in the office, college, field and their routine domestic chores.

Statistical analysis of the data: The inferences were drawn with the help of suitable statistical tools where over felt necessary.

Background information: The overall prevalence of overweight and obesity in Hathras city was ascertained 42.3%. More then two fifth females from all the categories belonged to age group 45 to 50 years and meant±SD values of the age were assessed 42.5±5.77 years for overweight and 42.5±5.43 years for obese females. Nearly three fifth overweight females (57.14%) possessed university education, whereas 37.54% obese females were secondary educated. Majority of the obese (88.74%) females were housewives, while more than half (57.14%) overweight females belonged to business class. In addition majority of the subjects were Hindu.

RESULTS AND DISCUSSION

Effect of nutrition education has been evaluated in order to provide feed back to the beneficiaries. The data of the post-nutrition education period were compared with pre education period and the effect of nutrition education programme was worked out.

Change in food habit: As majority of the females (95.33%) were vegetarian at the initial phase of the study (Table 1), but this habit was not changed at the final stage (93.33%). The nutrition education has no impact to change the food habit of the females ($Chi^2 = 0.792$, df = 1, p>0.05NS). It was observed that there was change in meal requirement of the females. There were 97.33% females at part intervention period against 92.00% at pre-intervention phase who required meal after feeling hungry. The change was found statistically significant (z = 2.605, p<0.01**). In addition feeling hunger during tension was significantly deteriorated ($Chi^2 = 9.968$, df = 2, p<0.01**); drinking tea or coffee was reduced ($Chi^2 = 11.056$, df = 1, p<0.01**). Liking for sweets ($Chi^2 = 13.996$, df = 3, p<0.01**); use of cold drink ($Chi^2 = 9.911$, df = 2, p<0.025**); nibbling between meals (z = 7.358, p<0.001***); participation in lunch party outside of the house and taking meal (z = 9.068, p<0.001***) were significantly changed after the nutrition education. Further habit of taking meal slowly ($Chi^2 = 12.811$, df = 1, p<0.001***) and taking meal with family members ($Chi^2 = 6.499$, df = 2, p<0.05*) were significantly enhanced. Instead of throwing left out foods, it was given to needy and poor hungry persons ($Chi^2 = 129.468$, df = 2, p<0.001***) and it was the best utilization of the left out foods.

Above mentioned food practices regarding food consumption were developed in the subjects due to nutrition education proved to them. Further use of salad (Table 1) increased significantly from 68.00-78.67%

Table 1: Food habit of overweight and obese females during pre and post implementation period of nutrition education

	Period of nutrition education						
	Pre (300)		Post (150)		Total (450)		
Food habit	No.	%	No.	%	No.	%	Statistical significance
Vegetarian	286	95.33	140	93.33	426	94.67	Chi^2 = 0.792, df-1, p>0.05NS
Non-vegetarian	14	4.67	10	6.67	24	5.33	
Meal requirement after feeling hungry	276	92.00	146	97.33	422	93.78	z = 2.605, p<0.01**
Feeling hunger during tension							
• Absolutely no.	200	66.67	99	66.00	299	66.44	Chi^2 = 9.968, df = 2, p<0.001***
• Less hunger	34	11.33	31	20.67	65	14.44	
• More hunger	66	22.00	20	13.33	86	19.11	
Drinking tea or coffee							
• Once daily	56	18.67	37	24.67	93	20.67	Chi^2 = 11.056, df = 2, p<0.01**
• Twice daily	179	59.67	99	66.00	278	61.78	
• More than two times	65	21.67	14	9.33	79	17.56	
Consumption of salad	204	68.00	118	78.67	322	71.56	z = 2.485, p<0.02**
Consumption of fruit							
• Daily	70	23.33	39	26.00	109	24.22	Chi^2 = 176.428, df = 3, p<0.001***
• Twice a week	37	12.33	102	68.00	139	30.89	
• Once a week	127	42.33	6	4.00	133	29.56	
• Never	66	22.00	3	2.00	69	15.33	
Habit of breakfast							
• Daily	83	27.67	44	25.33	127	28.22	Chi^2 = 31.392, df = 2, p<0.001***
• Some times	125	41.67	94	62.67	219	48.67	
• Never	92	30.67	12	8.00	104	23.11	
Frequency of meal							
• Two times daily	295	98.33	129	86.00	424	94.22	Chi^2 = 27.941, df = 1, p<0.001***
• Three times daily	5	1.67	21	14.00	26	5.78	
Liking for sweets							
• No	112	37.33	70	46.67	182	40.44	Chi^2 = 13.996, df = 3, p<0.01**
• Some times	104	34.67	59	39.33	163	36.22	
• Once a week	58	19.33	19	12.67	77	17.11	
• Twice a week	26	8.67	2	1.33	28	6.22	
Use of cold drink							
• Mattha or lemon water	36	12.00	21	14.00	57	12.67	Chi^2 = 9.911, df = 2, p<0.025**
• Soft drink	61	20.33	13	8.67	74	16.44	
• Nothing	203	67.67	116	77.33	319	70.89	
Frequency of cold drink							
• Nil	203	67.67	116	77.33	319	70.89	Chi^2 = 4.586, df = 2, p>0.05NS
• Once a day	72	24.00	26	17.33	98	21.78	
• Twice a day	25	8.33	8	5.33	33	7.33	
Nibbling between meals	98	32.67	11	7.33	109	24.22	z = 7.358, p<0.001***
Taking meal outside of the house	88	29.33	4	2.67	92	20.44	z = 9.068, p<0.000***
Participation in lunch party out side of the house							
• Professional or service related	31	10.33	Nil		31	6.89	-
• Kitty party	12	4.00	Nil		12	2.69	
Use of left out food							
• Eat	121	40.33	12	8.00	133	29.56	Chi^2 = 129.468, df = 2, p<0.001***
• Thrown away	104	34.67	16	10.67	120	26.67	
• Give to other	75	25.00	122	81.33	197	43.78	
Speed of taking meal							
• Fast	130	43.33	39	26.00	169	37.56	Chi^2 = 12.811, df = 1, p<0.001***
• Slow	170	56.67	111	74.00	281	62.44	
Companion during taking meal							
• Alone	135	45.00	62	41.33	197	43.78	Chi^2 = 6.499, df = 2, p<0.05*
• With family members	141	47.00	84	56.00	225	50.00	
• During viewing TV	24	8.00	4	2.67	28	6.22	

N.B. Number of subjects is given in parenthesis; *Just significant; **Moderately significant; ***Highly significant; NS = Statistically insignificant (Not significant)

after the education programme (z = 2.485, p<0.02**). Even the use of fruits daily (26.00%) or twice a week (68.00%) elevated to this level from 23.33% (daily) and 12.33% (twice a week) respectively. This finding showed significant change in the habit of using fruits (Chi^2 = 176.428, df = 3, p<0.001***). In an earlier study Srivastava and Madhu (2005) suggested low carbohydrate diet in management of obesity by providing adequate quantity of salads and non-starchy vegetables. Davigneaud *et al.* (2007) mentioned that energy context of fibre per unit weight is low. Consequently, inclusion of fibre in a diet reduced energy density. Dietary fibre tends to reduce dietary intake by slowing digestion and absorption of nutrients and by increasing the production of gut hormones enhancing satiety feeling. Moreover, some types of fibre reduce the overall absorption of fat and protein.

Initially, nearly one third females (30.67%) were not taking breakfast (Table 1), but this habit was reduced to 8.00% at post phase of the nutrition education. More than three fifth females (62.67%); followed by 29.33% used to take breakfast frequently or daily at post phase instead of 41.67 and 27.67% respectively from the initial phase (Chi^2 = 31.392, df = 2, p<0.001***). Huenemann *et al.* (1996) noted a reduction in the number of breakfast eaten by the obese subjects.

The frequency of food consumption plays on important role in the genesis of obesity. It is known that taking one meal per day opposed to two or three has metabolic consequences independent of calorie intake. Epidemiological studies have shown a clear negative correlation between number of meals and obesity, therefore the meals, the greater the tendency toward obesity (Fabray *et al.*, 1966). The frequency of eating also changes the metabolism of glucose and concentration of Cholesterol. Cohn (1964) found that when normal volunteers ate several small meals a day, they had lower concentrations of cholesterol than when the same total intake was eaten in a few large meals. This reduction of cholesterol with frequent ingestion of small meals has been confirmed in other studies (Young *et al.*, 1972). Glucose tolerance curve were also improved when eating three or more meals as compared with one or two large meals. In brief it can be said that frequency of eating is inversely related with obesity Table 1 also shows that overwhelming majority of the overweight and obese subjects (98.33%) consumed meal two times in a day; followed by three times in a day before implementation of nutrition education. The re after providing nutrition education to these females, 1400% of them used to take meal three times daily. The figured showed that 12.33% more females adopted three times meal pattern instead of two times a day. This quality of meal diversion is found statistically significant (Chi^2 = 27.941, df = 1, p<0.001***). This habit will certainly prove a green signal towards improvement of obesity in the females.

Traditional life styles are generally associated with gain in body weight with age. However, modernization apparently has profound effect on body weight. Rapid urbanization and industrialization change in life styles and eating habits. Sedentary occupation contribute to energy imbalance. A sedentary life style favours a positive energy balance and weight gain. The relationship between physical inactivity and obesity however complex, several confounders are likely to complicate the picture such as physical fitness, opportunities for exercise, diet and temporal relationships between exercise and meals.

In the present study, it was observed that initially 59.00% subjects did not perform any kind of physical exercise, while after nutrition education 14.00% more females were encouraged to perform some sorts of physical exercise (Table 2). Consequently at post implementation period , walking, yoga, cycling and gym were performed

Table 2: Type and frequency of physical exercise practiced by the females during pre and post period of implementation of nutrition education

Type and frequency of physical exercise	Period of nutrition education: Pre (300) No.	Pre (300) %	Post (150) No.	Post (150) %	Total (450) No.	Total (450) %	Statistical significance
Type ≠							
Walking	69	23.00	40	26.67	109	24.22	Chi^2 = 7.531, df = 1, p<0.01**
Yoga	72	24.00	58	38.67	130	28.89	
Cycling and gym	3	1.00	4	2.67	7	1.56	
Sports	5	1.67	2	1.33	7	1.56	
Dancing	1	0.33	-	-	1	0.22	
None	177	59.00	68	45.33	245	54.44	
Frequency							
Daily	93	31.00	57	38.00	150	33.33	Chi^2 = 15.101, df = 3, p<0.01**
Five days in a week	2	0.67	8	5.33	10	2.22	
Two days in a week	28	9.33	17	11.33	45	10.00	
Never	177	59.00	68	45.33	245	54.44	

≠ Multiple responses were obtained; Number of subjects is given in parenthesis

by 26.67, 38.67 and 2.67% females in comparison to 23.00, 24.00 and 1.00% females at pre implementation period. The statistical analysis evidenced that there was significant increase in physical activities due to implementation of nutrition education programme (Chi^2 = 7.531, df = 1, p<0.01**). Even the frequency of physical activities were also increased (Chi^2 = 15.101, df = 3, p<0.01**). The frequency of physical activities at post intervention period reached to 38.00% for daily; 5.33% for five days in a week and 11.33% for two days in a week from 31.00, 0.67 and 9.33% respectively at pre intervention period. Sahlin *et al*. (2008) mentioned that with physical exercise muscles consume energy derived from both fat and glycogen. Due to large size of leg muscles walking, running and cycling are the most effective means of exercise to reduce body fats exercise effects macronutrient balance. During moderate exercise, there is a shift to greater use of fat as a fuel.

Conclusion: In the nut shell, the role of nutrition education was found significantly effective to enhance physical exercise (regular walking and practicing yoga); practicing good food habits along with adequate use of salad, fruits and green leafy vegetables; habit of taking breakfast regularly and required quantity of diet in more frequency by avoiding sweet dishes, fatly fried items and cold drinks.

REFERENCES

Barness, L.A., J.M. Opitz and G. Barness, 2007. Obesity: Genetic molecular and environmental aspect. Am. J. Med. Genel. A, 143 A: 3016-34.

Cohn, C., 1964. Feeding patterns and some aspects of cholesterol metabolism. Fed. Proc., 23: 76-81.

Duvigneaud, N., K. Wigndaele, L. Malton, R. Phillippaerts, J. Lefevre, M. Thomis, C. Delecluse and W. Diquet, 2007. Dietary factors associated with obesity indicators and levels of sports participation in flemish adults: A cross sectional study. Nutr. J., 6: 26.

Fabray, P., S. Hejda, K. Cerney, K. Osanoova and J. Peacher, 1966. Effect of meal frequency in school children: Changes in weight, height proportion and skinfolds thickness. Am. J. Clin. Nutr., 18: 358-361.

Foster, G.D., H.R. Wyatt and J.R. Hill, 2003. A randomized trial of a low carbohydrate diet for obesity. N. Engl. J. Med., 348: 2082-2090.

Huenemann, R.L., M.C. Hampton, L.R. Shapiro and A.R. Behnke, 1996. Adolescent food practices associated with obesity. Fed. Proc., 25: 4-10.

Krauss, R.M., R.H. Ecked and B. Howard, 2000. AHA dietary guide lines: Rev. 2000, A statement for health care professionals from the Nutrition Committee of the American Heart Association. Circulation, 102: 2284-2299.

Mohanty, N., S.C. Mohapatra, P. Srivastava and J.N.P. Gupta, 1994. The measurement of performance skills of primary maternal child health workers: An Indian Experiment. In. J. Com. Med., 19: 59-63.

Sahlin, K., E.K. Sallstedt, D. Bishop and M. Tonkonogi, 2008. Turning down lipid oxidation during heavy exercise-what is the mechanism? J. Physiol. Pharmacol., 59 (Suppl): 19-30.

Sidhu, S. and H. Tatla, 2002. Prevalence of overweight and obesity among adult urban females of Punjab. A cross sectional study. Anthropologist (Special issue) I: 101-103.

Sidhu, S., A. Kaur and Prabhajit, 2005. Prevalence of overweight and obesity among urban and rural adult females of Punjab. Pub. Med. U.S. National Library of Medicine, 63: 341-345.

Srivastava, S. and S.V. Madhu, 2005. Low carbohydrate in management of obesity-A Short Review. Int. J. Diab. Dev. Countries, 25: 42-45.

Stern, L., N. Iqbal and P. Sheshadri, 2004. The effects of low corbohydrate versus conventional weight loss diets in severely obese adults : One year follow up of a randomized trial. Ann. Intern. Med., 140: 778-785.

Strycher, I., 2006. Diet in the management of weight loss. CMAJ, 174: 56-63.

Subramanyam, V., R. Jayashree and M. Rafi, 2002. Prevalence of overweight and obesity in affluent adolescent girls in Chennai in 1981 and 1998 from the Kanchi Kama Koti Childs Trust Hospital, 12-A Tamil Nadu (India).

Vaharatian, A., 2009. Prevalence of overweight and obesity among women of child bearing age. Matern. Chi. Hlth. J., 13: 268-273.

Wood House, R., 2008. Obesity in Art; A brief overview. Front Horn Res., 36: 271-86.

Young, C.M., L.F. Hutter, S.S. Scenlan, C.E. Rand, L. Lutwek and V. Simko, 1972. Metabolic effects of meal frequency on normal young men J. An. Diet. Assoc., 61: 391-398.

Pakistan Journal of Nutrition 9 (11): 1052-1057, 2010
ISSN 1680-5194

Total Antioxidant Capacity, Nutritional Composition and Inhibitory Activity of Unripe Plantain (*Musa paradisiacae*) on Oxidative Stress in Alloxan Induced Diabetic Rabbits

C.O. Eleazu[1], P.N. Okafor[2] and Ikpeama Ahamefuna[1]
[1]Department of Biochemistry, National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria
[2]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria

Abstract: The antioxidant composition of unripe plantain and its free radical scavenging activity on alloxan induced diabetic rabbits and on DPPH radical was investigated. 10 male rabbits weighing between 1.58 and 1.88 kg were used for this study. Diabetes was induced in the experimental rabbits with alloxan (35 mg/kg body weight. ip). Group 1 rabbits served as the control groups and they received normal rabbit feeds. Group 2 rabbits were the animals of group 1 which were made diabetic by the injection of alloxan and they also received normal rabbit feeds while group 3 rabbits (test groups) were the animals of group 2 which were later fed with unripe plantain at a dosage of 25 kg/kg body weight/day for 4 weeks. The duration of the experiment was 7 weeks and the weights of the animals in each group were recorded daily throughout the experiment while the blood glucose levels, malonaldehyde, catalase and glutathione were recorded on a 2 weeks interval. The results show that the diabetic rabbits placed on unripe plantain diet had an increase in their body weights, glutathione and catalase levels but a decrease in malonaldehyde and blood glucose levels after 4 weeks of unripe plantain intake when compared with the control ($p<0.05$). Correlation analysis carried out revealed that glutathione correlated negatively with malonaldehyde and glucose ($r = -0.77$ and -0.89), but positively with catalase and body weight ($r = 0.60$ and 0.70). Malonaldehyde correlated negatively with catalase and body weight ($r = -0.44$ and -0.72) but positively with glucose ($r = 0.86$). The antioxidant composition of the methanolic extracts of the unripe plantain flour as determined by the quantities of peroxidase and quercetin present was 52±0.00% peroxidase and 5.32 ug/ml quercetin while its free radical scavenging activity on DPPH radical was 78.57±0.00%. Analysis of the proximate and phytochemical composition of the unripe plantain flour showed that it contained 3.16±0.04% protein, 0.21±0.003% lipid, 52±2.82% moisture, 5.5±0.42% ash, 1.58±0.04% tannin, 1.82±0.05% saponin, 1.37±0.05% alkaloid and 0.98±0.00% flavonoid. These findings suggest that raised blood glucose level in diabetics could deplete cells of their antioxidant status by decreasing the glutathione and red cell catalase levels leading to an increased malonaldehyde level which is a marker of oxidative stress. In addition, unripe plantain intake by a diabetic could exert a free radical scavenging activity by restoring the altered antioxidant status since itself could serve as a natural source of antioxidants.

Key words: Diabetes, nutritional composition, rabbits, unripe plantain flour, free radical scavenger, oxidative stress, total antioxidant capacity

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases with hyperglycemia. World wide, an estimated 150 million people are affected by diabetes mellitus (Expert committee on the diagnosis and classification of diabetes mellitus, 1998) and this number is likely to reach 300 million by the year 2025 if successive strategies are not implemented for its prevention and control (King *et al*., 1999).

In recent studies, some evidence suggest that oxidative stress may play some role in the etiology of diabetes and its complications (Shin, 1998). Nourooz-zadeh *et al*. (1997) has reported an altered balance between Reactive Oxygen Species (ROS) production and antioxidants.

Though insulin therapy is used for the management of the disease, there are still draw backs like insulin resistance (Piedrola *et al*., 2001), as well as of its high cost which are not affordable in the poor economic community. Treatment with sulphonylureas and biguanides are also associated with side effects (Rang *et al*., 1991).

In India, use of herbal drugs based on *Ayurvada* has been commonly practiced for a long time and it is less

Corresponding Author: O. Eleazu Chinedum, Department of Biochemistry, National Root Crops Research Institute, Umudike, Umuahia Abia State, Nigeria

expensive. The herbal drugs are considered to be less toxic with fewer side effects when compared with synthetic drugs (Geetha *et al*., 1996; Rao *et al*., 2003).
In addition, dietary management of diabetes has been helpful and such diets used in the management of diabetes include beans, breadfruit and in Nigeria, unripe plantain diet.
However the mechanism by which unripe plantain flour ameliorates diabetes mellitus has not been fully investigated. There's indication that this could be through antioxidant activity since some of the phytochemical constituents could serve as antioxidants. Also the unripe plantain could have a low glycaemic response when consumed. This present work is aimed at investigating the above.

MATERIALS AND METHODS

Chemicals: Quercetin and DPPH (2,2-diphenyl-1-picrylhydrazyl) used were products of Sigma Chemical Company (UK). Peroxidase used was purchased from Horseradish. All other chemicals used were purchased from Associated Laboratories, Aba, Abia State, Nigeria.

Plant materials: Unripe plantain used was bought locally from the market in Umuahia, Abia State, Nigeria. It was thoroughly washed, peeled and freeze dried in a freeze drier for 48 h.

Preparation of plant materials for analysis: The peeled portion of the unripe plantain was ground into flour using a food processor and the flour was then used for analysis.

Proximate composition of unripe plantain flours: Moisture, crude protein, crude fat and total carbohydrates were analyzed according to the AOAC methods (1990). The values reported are means of triplicate samples with their standard deviations.

Phytochemical composition of unripe plantain flour: The gravimetric method of Harbone (1973) was used in the determination of the total alkaloid content while the AOAC method (1984) was used in the determination of other phytochemical constituents of the sample.

Assay of DPPH radical scavenging activity: The free radical scavenging activity of the plantain extract was determined using the modified method of Blois (1985). 1 ml of different concentrations (500, 250, 125, 62.5, 31.25 ug/ml) of extracts and standard quercetin were added to 1 ml of 0.3 mm DPPH in methanol to bring the final concentration of 250, 125, 31.25 and 15.62 ug/ml. The mixture was vortexed and incubated in a dark chamber for 30 min and the absorbance read at 517 nm against a DPPH control which contained 1 ml of methanol.

The Percentage Inhibition was calculated as:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times \frac{100}{1}$$

Assay of total antioxidant activity: The total antioxidant activity was measured according to the method described by Hsu *et al*. (2003). 0.2 ml of peroxidase + 0.2 ml of H_2O_2 (50 um) + 0.2 ml ABTS (100 um) + 1 ml distilled water were mixed together and left in the dark to form a bluish green complex.
After adding 1 ml of methanolic plantain flour extract, the absorbance was measured at 734 nm to represent the total antioxidant activity.

Animal experiments

Selection of animals and their care: 10 matured rabbits weighing between 1.58 and 1.88 kg were used for this experiment. Animals were acclimatized for a period of 7 days to the laboratory conditions prior to the experiment. Rabbits were housed in colony cages with 2 rabbits per cage at room temperature with 12 h light and dark cycle and they had free access to drinking water and their diets.

Chemicals: Alloxan used was obtained from Sigma and Aldrich. Malonaldehyde derivative (1,1,3,3 tetraethoxypropane) and Stock Glutathione used were also obtained from Sigma and Aldrich Chemical Company, UK. All other chemicals used for the animal experiments were bought from Associated Laboratories, Aba, Nigeria and were of analytical grade.

Induction of diabetes: Rabbits were fasted for 24 h before injection of a freshly prepared solution of alloxan intra-peritoneally at a dosage of 35 mg/kg body weight. This single dose of alloxan produced type 1 diabetes having fasting blood sugar level of 155±10.71 mg/dl after 10 days of injection of alloxan and this diabetic state was maintained throughout the duration of the experiment.

Experimental procedure

The rabbits were divided into 3 groups as follows:
(Group 1) Control group: The animals of this group received normal rabbit feeds. After feeding them for about 1 week, their body weights and fasting blood sugar levels were taken. Other parameters which included glutathione, malonaldehyde and whole blood catalase levels were also taken and recorded.

(Group 2) Diabetic rabbits without unripe plantain feed: At the expiration of 1 week, alloxan was injected intraperitoneally into the control group and they formed group 2 animals. The animals were confirmed diabetic after estimation of their fasting blood sugar level, 2 weeks after injection of alloxan. An animal was

considered to be diabetic if it had a fasting blood sugar level > 115 mg/dl. Other parameters which included body weight, catalase, plasma glutathione and malonaldehyde were also taken and recorded.

(Group 3) Diabetic rabbits after unripe plantain feed: At the expiration of 2 weeks of induction of diabetes into the animals of group 2, they were force fed with unripe plantain flour for a period of 4 weeks and they thus formed the animals of group 3. At the end of 4 weeks, their fasting blood glucose levels was estimated and recorded. Other parameters which included body weight, catalase, glutathione and malonaldehyde levels were also taken and recorded.

Determination of plasma malonaldehyde (MDA): The method of Health and Parker (1968) was used with slight modification. 0.2 ml of blood plasma was added to 3 ml of glacial acetic acid followed by 3 ml of thiobarbituric acid solution. The mixture was placed in boiling water for 15 min, allowed to cool before being read spectrophotometrically at 532 nm.

Preparation of thiobarbituric acid (TBA): 2% Sodium hydroxide was prepared by dissolving 2 g of sodium hydroxide in 100 ml of water. Then 1% TBA was prepared by dissolving 1 g of TBA in the 100 ml of the 2% sodium hydroxide. The standard curve was plotted using the MDA derivative (1,1, 3, 3 tetraethoxypropane).

Determination of whole blood glutathione: The principle was based on the determination of reduced glutathione in each dilution by the measurement of the absorbance of colored solution developed within 5 min of the generation of Elman's reagent at 430 nm wavelength.

Determination of plasma glucose: The principle of oxidation of β-D glucose to β-D glucono 1, 5 lactone with the release of hydrogen peroxide by glucose oxidase which later hydrolyses gradually to β-D gluconic acid was employed. The absorbance of the mixture was measured at 625 nm using ortholidine as the color reagent.

Determination of red cell catalase activity: The principle of Cohen *et al.* (1970) was made use of here by monitoring the rate of enzyme catalyzed decomposition of hydrogen peroxide (H_2O_2) using Potassium tetraoxomanganateVII ($KMnO_4$). 50 microlitre of sample was added to a test tube. H_2O_2 was then added to the tube and incubated on ice for 3 min. H_2SO_4 was used to stop the reaction. Finally, $KMnO_4$ was added and the absorbance recorded at 480 nm. In this assay,

$$1 \text{ unit of enzyme activity} = \frac{K}{0.00693}$$

where

$$K = \frac{So}{S_2} \times \frac{2.3}{t}$$

Where So = Absorbance of standard-absorbance, S_2 = Absorbance of standard-absorbance of sample. T = Time interval. The measured activities were normalized with the protein content of each sample.

Statistical analysis: Statistical analysis was conducted using the mean ± standard deviation of three experiments. The experimental design used was Completely Randomized Design while results were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Proximate composition of unripe plantain flour: In the study carried out, the proximate composition of the locally consumed unripe plantain flour showed that it contained low quantities of ash which reflected the mineral contents of the plantain (Table 1). Plantains have been reported to contain low quantities of minerals (Ketiku, 1973).

Table 1: Proximate composition of unripe plantain flour

Proximate analyzed	Percentage composition
Ash	5.50±0.420
Carbohydrate	39.14±0.212
Protein	3.15±0.042
Lipid	0.21±0.028
Moisture	52.0±2.8

Each value in the table was obtained by calculating the mean ± std of 3 experiments carried out on the unripe plantain

The low fat contents obtained in the unripe plantain flours (Table 1) were in accordance with previous reports (Agunbiade *et al.*, 2006).

The low crude protein content obtained in the plantain flours (Table 1) were also in accordance with previous studies (Brakohiapa *et al.*, 2001). Since a healthy adult needs about 0.75 g of protein per kg per day, plantains alone cannot meet adult protein diet.

The low total carbohydrate obtained in the unripe plantain flour would be expected since unripe plantain contains large amount of starch and low sugar in its green stage (Table 1). Similar results have been reported by Ahenkora *et al.* (1998).

The moisture content was also found to be high and this is in agreement with earlier reports (Ketiku, 1973).

Phytochemical composition of unripe plantain flour: The phytochemical composition of the unripe plantain flour showed that it contained significant quantities of saponins, flavonoids, tannin and alkaloids (Table 2).

Saponins are known to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic

permeabilization of the intestine) properties (Price *et al*., 1987). However, the levels of saponin in the flour are quite too low to cause any deleterious effects.

Flavonoids, alkaloids and tannins are polyphenolic compounds with antioxidant properties. Phenolics have been associated with antioxidant properties of food (Robbins, 2003). It has been reported that phenolic compounds in plants possess antioxidant activity and may help protect cells against the oxidative damage caused by free radicals (Kirkosyan *et al*., 2003).

The present study shows that unripe plantain flour contains considerable amount of phenolics and this implies that it may be useful in relation to diseases involving free radical reactions.

Antioxidant activity of unripe plantain flour: The antioxidant activity of the methanolic extract of unripe plantain flour as determined in this study is presented in Table 3. The extract of the unripe plantain flour showed a remarkable antioxidant activity and this would be expected since analysis showed that it contained phenolics and phytochemicals which are high potency antioxidants with free radical scavenging activities. The results obtained show unripe plantain flour to be a potential natural source of antioxidants that could be of medicinal purposes in the treatment of ailments implicating free radicals and oxidative stress.

Table 2: Phytochemical composition of unripe plantain flour

Phytochemical	Percentage composition
Tannin	1.577±0.004
Alkaloid	1.37±0.048
Saponin	1.827±0.0042
Flavonoid	0.981±0.0014

Each value in the table is the average of 3 experiments ± standard deviation

Table 3: Total antioxidant activity of unripe plantain flour

Antioxidant	Activity
Peroxidase	52±0.00%
Quercetin	5.32ug/ml

The results are the means of triplicate experiments ± standard deviation

Table 4: Inhibitory activity of unripe plantain on DPPH radical

Free radical	Percentage inhibition
DPPH	78.57±0.06

The value in the table was derived by calculating the average of 3 experiments ± standard deviation

Inhibitory activity of unripe plantain flour: The high scavenging activity of the methanolic extract of the unripe plantain flour on DPPH radical is a major significant finding in this study (Table 4). This is attributable to the phenolic content and presence of other phytochemicals in the unripe plantain. However, we could not prove if the free radical scavenging activity came solely from the phenols present or other phytochemicals or a combination of both.

Animal experiments: There was a significant reduction in the reduced glutathione levels of the diabetics when compared with the control (Table 5). This depletion in blood glutathione is attributable primarily to the alloxan injected in the rabbits, a xenobiotic and an inducer of diabetes.

Both xenobiotics and normal metabolism are known to deplete antioxidants as they are consumed in the course of scavenging reactive species generated. The depletion in glutathione to the level that was observed in this work could lead to a devastating decrease in the total antioxidant status of the animals because glutathione helps in recycling cellular antioxidants, inhibits free radical damage and plays a key role in the detoxification of harmful compounds (Robert *et al*., 2000). This agrees with earlier works carried out by Dominquez *et al*. (1998) and Polidori *et al*. (2000) who reported reduced total plasma antioxidant capacity in uncontrolled diabetes. However, unripe plantain intake by the diabetic rabbits increased their glutathione status to near the control level and this is remarkable as this implies that unripe plantain diet could have an ameliorating effect on the altered antioxidant status of a diabetic.

The concentration of plasma Malonaldehyde (MDA) was shown to be significantly increased in diabetic rabbits without unripe plantain when compared with the control (Table 4). This was also attributed to the alloxan that was injected into the rabbits. Ceriello *et al*. (1998) have reported that diabetic patients show during the postprandial period, an increase in plasma malonaldehyde levels. However, the diabetic rabbits, when placed on unripe plantain diet also remarkably had a decrease in their plasma malonaldehyde levels (Table 5) when compared with the control, thus indicating the free radical scavenging activity of unripe plantain on oxidative stress in diabetics.

Table 5: Comparison of some parameters of oxidative stress in alloxan induced diabetic rabbits before and after unripe plantain intake

Parameter	Non-diabetic rabbits (Control)	Diabetic rabbits b/f unripe plantain intake	Diabetic rabbits after unripe plantain intake	p-value
GSH (mg/ml)	61.08±11.16	27.09±3.18	48.09±9.39	<0.05
MDA (mg/ml)	0.115±0.02	0.437±0.15	0.129±0.02	<0.05
Catalase (umol/min/ml)	51.78±11.15	155.8±10.71	68.4±12.58	<0.05
Glucose (mg/dl)	55.51±12.65	155.8±10.71	68.4±12.58	<0.05
Weight (kg)	1.73±0.15	0.86±0.05	1.317±0.098	<0.05

Reported values are the means ± standard deviations (n = 10). NS = Not Significant; S = Significant; GSH = Glutathione; MDA = Malonaldehyde; b/f = before

The depletion of whole blood catalase activity after injection of alloxan is another significant finding in this study (Table 5). The decreased concentration of red cell catalase is attributable in part to the reduced synthesis of this antioxidant enzyme (which functions in the detoxification of hydrogen peroxide) whose concentrations would have fallen with the alloxan that was injected into the animals. Some studies have reported no alterations in the activity of red cell catalase in diabetics (Dohi *et al*., 1998). However, this is in agreement with earlier reports by Udoh *et al*. (2007) and Tagami *et al*. (1992) who reported a decreased red cell catalase activity in diabetics. It is important to note at this point that the drastic decrease in the antioxidant status of the body could precipitate "oxidant stress" with a concomitant attack of reactive oxygen species or free radicals on cells of some target tissues or organs of the body. However, the diabetic rabbits placed on unripe plantain diet had an increase in their catalase concentrations and this again indicates the ability of unripe plantain diet to restore the altered antioxidant status of diabetics.

The concentration of fasting blood glucose was increased in the alloxan induced diabetic rabbits. Alloxan is known to destroy the β-cells of the islets of the langerhams of the pancreas that function in the regulation of insulin secretion and thus leads to an increase in the concentration of blood glucose. However, this parameter was decreased significantly in the diabetic rabbits placed on unripe plantain diet. This is in agreement with earlier works done by Gomathy *et al*. (1990) who reported a hypoglycemic action of the pectin present in the juice of plantain. Chhanda *et al*. (2006) have also reported that diminished serum insulin level in streptozotocin induced diabetic rats was recovered significantly after co-administration of methanolic extracts of *Euglena Jambolana* and *Musa Paradisiaca*. They noted that the fasting blood sugar level came towards the control level gradually after supplementation of the seed of *E. jambolana* and *M. paradisiaca* in separate ways.

The alloxan induced diabetic rabbits had a marked loss in body weight (Table 5). This would be expected as one of the effects of diabetes is body weight loss. With the destruction of the pancreatic cells by alloxan, there's deficiency of insulin leading to increased synthesis of ketone bodies which are excreted in urine. The increased synthesis of ketone bodies coupled with increased lipolysis leads to a severe body weight loss. However, the diabetic rabbits placed on unripe plantain diet had a remarkable gain in body weight (Table 5).

Correlation analysis carried out revealed that glutathione correlated negatively with malonaldehyde and glucose (r = -0.77 and -0.89), but positively with catalase and body weight (r = 0.60 and 0.70). Malonaldehyde correlated negatively with catalase and body weight (r = -0.44 and -0.72) but positively with glucose (r = 0.86). The free radical scavenging activity of unripe plantain in diabetics has been demonstrated in this study. There's indication that unripe plantain flour mimics insulin action by binding to specific receptors in the cell membrane of tissues (in a way similar to insulin) possibly at the α-subunits of receptors that are transduced to the β-subunits, promoting the rapid autophosphorylation of a specific tyrosine residue of each β-subunit and inducing a conformational change, one of which is decreased oxidative stress through the restoration of altered antioxidant status. This is approached from a biochemical point of view and is subject to further confirmation.

In addition, it has been found to be a good source of antioxidants and this property could be included to the purpose for which its been utilized in diabetics.

ACKNOWLEDGEMENT

The authors are grateful to Mr Arukwe of Biochemistry Department, Abia State University Uturu, Mr Egbuedo of Soil Science Department, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State and Dr and Mrs I.N. Eleazu who contributed in one way or the other to make this work a success.

REFERENCES

Agunbiade, S.O., J.O. Olanlokun and O.A. Olaofe, 2006. Quality of chips produced from rehydrated dehydrated plantain and banana. J. Nutr., 5: 471-473.

Ahenkora, K., M.A. Kyei, E.K. Marfo and B. Banful, 1998. Nutritional composition of false horn Apantuba plantain during ripening and processing. J. Food Chem., pp: 455-458.

Association of Official Analytical Chemists, 1990. Official Methods of Analysis. W Horwitz (Ed) 13th Edn., pp: 233-234.

Association of Official Analytical Chemists, 1984. Official Methods of Analysis. 14th Edn., pp: 242-245.

Agunbiade, S.O., J.O. Olanlokun and O.A. Olaofe, 2006. Quality of chips produced from rehydrated dehydrated plantain and banana. J. Nutr., 5: 471-473.

Blois, M.S., 1985. Antioxidant determination by use of stable free radicals. Nature, 29: 1199-1200.

Brakohiapa, L.A., I.K. Quaya, A.G. Amoah, E.K. Harrison, D.O. Kennedy, Y. Kido and E. Ofei, 2001. Noguchi Memorial Institute for Medical Research. University of Ghana Region, Accra, pp: 220-221.

Ceriello, A., S. Lizzio, N. Bortolotti, A. Russo, E. Mortz, L. Tonutti, A. Crescentini and C. Taboga, 1998. Meal generated oxidative stress in type 2 diabetic patients. Diabetes Care, 21: 1529-1533.

Chhanda, M., M. Rajkumar and G. Debidas, 2006. Comparative study on the antihyperglycemic and antihyperlipidemic effects of separate and composite extracts of seed of *Euglena Jambolana* and root of *Musa Paradisiaca in* streptozotocine induced diabetic male albino rats. J. Pharmacol. Therapeutics, 85: 27-33.

Cohen, G., D. Dembiec and J. Marcus, 1970. Measurement of catalase activity in tissue extracts. Anal. Biochem., 34: 30-38.

Dohi, T., K. Kawamura, K. Morita, H. Okamola and A. Tsiyimolo, 1998. Alterations of plasma selenium concentrations and the activities of tissue peroxide metabolism enzymes. Streptozotocin induced diabetic rats. Horm. Metab. Res., 20: 671-675.

Dominquez, C., E. Ruiz, M. Gussinye and A.C. Carrascosa, 1998. Oxidative stress at onset and early stages of type1 diabetes in children and adolescents. Diabetes Care, 21: 1736-1742.

Expert Committee on Diagnosis and Classification of Diabetes Mellitus, 1998. Diabetes Care, pp: 215-219.

Geetha, G.F., A.M. Ferraris, M. Rolfo, R. Mangerini, S. Arena and H.N. Kirkman, 1996. Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocyte blood. Am. J. Clin. Nutr., 27: 1026-1034.

Gomathy, R., I. Vijayalekshmi and P.A.C. Kurup, 1990. Hypoglycemic action of the pectin present in the inflorescence stalk of plantain (Musa Sapentum)-Mechanism of action. J. Biosci., 15: 297-303.

Harbone, J.B., 1973. Comparative biochemistry of the flavonoids. New York Academic Press, pp: 221-222.

Health, R.L. and L. Parker, 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid oxidation. Arch. Biochem. Biophys., 125: 189-198.

Hsu, CL., W. Chen, Y.M. Weng and C.Y. Tseng, 2003. Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. Food Chem., 83: 85-92.

Ketiku, A.O., 1973. Chemical composition of Unripe Plantain (*Musa Paradisiaca*). J. Sci. Food Agric., 24: 703-707.

King, H., H.E. Aubert and W.H. Herman, 1999. Global burden of diabetes, 1995-2025. Diabetes Care, 21: 1414-1431.

Kirkosyan, A., E. Seymour, O.B. Kaufman, S.E. Warber and S.C. Chang, 2003. Antioxidant capacity of polyphenolic extracts from leaves of crataegus Laevigata and Crataegus monogyna (Hawthorn) subjected to drought and cold stress. J. Agric. Food Chem., 51: 3973-3976.

Nourooz-Zadeh, J.A., J. Rahimi, J. Tajaddini-Sarmadi, H. Tritshler, P. Rosen, B. Halliwell and D.J. Betteridge, 1997. Relationships between plasma measures of oxidation stress and metabolic control in non-insulin dependent diabetes mellitus. Diabetologia, 40: 647-653.

Piedrola, G., E. Novo, F. Escober and R. Garcia, 2001. White blood count and insulin resistance in patients with coronary artery disease. Ann. Endocrinol., 62: 7-10.

Polidori, M.C., P. Mecocci, W. Stahl, B. Parente, P. Cecehelti, A. Cherubini, P. Cao, H. Sies and U. Sienin, 2000. Plasma levels of lipophilic antioxidants in very old patients with type 2 diabetes. Diabet. Metab. Res. Rev., 316: 15-19.

Price, K.R., I.T. Johnson and C.R. Fenwic, 1987. The chemical and biological significance of saponins in food and feeding stuff. Unpublished Crit. Rev. Food Sci. Nutr., 26: 27-135.

Rang, H.P., M. Dale and J.M. Ritter, 1991. The endocrine system. Pharmacology. in: Pharmacology. Longman Group Ltd, UK., pp: 504-508.

Rao, B.K., P.R. Sudarshan, M.D. Rajsekher, N. Nagaraju and C.A. Rao, 2003. Antidiabetic activity of *Terminalia Pallida* fruit in alloxan induced diabetic rats. J. Ethnopharmacol., 85: 169-172.

Robbins, R.J., 2003. Phenolic acids in foods. An overview of analytical methodology. J. Agric. Food Chem., 51: 2886-2887.

Robert, K.M., K.G. Darry and A.M. Peter, 2000. Harpers Biochemistry. 23rd Edn., pp: 150-151.

Shin, S.H., 1998. Oxidative stress and diabetic vascular complications. Recent advances in pathogenesis and management of diabetes mellitus. 1st Edn., Elsvier Science Company, Singapore, 633: 3-8.

Tagami, S., T. Kondo, K. Yoshidfa, J. Hirokaw, Y. Ohsuka and Y. Kaweasmi, 1992. Effect of insulin on impaired antioxidant activities on aortic endothelial cells from diabetic rabbits. Metabol., 41: 1053-1058.

Udoh, A.E., I. Ntu, O. Essien and M. Ndon, 2007. Red cell catalase activity in diabetics. J. Nutr., 6: 511-515.

Pakistan Journal of Nutrition 9 (11): 1058-1060, 2010
ISSN 1680-5194

Synthesis of 1, 3- Propanediol from Sorghum

C.M. Obele[1], O. Ogbobe[2] and I.F. Okonkwo[3]
[1]Department of Polymer and Textiles Engineering, Nnamdi Azikiwe University, Awka, Nigeria
[2]Department of Polymer and Textiles Engineering, Federal University of Technology, Owerri, Nigeria
[3]Department of Agricultural and Bio-Resources Engineering, Nnamdi Azikiwe University, Awka, Nigeria

Abstract: 1, 3-Propanediol (PDO) is the commonest diol used in polyester synthesis, polyurethanes and cyclic compounds. Starch was prepared from white sorghum. Dual enzyme technique was used to convert starch to sugar. Then glycerol was formed by fermentation of the sugar by yeast after 72 h. Mixed culture of *Escherichia coli* and *Klebsiella* specie was used to inoculate the glycerol obtained for final conversion to 1, 3-propanediol. The mixture was left for 72 h to ensure complete conversion. The reaction temperature for fermentation was 37°C. The expression of *Klebsiella* sp-diol dehydratase in E-*coli* catalyzed the conversion of glycerol to 1, 3-propanediol. The sample was then re-filtered, distilled and condensed to obtain the pure, bio-PDO which is colourless and odourless. Biologically produced 1, 3-propanediol was characterized and the result is as follows: specific gravity = 0.9992, purity 99.92%, boiling point 121-124°C. The PDO synthesized was found to be of comparable purity to chemically produced PDO.

Key words: 1, 3-Propanediol, bacteria, starch

INTRODUCTION

1, 3-Propanediol (PDO) is one of the commonest diol used in polyesters, manufacture of polyurethane and cyclic compounds. Recently, it has found application in cosmetics as a preservative (WIPO WO/2008/061187). 1, 3-propanediol could be biologically synthesized from cereals such as maize, millet, sorghum etc. Chemically synthesized PDO generally contain impurities from the chemical processes used to generate them. Many of such impurities are known to be harmful irritants and even toxic in some cases. In polyester synthesis, the use of chemically synthesized PDO and a chemically based diacid will not yield biodegradable polyester. These days, the concern for the disposal of non-degradable synthetic polymers has lead to increased interest in Biobased monomers and polymers which are biodegradable.

Biologically synthesized PDO has higher purity than chemically synthesized PDO. The production of PDO through fermentation was first discovered because of a major impurity in glycerol. In 1895, Noyes and Watkins discovered that glycerol that was unsuitable for certain applications contained greater than 1% of an unknown impurity. The onset of First World War and the need for high purity glycerol for trinitro glycerine for explosives made the identification of the impurity a major concern. Voisenet identified the compound as PDO in 1914 and it was determined to be product of anaerobic fermentation of the glycerol.

PDO could be prepared from hydrocarbons such as glycerol, reacted in the presence of carbon monoxide and hydrogen over periodic table group catalyst (US Patent 6,140,546; US patent 6,284,930).

Some conventional chemical routes to PDO include The Degussa process which consists of the following three steps:

1. Oxidation of propylene to acrolein

$$CH_2 = CH\ CH_3 + O_2 \longrightarrow CH = CH\ CHO$$

2. Selective hydration to 3-hydroxypropionaldehyde (3-HPA)

$$CH_2 = CHCHO + H_2O \longrightarrow OCHCH_2CH_2OH$$

3. Catalytic hydrogenation to 1, 3-propanediol (PDO)

$$OCH\ CH_2CH_2OH + H_2 \longrightarrow HO\ (CH_2)_3\ OH$$

Shell chemical uses a two step process:

1. Hydroformylation of ethylene oxide to 3-HPA

$$OCH_2\ CH_2 + CO + H_2 \longrightarrow OCHCH_2\ CH_2OH$$

2. Catalytic hydrogenation to 1, 3-propanediol (PDO)

$$OCHCH_2\ CH_2\ OH + H_2 \longrightarrow HO\ (CH_2)_3\ OH$$

These methods, apart from generating waste streams are expensive. As a result, several processes for biological production of PDO have been reported (EP 1204755; EP 107, 6708; WO 0112833; WO 0111070).

Corresponding Author: C.M. Obele, Department of Polymer and Textiles Engineering, Nnamdi Azikiwe University, Awka, Nigeria

Glucose from cereals such as maize and sorghum could be converted to glycerol using dual enzyme technique. Bacterial strains which are able to convert glycerol into 1, 3-propanediol are found in the species of *Klebsiella*, *citrobacter*, *clostridium* and *lactobacillus*. The conversions of glucose to PDO occur in nature in two stages; first by yeast to an intermediate product, glycerol then by bacteria to PDO or 3G.

Kurian *et al*. (2005) utilized maize as their renewable sugar source for PDO synthesis. They reported the use of genetically engineered *Klebsiella pneumonia* and *E-coli* for the conversion of glycerol to PDO. In this research, sorghum has been used as the renewable sugar source. There has been no report on the use of sorghum for PDO synthesis. *Klebsiella* species and *E-coli* have been used in this work. Sorghum is a genius of numerous species of grasses which belong to the family poaceae. The most popular specie is sorghum bicolor.

In Nigeria and South Africa, sorghum is industrially used for the production of lager beer (Doggett, 1988; Dufour *et al*., 1992). Sorghum grain comes in three major varieties: white, yellow and red. Agu *et al*. (1995) reported that the white variety has better brewing properties. Sorghum starch has been successfully applied for the production of bio-ethanol. In India and other places, sweet sorghum stalks are used for producing bio-fuel.

MATERIALS AND METHODS

Preparation of starch: White sorghum purchased from a local market was sorted and the foreign bodies and half grains removed. It was winnowed to remove the husks and dusts. The clean grains were steeped for twelve (12) hours changing the water every six (6) hours. The steep water was drained off, crushed and soaked in fresh-water. The germs which float were skimmed off. The endosperm was then milled to amorphous sized particles and sieved with nylon cloth to separate the raw starch from the fiber.

The starch was then allowed to settle and the clear water skimmed off. The starch was then spread evenly on a thermos hot air oven trays and dried overnight at sixty five (65) degree Celsius. The dried starch sample was used for the work.

Synthesis of 1, 3-Propanediol (PDO): 50 grams of starch was dissolved in 450 ml of process water. pH of the slurry was checked with a Hanna digital table pH meter and adjusted to 6.4 with 1 gram of calcium hydroxide. 0.2 grams of calcium sulphate was added and the starch hydrolyzed using dual enzyme conversion technique by the infusion-decoction method of mashing. 2 mls of protease was pipetted into the mash and its temperature maintained at 50°C for the protein content of the mash (if any) to be converted to amino acids. 2 mls of termamyl (a bacterial alpha amylase) was pipetted into it. The mash was heated up gradually to 95°C. While raising the mash temperature to 95°C, the starch gelatinized and the gel liquefied by the bacterial alpha amylase added.

At 95°C, the mash was rested for 5 min for the liquefaction to be completed.

200 mls of chilled process water was added gradually into the mash, reducing the mash temperature to 60°C. The pH was adjusted to 5.6. The mash was rested at 60°C for 15 min. Within this period of rest the rate of starch degradation to simple sugar was monitored. After saccharification, the mash was heated up to 78°C and rested at that for 3 min for the exogenous enzymes used to be inactivated. It was then filtered, cooled to 20°C and the original gravity measured with a saccharometer. It was cooled further to 10°C and after measuring the volume, it was pitched with yeast slurry (saccharomyces uvarum) and fermented at 37°C for 72 h under constant shaking with orbital shaker. The fermented "must" or" wort" as it is called was then filtered and inoculated with cultures of *Escherichia Coli* (*E. coli*) and Klebsiella specie. The entire mixture was stirred with orbital shaker set at 150 rpm for 72 h. At the end 72 h of agitation, the PDO synthesized was filtered. The filtrate was then distilled out with heat supplied by a heating mantle and the PDO condensed with a spiral condenser. The distillate was cooled further to 20°C and its specific gravity measured with a 25 ml specific gravity bottle.

Characterization of 1, 3-Propanediol

Boiling point: This was determined using fusion tube attached to a thermometer. The temperature is noted at the point the liquid begins to boil.

Gravity: This was determined using a 20 to 30cp Saccharometer.

Specific gravity: Specific gravity bottle was employed for the determination of gravity.

Determination of purity: Determination of purity of PDO was done using ultraviolet spectrophotometric method of world intellectual patent organization WIPO, 2008. The spectrophotometer was set at different wavelengths namely; 220 nm, 240 nm and 275 nm. The samples were read and the optical density noted.

Table 1: Purity of the samples under different wavelengths

Wavelengths (nm)	Expected absorbance	Absorbance observed
220	<0.200	-0.291
250	<0.075	-0.294
275	<0.075	-0.298

RESULTS AND DISCUSSION

The boiling point of PDO synthesized was found to be 121-124°C. This is comparable with 1, 3-Propanediol synthesized from maize (122°C). The gravity was found to be 24.34° P. Both gravity and specific gravity determines the solid content of the PDO sample before and during fermentation. They measure the level of solid in the sample that is available for fermentation. The specific gravity was 0.9992. The purity of the sample as read under different wavelengths is given in Table 1.

These values of absorbance observed helps to confirm the purity level of PDO synthesized. Sorghum-based PDO has similar properties with maize based PDO. Sorghum based PDO has been found to be of comparable purity to chemically synthesized PDO with purity level of 99.96%. White sorghum has been found to give better appearance (colour) than red and yellow varieties. Sorghum is commercially available in Nigeria and the prize is affordable. Therefore, the study recommends the use of sorghum in place of maize which has various uses and exorbitant in price, in biological synthesis of 1, 3-Propanediol.

REFERENCES

Agu, R.C., M.U. Okenchi, G. Aneke and A.H. Onwumelu, 1995. Brewing properties of Nigerian white and yellow malted sorghum varieties mashed with external enzymes. World J. Microbiol. Biotechnol., II: 591-592.

Doggett, H., 1988. Sorghum. 2nd Edn., Longman Scientific and Technical London.

Dufour, J., P. Melotte and S. Srebmik, 1992. Sorghum malts for the production of a lager beer. ASBC J., 50: 110-119.

EP 107, 6708, Method of production of 1, 3-Propanediol by Recombinant organisms comprising genes for vitamin B-12 Transport.

EP 1204755, Process for the Biological production of 1, 3-Propanediol.

Kurian, J., R. Muby and R. Miller, 2005. Dupont Sorona Polymer: A new sustainable platform for growth. Paper presented at Global Environmental Conference.

US Patent 6,284,930, Process for the production of Hydroxypropanol.

US Patent 6,140,546, Process for the production of 1, 3-Propanediol.

WIPO WO/2008/061187

WO 0111070, 1, 3-Propanediol and polymer Derivatives from a fermentable carbon source.

WO 0112833, Process for Biological Production of 1,3-Propanediol with high liter.

Pakistan Journal of Nutrition 9 (11): 1061-1068, 2010
ISSN 1680-5194

Incidence of Antibiotic Resistance in Some Bacterial Pathogens from Street Vended Food in Ogbomoso, Nigeria

I.C. Oladipo and O.D. Adejumobi
Department of Science Laboratory Technology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria

Abstract: This study was conducted to examine the level of bacterial contamination in some selected cooked food in Ogbomoso, Nigeria and to determine the antibiotic susceptibility profile of the bacterial contaminant. A total of nine (9) organisms were isolated, the isolates were subjected to various biochemical tests and the isolates were identified as *Bacillus licheniformis*, *Aeromonas hydrophila*, *Enterobacter aerogenes*, *Bacillus cereus*, *Proteus mirabilis*, *Pseudomonas putida*, *Proteus vulgaris*, *Pseudomonas chlororaphi* and *Proteus morganii*. Survival of isolates at different temperature ranges of 50-80^{0}C was determined and it was discovered that as the temperature increased the growth of the isolates decreased. Survival of isolates at different pH ranges was determined using Spectrophotometer at wavelength of 560 nm as the pH changed to basicity from acidity growth of isolates increased. Effect of different concentration of Sodium Chloride (NaCl) on the growth of isolates shows that the rate of growth of isolates decreased as the concentration of NaCl increased. Finally, antibiotic susceptibility test was conducted and the result indicated 53.85% resistance while 46.15% are sensitive to the antibiotics.

Key words: Antibiotics, pH, NaCl, temperature, street vended food, bacterial contaminant

INTRODUCTION

The term 'street food' refers to a wide variety of ready-to-eat foods and beverages sold and sometimes prepared in public places, relatively cheap and easily accessible (Mensah *et al*., 2002; FAO, 1989). Street food may be consumed where it is purchased or can be taken away and eaten elsewhere, the consumers who depend on such food are more interested in its convenience than in question of its safety and hygiene (Barro *et al*., 2002b; Collins, 1997; Mensah *et al*., 2002).

The consumption of street food is common in many Countries where unemployment is high, salaries are low, work opportunities and social programmes are limited and where urbanization is taking place. Street food vendors benefits from a positive cash flow, often evade taxation and can determine their own working hours (Collins, 1997). The hygiene aspects of vending operations are a major source of concern for food control officers, for examples, stands and often crude structures and running water may not be readily available. Also, toilets and adequate washing facilities are rarely available. The washing of hands, utensils and dishes is often done in buckets or bowls. Disinfection is not usually in no organized sewage disposal, so food is not adequately protected from flies and refrigeration is usually unavailable (Mensah *et al*., 1999).

In addition, street foods (cooked) are subjected to cross contamination from various sources such as utensils, knives, raw foodstuffs, flies that are sporadically landing on the foods, by vendors bare hand serving occasionally, food handling by consumers (Marks *et al*., 1998; Bryan, 1988; Gorris, 2005). Ready-to-eat foods (street food) are processed (peeled, squeezed, cut up and/or cooked) and readily available for purchase and consumption. However, street foods have been implicated in the transmission of foodborne disease (Chomvarin *et al*., 1993; Gillespie *et al*., 2000; Fang *et al*., 2003).

Foodborne illness is a major international health problem and an important cause of reduced economic growth (WHO, 1983). Foodborne illness of microbial origin is major cause of death in developing Countries (WHO, 2002a,b; Rehydration Project, 2004). The problems of food safety in the industrialized world differ considerably from those faced by developing Countries. Whereas, in developing countries traditional methods of processing and packaging, improper holding temperature, poor personal hygiene of food handlers are still observed during food marketing and technology (Barro *et al*., 2002a, 2002b; Mensah *et al*., 2002).

The use of antibiotic(s) after the intake of the organism(s) may not be effective as the organisms may be susceptible or resistant to it. Resistance to antibiotics in foodborne pathogens may create problems for disease or illness treatment while antibiotic susceptibility leads to healing of the illness which the organism(s) caused. Traveler's diarrhea is a major inconvenience to visitors arriving in developing Countries from more industrialized areas (Dupont *et al*., 1982).

Corresponding Author: I.C. Oladipo, Department of Science Laboratory Technology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria

Foodborne illnesses affect people's health and well being as well as have an economic impact on individuals and nations. Diarrhea disease has been a major public health problem causing high morbidity and mortality among children especially in Thailand for many years (Bureau, 2004). Foodborne illness outbreaks from enteropathogenic bacteria, such as *Salmonella, Vibrio cholerae, V. parahaemolyticus* and *Staphylococcus aureus*, are common causes of foodborne infection throughout the world including Thailand (Chomvarin *et al*., 1993; Mosupye and Von Holy, 1999; Adams and Moss, 2000; Bangtrakunonth *et al*., 2004; Meldrum *et al*., 2006).

Salmonella caused diseases ranging from diarrhea to septicemia. *Salmonellosis* from contaminated food generally causes diarrhea. *Aeromonas species* are an emerging important pathogens causing diarrhea and can be found in food and water (Kirov, 1993). They asses (I) the safety of food for consumers, (ii) adherence to good manufacturing practices, (iii) the keeping quality (shelf life) of perishable foods and (iv) the suitability of a food or ingredient for a particular purpose (Montville and Matthews, 2005). Therefore, isolation of relevant bacterial pathogens and indicator organisms is used to evaluate microbiological safety and quality of food.

Food contamination with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among foodborne pathogens has increased during recent decades (Chui *et al*., 2002; Davis *et al*., 1999; Garau *et al*., 1999; Threfall *et al*., 2000).

In addition, the lack of stringent controls on antimicrobial usage in human health and particularly in animal production systems increases the risk of antibiotic resistant foodborne microbes. Also, *Enterococci* are common components of the micro-floral in soil, on plants and in water. These organisms are particularly challenging to eliminate because of their ability to adapt to environmental stresses. Thus, it is not surprising that antimicrobial resistant variants of *Enterococci* have been found within probiotic formulations (Giraffa, 2002). More so, in the clinical environment, *Enterococci* can persist for long periods of time on surfaces and can readily be transferred among patient population (O'Connell and Humphreys, 2000).

According to street foods studies carried out in Africa, their tremendous unlimited and unregulated growth have placed a severe strain on city resources such as water, sewage system and interferences with the city plan through congestion and littering adversely affecting daily life (Canet and N'Daiye, 1996; Barro *et al*., 2002a). FAO and several authors stipulated that street vended food raise concern with respect to their potential for serious food poisoning outbreak (Estrada-Garcia *et al*., 2002, 2004; Collins, 1997; King *et al*., 2000; Tjoa *et al*., 1977; Umoh *et al*., 1984), due to improper use of additives, the presence of pathogenic bacteria, environmental contaminants and improper food handling practices based on un-respect of good manufacturing practices and good hygiene practices (Barro *et al*., 2002b; Canet and N'Daiye, 1996).

Vendors are often poor level education, unlicensed, untrained in food hygiene, technology and work under crude unsanitary conditions (Barro *et al*., 2006; Muinde and Kuria, 2005). In the context of poverty, street food accounts for a part of the family income, daily diet and so contribute towards meeting nutritional requirements (Chakravarty and Canet, 1996), following example of the most developing Countries urbanization in West Africa generates many concerns as the difficult access to potable water, presence of different waste everywhere, lack of efficient drainage system, indeed in most Countries, organic wastes and sewage are discarded on the street which causes contamination through flies. Thus, street food importance has consequence such as its association to epidemic and disease outbreak in case of microbiological quality failure (Barro *et al*., 2005; Cardinale *et al*., 2005; Estrada-Garcia *et al*., 2004; WHO, 2002b).

Traveler's diarrhea may be an important factor inhibiting tourism to developing Countries. In visitors to Mexico, approximately 80% of diarrhea is of bacteria origin (Dupont *et al*., 1982). Epidemiological evidence has implicated food as an important vector of enteropathogenic bacteria (Ericson *et al*., 1980; Tjoa *et al*., 1979). Several authors have reported low level of enteropathogenic organisms in foods in various Countries including Sweden (Danielsson *et al*., 1979), the United States (Sack *et al*., 1977) and the Philippines (Echeverria *et al*., 1978).

Jiwa *et al*. (1981) reported that enterotoxigenic organisms of many general were found in food and water in an Ethiopian community e.g. *E. coli*. Also, restaurant foods from Guadalajara (Mexico) were generally more contaminated with coliforms, the food samples from mexican restaurant in Houston contained enterotoxigenic bacteria including *Enterobacter aerogenes, Klebsiella pneumoniae* and was unable to isolate *Salmonella* or *Shigella*.

Antibiotic resistance of *E. coli* and *Salmonella* isolates was determined by the disk diffusion method using the standard procedure of the Clinical Standard Institute (CLSI, formerly NCCLS) (NCCLS, 2004). The isolates were classified as susceptible, intermediate, or resistant according to interpretation of the zone diameter standards recommended by CLSI (CLSI, 2005). Isolates were screened for antibiotic resistance against 15 antibiotics and 50.5% of the *Salmonella* isolates were found to be resistant to at least one antibiotic, on the

other hand 83.8% of *E. coli* isolates were resistant to at least one antibiotic. Also, multiresistance (resistance to at least three different classes of antibiotics) was detected in 20.9 of *Salmonella* isolates (Thi Thu Hao Van *et al*., 2007).

Moreso, a study which was carried out by Chariya *et al.* (2006) to investigate the microbiological quality of ready to eat food in the Municipality of Khon Kaen, Thailand was recorded that four categories of 186 food samples were collected (I) high heat food (ii) low heat food (iii) no heat food (iv) on-site prepared fruit juices and beverages; 73% (145) were recorded to failed to meet acceptable and morphological standards, including fruit juice and beverages (100%), no heat food (91.7%), low heat food (81.7%) and high heat food (57.9%). Also, pathogenic bacteria were found in 6.5% of food samples, *Salmonella*, *Vibrio cholerae* and *Aeromona hydrophila* were found in 4.3, 1.6 and 0.5% of the total food samples respectively and *Staphylococcus aureus* were found in 2.7% of the samples which resulted in that more than half of the ready-to-eat foods tested in Khon Ken did not meet microbiological national standards and many kind of enteropathogenic bacteria were found which may be a source of foodborne disease (Kirov, 1993; Fang *et al*., 2003; Gillespie *et al*., 2000; Ohasshi *et al*., 1978; Mosupye and Holy, 1999).

Gillespie *et al.* (2000) studied and isolated 146 raw (minced, chicken, beef) and cooked (red meat, chicken) meat sampled which was analyzed for the presence of *Listeria spp*. The isolates were characterized by morphological, cultural and biochemical tests according to Bergey's Manual and was later confirmed by API-*Listeria* kit. It was recorded that out of a total of 146 meat samples, 79 (54.10%) were found to be contaminated with *Listeria spp*; with the highest incidence (86.4%) occurring in raw minced meat. *Listeria monocytogenes* was isolated from 9 (6.16%) of the 79 samples examined by them. Other species isolated were *L. innocua* 68 (46.57%), *L. welshimeri* 1 (0.69%) and *L. murrayi* 1 (0.68%). In their conclusion, it was later discovered that *Listeria* strains isolated from meat and meat products were mostly resistant to cephalothin and nalidix acid but exhibited a high degree of susceptibility to kanamycin, chloramphenicol and tetracycline. The objectives of this study therefore are to isolate and characterize some bacterial pathogens from street vended food, evaluate the antibiotic susceptibility profile and the physiological properties of the isolates.

MATERIALS AND METHODS

The study was carried out in science laboratory Technology Dept., Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria between January, 2009 and December 2009.

Collection of samples: Street vended food samples were purchased from different selling point in Ogbomoso, South West Nigeria in January, 2009.The samples are moinmoin, yam, spaghetti, beans, bean cake, rice, indomie. The samples were already cooked and ready to be eaten, the samples were purchased and aseptically transported to the laboratory for analysis.

Isolation of microorganisms: 10 g of each vended street food sample was mashed in sterile mortal and pestle and was serially diluted. 1 mL of an appropriate dilution was inoculated on sterile MacConkey agar and Nutrient agar, the plates were incubated for 24 h at 37°C. After 24 h, sterile wire loop was used to pick the isolate from the plate and was streaked on a freshly prepared nutrient agar then incubated for 24 h at 37°C in order to get pure culture. The routine laboratory method of Cruickshank *et al*. (1975) was used to characterize different isolates. The isolates were identified using their macroscopic, cellular, physiological and biochemical characteristics.

Antibiotics susceptibility test: Sterile nutrient agar medium was poured into sterile petri dishes and allowed to solidify. A suspension of the isolated organisms was transferred into petri-dishes accordingly and swab over the entire plate, it was then incubated for 1 h at 37°C and a forcep was used to transfer each sensitivity disc on the plate and incubated for 24 h at 37°C. The antibiotics used included Amoxyllin, Streptomycin, Chloramphenicol Tetracycline, Gentamycin, Ofloxacin, Augmentin, Ciprofloxacin, Cotrimoxazole, Nitrofurantion, Ampiclox, Cefroxine and Erythromycin.

Survival of isolates at different temperatures ranges: Nutrient broth was prepared and dispensed into series of screw-capped bottles and sterilized. It was allowed to cool and the test organisms were inoculated into it, then incubated at different temperature ranges (50, 60, 70 and 80°C) for 24 h after which cecil 2031 (automatic) spectrophotometer was used to detect increase or decrease in turbidity of the growth medium.

Growth of isolate at different pH ranges: Nutrient broth was prepared and the pH was adjusted using O.IM phosphate buffer of different pH to adjust the pH of the broth to 3.0, 5.0, 7.0 and 9.0. It was then dispensed into screw capped bottles and then sterilized in the autoclave at 121°C for 15 min. After cooling, the various test isolates were inoculated into it and incubated at 30°C for 48 h. Growth was detected by increase turbidity using Cecil 2031 (automatic) spectrophotometer. Uninoculated tubes serve as control. This test was done to detect the best pH that favours growth and metabolism as indicated by the increased turbidity (Schillinger and Lucke, 1989).

Growth of isolates in different concentration of NaCl: Nutrient broth containing 2% (w/v), 3% (w/v), 4% (w/v) and 5% (w/v) NaCl was prepared and sterilized at 121°C for 15 min. 20 ml of the broth was the dispensed into sterile screw capped vials aseptically. After cooling, the tubes were inoculated with the test organisms and incubated for 24 h at 30°C increased turbidity of the medium was recorded as positive for growth while a negative result shows no turbidity. Uninoculated tubes serve as control (Schillinger and Lucke, 1987).

RESULTS

A total of eleven (11) organisms were isolated from street food in Ogbomoso. *Bacillus licheniformis* and *Aeromonas hydrophila* was found present in Beans, *Enterobacter aerogenes* in Moinmoin, *Bacillus cereus* and *Proteus mirabilis* was present in rice, *Proteus mirabilis* and *Pseudomonas putida* in Spaghetti, also, *Proteus vulgaris* in Bean cake, *Pseudomonas chlororaphi* and *Pseudomonas putida* in Indomie and *Proteus morganii* was found in Yam (Table 1).

The isolates were differentiated on the basis of the cultural and cellular morphological studies, after which they were subjected to various biochemical and physiological test and the isolates were identified to be *Bacillus licheniformis*, *Aeromonas hydrophila*, *Enterobacter aerogenes*, *Bacillus cereus*, *Proteus mirabilis*, *Pseudomonas putida*, *Proteus vulgaris*, *Pseudomonas chlororaphi* and *Proteus morganii*.

Table 1: List of sources of isolates

Code	Source	Isolates
B_2a	Beans	*Bacillus licheniformis*
B_2b	Beans	*Aeromonas hydrophila*
Moi_2	Moinmoin	*Enterobacter aerogenes*
$W.R_1$	Rice	*Bacillus cereus*
$W.R_2$	Rice	*Proteus mirabilis*
$Spag_1$	Spaghetti	*Proteus mirabilis*
$Spag_2$	Spaghetti	*Pseudomonas putida*
BC_2	Bean Cake	*Proteus vulgaris*
Ind_1	Indomie	*Pseudomonas chororaphi*
Ind_2	Indomie	*Pseudomonas putida*
Ya	Yam	*Proteus morganii*

Antibiotic susceptibility of all the isolated organisms were determined by agar diffusion method. All isolates were found resistant to Erythromycin (ERY), almost all isolates are resistant to Chloramphenicol (CHL) except *Bacillus licheniformis* with 10.5 mm. *Pseudonomas chlororaphii* and *Pseudomonas putida* with the zone of inhibition of 9.0 mm each were found sensitive to Nitrofurantion (NIT) while others were resistant; also, *Bacillus licheniformis* and *Bacillus cereus* were found sensitive to Streptomycin (STR) with 8.0mm and 13.5 mm respectively while others were resistant. Some isolates were found resistant and some sensitive to Cotrimaxazole (COT). Most isolates were resistant to Ceftrazone (CEF) except *Aeromonas hydrophila* and *Proteus mirabilis* with zone of inhibition of 11.0 mm each. For Amoxylin (AMX) almost every isolates were sensitive except *Aeromonas hydrophila* and *Proteus mirabilis*; *Enterobacter aerogenes, Proteus mirabilis* and *Proteus vulgaris* were found to be resistant to Ofloxacin (OFL) while other isolates were sensitive to it ; *Bacillus licheniformis, Proteus vulgaris* and *Proteus morganii* were resistant to Gentamycin (GEN); *Pseudomonas chlororaphi* was resistant to Pefloxacin (PEF) while the other isolates were found to be sensitive, almost all isolates were sensitive to Ciprofloxacin (CPX) except *Enterobacter aerogenes* and *Proteus vulgaris* that were resistant. *Bacillus licheniformis, Bacillus cereus* and *Proteus mirabilis* were resistant to Augmentin (AUG) while other isolates were sensitive to it. *Enterobacter areogenes, Proteus vulgaris, Psedomonas chlororaphi* and *Psedomonas putida* were sensitive to Tetracycline (TET) with zones of inhibition of 11.0, 12.5, 11.5 and 11.0 respectively some while other isolates were résistant to it (Table 2).

Spectrophotometer at a wavelength of 560nm was used to determine the effect of different pH ranges on the growth of isolates and it was found that rate of growth of the isolates was increasing as the pH of the medium changed from basic to acidic. The Optical Density (OD) reading shows that as pH increased from 3 to 9, *Bacillus licheniformis* increased from 0.065 to 1.564, *Aeromonas hydrophila* increased from 0.136 to 1.288, *Enterobacter aerogenes* increased from 0.109 to 1.291 and *Bacillus cereus* from 0.157 to 1.355 (Table 3).

Table 2: Antibiotic susceptibility profile of the isolates

Isolates	AMX	OFL	STR	CHL	CEF	GEN	PEF	COT	CPX	ERY	NIT	AUG	TET
Bacillus licheniformis	14.5	14.0	8.0	10.5	-	-	15.5	-	18.5	-	-	-	-
Aeromonas hydrophila	-	11.0	-	-	11.0	12.0	17.5	17.5	18.0	-	-	15.0	-
Enterobacter aerogenes	11.5	-	-	-	-	13.5	17.5	12.5	-	-	-	12.0	11.0
Bacillus cereus	15.5	18.5	13.5	-	-	12.0	19.0	-	18.5	-	-	-	-
Proteus mirabilis	-	-	-	-	11.0	-	14.5	-	16.0	-	-	-	-
Proteus vulgaris	11.0	-	-	-	-	14.5	19.0	-	-	-	-	13.5	12.5
Pseudomonas chlororaphi	14.0	13.5	-	-	-	18.5	-	-	18.5	-	9.0	13.5	11.5
Pseudomonas putida	11.5	13.0	-	-	-	16.5	19.0	-	15.5	-	9.0	10.5	11.0
Proteus morganii	11.5	15.5	-	-	-	-	15.5	10.0	15.0	-	-	8.0	-

Note: (-) Resistant

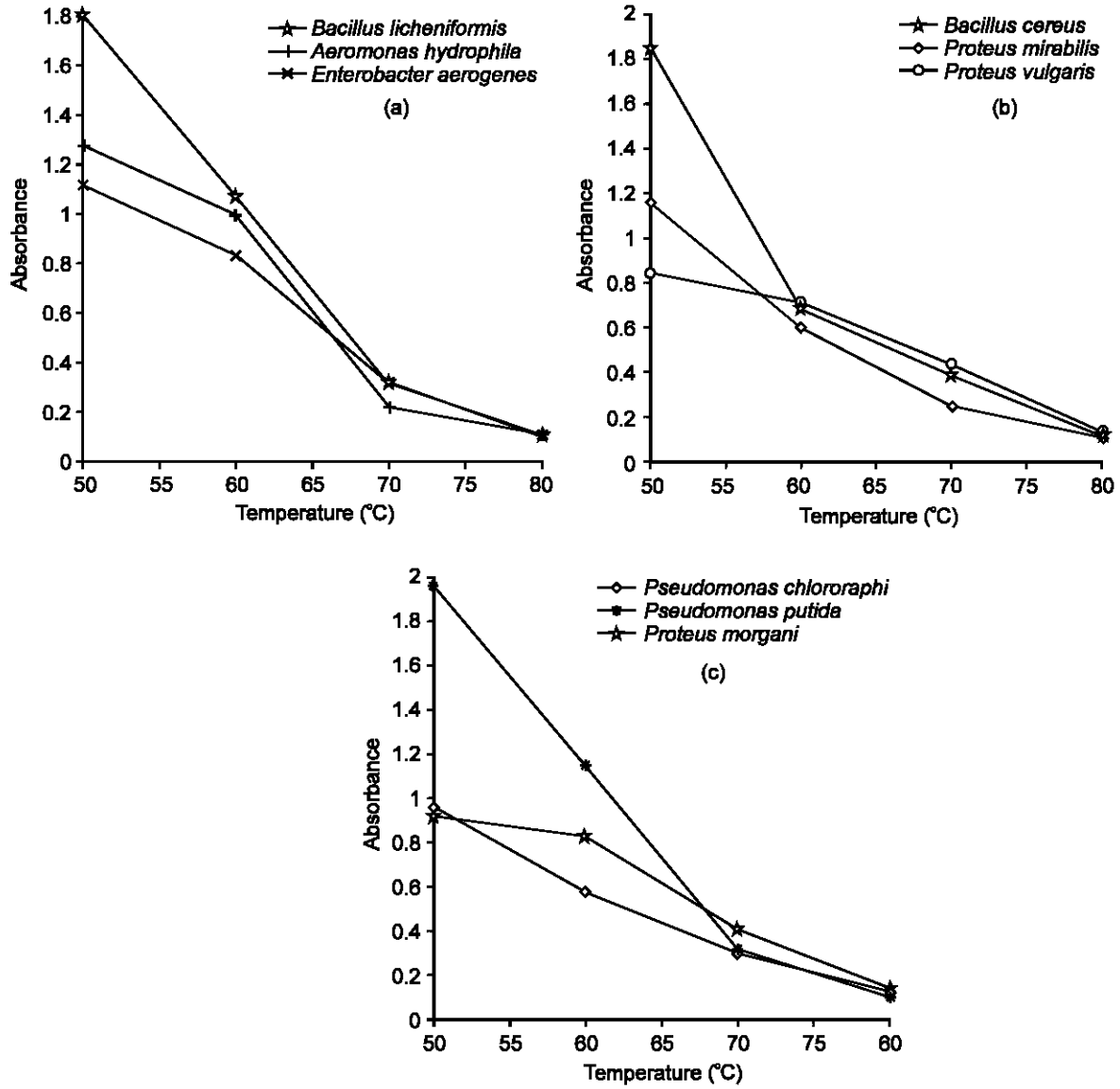

Fig. 1: Growth of isolates at different temperature ranges

Table 3: Growth at different pH ranges

Isolates	Growth of Isolates at different pH ranges (OD at 560 nm)			
	3	5	7	9
Bacillus licheniformis	0.065	0.936	1.360	1.564
Aeromonas hydrophila	0.136	1.100	1.253	1.288
Enterobacter aerogenes	0.109	1.140	1.242	1.291
Bacillus cereus	0.157	1.228	1.310	1.355
Proteus mirabilis	0.149	0.358	1.334	1.472
Proteus vulgaris	0.250	0.980	1.024	1.048
Pseudomonas chlororaphi	0.195	0.825	0.894	0.896
Pseudomonas putida	0.108	0.812	0.902	1.038
Proteus morganii	0.110	0.608	1.176	1.253

The effect of different temperature ranges on growth rate of the isolates was determined and it was found that rate of growth of the isolates was decreasing as the temperature of the medium was increasing. The Optical Density (OD) reading shows that as temperature increased from 50-80°C, *Bacillus licheniformis* decreased from 1.800 to 0.101, *Enterobacter aerogenes* decreased from 1.112 to 0.101, *Proteus mirabilis* decreased from 1.152 to 0.103 and *Pseudomonas chlororaphi* from 0.958 to 0.120 (Fig. 1a, 1b and 1c) etc. Effect of different concentration of Sodium Chloride on the test isolates was determine by using Spectrophotometer at wavelength of 560 nm, it was found that as the rate of concentration of NaCl increased, the rate of growth of isolates decreased. The Optical Density (OD) reading shows that as concentration of sodium chloride increased from 2-5%,

Table 4: Survival of isolates at different concentration of sodium chloride (NaCl)

	Growth of isolates at different NaCl ranges (OD) at 560 nm			
Isolates	2%	3%	4%	5%
Bacillus licheniformis	1.516	1.059	1.054	0.467
Aeromonas hydrophila	1.961	1.074	0.800	0.615
Enterobacter aerogenes	0.947	0.883	0.732	0.695
Bacillus cereus	1.971	0.652	0.545	0.120
Proteus mirabilis	1.213	1.089	0.879	0.731
Proteus vulgaris	1.028	0.891	0.697	0.558
Pseudomonas chlororaphi	1.157	1.145	1.100	0.956
Pseudomonas putida	1.124	0.936	0.574	0.467
Proteus morganii	1.523	1.035	0.527	0.101

Aeromonas hydrophila decreased from 1.961 to 0.615, *Proteus vulgaris* decreased from 1.028 to 0.558, *Pseudomonas putida* decreased from 1.124 to 0.467 and *Proteus morganii* from 1.523 to 0.101 and so on (Table 4).

DISCUSSION

The result of this study demonstrated that the food samples vended for consumer consumptions were contaminated by pathogenic bacteria which if ingested may be deleterious to consumers' health and may lead to foodborne illness or disease.

Antibiotic susceptibility results indicated 53.85% resistance and 46.15% sensitivity among vended food isolates. The prevalence of antimicrobial resistance among foodborne pathogens has increased during recent decades (Boonmar *et al*., 1998a,b; Chui *et al*., 2002; Davis *et al*., 1999; Threfall *et al*., 2000), possibly as a result of selection pressure created by the use of antimicrobials in food-producing animals (Aarestrup 1999; Angulo *et al*., 2000; Bywater, 2004; Teuber, 2001; Van den Bogaard and Stobberingh, 2000). The coexistence of resistance genes with mobile elements such as plasmids, transposons and integrons facilitates the rapid spread of antibiotic resistance genes among bacteria (Sunde, 2005). Also, high rates to antibiotics resistance of bacteria may possibly resulted from inappropriate or uncontrolled use of antibiotics in farming practices, so it is necessary to pay more attention to food hygiene practices to reduce or eliminate the risk from antibiotic resistance and pathogenic bacteria originating from food. In addition, the use of antibiotics in animal feeds need to be regulated strongly to minimize the opportunity for organisms to develop resistance (Thi Thu Hao Van *et al*., 2007).

Most of the organisms isolated have being reported to adapt to environmental stress and as a result, it is always a challenge to eliminate them from the environment. This fact is reflected in the physiological study of the isolates. Increasing physiological parameters such as teperature and sodium chloride did not eliminate but rather reduced the rate of growth of the vended food isolates.

The results of this study have illustrated the extent of antibiotic resistance in all the isolated organisms found. It is necessary to pay more attention to food hygiene practices to reduce or eliminate the risk from foodborne pathogens; especially those that are originated from street food. Also, strict implementation of food sanitation code and license for street food vendors is needed to make the consumers save. If possible, public health authorities should intensify efforts to monitor conditions of sanitation and hygiene in establishment serving food and drink to the public.

So, food safety education is a critical part of the overall strategy to reduce the incidence of foodborne illness and complements regulatory and other activities. However, meeting the huge challenge of food safety in the 21st Century will require the application of new methods to identify, monitor and access foodborne hazard. Both traditional and new technologies for assuring food safety should be improved and fully exploited. This need to be done through legislative measures where suitable, but much greater reliance on voluntary compliance and education of consumers and professional food handlers.

Finally, it is necessary for public health organizations to be concerned since microorganisms causing foodborne hazards and food spoilage can be isolated from raw materials and finished products; thus reduction of contamination is an achievable policy objective.

REFERENCES

Aarestrup, F.M., 1999. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. Int. J. Antimicrob. Agents., 12: 279-285.

Adams, M.R. and M.O. Moss, 2000. Food microbiology. 2nd Edn., Cambridge: Royal Society of Chemistry.

Angulo, F.J., K.R. Johnson, R.V. Tauxe and M.L. Cohen, 2000. Origins and consequences of antimicrobial-resistant nontyphoidal *Salmonella*: Implications for the use of fluoroquinolones in food animals. Microb. Drug Resist., 6: 77-83.

Bangtrakunonth, A., S. Pornreongwong and C. Pulsrikarn, 2004. Salmonella serovars from humans and other sources in Thailand, 1993-2002. Emerg. Infect. Dis., 10: 131-136.

Barro, N., C.A.T. Ouattara, A.P. Nikiema, A.S. Ouattara and A.S. Traore, 2002a. Evaluation de la qualite microbiologique de quelques aliments de me dans la ville de Ouagadougou au Burkina Faso. Cah. Sante, 12: 369-374.

Barro, N., P. Nikiéma, C.A.T. Ouattara and A.S. Traoré, 2002b. Evaluation de l'hygiène et de la qualité microbiologique de quelques aliments rue et les caractéristiques des consommateurs dans les villes de Ouagadougou et de Bobo-Dioulasso (Burkina Faso). Rev. Sci. Tec. Sci. Santé, 25: 7-21.

Barro, N., L. Sangare, M.C. Tahita, C.A.T. Ouattara and A.S. Traore, 2005. Les principaux agents du peril fecal identities dan les aliments de me et ceux des cantines Burkina Faso et les risques de maladies assoclees.colloque Regional Scientifique et Pedagogique: Maitrise de procedes en Vue d'ameliorer in quality et la securite des aliments, utilization does oam, analyses des resques en Agroalimentaires. Du 8 au 10 novembre 2005 a Ouagadougou UO/AUF/SP3A/CIDEFA.

Barro, N., A.R. Bello, A. Savadogo, C.A.A. Ouattara, A.J. Ilboudo and A.S. Traore, 2006. Hygienic status assessment of dishwater, utensils, hands and pieces of money in street foods vending sites in Ouagadouguo; Burkina Faso. Afr. J. Biotech., 5: 1107-1112.

Boonmar, S., A. Bangtrakulnonth and S. Pornninangwong, 1998a. Predominant serovars of Salmonella in humans and foods from Thailand. J. Vet. Med. Sci., 60: 877-880.

Boonmar, S., A. Bangtrakulnonth, S. Pornruangwong, S. Samosornsuk, K. Kaneko and M. Ogawa, 1998b. Significant increase in antibiotic resistance of *Salmonella* isolates from human beings and chicken meat in Thailand. Vet. Microbiol., 62: 73-80.

Bryan, F.L., 1988. Risk associated with practices, procedures and processes that lead to outbreaks of foodborne disease. J. Food Prot., 51: 663-673.

Bureau of Epidermiology, 2004. Situation of diarrhea disease. Bangkek: Department of Disease Control, Ministry of public Health, Thailand.

Bywater, R.J., 2004. Veterinary use of antimicrobials and emergence of resistance in zoonotic and sentinel bacteria in the EU. J. Vet. Med. B, 51: 361-363.

Canet, C. and C. N'Daiye, 1996. L'alimentation de rue en Afrique. Food. Nutr. Agric., 17/18: 4-13.

Cardinale, E., J.D. Perrier Gros-Claude, F. Tall, E.F. Gueye and G. Salvat, 2005. Risk factors for contamination of ready-to-eat street-vended poultry dishes in Dakar, Senegal. Int. J. Food Microbiol., 103: 157-165.

Chakravarty, I. and C. Canet., 1996. Street food in Calcutta. Food, Nutr. Agric., 17/18: 7.

Chariya Chomvarin, Orn-Anong Ratchtrachenchai, Yingrit Chantarasuk, Sugunya Srigulbutr, Kunyaluk Chaicumpar, Wises Namwat and Daroon Kotimanusvanij, 2006. Characterization of diarrheagenic *E. coli* Isolated from Food in Khon Kaen, Thailand. South East Asian J. Trop. Med. Public Health, 36: 931-939.

Chomvarin, C., D. Kotimanusvanij and A. Rhompruk, 1993. Study on the correlation between the enterotoxin producing staphylococcus aureus isolated from prepared food and cooks. Srinagarind Hosp. Med. J., 6: 231-242.

Chui, C.H., T.L. Wu, L.H. Su, C. Chu, J.H. Chia, A.J. Kuo, M.S. Chien and T.Y. Lin, 2002. The emergence in Taiwan of fluoroquinelone resistance in Salmonella enterica serotype cholerasuls. N. Engl. J. Med., 346: 416-419.

CLSI, 2005. Performance standard for antimicrobial susceptibility testing; 15th Informational supplement. Clinical and Laboratory Standards Institute, Wagne, PA.

Collins, J.E., 1997. Impact of changing consumer lifestyle in the emergence/reemergence of foodborne pathogens. Emerg. Infect. Dis., 3: 471-479.

Cruickshank, R., J.P. Duguid, B.P. Marmion and H.A. Swainr, 1975. Medical Microbiology, vol. 2, The Practice of Medical Microbiology. Edinburgh, London and New York: Churchill Livingst one.

Danielsson, M.L., R. Mollby, H. Brag, N. Hanson, P. Johnson, P.E. Olsson and Wadsrom, 1979. Enterotoxigenic enteric bacteria in foods and outbreaks of foodborne disease in Sweden. J. Hyg. Camb., 83: 33-40.

Davis, M.A., D.D. Hancock, T.E. Besser, D.H. Rice, J.M. Gay, C. Gay, L. Gearhart and R. Difiacomo, 1999. changes in antimicrobial resistance among Salmonella enterica serovar. Infect. Dis., 5: 802-806.

Dupont, H.L., D.G. Evans, N. Rios, F.T. Cabuda, D.J. Evans Jr. and M.W. Dupont, 1982. Prevention of traveler's diarrhea with trimethoprim-sulfamethoxazole. Rev. Infect. Dis., 41: 533-539.

Echeverria, P., L. Verhaert, V. Basaca-sevilla, T. Banson, J. Cross, F. Orskov and I. Orskov, 1978. Food and water in a community in the Philippines. J. Infect. Dis., 138: 87-90.

Ericson, C.D., L.K. Pickering, P. Sullivan and H.L. Dupent, 1980. The role of location of food consumption in the prevention of traveler's diarrhea in Mexico. Gastroenterology, 79: 812-816.

Estrada-Garcia, T., C. Lopez-sancedo, B. Zamarripa-Ayala, M.R. Thompson, L. Guitierrez-cogco, A. Mancera-Martinez and A. Escobar-Gutierrez, 2004. Prevalence of *Escherichia coli* and *Salmonella* spp. in street vended food of open markets (tianguis) and general hygienic and trading practices in Mexico city. Epidemiol. Infect., 132: 1181-1184.

Estrada-Garcia, T., J.F. Cerna, M.R. Thompson and C. Lopez-Sancedo, 2002. Faecal contamination and enterotoxigenic *Escherichia coli* in street vended chili sauces in Mexico and its public health relevance. Epidemiol. Infect., 129: 223-226.

Fang, T.J., Q.K. Wei, C.W. Liao, M.J. Hang and T.H. Wang, 2003. Microbiological quality of 18 degrees ready-to-eat food products sold in Taiwan. Int. J. Food Microbiol., 80: 241-250.

Food and Agriculture Organization (FAO), 1989. Street foods. Report of an FAO expert consultation, Yogyakarta, Indonesia.

Garau, J., M. Xercavins, M. Podriguez-Carballeira, J.R. Gomez-vera, I. Coll, D. Vidal, T. Wovet and A. Ruiz-Breman, 1999. Emergence nad dissemination of quinoline resistant *Escherichia coli* in the community. Antimicrob. Agents Chemother., 43: 2736-2741.

Gillespie, I., C. Little and R. Mitchell, 2000. Microbiological examination of cold ready-to-eat sliced meats from catering establishments in the United Kingdom. J. Appl. Microbiol., 88: 467-474.

Giraffa, G., 2002. Enterococci from foods. FEMS Microbiol. Rev., 26: 163-171.

Gorris, L.G.M., 2005. Food safety objective: an integral part of food chain management. Food Centr., 16: 801-809.

Jiwa, S.F., K. Krovocek and T. Wadstrom, 1981. Enterotoxigenic bacteria in food and water from an Ethiopian community. Appl. Environ. Microbiol., 41: 1010-1019.

King, L.K., B. Awumbila, E.A. Canacoo and S. Ofosu-Amaah, 2000. An assessment of the safety of street foods in the Ga district of Ghana; implication for the spread of zoososes. Acta Tropica, 76: 39-43.

Kirov, S.M., 1993. The public health significance of *Aeromonas* spp. in foods. Int J. Food Microbiol., 20: 179-198.

Marks, H.M., M.E. Coleman, C.T. Lin and T. Roberts, 1998. Topics in microbial risk assessment dynamic flow tree process. Risk Anal., 18: 303-328.

Meldrum, R.J., R.M. Smith, P. Ellis and J. Garside, 2006. Microbiological quality of randomly selected ready-to-eat foods sampled between 2003 and 2005 in Wales, UK. Int. J. Food Microbiol., 108: 379-400.

Mensah, P., D. Yeboah-Manu, K. Owusu-Darko and A. Ablordey, 2002. Street foods in Accra, Ghana: How safe are they? Bull. W.H.O., 80: 546-54.

Mensah, P., K. Owusu-Darko, D. Yeboah-Manu, A. Ablordey, F.K. Nkrumah and H. Kamiya, 1999. The role of street food vendors in the transmission of enteric pathogens. Ghana Med. J., 33: 19-29.

Montville, T.J. and K.R. Matthews, 2005. Food Microbiology: An introduction. Washington: ASM Press.

Mosupye, F.M. and A. Von Holy, 1999. Microbiological quality and safety of ready-to-eat-street-vended-foods in Johannesburg, South Africa. J. Food Prot., 62: 1278-1284.

Muinde, O.K. and K. Kuria, 2005. Hygienic and Sanitary practices of vendors of street foods in Nairobi, Kenya. AAJFAND online www.ajfand.net, 5: 1-13.

NCCLS, 2004. Performance standards for antimicrobial disk and dilution susceptibility tests for bacterial isolated Approved standard M31-A2. Nationnal committee for clinical laboratory standards, Wayne, PA.

O'Connell, N.H. and H. Humphreys, 2000. Intensive care unit design and environmental factors in the acquisition of infection. J. Hosp. Infect., 45: 255-262.

Ohasshi, M., H. Murakami, Y. Kudoh and S. Sakai, 1978. Manual for the laboratory diagnosis of bacteria food poisoning and the assessment of the sanitary quality of food. Tokyo. SEAMIC Publication.

Rehydration Project, 2004. http.//.www.rehydrate.org/facts/child_deaths.

Sack, R.B., D.A. Sack, I.J. Mehlman, F. Orskor and Orskor, 1977. Enterotoxigenic *Escherichia coli* isolated from food. J. Infect. Dis., 135: 313-317.

Schillinger, U. and F.-K. Lücke, 1987. Identification of lactobacilli from meat and meat products. Food Microbiol., 4: 199-208.

Schillinger, U. and F.-K. Lücke, 1989. Antibacterial activity of Lactobacillus sake isolated from meat. Appl. Environ. Microbiol., 55: 1901-1906.

Sunde, M., 2005. Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. J. Antimicrob. Chemother., 56: 1019-1024.

Thi Thu Hao Van, Genge moutafis, Linh Thuoc Tran and J. Peter Coloe, 2007. Antibiotic resistance in foodborne bacterial contaminants in Victrian.

Threfall, E.J., L.R. Ward, J.A. Frost and G.A. Willshaw, 2000. The emergence and spread of antibiotic resistance in foodborne bacteria. Int. J. Food Microbiol., 62: 1-5.

Teuber, M., 2001. Veterinary use and antibiotic resistance. Curr. Opin. Microbiol., 4: 493-499.

Tjoa, W.S., H.L. Dupont, P. Sullivan, L.K. Pickering, A.H. Holguin and J. Celarte, 1977. Location of food consumption and traveler's diarrhea. Am. J. Epidermiol., 106: 61-66.

Tjoa, W.S., H.L. Dupont, P. Sillivan, L.K. Pickering, A.H.H. Holguin, J. Olarte, D.G. Evans and G.J. Evans Jr., 1979. Location of food consumption and traveler's diarrhea. Am. J. Epidemiol., 106: 61-66. WHO (World Health Organization), 2002.

Umoh, V.J., A. Dagan and J.U. Umoh, 1984. Isolation of Yersinia enterocolitica from milk and milk products in Zaria. Nig. Int. J. Zoonoses, 11: 223-228.

Van den Bogaard, A.E. and E.E. Stobberingh, 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. Int. J. Antimicrob. Agents, 14: 327-335.

World Health Organization, 1983. The role of food safely in health and development. Report of the Joint FAO/WHO expert Committee in Food Safety. Genera.

World Health Organization (WHO), 2002a. WHO global strategy for food safety food for better health. World Heath Organization, Genera sitzerland.

World Health Organization (WHO), 2002b. Foof safety and foodborne illness. Fact Sheet, nE237, pp: 7.

Pakistan Journal of Nutrition 9 (11): 1069-1073, 2010
ISSN 1680-5194

The Chemical Composition of Pigeon Pea (*Cajanus cajana*) Seed and Functional Properties of Protein Isolate

Abdel Rahman Sirelkhatim Mohammed Eltayeb[1], Ali Osman Ali[2] and Rea Haron[1]
[1]Faculty of Agriculture and Fish Sceince, Elnailan University, Sudan
[2]Department of Food Science and Technology, University of Gezira, Sudan

Abstract: Pigeon pea obtained from Khartoum North market were milled at the milling laboratory Food Research Center in Shambat. The chemical composition and functional properties were carried out in the Food Analysis Laboratory, Department of Food Science and Technology, University of Gezira. The proximate analysis in terms (%) of moisture (8), crude protein (21), crude fat (1.7), ash (3.2) and fiber contents (2.5). A protein isolate from defatted Pigeon pea seed flour was extracted and evaluated for its functional properties. The water retention capacity of the isolate (250.3 ml/100 g). The fat absorption capacity was (130 ml/100 g). At the pH 3 gave the highest foam volume (130%). The emulsification capacity (120%) was highest at the pH 4.5 was recorded. Other functionalities such as formation of gels are also reported. Pigeon pea seed protein isolate can be considered of great potential for incorporation into human food products in the Sudan as well as for promotion the functional property in different food.

Key words: Pigeon pea, food protein, fat absorption capacity

INTRODUCTION

World demand for proteins is increasing and so more food protein is required from both conventional and new sources of protein. Accepting that all proteins will have nutritional value, then in both cases successes in the food industry, requires that the protein have good functional properties to be acceptable as a food ingredient.

Market potential for new proteins is great both for supplementation of existing foodstuff and fabrication of new food-stuff. Therefore very important for protein biochemist to understand what functional properties are and how they can be improved in both existing proteins and new proteins.

No one legume or cereal can provide adequate amounts of all nutrients to meet the nutritional requirements of a child. However, even before knowledge on protein content, protein quality, digestibility and the nutrient requirements of humans, to become available it was recognized that mixing legumes with cereal in the diet could improve overall nutrition. The present and newly drive knowledge in these area makes it possible into blend, mix or fortify one food material with others, so that the resulting fortified mix has not only better nutritional quality but also the necessary attributes for consumer acceptance (Hickey and King, 1997).

The plant proteins have been widely used as meat and cereal extenders in recent years nutritional adequate and the unique functional properties of these proteins. However, plant proteins perform an important role in the world food supply.

Pigeon pea is a member of the family Fabaceae. The cultivation of the pigeon pea goes back at least 3000 years. The centre of origin is most likely Asia, from where it traveled to East Africa. Today pigeon peas are widely cultivated in all tropical and subtropical regions of both the old and new world, with temperature range 20-40°C.

In Sudan is traditionally grown along irrigated channel in Gezira, central Sudan or demarcate small farm holding in north Sudan along the Nile.

The objectives of this study were as follows:

- To examine the chemical composition of pigeon pea
- To examine the functional properties of pigeon pea seed protein isolate.
- Focus attention and its compatibility in food system.

MATERIALS AND METHODS

Source of material: Pigeon pea obtained from Khartoum North market were millet at Food Research Center in Shambat. The chemical composition and functional properties were carried out in the Food Analysis Laboratory, Department of Food Science and Technology, University of Gezira.

Sample preparation: Pigeon pea seeds were washed and dried before milling and pass through a 60mm mesh sieve (British Standard), the flour was extracted from defatted flour with n-hexane in a sox let for 9 h at room temperature (27±2°C).

Corresponding Author: Abdel Rahman Sirelkhatim Mohammed Eltayeb, Faculty of Agriculture and Fish Sceince, Elnailan University, Sudan

Proximate analyses: Proximate analysis was carried out on raw pigeon pea flour for moisture content, crude protein (Kjeldahl method), crude fat (sox let extraction), crude fiber and ash were determined according to AOAC (1984). Total carbohydrate was obtained by difference.

Determination of water and oil absorption capacity: Water absorption capacity was determined using the method of Sathe and Salunkhe (1981) with slight modifications. 10 mL of distilled water was added to 1.0 g of the sample in a beaker. The suspension was stirred using a magnetic stirrer for 5 min. the suspension obtained was thereafter centrifuged at 3555 rpm for 30 min and the supernatant measured in a 10 mL graduated cylinder. The density of water was taken as 1.0 g/cm^3. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant. The same procedure was repeated for oil absorption except that oil was used instead of water.

Determination of the gelation concentration: The least gelation concentration was determined by the method of Sathe and Salunkhe (1981). East tubes containing suspensions of 2, 4, 6, 8 up to 20% (w/v) flour in 5 ml distilled water were heated for 1 h in boiling water, followed by cooling in ice and further cooling for 2 h at 4°C. The least gelation concentration was the one at which the sample did not fall down or slip when the test tube was inverted.

Determination of foaming properties: The foam capacity and stability were studied by the method of Coffman and Garcia (1977). A known weight of the mucuna sample was dispersed in 100 mL distilled water. The resulting solution was homogenized for 5 min at high speed. The volume of foam separated was noted. The total volume remaining at interval of 0.00, 0.30, 1, 2, 3, 4 up to 24 h was noted for the study of foaming stability.

$$\text{Foaming capacity (\%)} = \frac{\text{Vol. after homogenization - Vol. before homogenization}}{\text{Vol. before homogenization}} \times \frac{100}{1}$$

$$\text{Foaming stability (\%)} = \frac{\text{Foam volume alter time (t)}}{\text{Initial foam volume}} \times \frac{100}{1}$$

Emulsion capacity: The emulsion capacity and stability were studied by the method of Coffman and Garcia (1977).

Emulsions were formed inside a 600 ml beaker using a continuous stirring apparatus. The apparatus consisted of a regulated/ stabilized 6 V power supply, burette, a stirrer, a beaker with emulsion and a digital millimeter. The stirrer was made up of stainless steel rod holding a Perspex bridge was fixed to a 6 V D.C. motor spindle by means of a plastic adaptor. The motor itself was driven by a regulated and stabilized 6 V D.C. power supply. The millimeter monitored the current drop by the stirrer motor to maintain a constant speed. The greater the viscosity of the emulsion, the greater will be the current drawn. The protein sample (0.2, 0.5, 0.75, 1.00 and 1.25 g) was dissolved in 25 ml of distilled water making 1, 2, 3, 4 and 5% Slurries (w/v), respectively.

Necessary pH adjustment was made to ensure maximum solubilization of the protein. The mixture was stirred for 30 min in order to disperse the sample. Oil was then added at a rate of 1.00 ml/s from a burette until emulsion collapsed indicated by a shape fall in motor current. The volume of oil added up to inversion point was noted and the emulsion capacity expressed as ml oil per g of sample. The emulsion stability was determined by allowing the emulsion prepared to stand in a graduated cylinder and the volume of oil separated at time of 0.00, 0.5, 1, 2, 3 up to 24 h was noted each case. The emulsion stability was determined by following the procedure used for emulsion capacity except that 100 ml of oil was added rather than adding oil until the emulsion breakdown.

The good capacity of FRC pigeon pea seed protein isolate qualifies for use in products such as confections, soups and sausage emulsions.

Wet ability was estimated according to the method of Coffman and Garcia (1977).

Two grams isolate powder were weighed in a sieve (20 mech) and transferred to a beaker containing 80 ml. distilled water without stirring the water. The behavior of the powder was observed on the water surface immediately after adding the sample. After 30 min. observation, the material was stirred sufficiently fast enough to form a vortex to reach the bottom of the beaker. The stirring continued for one min. after which the grade describing wet ability was recorded as excellent, good fair or poor according to the time and behavior of the dispersion.

RESULTS AND DISCUSSION

Chemical composition: The results of proximate analysis of the pigeon pea flour are shown in Table 1. The pigeon pea is higher content of protein (21%). The protein content was within the range of 19-23% as reported by Duke (1981). The moisture content is in the range of 8-15% that was reported by NAS (1980).

Water retention capacity (WRC): WRC of Pigeon pea seed protein isolate was (250.333 ml/100 g) (Table 2). This result of protein isolate is similar to value reported by Lin *et al.* (1987) on sunflower meal products and less than the value of watermelon (320 ml/100 g) reported by Hayat *et al.* (1999). These result less than double the value (140) reported by Elkhatim (1994) for cottonseed protein isolate. The WRC was reported to increase with increasing level of protein content (Rhee *et al.*, 1981; Kinsella, 1979; Lin and Leeder, 1974).

Table 1: Proximate chemical composition of pigeon pea

Component	Percentages
Moisture	8.0
Protein	21.0
Fat	1.7
Fiber	2.5
Ash	3.2
CHO by difference	

Table 2: Water and oil absorption of pigeon pea protein isolate

	Pigeon pea	
	Protein (g/100 g)	Average
Water absorption	250	250.3
	252	
	249	
Oil absorption (%)	131	130
	129	
	130	

WRC is a critical function of protein various food products like soups, dough and baked product (Sosulski *et al*., 1976).

Fat absorption capacity (FAC): The result of FAC are presented in Table 2. The FAC is (130 ml/100 g). The value of FAC differ depending of the nature of the oil used for the study (Booma and Prakash, 1990). Akobundu *et al*. (1982) reported a value of 2.1 ml/g FAC watermelon seed flour and 2.1 ml/g for cottonseed protein isolate and 2.3 ml/g for egg protein. FAC of casein was 0.7 ml/g as reported by Booma and Prakash (1990).

Emulsion capacity: The EC of Pigeon pea seed protein isolate was more efficient in emulsifying the oil at the pH 4.5 (120 ml oil/g isolate) (Fig. 1). Ramanatham *et al*. (1978) reported that groundnut and soy protein isolate were more efficient in emulsifying oil at pH 3.0 (100 ml oil/g sample) than at alkaline pH 8.0 (82 oil/g sample). The experimental conditions, such as equipment design, shape of the container, temperature, speed of blending, nature of blades in the blender, rate and mode of addition, pH, protein, solubility, concentration, preence of salt and water, would all individually contribute to the emulsifying capacity of proteins (Kinsella, 1976).
The Emulsion Stability (ES) of the isolate taken at the time is 0.25, 0.5, 0.75, 1, 2, 12, 24, 48h, this suggests the isolate has stable activity which is not affected by heat treatment (Fig. 2). The decrease of emulsion increase when the time (h) increase.

Foaming capacity: The effect of pH on FC is shown in Fig. 3. Maximum increase in foam volume (170) was observed at the pH 3. The FC of cottonseed protein isolate was found to be better at pH 7.0 than at pH 4.0 while its maximum solubility was at pH 7. The FS of Pigeon pea seed protein isolate taken at extreme pH 3, 4.5, 6, 7.5 and 9 presented in Fig. 4. It can be seen that the falling rate of FS at the pH 3 compared with the others pH. Generally the FS decreased gradually at the first min standing as about 50% of the initial foam

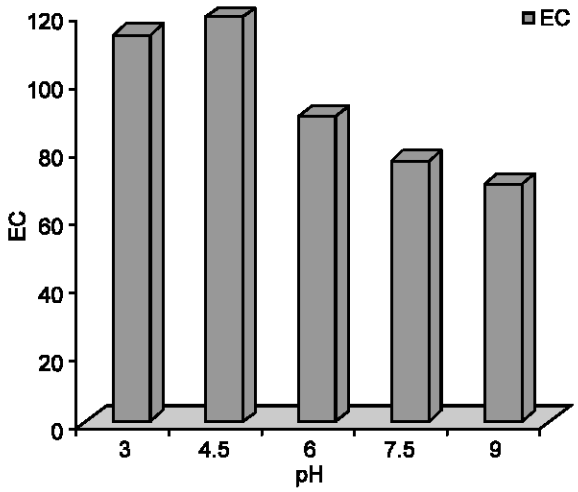

Fig. 1: Emulsion capacity

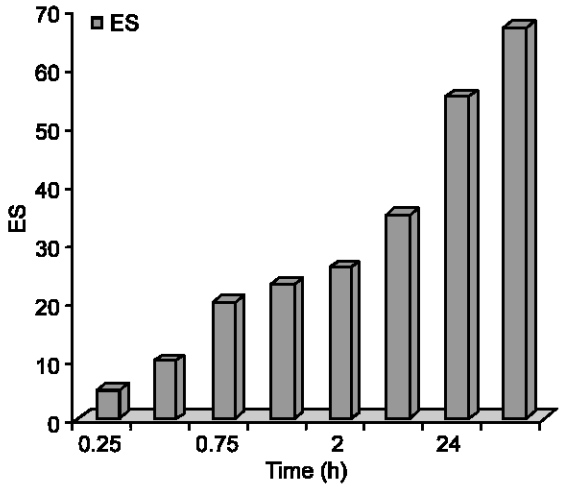

Fig. 2: Emulsion stability

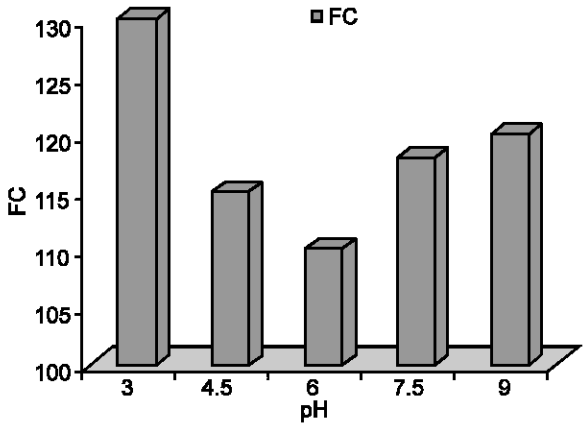

Fig. 3: Foaming capacity

collapsed. After 15-20 min standing the remaining foam was stable nearly for one hours.

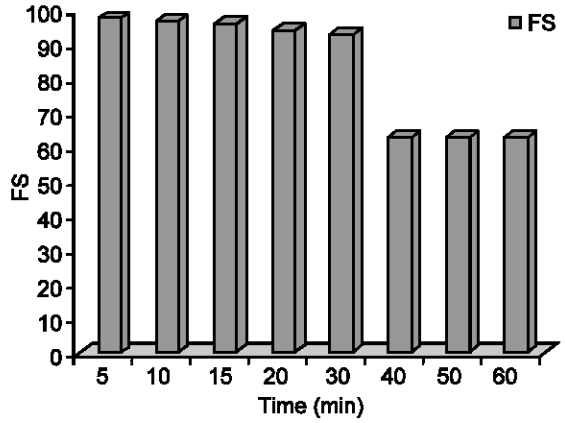

Fig. 4: Foaming stability

Table 3: Least gelation concentration (%) of pigeon pea protein isolate

Sample	Concentration (%) at nitrate pH	Gel formation
Pigeon pea	2-14	Liquid
	16 and above	Viscous

Having seed foaming properties, may be useful for in food systems to improve textural and leavening characteristics such as ice-cream, cake topping and confectionery products.

Gelation: The gelling property of pigeon pea protein isolate was determined at various solid desperation concentration. Gelling of protein depends on protein concentration; pH balance of cat ions and anions (Meganne and Ralph, 1987). Viscous gel formation did not occur at below a concentration 16% at neutral pH show that at Table 3.

The gelation formed at 16% these values compared favorably with those reported for African yam bean (16 to 20% by Abbey and Ayuk, 1991).

Gelation properties are interrelated to water absorption capacities hence the low water absorption capacity recorded by the protein isolates flours could explain the deficient gel formation capacity. Gelation takes place more readily at higher protein Concentration becauce of greater intermolecular contact during heating. High protein solubility is always necessary for gelation as Observed by Wiltoon *et al.* (1997).

Wettability: The wettability of the pigeon pea protein isolate was good since is took 27 min. for complete wetness. This time of witting was less than that of watermelon protein isolate reported by Hayat *et al.* (1999) and also less than cotton seed protein isolate reported by Elkhatim (1994).

Conclusion and recommendations: (WAC) of pigeon pea protein isolate is highly show that in Table 2. This is a critical function to great use in bakery products.

The pigeon pea protein isolate added at various ratio to produced ice cream due to its highly foaming properties at neutral pH show at Fig. 3 and 4.

The high protein content of the pigeon pea, could be used as a protein supplement to increase the protein content of any convenience food making a highly nutritious probably low cost product.

In addition to the nutritional contribution, the pigeon pea and protein isolate was found to exhibit some good functional properties which it a possible good quality protein source for food application for instance. The high solubility of the isolate of both acidic acid position in terms of being used as functional ingredient for its emulsification, foaming, water and gelling properties in various food systems such as meat products, salad dressing, dairy product etc.

The possible used of the protein isolates can be summarized as follows:

- It could be incorporated into liquid food and beverage due to its good solubility at both the acidic and alkaline pH.
- The water retention capacity of the isolate makes it of great use in bakery products as well as in meat product.
- The fat absorption capacity for the isolate allows its use in sausages where it can be a good alternative to casein.
- The isolate can be used to enhance and stabilized fat emulsion in chapped and comminuted meats, cake batters, milk, mayonnaise and frozen desse.
- It could also be used in whipped toppings, chiffon deserts and ice cream specialties due to its foaming proprieties.
- Its gelation properties can be utilized in cheese and other milk products.
- Its good wettability allows its use in texture and/or comminuted meats as well as bakery product.

REFERENCES

Abbey, B.W. and E.J. Ayuk, 1991. Functional properties of African yam bean flour (*Sphenostylis sternocarpa*). Nig. J. Nutr. Sci., 12: 44-46.

Akobundu, E.N.T., J.P. Cherry and J.G. Simmon, 1982. Chemical functional and nutritional properties of Egusi (*Colocynthis citrullus* L.) seed protein product. J. Food Sci., 47: 829-835.

AOAC, 1984. Official Methods of Analysis, 14th Edn., Association of Analytical Chemists, Washington, DC.

Booma, K. and V. Prakash, 1990. Functional properties of the flourand the major protein fraction from sesame seed. sunflower and safflower seed. Acta Alimentaria, 19: 163-176.

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

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

Elkhatim, M.N., 1994. Functional properties of Edible Grade Cotton Seed Protein Isolate. M.Sc. Thesis, University of Khartoum, Sudan.

Hayat, A.H., A.M.N. Abdel Azim and R.A. Abdel Halim, 1999. Functional properties of Watermelon (*Citrullus lanatus*) seed protein isolate.

Hickey, M. and C. King, 1997. Common families of flowering plants. Cambridge: Cambridge University Press.

Kinsella, J.E., 1979. Functional properties of soy proteins. J. Am. Oil Chemists Soc., 56: 242-246.

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

Lin, M.P. and G.J. Leeder, 1974. Mechanism of emulsifier action in an ice cream system. J. Food Sci., 39: 108-111.

Lin, M.J.Y., E.S. Humbert and F.W. Sosulski, 1987. Certain functional properties of sunflower meal products. J. Food Sci., 39: 3632-670.

Meganne, O.W. and L.P. Ralph, 1987. Functional properties of protein concentrates from pressed jojoba meal. Cereal Chemists, 64: 94-97.

NAS, 1980. Firewood crops. Shrub and tree species for energy production. National Academy of Sciences, Washington, DC.

Ramanatham, G., L.H. Ron and L.N. Urs, 1978. Emulsification properties of groundnut protein. J. Food Sci., 43: 1270-1278.

Rhee, K.C., C.K. Kuo and E.W. Lusas, 1981. Texturization. In: Protein Functionality in foods (Ed J.P. Cherry). ACS Symp. Ser. 147. American Chemical Society, Washington, DC.

Sathe, S.K. and D.K. Salunkhe, 1981. Functional properties of great northern bean (*Phaseolus vulgris*) proteins: Emulsion, foaming, viscosity and gelation properties. J. Food Sci., 46: 71-75.

Sosulski, F.W., E.S. Humber, E.S. Bui and J.I. Jones, 1976. Functional properties of rapeseed flours concentrates and isolates. J. Food Sci., 41: 1349-1351.

Wiltoon, P., R. Larry, K. Beuchat and P. Dixon, 1997. Functional properties of cowpea (*Vigna unguiculata*) flour as affected by soaking, boiling and fungal fermentation. J. Food Sci., 45: 480-486.

Pakistan Journal of Nutrition 9 (11): 1074-1077, 2010
ISSN 1680-5194

Evaluation of *in-vitro* Antioxidant Activities of Methanol Extracts of *Persea americana* and *Cnidosculus aconitifolius*

M.F. Asaolu[1], S.S. Asaolu[2], J.B. Fakunle[3], B.O. Emman-Okon[3], E.O. Ajayi[3] and R.A. Togun[4]
[1]Department of Biochemistry, University of Ado-Ekiti, P.M.B. 5363, Ado- Ekiti, Nigeria
[2]Department of Chemistry, University of Ado-Ekiti, P.M.B. 5363, Ado-Ekiti, Nigeria
[3]Department of Chemical Pathology, Obafemi Awolowo University, Ile-Ife, Nigeria
[4]Department of Heamatology and Immunology, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract: The present study was undertaken to investigate the antioxidant activities of *Persea americana* and *Cnidosculous aconitifolius*. The *in-vitro* antioxidant activity of the methanol extracts of the leaves of *Persea americana* and *Cnidosculous aconitifolius* was evaluated using various experimental methods such as 1,1-diphenyl-2-picryl-hydrazyl (DPPH), nitric oxide and reducing power radical- scavenging activity assay. Phytochemical screening as well as the amounts of total phenol and flavonoids were also determined. The present study revealed that both the methanol extracts of the leaves of *Persea americana* and *Cnidosculous aconitifolius* possess significant antioxidant activities. However, *Persea americana* was found to have higher radical scavenging activity than *Cnidosculous aconitifolius* but the phenol content of *Cnidosculous aconitifolius* was higher than that of *Persea americana* whereas *Persea americana* was observed to possess more flavonoids than *Cnidosculous aconitifolius*.

Key words: Methanol extracts, radical-scavenging activity, antioxidant activities, phytochemical screening

INTRODUCTION

The development of a wide range of diseases such as malaria, diabetes, cardiovascular diseases and Parkison's diseases to mention few have been attributed to oxidative stress (Kremsner *et al*., 2000; Austin *et al*., 1994; Liao *et al*., 1997). Free radicals which are the products of oxidative stress (Utpal *et al*., 2008) are aggravated in these disease states leading to decrease in the antioxidant defense system.

Antioxidants have been found to play a major role in protecting the human body against damage induced by reactive free radicals (Halliwell and Gutteridge, 1990; Mates *et al*., 1999) by reacting with free radicals, chelating and also by acting as oxygen scavenger (Shahidi and Wanasundara, 1992; Buyukokuroglu *et al*., 2001).

Many studies have revealed that natural products and their derivatives possess efficient antioxidative characteristics (Rhee *et al*., 2009; Asaolu *et al*., 2010b). Besides, flavonoids and phenols, which are widely distributed in plants are known for their anticarcinogenic, antioxidant, inflammatory as well as free radical scavenging potentials (Miller, 1996; Frankel, 1994).

Worldwide, interest in natural products as antioxidants in reducing free radical induced tissue damage has led to a greater appreciation of the therapeutic potentials of plants. The use of natural antioxidants symbolizes safety in contrast to the synthetic products (Patel *et al*., 2010).

The presence of some phytochemicals and antioxidants has been reported in *Persea americana* and *Cnidosculous aconitifolius* which explained the hypotensive action of these plants in the treatment and management of cardiovascular diseases (Asaolu *et al*., 2010a, b, c). In this study, our purpose is to investigate and compare the *in vitro* antioxidative capacities of methanol extracts of *Persea americana* and *Cnidosculous aconitifolius*.

MATERIALS AND METHODS

Plant materials

Collection and identification of plant materials: Fresh leaves of *Persea americana* and *Cnidosculous aconitifolius* were collected from a farm in Ado-Ekiti, Ekiti State, Nigeria. Taxonomic identification of the plants was made in the Department of Plant Science, University of Ado-Ekiti, Nigeria.

Preparation of extracts: The leaves of *Persea americana* and *Cnidosculous aconitifolius* were washed with distilled water, air dried and ground into fine powder using a blender. The powdered leaves were defatted with n-hexane in soxhlet extractor and then extracted with methanol at room temperature. The extracts were concentrated using rotary evaporator.

Analysis: The preliminary phytochemical screening of the methanol extracts of *Persea americana* and *Cnidosculous aconitifolius* was carried out using the

Corresponding Author: M.F. Asaolu, Department of Biochemistry, University of Ado-Ekiti, P.M.B. 5363, Ado-Ekiti, Nigeria

method described by Sofowora (1986). Total phenols and flavonoids contents of the extracts were also determined (Ebrahimzadeh *et al*., 2009). Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of the extracts was performed as described by Batubara *et al*. (2009) while nitric oxide radical scavenging activity assay was carried out using the method of Green *et al*. (1982). The reducing power of the methanol extracts of the leaves of *Persea americana* and *Cnidosculous aconitifolius* was evaluated according to the method of Okhawa *et al*. (1979).

Statistical analysis: Data were expressed as mean±SEM. The significant differences between *Persea americana* and *Cnidosculous aconitifolius* were assessed by Student's t test. A probability value of $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The results obtained from the phytochemical screening of the leaves of *Persea americana* and *Cnidosculous aconitifolius* showed the presence of saponins, tannins, flavonoids, alkaloids, phenols, anthraquinones, triterpenes and phlobatannins in these plants (Table 1). In DPPH radical-scavenging activity assay, the radical scavenging activity of the methanol extract of the leaves of both *Persea americana* and *Cnidosculous aconitifolius* increases with increasing concentration (Table 3) but was less than that of BHA which served as the reference compound. This may account for the uses of DPPH as a substrate in evaluation of antioxidative activity of antioxidants (Duh and Yen, 1995). However, the scavenging effect was higher in *Persea americana* than in *Cnidosculous aconitifolius* (Tables 3 and 4). It can therefore be concluded that the extracts of the leaves of *Persea americana* and *Cnidosculous aconitifolius* exhibit a noticeable effect on scavenging free radicals.

Tables 5 and 6 presents the percentage inhibition of nitric oxide generation by methanol extracts of *Persea americana* and *Cnidosculous aconitifolius* respectively. The antioxidant activity of methanol extracts of the leaves of both *Persea americana* and *Cnidosculous aconitifolius* was much weaker than that of quercetin which was used as a standard. Similar observation has been reported for other medicinal plants (Komal *et al*., 2010). The ability of the methanol extract of *Persea ameriana* to effectively scavenge free radical was higher compared with that of *Cnidosculous aconitifolius*.

The reducing power by methanol extract of *Persea americana* and *Cnidosculous aconitifolius* is as presented in Tables 7 and 8 respectively. The antioxidant activity of methanol extract was less than that of ascorbic acid.

Table 1: Phytochemical screening of methanol extracts of the leaves of *Cnidosculous aconitifolius* and *Persea americana*

Phytochemicals	*Cnidosculous aconitifolius*	*Persea americana*
Saponins	+++	++
Tannins	+	+
Flavonoids	++	++
Glycosides	ND	ND
Alkaloids	+++	+++
Phenols	++	+++
Anthraquinones	++	+++
Triterpenes	+	++
Steroids	ND	ND
Phlobatannins	+	++
Cardenolides	ND	ND

Table 2: % phenolic and flavonoid contents of methanol extracts of the leaves of *Cnidosculous aconitifolius* and *Persea americana*

Plant	Phenol (%)	Flavonoids (%)
Cnidosculous aconitifolius	1.80±0.43	0.35±0.11
Persea americana	1.22±0.52	0.58±0.09

Data are expressed as mean±SEM of triplicate tests

Table 3: Percentage DPPH radical scavenging activity of methanol extracts of the leaves of *Persea americana* and Butylated Hydroxyl Anisole (BHA)

Concentration (µg/ml)	% Inhibition by methanolic extract of *Persea americana*	% Inhibition by BHA
100	40.58±3.22	60.11±4.32
200	53.44±5.29	70.30±3.81
300	61.23±3.89	75.55±4.00
400	68.38±3.61	83.22±2.10
500	73.49±2.81	89.10±3.11
600	80.11±5.67	92.12±4.30

Data are expressed as mean±SEM of triplicate tests

Table 4: Percentage DPPH radical scavenging activity of methanol extracts of the leaves of *Cnidosculous aconitifolius* and Butylated Hydroxyl Anisole (BHA)

Concentration (µg/ml)	% Inhibition by methanolic extract of *Cnidosculous aconitifolius*	% Inhibition by BHA
100	30.33±2.11	40.23±1.90
200	41.34±3.22	50.11±2.14
300	52.46±2.81	60.22±1.14
400	59.45±2.51	71.34±4.88
500	61.67±5.98	79.23±2.14
600	72.78±6.54	83.14±4.23

Data are expressed as mean±SEM of triplicate tests

Generally, the results of the present study indicate that the methanol extracts of the leaves of both *Persea americana* and *Cnidosculous aconitifolius* showed strong antioxidant activity. This might be as a result of the presence of some phytochemicals most importantly flavonoids and phenols which are well known

Table 5: Percentage Nitric oxide radical scavenging activity of methanol extracts of the leaves of *Persea americana* and curcumin

Concentration (µg/ml)	% Inhibition by methanolic extract of *Persea americana*	% Inhibition by curcumin
40	20.89±6.82	44.33±1.89
80	38.11±4.18	55.49±2.67
100	58.41±2.18	63.34±3.62
120	65.33±1.21	71.38±2.10
140	70.44±3.42	80.14±3.90
160	75.31±4.11	85.77±4.20

Data are expressed as mean±SEM of triplicate tests

Table 6: Percentage Nitric oxide radical scavenging activity of methanol extracts of the leaves of *Cnidosculous aconitifolius* and curcumin

Concentration (µg/ml)	% Inhibition by methanolic extract of *Cnidosculous aconitifolius*	% Inhibition by curcumin
40	18.49±2.18	40.21±2.33
80	33.64±3.11	51.31±3.84
100	52.14±4.31	60.41±2.00
120	61.22±2.17	68.81±1.95
140	68.21±1.48	75.22±4.80
160	73.14±2.33	82.19±3.18

Data are expressed as mean±SEM of triplicate tests

Table 7: Percentage antioxidant activity of methanol extracts of the leaves of *Persea americana* and ascorbic acid in reducing power method

Concentration (µg/ml)	Reducing power of methanol extract of *Persea americana*	Reducing power of methanol extract of Ascorbic acid
50	42.17±2.14	51.92±2.18
100	51.34±2.64	59.22±3.27
150	65.22±5.36	70.18±2.14
200	72.31±4.11	72.48±4.22
250	83.47±3.18	90.50±3.81
300	90.12±2.41	96.00±4.81

Data are expressed as mean±SEM of triplicate tests

Table 8: Percentage antioxidant activity of methanol extracts of the leaves of *Cnidosculous aconitifolius* and Ascorbic acid in Reducing Power method

Concentration (µg/ml)	Reducing power of methanol extract of *Cnidosculous aconitifolius*	Reducing power of methanol extract of Ascorbic acid
50	31.21±2.81	43.16±4.18
100	43.11±1.89	47.38±3.01
150	55.22±4.10	65.14±4.08
200	70.05±3.21	67.98±3.21
250	75.14±2.38	85.84±5.24
300	85.22±3.18	90.49±4.21

Data are expressed as mean±SEM of triplicate tests

antioxidants. Phenols in plants are known for their scavenging ability as a result of the presence of hydroxyl groups in them. In some plants, a significant association has been reported between phenols and antioxidant activity (Gulcin *et al*., 2002) as phenols help in stabilizing lipid peroxidation (Yen *et al*., 2005). However, antioxidative activity of methanol extract of the leaves of *Persea americana* was much more effective than that of *Cnidosculous aconitifolius* but less effective than that of ascorbic acid, quercetin and BHA. This may be due to the presence of some chemicals in the methanol extract of the leaves of *Persea americana* which play a significant role in the antioxidant capacity. This needs to be investigated.

REFERENCES

Asaolu, M.F., S.S. Asaolu, I.G. Adanlawo, B.T. Aluko, Y.R. Alli-Smith, Y. Ibitoye and A.M. Abidakun, 2010a. Comparative chemical compositions of the leaves of some selected antihypertensive medicinal plants in Nigeria. Der Pharm. Chem., 2: 11-15.

Asaolu, M.F., S.S. Asaolu and I.G. Adanlawo, 2010b. Evaluation of phytochemicals and antioxidants of four botanicals with antihypertensive properties. Int. J. Pharm. Bio. Sci., 1: 1-7.

Asaolu, M.F., S.S. Asaolu, A.O. Oyeyemi and B.T. Aluko, 2010c. Hypolipemic effects of methanolic extracts of *Persea americana* seeds in hypercholestrolemic rats. J. Med. Med. Sci., 1(4).

Austin, M.A., J.E. Hokanson and J.D. Brunzell, 1994. Oxidative stress and ageing. Curr. Opin. Lipid., 5: 395-403.

Batubara, I., T. Mitsunaga and Ohashi, 2009. Screening anti-acne potency of Indonesian medicinal plants: Antibacterial, lipase inhibition and antioxidant activities. J. Wood Sci., 55: 230-235.

Buyukokuroglu, M.E., I. Gulcin, M. Okatay and O.I. Kufrevioglu, 2001. *In-vitro* antioxidant properties of dantrolene sodium. Pharmacol. Res., 44: 491-494.

Duh, P.D. and G. Yen, 1995. Changes in antioxidant activity and components of methanolic extracts of peanut hulls irradiated with ultraviolet light. Food Chem., 54: 127-131.

Ebrahimzadeh, M.A., S. Eshanifar and B. Eslami, 2009. *Sambucus ebulus elburensis* fruits: A good source for antioxidants. Phcog. Mag., 4: 447-450.

Frankel, J.L., 1994. Nutritional benefits of flavonoids. Paper presented at International conference on food factors: Chemistry and Cancer Prevention. Hamamatsu, Japan; Abstract C6-2.

Green, L.C., D.A. Wanger, J. Glogowski, P.L. Skipper, J.K. Wishnok and S.P. Tannenbaum, 1982. Antioxidant properties. Biochem., 126: 131-138.

Gulcin, I., M.E. Buyulcokuroglu, M. Oktay and O.I. Kufrevioglu, 2002. On the *in vitro* antioxidative properties of melatonin. J. Pineal. Res., 33:167-171.

Halliwell, B. and J.M.C. Gutteridge, 1990. Role of free radicals and catalytic metal ions in human disease: An overview. Method Enzymol., 186: 1-85.

Komal, M., K. Parimal and S.S. Khadabadi, 2010. *In vitro* antioxidant activity of methanolic extract of *Erythrina indica*. Der Pharm. Lettr., 2: 16-21.

Kremsner, P.G., B. Greve, B. Lell, D. Luckner and D. Schmid, 2000. Malaria anaemia in African children associated with high oxygen radical production. Lancet, 355: 40-41.

Liao, L., R.M. Starzyk and D.N. Granger, 1997. Arterioscler. Thromb. Vasc. Biol., 17: 437-444.

Mates, J.M., C. Perez-Gomez and I. Nunez de Castro, 1999. Antioxidant enzymes and human diseases. Clin. Chem., 32: 595-603.

Miller, A.L., 1996 Antioxidant flavonoids: Structure, function and clinical usage. Alt. Med. Rev., 1: 103.

Okhawa, H., W. Ohishi and K. Yagi, 1979. Analytical Biochemistry, 95: 351.

Patel, R.V., P.R. Patel and S.S. Kajal, 2010. Antioxidant activity of some selected medicinal plants in Western Region of India.

Rhee, M.H., H.J. Park and J.Y. Cho, 2009. Salicornia herbacea: Botanical, chemical and pharmacological review of halophyte marsh plant. J. Med. Plants Res., 3: 548-555.

Shahidi, F. and P.D. Wanasundara, 1992. Phenolic antioxidants. Crit. Rev. Food. Sci. Nutr., 32: 67-103.

Sofowora, A., 1986. The state of medicinal plant research in Nigeria. Ibadan University Press, pp: 113-160.

Utpal, B., A. Sahu, S. Ali, L. Kasoju, A. Singh and H. Sharanabasava, 2008. Oxidative stress and free radical changes. Food Res. Int., 41: 1-15.

Yen, W.J., L.W. Chang and P.D. Duh, 2005. Antioxidant activity of peanut seed testa and its antioxidative component, ethyl protocatechuate. Food Sci. and Tech., 38: 193-200.

Pakistan Journal of Nutrition 9 (11): 1078-1083, 2010
ISSN 1680-5194

Bacteriological and Physico-Chemical Quality of Wheaten White Bread Flour Made for Nigerian Market

A.R. Akpe[1], P.O.A. Usuoge[2], O.I. Enabulele[3], F.I. Esumeh[1], H.A. Obiazi[1], I.N. Amhanre[1] and O.M. Omoigberale[1]
[1]Department of Microbiology, Ambrose Alli University, PMB 14, Ekpoma, Edo State, Nigeria
[2]Bendel Feed and Flour Mill, Ewu, Edo State, Nigeria
[3]Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria

Abstract: Bacteriological and physico-chemical quality changes in wheaten white bread flour made for Nigerian market were investigated during storage at room temperature for four months. During storage, bacterial count decreased; between day 15 and day 105, count decreases from 45.0 X 10^3 cfu/g to 1.0 X 10^3 cfu/g for flour brand 1 and between day 60 and day 105, count decreases 12.5 X 10^3 cfu/g to 3.5 X 10^3 cfu/g for flour brand 3. Statistically, bacterial counts in the different brands of flour during storage show a significant difference. Total coliform count in flour brand 1 decreases d from 4.60 MPN/g (day 15) to zero (day 105) in storage. Significant count in coliform count was obtained for flour brand 1 and flour brand 2 but no significant difference was observed for flour brand 3 and 4 during storage. *Staphylococcus albus*, *Klebseilla pneumoneae* and *Bacillus subtilis* were detected and isolated. Lower pH of below pH 6.0 were recorded at day 105 for flour brands 1, 2 and 4 and the ash content of the various brands of flour was above 0.65% recommended for Nigerian flour with effect from day 90 of storage. Protein, gluten, fat, moisture, and carbohydrate contents were within the acceptable limit values for Nigerian flour.

Key words: Bacteriological, physico-chemical, wheaten, flour, bread

INTRODUCTION

Wheat flour is the clean, soft and dry product derived from milling or grinding of clean fully moistured wheat (*Triticum* species) grains (SON, 2000). Wheat is unique among the grain because it has the potential to produce gluten, a protein that gives dough its strength and elasticity; so it is an important element in the texture of baked food products.

More than 90% of the wheat flour we eat is white or refined flour, which consists of only the ground endosperm of the wheat kernel (Badsha *et al*., 2005). White flour is popular because it produce lighter baked good that whole-wheat flour and has unequaled ability to produce gluten.

There are several commercial grade of flour and flour is made from different blends of wheat. The composition of flours is therefore variable and varies from one region (country) to another. The 'all purpose' white flour, which is common in most countries, may differ according to geographic region, milling process and quality of the wheat (Quaglia, 1984).

The quality of the flour and storage condition after milling is very important in the shelf life of the flour. Wheaten white flour being, a food product with high nutritional content can harbour a variety of bacteria including pathogenic and non-pathogenic forms. As the vigorous cleaning processes to which wheat is subjected in the mill cannot remove all the bacteria, wheaten flour invariably possess a bacterial population derived from the grain. The number of bacteria present will be related to the bacteriological status of the wheat but also depends on the grade of the flour; high grade wheat flour (white flour) produced from the endosperm near the center of the kernels and which are soon removed from contact with the outer skin of the original site of the bacteria will contain significantly fewer bacteria than the low grade flours (Kent-Jones and Amos, 1967). Flour is susceptible to spoilage especially when stored improperly or for too long, it can develop an off flavour or even result in low quality product when used for baking. Hence most flour produced in temperate regions are required to be stored for short periods, for example, the shelf life used by most flour producing industries ranges between 3-4 months (Mashood *et al*., 2005). Changes in the physico-chemical properties of flour during storage have been widely documented (Sur *et al*., 1993; Kent-Jones and Amos, 1967; Hruskova and Machova, 2002). There is very little or no information on the Bacteriological and physico-chemical quality of flour in the Nigerian market. This survey is intended to augment the scarce information on the bacteriogical and physico -chemical quality of Nigerian flour.

MATERIALS AND METHODS

Sample collection: Freshly milled wheaten white flours ready for packaging were collected from four mills

Corresponding Author: A.R. Akpe, Department of Microbiology, Ambrose Alli University, PMB 14, Ekpoma, Edo State, Nigeria

located at Lagos, Sapele, Ewu and Kano, all in Nigeria. Two samples were collected from each location in clean polythene bags and properly sealed. The samples were taken to the laboratory where they are maintained at 28°C±2 and analyses at 15days interval for a period of 4 months to determine their bacteriological qualities and physico-chemical properties. This period was based on the assumed shelf-life of 3-4 months of the flour by the millers.

Bacteriological analysis: The various types and numbers of bacteria associated with wheaten white bread flour were enumerated and quantified according to the method described by Harrigan and McCane (1976). Ten fold serial dilutions were carried out and 1 ml of appropriate dilutions was aseptically plated on nutrient agar (Biotec) using the pour plate technique for total aerobic bacterial count. Emerging colonies were recorded as colony forming unit per gram.

The Most Probable Number (MPN) of coliforms in the samples were determined by weighing 10 grams of into 90ml of sterile distilled water from which the 3 sets of tubes of 10 ml MacConkey broth (double strength) were inoculated with 10 ml flour suspension, 3 sets of 5 ml MacConkey broth (single strength) with 1 ml flour suspension and another 3 sets of 5 ml MacConkey broth (single strength) with 0.1 ml of the flour suspension. All media were incubated at 35°C for 24 h. Positive tubes were noted and Most Probable number of coliform estimated using McCrady's probability table and recorded as MPN/10 g of sample. Emerging colonies on the nutrient agar were recorded as colony forming unit per gram (cfu/g).

Positive MPN tubes were subcultured onto fresh McConkey broth and Peptone water and incubated at 44°C for 24 h to detect the presence of *E. coli*. Indole test was carried out on the peptone water culture thereafter.

Characterization and identification isolates: Positive MPN tubes were subcultured to MacConkey agar and incubated at 37°C for 24 h. Thereafter discrete colonies from these and nutrient agar plates were characterized and identified using colonial, morphological and biochemical characteristics described by Vanderzannt and Splittoesser (1993).

Determination of physico-chemical properties of flour

pH: A pH meter (JENWAY 3310) was used to determine the pH of 10% suspension of flour in water after standardizing with buffer at pH 7. A standard buffer 7 powder was prepared into 200 ml solutions with distill and ionize in a volumetric flask. The buffer solution was poured into a beaker and the pH electrodes immersed in and regulated to stabilize at pH 7. There after, the electrodes were removed and introduced into the filtrate from the 10% flour suspension and allowed to stabilize and the final pH reading to be taken.

Moisture: Moisture content was determined using the dry oven method (Polmeranz and Meloan, 1996).

Gluten: Extraction of gluten was done according to the ICC (international cereal chemistry) -Standards No 106/1.

Protein: Analysis of protein content was done using the Kjeldahl method. The sample was heated in sulphuric acid and digested until the carbon and hydrogen are oxidized and the protein nitrogen is reduced and transformed into ammonium sulphate. The concentrated sodium hydroxide is added and the digest heated (distillate) to drive off the liberated ammonia into a known volume of standard acid solution. The unreacted acid is determined and the results are transformed by calculation with factor 5.7 into a percentage of protein in the flour sample.

Carbohydrate: This was estimated according to the ICC -standard No. 123, method for the determination of starch content by hydrochloric acid dissolution.

Fat: Extraction of fat was performed by the Soxtec method in automatic fat extraction unit using diethyl ether.

Ash: Determination of flour ash was carried out according to the ICC-standards No. 104, for the determination of flour ash at 900°C.

Statistical analysis: Changes in bacteriological and physico-chemical qualities over the duration of storage for the different flour brands were analyzed for statistical significance using the chi-square goodness of fit. Differences in the above qualities among the different flour brands were tested for statistical significance using the Single Factor Analysis of variance (ANOVA). Where significant differences were detected, the Duncan's Multiple Range (DMR) test was used to separate means on the basis of significance. All statistical tests were carried out using the "SPSS10.0 package".

RESULTS

Results of the Bacteriological and Physico-chemical changes of wheaten white bread flour produced for Nigerian Market during storage are shown in Tables 1-11 below. Moisture content of the various brands of flour ranges between 11.97% (brand 3) to 13.56% (brand 4) (Table 11). Total aerobic bacterial counts of the individual brands of flour decreases during storage (Table 1). Flour brands 1 shows a decrease from 45.0 x 10^3 cfu/g at day 15 to 1.0 x 10^3 cfu/g at day 105. Bacterial counts in the flour brands during storage show significant difference but no significant difference ($p = 0.27$) was noticed in average aerobic bacterial count

Table 1: Total aerobic bacterial count (CFU/g X 10^3) wheaten white bread flour during storage

Flour	Storage Periods								
Brands	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105	significant
1	6.25±0.0	45.0±0.0	21.5±1.5	15.0±0.0	2.0±0.0	1.5±0.5	1.0±0.0	1.0±0.5	p<0.001
2	13.5±1.5	15.5±0.5	6.5±1.5	2.0±0.0	0.002	15.5±0.5	7.0±1.0	2.5±0.5	p<0.01
3	15.1±1.5	5.0±1.0	12.0±0.0	3.5±0.5	12.5±0.5	6.5±0.5	5.0±1.0	3.5±0.5	p<0.01
4	15.6±0.4	28.0±1.0	8.5±0.5	1.0±0.0	5.5±0.5	8±0.0	9.0±1.0	2.0±0.0	p<0.001

Note: p<0.01 = significantly different, p< 0.001 = highly significantly different

Table 2: Total coliform count (mpn/g) wheaten white bread flour during storage

Flour	Storage Period								
brands	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105	significant
1	11.0±1.0	4.1±0.0	4.6±0.0	4.4±0.2	4.4±0.2	2.5±0.1	1.5±0.5	No Growth	p<0.05
2	1.3±0.2	10.0±0.0	2.55±0.15	2.4±0.0	No Growth	1.05±0.05	1.5±0.05	2.0±0.0	p<0.01
3	1.5±0.0	No Growth	1.00±0.0	4.7±0.0	1.0±0.5	0.35±0.05	1.5±0.5	2.5±0.05	p<0.01
4	1.5±0.0	1.75±0.25	1.4±0.0	2.4±0.0	2.3±0.0	2.35±0.05	No Growth	No Growth	p<0.001

Note: p<0.05 = significantly different, p<0.01 = significantly different, p<0.001 = highly significantly different

among the various flour brands. Average bacterial counts in the different brands of flour ranges from 7.813 x 10^3 cfu/g (brand 2) to 11.565 x 10^3 cfu/g (brand 1) (Table 12).

Average total coliform counts for the various brands of flour ranges from 4.06MPN/g (brand 1) to 1.57MPN/g (brand 2) (Table 11) but shows no significant difference (p = 1.80). coliform counts of the individual brands of flour during storage show significant difference for brand 1 (Chi^2 = 142.596) and brand 2 (Chi^2 = 18.727) but no significant difference was detected in brand 3 and 4 (Table 2). Three bacterial genera were isolated (Table 3). The difference in the moisture content of the individual brands of flour is highly significant (p = 21.966) but there is no significant difference in moisture content of flour during storage. There was no significant difference (p = 0.479) in pH of individual flour. The pH ranges from 6.03 (brand 1) to 6.12 (brand 3) (Table 11). Protein and gluten content of the individual flour shows highly significant difference (p = 18.517). Protein and gluten for brand 2 is 11.47% and 10.23% and for brand 4 is 10.24 and 8.64 respectively. Gluten content correlates with the protein content. Carbohydrate content was between 65-66% in all the brands of flour with no significant difference (p = 0.248). Ash content increases for the individual brands of flour during storage, but statistically, there is no significant difference (Table 9). However, there is a high significant difference (p = 7.297) in the ash of the different brands of flour with the range of 0.56% (brand 1) to 0.80% (brand 4) (Table 11). Fat content of the different brand of flour ranges from 0.92% (brand 3) to 0.98% (brand 4), no significant difference (p = 0.915) in the fat content of the various flour brands.

DISCUSSION

Wheaten white bread flour is industrially milled and is expected to contain minimal microbial load. The flour however is not usually treated with any ant microbial agent, but the wheat from which the flour is gotten and the flour itself is subjected to vigorous screening and conditioning during processing. The screening and passage of the flour through the Entelator reduces the levels of vectors such as weevils, ants and maggots.

Statistically, average total bacterial counts from the different brands of flour showed no significant different (p = 0.27) but there was significance in the counts during storage, with flour brands 1 and 2 having a decrease in total bacterial count from 45.0 x 10^3 cfu/g and 15.5 x 10^3 cfu/g at day 10 to 1.0 x 10^3 cfu/g and 2 cfu/g at day 105 respectively. This correlates previous studies that bacterial count and pH values decrease during storage, (Kent-Jones and Amos, 1967). The intermittent decrease in aerobic bacterial counts in the flour brands (2, 3 and 4) can be associated with the decrease in pH and the decimal reduction in the moisture content of the flour. The sudden drop in pH from 6.14 (day 45) to 5.94 (day 60) in Brand 2 (Table 5) resulted to very few bacterial count (Table 1) in flour brand 2 at day 60. At day 105, flour Brand 3 has the highest bacterial count of 3.5 x 10^3 cfu/g compared to the other Brands. This could be attributed to the high pH value (above 6) of Brand 3 compared to the other Brands with pH value lower than 6.0. Brand 1 with pH of 5.64 has the bacterial count of 1.0 x 10^3 cfu/g at day 105.

Bacterial counts of the various flours at later period of storage (from day 90) (Table 1) were in the acceptable limit of 10^4 cfu/g for Nigerian white flour (SON, 2000).

Three bacterial isolates were identified; *Bacillus subtilis, Klebseilla pneumoneae* and *Staphylococcus albus* (Table 3). *Bacillus subtilis* was present in all the Brands and throughout the storage period. This is in agreement with previous study of Sorokulova *et al.* (2003) where they reported that about 10 strains of *Bacillus subtilis* are capable of surviving storage of flour and could actually cause roping in baked bread. During baking of the

Table 3: Bacteria associated with whiten white bread flour during storage

	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105
	Brands	Brands	Brands	Brands	Brands	Brands	Brands	Brands
Bacterial Species	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
Bacillus subtilis	+ + + +	+ + + +	+ + + +	± ± ± ±	- - ± ±	± ± ± ±	+ + + +	± + + +
Klebseilla pneumoneae	± + + +	± ± - +	+ + + +	+ + + +	+ - ± +	+ + ± ±	+ + + -	- + + -
Staphylococcus albus	+ + - -	- - - -	- - - -	- - - -	- - - -	- - + ±	- - - -	- - - -

+ = Present, ± = Relatively present, - = Absent

Table 4: Changes in moisture content (%) of wheaten white bread flour during storage

Flour	Moisture content (%) at								
Brands	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105	significant
1	12.92±0.02	12.85±0.01	12.48±0.37	12.26±0.06	12.92±0.05	13.03±0.00	13.16±0.06	12.97±0.16	p>0.05
2	13.00±0.06	12.67±0.04	12.53±0.19	12.15±0.01	12.79±0.02	12.98±0.09	13.00±0.01	13.00±0.01	p>0.05
3	11.93±0.08	11.89±0.31	11.25±0.05	11.60±0.44	12.02±0.05	12.27±0.40	11.91±0.10	11.92±0.08	p>0.05
4	13.65±0.08	13.23±0.01	13.19±0.01	13.22± 0.02	13.71±0.03	13.82±0.13	13.80±0.07	13.85±0.00	p>0.05

Note: p>0.05 = not significantly different

Table 5: Changes in ph of wheaten white bread flour during storage

Flour	pH of flour at								
Brands	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105	significant
1	6.45±0.02	6.00±0.00	6.01±0.00	6.10±0.05	5.76±0.03	6.07±0.15	6.20±0.01	5.64±0.02	p>0.05
2	6.20±0.01	6.01±0.01	6.01±0.01	6.14±0.01	5.94±0.01	6.14±0.04	6.18±0.01	5.77±0.01	p>0.05
3	6.21±0.01	6.03±0.01	6.0±0.00	6.04±0.06	6.11±0.01	6.21±0.04	6.27±0.01	6.05±0.03	p>0.05
4	6.05±0.02	6.00±0.00	5.95±0.00	6.13± 0.01	6.09±0.03	6.14±0.03	6.14±0.02	5.89±0.08	p>0.05

Note: p>0.05 = not significantly different

Table 6: Changes in carbohydrate content (%) of wheaten white bread flour during storage

Flour	Carbohydrate content (%) at								
Brands	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105	significant
1	66.64±0.04	68.97±0.00	68.42±0.55	65.13±0.00	66.51±0.28	60.78±2.21	62.65±0.15	64.33±0.03	p>0.05
2	66.65±0.00	66.57±0.28	68.15±0.28	64.46±0.13	65.60±0.37	63.22±0.27	62.75±0.05	66.35±0.03	p>0.05
3	60.40±0.20	67.97±0.10	68.15±0.28	65.78±0.10	69.20±0.27	62.95±0.55	63.75±0.25	68.70±0.20	p>0.05
4	64.22±0.20	68.15±0.83	68.97±0.37	66.69±0.09	65.60±0.09	65.96±1.92	64.40±0.20	66.30±0.10	p>0.05

Note: p>0.05 = not significantly different

Table 7: Changes in protein content (%) of wheaten white bread flour during storage

Flour	Protein content (%) at								
Brands	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105	significant
1	11.65±0.04	11.27±0.03	11.55±0.00	11.45±0.05	11.49±0.02	11.46±0.06	11.44±0.05	11.38±0.02	p>0.05
2	11.35±0.00	11.60±0.07	11.64±0.05	11.45±0.03	11.45±0.05	11.50±0.02	11.34±0.04	11.45±0.05	p>0.05
3	11.10±0.05	11.24±0.13	11.41±0.06	11.48±0.08	11.18±0.02	11.21±0.01	10.98±0.01	11.03±0.02	p>0.05
4	9.93±0.08	10.09±0.01	10.36±0.01	10.12± 0.07	9.96±0.04	10.02±0.02	9.96±0.02	9.85±0.05	p>0.05

Note: P>0.05 = not significantly different

dough, the vegetative forms of bacteria that are present or all but a few of them will be killed in most circumstances. Bacterial spores are much more resistant and even when the middle of the loaf attains 110^{0}C the maximum, a significant number survives. When the bread is cold and conditions become favourable, the spores develop into vegetative forma and continue their activity and the bread will become 'ropy'. The stickiness and ropiness of the diseased bread is due to the production by the organisms of gums and sugars from the starch (Kent-Jones and Amos, 1967; Sorokulova *et al*., 2003).

There is no significant difference in the average total coliform counts in the different (p = 1.885) brands of flour (Table 11). Average total coliform counts for the different brands of flour ranges from 1.57MPN/g (brand 3) to 4.06MPN/g (Brand 1) (Table 11). However, there was intermittent decrease in coliform count in the flour brands. Brand 1 shows a decrease from 11.0MPN/g (day 0) to no growth at day 105. The gradual decrease in the total coliform count as the storage progresses corresponds with the previous study of Kent-Jones and Amos (1967) that during storage of flour, bacterial count decrease with the blood (Pathogenic) organisms dying off. Coliform is an indicator of contamination, findings revealed that the bulk of wheat imported to Nigeria for flour production are usually contaminated from the field and even the vigorous screening process of the wheat in

Table 8: Changes in gluten content of wheaten white bread flour during storage

Flour	Gluten content (%) at								
Brands	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105	significant
1	10.40±0.00	9.96±0.05	10.00±0.00	9.75±0.15	9.68±0.16	10.02±0.02	10.00±0.00	9.90±0.01	p>0.05
2	10.05±0.00	9.98±0.02	10.02±0.02	10.04±0.00	10.28±0.08	10.02±0.02	10.01±0.01	10.05±0.05	p>0.05
3	10.09±0.06	9.95±0.05	10.00±0.00	10.10±0.00	10.00±0.02	10.25±0.05	10.15±0.05	9.98±0.08	p>0.05
4	8.94±0.14	8.90±0.15	8.75±0.25	8.50±0.00	8.55±0.05	8.50±0.00	8.55±0.05	8.45±0.05	p>0.05

Note: p>0.05 = not significantly different

Table 9: Changes in ash on dry matter content of wheaten white bread flour during storage

Flour	Ash on dry matter content (%) at								
Brands	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105	significant
1	0.60±0.00	0.61±0.02	0.63±0.01	0.64±0.01	0.59±0.02	0.62±0.00	0.67±0.01	0.67±0.02	p>0.05
2	0.50±0.00	0.62±0.00	0.65±0.01	0.65±0.01	0.60±0.02	0.65±0.02	0.69±0.01	0.66±0.01	p>0.05
3	0.68±0.02	0.69±0.01	0.70±0.00	0.73±0.03	0.67±0.03	0.71±0.02	0.69±0.01	0.71±0.01	p>0.05
4	0.74±0.03	0.76±0.03	0.79±0.07	0.84±0.04	0.78±0.06	0.81±0.04	0.83±0.05	0.88±0.03	p>0.05

Note: p>0.05 = not significantly different

Table 10: Changes in fat content (%) of wheaten white bread flour during storage

Flour	Fat content (%) at								
Brands	DAY 0	DAY 15	DAY 30	DAY 45	DAY 60	DAY 75	DAY 90	DAY 105	significant
1	0.92±0.00	0.94±0.04	0.93±0.01	0.95±0.01	1.08±0.01	0.84±0.03	0.92±0.01	0.93±0.01	p>0.05
2	0.95±0.01	1.04±0.01	0.85±0.01	0.87±0.01	1.08±0.02	0.95±0.04	0.86±0.01	0.88±0.03	p>0.05
3	1.07±0.00	0.86±0.04	0.83±0.03	0.81±0.01	1.05±0.01	0.89±0.06	1.02±0.03	0.84±0.02	p>0.05
4	1.02±0.02	0.95±0.02	0.94±0.03	0.94±0.02	1.02±0.02	1.02±0.02	1.04±0.01	0.94±0.02	p>0.05

Note: p>0.05 = not significantly different

Table 11: Average summary on quality evaluation of individual brands of flour

Parameters	Brand 1 Mean±SD	Brand 2 Mean±SD	Brand 3 Mean±SD	Brand 4 Mean±SD	significant
Moisture	12.82^{b}±0.11	12.77^{b}±0.31	11.97^{a}±0.58	13.56^{c}±0.29	p<0.001
pH	6.03±0.09	6.07±0.04	6.12±0.04	6.05±0.03	p>0.05
Carbohydrate	65.31±0.97	65.46±0.65	65.87±1.13	66.26±1.12	p>0.05
Protein	11.46^{b}±0.04	11.47^{b}±0.04	11.09^{a}±0.15	10.24^{a}±0.11	p<0.001
Gluten	9.96^{b}±0.08	10.23^{b}±0.37	10.28^{b}±0.48	8.64^{a}±0.19	p<0.001
Ash	0.56^{a}±0.07	0.63^{b}±0.06	0.69^{c}±0.07	0.80^{c}±0.02	p<0.001
Fat	0.94±0.02	0.94±0.03	0.92±0.04	0.98±0.11	p>0.05
Bacterial count (X 10^{3} CFU/g)	11.66±5.47	7.81±2.22	7.89±1.62	8.86±3.41	p>0.05
Coliform count (MPN/g)	4.06±1.15	2.60±3.10	1.57±1.48	1.59±1.03	p>0.05

Note: Those with similar alphabet are not significantly different from each other. p>0.05 = not significantly different, p<0.05 = significantly different, p<0.001 = highly significantly different

the mill may not be able to remove all the coliforms. Total coliform count for flour is expected not to be above 100cfu/g (SON, 2000).

The average fat content in the different brands flour shows no significant difference (p = 0.915) (Table 10). The value obtained for fat is acceptable as regarded <1.5% fat content for Nigerian white wheat flour (SON, 2000). Intermittent decrease was noticed in the protein content of the various brands of flour during storage. Flour brand 4 shows decrease in protein content from 10.02% (day 75) to 9.85% (day 105) and flour brand 2 shows a decrease in protein content from 11.64% (day 30) to 11.34% (day 90). The decrease noticed in the protein content of the flour corresponds with earlier reports that protein content flour decreases during storage (Sur *et al*., 1993; Hruskova and Machova, 2002). The changes in protein content of the flour was however not significant, but average protein content for the individual brands of flour shows highly significant difference (p = 18.517) with brand 1 having 11.46% and brand 4; 10.24% (Table 11). Gluten content was seen to correlate with the total protein content as it also decreased slightly with storage (Table 8 and 11). This finding corresponds with previous reports of Sur *et al*. (1993) and Hruskova and Machova (2002).

Conclusion: Dough functionality depends on its chemical composition as well as on the micro-floral activity (Vazquez-Chavez and Guerrero-Lagarreta, 2002). The acceptability of a food item depends on the availability of durable and measurable quality indices. The finding despite the effort put by the flour millers to observe the standards as recommended for Nigeria wheat flour grade, there seemed to be continuous problem of bacteriological and physico-chemical qualities. The data revealed that brand 4 is low-grade flour made from low-grade wheat, though it has physico -chemical properties other than ash within the acceptable limit value of Nigerian market.

Table 12: Summary on quality evaluation of individual brand of flour

Parameters	Brand 1 Mean±SD	Brand 2 Mean±SD	Brand 3 Mean±SD	Brand 4 Mean±SD	Significant
Moisture	12.82b±0.11	12.77b±0.31	11.97a±0.58	13.56c±0.29	P<0.001
pH	6.03±0.09	6.07±0.04	6.12±0.04	6.05±0.03	P>0.05
Carbohydrate	65.31±0.97	65.46±0.65	65.87±1.13	66.26±1.62	P>0.05
Protein	11.46b±0.04	11.47b±0.04	11.09b±0.15	10.24a±0.11	P<0.001
Gluten	9.96b±0.08	10.23b±0.37	10.28b±0.48	8.64a±0.19	P<0.001
Ash	0.56a±0.07	0.63b±0.06	0.69c±0.007	0.08c±0.02	P<0.001
Fat	0.94±0.02	0.94±0.03	0.92±0.04	0.98±0.02	P>0.05
Bacterial count	11.66±5.47	7.81±2.22	7.89±1.622	8.86±3.41	P>0.05
Total Coliform	4.06±1.15	2.60±3.1	1.57±1.48	1.59±1.03	P>0.05

Note: Those with similar alphabet are not significantly different from each other. P>0.05 = Not Significant. P<0.001 = Highly Significantly Different

The finding of *Bacilus* and *Klebseilla* species throughout the storage period revealed that more effort is required by the millers on their diligence to minimize microbial load and thus increase the shelf life of the bread flour. Adequate monitoring of wheat from source of purchase to delivery will go a long way on reducing the contamination of wheat before getting to the mill.

REFERENCES

Badsha, K. A., J. Abdul, K. Maazulla, B. Nizakat, C.M. Ashraf and K. Saeed, 2005. Evaluation of physical and chemical characteristics of newly evolved wheat cultivars. J. Sci. Food and Agric., 85: 1061-1064.

Harrigan, W.F. and M.E. McCane, 1976. Laboratory Methods in Food and Dairy Microbiology. Academic Press, New York, pp: 452.

Hruskova, M. and D. Machova, 2002. Changes of wheat flour properties during short-term storage. Czech J. Food Sci., 20: 125-130.

Kent-Jones, D.W. and A.J. Amos, 1967. Modern Cereal Chemistry. Food Trade Press, London.

Mashood, S.B., N. Muhammad, A. Saeed and S. Kamran, 2005. Effect of moisture and packaging on the shelf life of wheat flour. Int. J. Food Safety, 4: 1-6.

Polmeranz, Y. and C.E. Meloan, 1996. Food Analysis; Theory and Practice. Third Edition. CBS Publishers and distributions (India), pp: 575-684.

Quaglia, G.B., 1984. Scienza e Technologia della Panificazione. Pinerolo Chiriotti, Italia.

SON (Standards Organization of Nigeria), 2000. Standards for Wheat Flour. NIS: 121.

Sorokulova, I. B., O.N. Reva, V. Smirnor, I.V. Pinchuk, S.V. Lapa and M.C. Urdaci, 2003. Genetic diversity and involvement in bread spoilage of Bacilllus stria isolated from flour and ropy bread. Letters in Appl. Microbiol., 37: 169.

Sur, R., H.P. Nagi, S. Sharman and K.S. Sekhon, 1993. Storage changes in the quality of sound and sprouted flour. Plant Foods Hum. Nutr., 44: 35-44.

Vanderzannt, C. and O.F. Splittoesser, 1993. Compendium of Methods for the microbiological Examination of Foods. 3rd Edn. American Public Health, Washington, D.C., pp: 60.

Vazquez-Chavez, L. and I. Guerrero-Lagarreta, 2002. Microbial population and bread making properties of wheat-amaranth sour doughs. Food Microbiology-General 1: Annual Meeting and Food Expo. Anahein, California.

Pakistan Journal of Nutrition 9 (11): 1084-1087, 2010
ISSN 1680-5194

Implementing Appropriate Safety and Emergency Strategies in Fitness Centres in Edo and Delta States

Elias O. Agwubike and Solomon Ogbouma
Department of Health, Environmental Education and Human Kinetics,
University of Benin, Benin City, Nigeria

Abstract: This study was set to investigate the extent to which safety and emergency equipment and measures have been instituted in selected fitness centres in Edo and Delta States with a view to identifying possible lapses in the current status vis a vis the recommendations of the American Heart Association/American College of Sports Medicine. A validated questionnaire and a checklist were used as the research instruments and 37 fitness centres were randomly selected from four major towns in the two states. The findings of the study helped to throw more light on the current state of play regarding safety and emergency equipment and measures. It revealed that fitness centres in Edo and Delta States are far from meeting the required recommendations for safety and emergency equipment and measures.

Key words: Safety measures, safety equipment, emergency measures, emergency equipment

INTRODUCTION

In the ancient Roman Empire, the wide use of the famous Latin quotation "mens sana in corpore sano" often translated as "a sound mind in a sound body", underlined the need to get fit, even among the ancient people. Though the popularity of Latinism is, today, a declining custom, the basic philosophy underpinning this phrase can still conveniently find its place in our current settings. This is evident in the ever increasing recognition and call for the need to keep fit and live healthy lives in order to function maximally in our increasingly tasking environment.

In response to the need to satisfy the desire to keep fit and live healthy lives, there has cropped up series of services including medical services, health and wellness-related services and traditional services. While the medical field is mainly concerned with providing laboratory-tested remedies in the form of chemotherapies to pathogenic disorders and other medical anomalies, the traditional health promoters are concerned with the provision of unorthodox remedies in the form of roots, herbs and other concoctions for the prevention and cure of diseases and the promotion of good health. The health and wellness-related service providers on their part are concerned with the promotion of the optimum health of the totality of the individual. Their services span beyond the bounds of disease prevention and curative interventions to encompass such spheres as physical, social, cultural, emotional and intellectual orientations (Wuest and Bucher, 2003). Among this latter group of service providers are those that operate fitness centres/clinics. With an assumed notion of what constitutes a healthy life, fitness centres/clinics set about providing services in the form of exercises/fitness programmes, nutrition counselling, body massage and a host of others, all geared towards enhancing the fitness status and the overall wellbeing of their clients (Koch, 2006; Wuest and Bucher, 2003).

Granted that indulgence in fitness programmes and physical activities can be deemed a commendable avenue for achieving a fit and healthy life, fitness activities, like most sports and physical activities are by definition, accident prone owing to the fact that they often involve vigorous movements, physical contacts and the use of equipment (Tukur, 2006; Dougherty *et al*., 1994; Hart and Ritson, 2000). To this end, the likelihood of accident and injury occurring in the course of engaging in physical activities and fitness programmes is ever present. While recognizing the need for safety consciousness in fitness programmes and physical activities, the nature of such programmes often makes it difficult, if not impossible, to keep them absolutely safe. This is because it is not possible to do so and still maintain the character and value of such programmes. However, taking cognizance of this fact and taking steps to at least reduce accidents to the barest minimum where they can not be totally eliminated should therefore be a priority in any fitness programme.

Statement of the problem: It goes without saying that there is currently an increased awareness of the relevance of fitness clinics in the promotion of good health and physical fitness. It is therefore not surprising that there has in recent times, been an increase in the establishment of fitness centres in our society.

Corresponding Author: Elias O. Agwubike, Department of Health, Environmental Education and Human Kinetics, University of Benin, Benin City, Nigeria

While it can be conceded that the increase in the number of fitness centres is a positive step towards improving the health and fitness status of the populace, the question however is, to what extent are these fitness centres implementing safety and emergency strategies to address incidents of accidents? To this end, this study was hinged on the need to assess the extent to which existing fitness centres in Edo and Delta States meet the set standards for fitness centres in terms of implementation of safety and emergency strategies as recommended by the American Heart Association/ American College of Sports Medicine.

MATERIALS AND METHODS

Subjects: A total of 37 fitness centres were selected from Benin City, Uromi, Warri and Asaba in Edo and Delta States: This involved 10 fitness centres from each of Benin City, Warri and Asaba and 7 from Uromi. In each of the selected fitness centres, the chief officer in charge of the centre constituted the respondent for the study. Thus from the 37 fitness centres, 37 respondents were conveniently selected as sample for the study.

Procedure: The selected towns in Edo and Delta States were selected using the purposive sampling technique owing to the fact that they amply suit the purpose of the study. After selecting the towns, the fitness centres used were selected using the systematic sampling technique. In doing that, the researchers drew up a list of all the fitness centres in the four towns and from the list, the first and every third fitness centres were picked for the study. Though this method was only applied to three of the towns, viz, Benin City, Warri and Asaba, in Uromi, all the 7 fitness centres in the list were used for the study.

Research instruments: A validated, self-developed, structured questionnaire and a checklist were used to generate data for the study. The questionnaire was made up of two sections, A and B. The first section labeled as section A contained items designed to elicit demographic information from the respondents. The second section labeled as section B contained 18 items designed in a modified Likert Scale format with response options in three scales viz: Yes, Undecided and No. The checklist was made up of 10 items designed to reflect the availability or non-availability of equipment and facilities within the fitness centres with a "yes" or "no" response options for the researchers to fill in.

Statistical analysis: Data obtained were analyzed using descriptive statistics with the application of Statistical Package for the Social Sciences (SPSS).

Research questions: The following research questions guided the study:

- What safety and emergency measures have been instituted to address the issue of accident and safety in these fitness centres?
- What safety and emergency equipment and facilities have been put in place in these fitness centres to address the issue of accidents?

Research question 1: What safety and emergency measures have been instituted to address the issue of accidents and safety in the fitness centres?

From Table 1, it is evident that majority of the respondents were undecided as to the level of safety and emergency measures that have been instituted in the fitness centres to address the issue of accidents. This can be observed where 51.4, 54.1, 73, 56.8, 51.4, 73, 73, 54.1, 59.4, 51.4 and 73%, respectively were undecided as to whether the centre has an on-site physician or is affiliated with a doctor; whether the staff are properly trained to identify the warning signs of fatigue or distress; whether staff members have cardiopulmonary resuscitation and first aid training; whether the fitness centres have visual emergency signals in strategic places; whether the staff are properly trained to handle emergencies that may arise; whether the fitness centres' atmosphere comfortable; whether the fitness centre is clean and well kept; whether the fitness centre is well lit; whether the equipment area uncluttered and whether staff members receive training in providing services to members with functional limitations or disabilities. Furthermore, 46, 45.9 and 43.3% of the respondents respectively were negative in their responses to questions on whether safety signs are visible; whether safety signs are written in fonts that are bold enough for people to see and whether the equipment are well maintained. On the average, a total of 49.7% of the respondents were undecided in their responses to questions on what safety and emergency measures have been instituted in the fitness centres to address the issue of accidents; 26.8% were negative in their responses while 23.5% were positive.

Research questions 2: What safety and emergency equipment have been put in place in these fitness centres to address the issue of accidents?

The analysis in Table 2 shows that the fitness centres surveyed were under equipped with safety and emergency equipment. This can be observed in the table where only 48.6% of the fitness centres had well equipped first aid box. In the same vein, only 16 of the fitness centres representing 43.2% had fire extinguishers and out of these, only 7 were functional. However, none of the fitness centres surveyed had automatic external defibrillator, while only 1 had a stethoscope. On the whole, safety and emergency equipment were 85.5% unavailable in the surveyed fitness centres while out of the ones available, 75.9% were functional.

Table 1: Safety and emergency measures

	Responses						
Measures	Yes	%	U	%	No	%	Total
Does the centre have on-site physician?	08	21.6	19	51.4	10	27	37(100%)
Is the centre affiliated with a doctor?	09	24.3	20	54.1	08	21.6	37(100%)
Are safety signs visible?	11	29.7	09	24.3	17	46	37(100%)
Are safety signs written in bold fonts?	11	29.7	09	24.3	17	45.9	37(100%)
Are the staff properly trained to identify the warning signs of fatigue or distress?	04	10.8	27	73	06	16.2	37(100%)
Do staff members have cardiopulmonary resuscitation and first aid training?	09	24.3	21	56.8	07	19	37(100%)
Does the fitness centre have visual emergency signals in strategic places?	06	16.2	19	51.4	12	32.4	37(100%)
Are the staff properly trained to handle emergencies when they arise?	04	10.8	27	73	06	16.2	37(100%)
Are routes free of temporary or permanent obstructions?	12	32.4	11	29.7	14	37.9	37(100%)
Are the equipment well maintained?	12	32.4	09	24.3	16	43.3	37(100%)
Is the fitness centre's atmosphere comfortable?	06	16.2	27	73	04	10.8	37(100%)
Is the fitness centre clean and well kept?	09	24.3	20	54.1	08	21.6	37(100%)
Is the fitness centre well lit?	08	21.6	22	59.4	07	19	37(100%)
Is the equipment display/storage area uncluttered?	07	18.9	19	51.4	11	29.7	37(100%)
Do staff members receive training in providing fitness services to members with functional limitations or disabilities?	04	10.8	27	73	06	16.2	37(100%)
Mean total	8.7	23.5	18.4	49.7	9.9	26.8	37(100%)

Table 2: Safety and emergency equipment

Equipment		A	%	N.A.	%	F	%	N.F.	%
Well equipped first aid box	37(100%)	18	48.6	19	51.4	18	100	0	0
Fire extinguisher	37(100%)	16	43.2	21	56.8	07	43.8	09	56.2
Telephone in the fitness room	37(100%)	03	8.1	34	91.9	02	66.7	1	33.3
Signs indicating where the telephones are	37(100%)	01	2.7	36	97.3	01	100	0	0
Signs indicating where the fire extinguisher is	37(100%)	01	2.7	36	97.3	01	100	0	0
Automatic external defibrillator	37(100%)	0	0	37	100	0	0	0	0
Blood pressure kit	37(100%)	03	8.1	34	91.9	03	100	0	0
Stethoscope	37(100%)	01	2.7	36	97.3	01	100	0	0
Mean total	37(100%)	5.4	14.5	31.6	85.5	4.1	75.9	1.3	24.1

A: Availability N.A. Non availability F: Functionality N.F. Non functionality

RESULTS AND DISCUSSION

Revealed in this study is the fact that safety and emergency measures and equipment have not been sufficiently put in place in the fitness centres to address the issue of accidents. As evidenced in the data analysis, equipment for safety and emergency are in acute shortage. In some cases, they were completely not available as in the case of automatic external defibrillator. In other cases, only a negligible few had the equipment as shown in the case of emergency plans, telephones, signs, blood pressure kits and stethoscopes. This situation, no doubt, negates the guidelines for safety and emergency preparation in fitness clinics as postulated by the American Heart Association/American College of Sports Medicine (2006).

While there is already a problem of availability, the study further revealed that not all of the very few safety and emergency equipment available were functional. This was revealed in the analysis where 21.6% of the available safety and emergency equipment were not functional. In a nutshell, safety and emergency equipment and facilities in the surveyed fitness centres, like most sports equipment and facilities, were grossly inadequate, corroborating the findings of Akinsanmi (1995), Mgbor (2005), Mgbor and Anyanjor (2005) and Ojeme (2005) where they all observed that sports equipment and facilities, though recognized to be crucial to the implementation of sports programme, are grossly inadequate. Meanwhile, the importance of an up-to-date safety and emergency plan and equipment to a health/fitness clinic has been elaborately spelt out in the recommendations of the American Heart Association/American College of Sports Medicine (2006). The findings showed a disappointing situation depicting a defective adherence to the set standard.

On what measures that have been taken in the fitness centres in Edo and Delta States to address accidents, the study revealed that apart from the acute shortage of safety and emergency equipment, adequate measures to address the possibility of accident occurrence have not been instituted in the fitness centres surveyed. This act of omission greatly undermines the importance of such measures in a fitness programme. This could therefore render the fitness trainer liable to possible litigation as expressed by Mitten (2000), Chen and Esposito (2004).

Conclusion and Recommendations: Inferring from data analyzed in this study, it can be taken that majority of fitness centres in Edo and Delta States lack safety and an elaborate and up to date emergency plan and equipment. Furthermore, majority of these fitness centres do not have in place elaborate safety and emergency measures instituted to checkmate the occurrence of accidents and their attendant injuries. In the light of this, it is therefore recommended that fitness centres in Edo and Delta States should observe the strictest safety principles and procedure in order to avoid accidents and injuries. This can be achieved through a strict compliance with all known safety rules in physical activities and providing adequate and up-to-date safety and emergency plan and equipment as well as a legitimized implementation process. Furthermore, it is recommended that an association like the American Heart Association/American College of Sports Medicine be set up to serve as a regulatory body for exercise science and fitness in this part of the world. Such association or regulatory body should design a benchmark defining guidelines and specifications suitable for fitness centres in this part of the world. Among such benchmarks will be the provision of adequate, functional and up-to-date equipment and facilities in the operation of any fitness programme as well as the levels of implementation process. When this is instituted, it is hoped that the standard of safety and emergency strategies and implementation process will be enhanced.

REFERENCES

Akinsanmi, T., 1995. Maintenance of sports facilities and equipment: The experience of Nigeria Colleges of Education in Nigeria. J. Nigeria Acad. Sports Administration, 1: 64-65.

American Heart Association/American College of Sports Medicine, 2006. Recommendations for Health/ Fitness Facilities. Retrieved March 21st, 2009 from http://www.circ.ahajournals.org.

Chen, S. and E. Esposito, 2004. Practical and critical legal concerns for sport physicians and athletic trainers. The Sports J., 7: 34-43.

Dougherty, N.J., D. Auxter, A.S. Goldberger and G.S. Heinzmann, 1994. Sports, Physical Activity and the Law. Champaign Ill: Human Kinetics.

Hart, J.E. and R.J. Ritson, 2000. Liability and Safety in Physical Education and Sports. USA: NASPE.

Koch, P., 2006. Essential considerations of fitness programme design. Retrieved March 21st, 2009 from http://www.doogen.com/myDoogen/modules.php.

Mgbor, M.O., 2005. The implementation of school sports in the UBE programme: Challenges and implications. J. Nig. Assoc. Phys., Health Educ., Recreation, Sports Dance, 2: 146-152.

Mgbor, M.O. and A. Anyanjor, 2005. The development of physical education in Nigeria: Issues and future directions. J. Nig. Assoc. Phys., Health Educ., Recreation, Sports Dance, 2: 172-178.

Mitten, M.J., 2000. Emerging legal issues in sports medicine: A synthesis, summary and analysis. St. John's Law Rev., 76.

Ojeme, E.O., 2005. Towards the effective implementation of the P.E and sports curriculum in the Universal Basic Education Programme. J. Nig. Assoc. Phys., Health Educ., Recreation, Sports Dance, 2: 1-8.

Tukur, I.T., 2006. Legal responsibility: A requirement for safe physical activity participation. J. Nig. Assoc. Phys., Health Educ., Recreation, Sports Dance, 3: 29-37.

Wuest, D.A. and C.A. Bucher, 2003. Foundations of Physical Ducation, Exercise Science and Sports. New York: McGraw Hill.

Pakistan Journal of Nutrition 9 (11): 1088-1093, 2010
ISSN 1680-5194

Quality Attributes of Soy-yoghurt During Storage Period

Manhal Mohammed Dafaalla Osman and Kamal Awad Abdel Razig
Department of Food Science and Technology, Faculty of Agriculture,
Al-Zaeim Al-Azhari University, P.O. Box 1432, Khartoum North 13311, Sudan

Abstract: The utilization of soybean milk in manufacturing yoghurt in Sudan was investigated. Soybean milk was prepared from grinded soybean seeds. Four samples of soy yoghurt product were used, namely sample A) 100% soymilk (sample B) 1:1 soymilk: cow milk), sample C (2:1 soymilk: cow milk) and sample D (1:2 soymilk: cow milk). The prepared soy yoghurt samples were stored at refrigerator temperature (10±2°C) followed by analyses at 0, 5, 10, 15 and 20 days intervals. The chemical compositions of soybean milk used were the total solids (13.15%), protein (7.64%), fat (2.75%), pH-value (6.70) and titratable acidity (0.20%).The type of milk significantly ($p \leq 0.05$) affected the pH-value of the soy yoghurt. Sample A was the highest (4.50), sample D the lowest (3.30) while sample B and sample C were at an intermediate position (3.50 and 3.70 respectively). Storage period significantly ($p \leq 0.05$) affected the pH-value of soy yoghurt, the highest value (4.30) was obtained at the beginning of the storage period, while the lowest value (3.00) at the end. The type of milk significantly ($p \leq 0.05$) affected the titratable acidity of the soy yoghurt. Sample A was the lowest (1.12%), Sample D was the highest (2.50%), while samples B and C were in an intermediate position. Storage period significantly ($p \leq 0.05$) affected the titratable acidity of soy yoghurt. The lowest titratable acidity (1.21%) was obtained at the beginning of storage period and the highest (2.60%) at the end. The type of milk significantly ($p \leq 0.05$) affected the wheying off of the soy yoghurt. Sample A was the highest (2.70 ml), sample D was the lowest (1.70 ml), while samples B and C were at an intermediate position. Storage period significantly ($p \leq 0.05$) affected the wheying off of soy yoghurt. The lowest wheying off (0.00 ml) was obtained at the beginning of storage period and the highest (4.00 ml) at the end. The chemical analyses of soy yoghurt samples at zero time processing, sample A) 100% soymilk (was the highest (31.20%) for total solids and protein content (16.70%),while sample D (1:2 soymilk: cow milk) was the lowest (15.70%) for total solids and protein content) 11.25). Sample B and C were at an intermediate position. Sample A was the lowest (2.20%) for fat content, sample D was the highest (3.25%),while samples Band C occupy an intermediate position. The sensory evaluation significantly ($p \leq 0.05$) affected by The type of milk and storage period revealed that, the best score ($p \leq 0.05$) in appearance (4.38), flavour (4.10), texture (4.22) and overall acceptability (4.28) in sample D(1:2 soymilk: cow milk), the worst was recorded by sample A (Soymilk 100%) for appearance (2.68), flavour (3.48), texture (3.36) and overall acceptability (3.44). The other samples were at an intermediate position. It was found that 10 days storage period at refrigerator temperature (10±2°C) was quite satisfactory to attain good quality soy yoghurt.

Key words: Soy milk, yoghurt, storage, cow milk

INTRODUCTION

Vegetable milk is prepared from food grains such as soya bean, sesame and groundnut. Soybean (*Glycine max*), a plant protein which is cheaper could serve as an alternative to cow milk. Soybean milk is richer in protein than most animal milk. It contains up to 40% protein compared with 1.0% to 5.6% protein content of most animal milk (Burton, 1985). Soymilk is processed from soybean. Intake of fermented soymilk improves the ecosystem in the intestinal tract by increasing the amount of probiotics (Chang *et al*., 2005). Soybean and its derivates represent an excellent source of high quality protein, with low content of saturated fat and a great amount of dietary fiber and bioactive components like the isoflavones. Soymilk and its fermented products constitute an alternative for lactose intolerant people. In this way, soy yoghurt could be a suitable vehicle for iron fortification, since it has high nutritional value and low-cost. However, calcium content of soymilk is lower that found in cow's milk, being necessary the addition of this mineral to improve its nutritional properties. The objective of this work is to prepare the milk from soybean seeds and to study their effects on quality of Sudanese soy yoghurt during storage period.

Corresponding Author: Manhal Mohammed Dafaalla Osman, Department of Food Science and Technology, Faculty of Agriculture, Al-Zaeim Al-Azhari University, P.O. Box 1432, Khartoum North 13311, Sudan

MATERIALS AND METHODS

The seeds were obtained from the local market. The starter culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were obtained from Khartoum Dairy Products Company Ltd. Plastic cups (250 ml volume) were purchased from retailers in the local market. Fresh cow milk was obtained from the University of Sudan Dairy Farm (Shambat).

Preparation of soymilk: Soybean milk was prepared by soaking kernels in 0.5% sodium bicarbonate solution for 16-18 h, drained, washed with tap water, grinded, steeped for 4-5 h in tap water (100 g soybean mixed with 100 ml tap water) and filtrated through cheese cloth to obtain the soymilk.

Preparation of soy-yoghurt: Four samples (A, B, C and D) were selected in which soymilk was kept in equal volumes after being filtered from impurities. Sample (A) 100% soymilk, sample (B)1:1 soymilk: cow milk, sample © 2:1 soymilk: cow milk and sample (D) 1:2 soymilk: cow milk. The volumes were pasteurized at 85°C for 10 min and then cooled to 45°C. Starter culture 3% of the milk volume was added in the form of (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). The milk mixture was placed in 250 ml cups and kept in an incubator at 45°C for 3-6 h. The cups were transferred to refrigerator and stored in a temperature of 10±2°C for 0, 5, 10, 15 and 20 days intervals. The Analyses were carried for physicochemical, rheological and sensory evaluation.

Chemical analyses: The pH-values of samples were determined according to Newlander and Atherton (1964), while the titratable acidity, total solids, protein and fat content were determined according to AOAC (1995).

Rheological properties: Wheying-off was measured by sucking off the water from the surface of the curd and poured in a graduated cylinder.

Sensory evaluation: The sensory evaluation was performed by 10 untrained panelists using procedure according to Ihekoronye and Nogddy (1985).

Statistical analyses: Data was subjected to statistical analyses using Statistical Analysis System program (SAS, 1988).

RESULTS AND DISCUSSION

Soybean milk: The results of chemical properties of soy milk were of total solids (13.15%), pH-value (6.70), titratable acidity (0.20%), protein content (7.64%) and fat content (2.75%).

Tuitemwong *et al.* (1993) found that, the soymilk has desirable characteristics as an ingredient for making soy yoghurt because of its high solid content (Moriguchi *et al.*, 1961). Angeles and Marth (1971) found a titratible acidity of 0.23-0.25% or a pH of 5.7. Wolf, (1978) concluded 60-70% of the total protein in the soybean is stored in the protein bodies. Deshpande *et al.* (2008) found that, the soymilk contains total solids (9.8%), fat (2.6%), protein (5.8%), pH value (6.0) and ash (0.6%). Lee *et al.* (1990) stated the variation in the results to the origin of soybean and to the processing condition.

Chemical properties of soy yoghurt

pH-value: The type of milk was significantly ($p \leq 0.05$) affected pH-values of the soy yoghurt (Table 1). Samples D (1:2 soymilk: cow milk) was the lowest (3.30) and sample A) 100% soymilk (the highest (4.50), while samples B (1:1 soymilk: cow milk) and C (2:1 soymilk: cow milk) were at an intermediate position (3.50 and 3.70) respectively.

Storage period significantly ($p \leq 0.05$) affected the pH-values, where they decreased gradually till the end of storage (Table 2 and Fig. 1). The highest pH-value (4.30) was obtained at the beginning of the storage period, whereas the lowest (3.00) at the end. Sugimoto and Van Buren (1970) and Buono (1988) found that, the pH-value of soy yoghurt to be 5.00. Moriguchi *et al.* (1961) stated high optimum pH-values of soy yoghurt varied form 5.0-6.0.

Table 1: Effect of type of milk on pH-value*, titratable acidity* and wheying off* of soy-yoghurt

	Samples					
Parameters	A	B	C	D	$LSD_{0.05}$	SE±
pH-value	4.50±0.08^{a}	3.50±0.04^{c}	3.70±0.06^{b}	3.30±0.02^{d}	0.8297	0.2633
Titratable acidity (% lactic acid)	1.12±0.01^{d}	1.70±0.04^{b}	1.41±0.03^{c}	2.50±0.05^{a}	0.1151	0.03651
Wheying-off (ml)	2.70±0.06^{a}	2.40±0.04^{c}	2.50±0.05^{b}	1.70±0.02^{d}	0.1819	0.05774

*Means±SD. Values having different superscript letter in rows are significantly different ($p \leq 0.05$). A = Soymilk 100%; B = Soymilk: cow milk 1:1; C = Soymilk: cow milk 2:1; D = Soymilk: cow milk 1:2

Table 2: Effect of storage period on pH-value*, titratable acidity* and wheying off* of soy-yoghurt

	Storage period (days)						
Parameters	0	5	10	15	20	$LSD_{0.05}$	SE±
pH-value	4.30±0.09^{a}	3.90±0.08^{b}	3.70±0.06^{c}	3.30±0.04^{d}	3.00±0.02^{e}	0.1819	0.05774
Titratable acidity (% lactic acid)	1.21±0.01^{e}	1.52±0.02^{d}	2.00±0.04^{c}	2.34±0.06^{b}	2.60±0.08^{a}	0.1151	0.03651
Wheying-off (ml)	0.00±0.00^{c}	1.80±0.01^{b}	2.40±0.03^{b}	3.00±0.05^{ab}	4.00±0.07^{a}	1.156	0.3670

*Means±SD. Values having different superscript letter in rows are significantly different ($p \leq 0.05$)

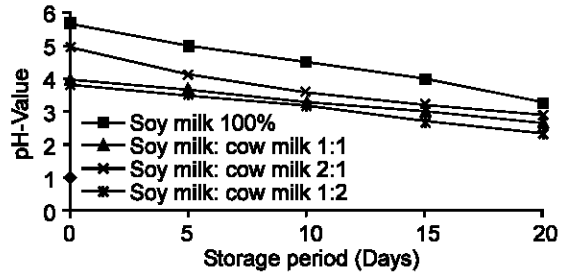

Fig. 1: Effect of storage period on pH-value of soy-yoghurt

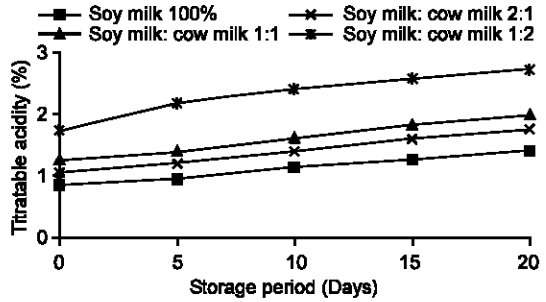

Fig. 2: Effect of storage period on titratable acidity (% lactic acid) of soy-yoghurt

Titratable acidity: The type of milk was significantly ($p \leq 0.05$) affected titratable acidity (Table 1). Sample A (100% soy milk) was the lowest (1.12%), Samples D (1:2 soymilk: cow milk) the highest (2.50), while sample B and C were at an intermediate position (1.70% and 1.41, respectively).

Storage period significantly ($p \leq 0.05$) affected titratable acidity of Sudanese soy yoghurt samples (Table 2 and Fig. 2). Titratable acidity increased gradually till the end of storage. The highest value (2.60%) was obtained at the end, while the lowest (1.21%) at the beginning of the storage.

Methods for the reduction of the flatulent sugars, raffinose and stachyose in soymilk and soybean products have been extensively investigated (Mital *et al*., 1973; Pinthong *et al*., 1980a,b; Buono, 1988; Buono *et al*., 1990a,b). Mital *et al.* (1973) stated the activity of the enzyme alpha-galactosidase (EC 3.2.1.22) in some lactic acid bacteria. The soy yoghurt contains oligosaccharides such as raffinose, stachyose produced during the fermentation process.

Wheying off: The type of milk was significantly ($p \leq 0.05$) affected the wheying off (Table 1). Sample A was the highest (2.70 ml), sample D the lowest (1.70%), while sample B and C were at an intermediate position (2.40 ml and 2.50 ml respectively).

Storage period significantly ($p \leq 0.05$) affected wheying off of Sudanese soy yoghurt samples (Table 2 and Fig. 3). The wheying off increased gradually till the end of storage. The highest value (4.00 ml) was obtained at the end, while the lowest (0.00 ml) at the beginning of the storage period. Lucey *et al*. (2000) found that, stabilizers are commonly used in cultured products to reduce whey separation.

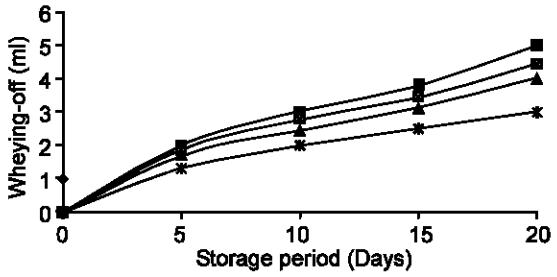

Fig. 3: Effect of storage period on wheying-off of soy-yoghurt

Chemical composition of soy yoghurt samples

Total solids: The type of milk significantly ($p \leq 0.05$) affected the total solids of the soy yoghurt. Sample A) 100% soymilk (recorded the highest content of total solids (31.20%) compared with sample D (1:2 soymilk: cow milk), that gave the lowest (15.70%). Samples B (21.80%) and C (29.40%) showed an intermediate position (Table 3).

Tuitemwong *et al*. (1993) found high total solid content of processed soy yoghurt ranged from 56.02-61.12%. The soymilk has desirable characteristics as an ingredient for making soy yoghurt because of its high solid content. Tamime and Robinson (1999) found the total solids of processed soy yoghurt ranged from 34.2-44.4%.

Protein content: The type of milk was significantly ($p \leq 0.05$) affected the protein content of soy yoghurt (Table 3). Sample A was the highest (16.70%), Sample D the lowest (11.25%), while sample B and Sample C, occupied an intermediate position (13.00% and 14.80%, respectively). Burrington (2000) noticed that, soy proteins are high in the amino acids glycine and arginine.

Fat content: The type of milk was significantly ($p \leq 0.05$) affected fat content of soy yoghurt. Sample D (Soymilk: cow milk 1:2) was the highest (3.25%) whereas sample A (Soymilk 100%) was the lowest (2.20%). While sample B and C showed an intermediate position (3.00% and 3.20, respectively). Tamime and Robinson (1999) reported that, the fat content of processed soy yoghurt varied from 1.2-2.2%. Friedman and Brandon (2001) found that, soy or soy product containing soy protein that meets requirements including low saturated fat and low cholesterol with a minimum of 4.25% of soy protein. Burrington (2000) noticed that, soy proteins are high in the amino acids glycine and arginine, which decrease cholesterol and lower fat levels. Dashiell *et al.* (1990) stated low cholesterol soy milk yoghurt.

Table 3: Chemical composition* (%) of soy-yoghurt

	Samples					
Parameters	A	B	C	D	$LSD_{0.05}$	SE±
Total solids content	31.20±0.09[a]	21.80±0.02[c]	29.40±0.06[b]	15.70±0.04[d]	0.1819	0.05774
Fat content	2.20±0.01[c]	3.00±0.01[b]	3.20±0.46[ab]	3.25±0.01[a]	0.3728	0.1183
Protein content	16.70±0.48[a]	13.00±0.26[c]	14.80±0.39[b]	11.25±0.15[d]	0.1409	0.04472

*Means±SD. Values having different superscript letter in rows are significantly different ($p \leq 0.05$). A = Soymilk 100%; B = Soymilk: cow milk 1:1; C = Soymilk: cow milk 2:1; D = Soymilk: cow milk 1:2

Table 4: Effect of type of milk on quality attributes* of soy-yoghurt

	Samples					
Quality attribute	A	B	C	D	$LSD_{0.05}$	SE±
Appearance	2.68±0.01[d]	3.37±0.04[b]	2.86±0.02[c]	4.38±0.05[a]	0.3728	0.1183
flavour	3.48±0.02[c]	3.58±0.05[b]	3.36±0.04[bc]	4.10±0.06[a]	0.0814	0.0258
Texture	3.36±0.01[c]	3.56±0.03[b]	3.46±0.02[bc]	4.22±0.04[a]	0.0006	0.0002
Overall acceptability	3.44±0.03[c]	3.88±0.05[b]	3.68±0.04[d]	4.28±0.07[a]	0.0006	0.0002

*Means±SD. Values having different superscript letter in rows are significantly different ($p \leq 0.05$). A = Soymilk 100%; B = Soymilk: cow milk 1:1; C = Soymilk: cow milk 2:1; D = Soymilk: cow milk 1:2

Table 5: Effect of storage period on quality attributes* of soy-yoghurt

	Storage period (days)						
Quality attribute	0	5	10	15	20	$LSD_{0.05}$	SE±
Appearance	3.26±0.01[d]	3.52±0.03[c]	3.86±0.05[a]	3.62±0.04[b]	2.28±0.02[e]	0.0006	0.0002
Flavour	3.58±0.03[e]	4.04±0.05[c]	4.30±0.06[a]	4.10±0.05[b]	3.68±0.02[d]	0.0006	0.0002
Texture	3.64±0.02[c]	3.88±0.03[b]	4.07±0.04[a]	3.86±0.03[b]	3.62±0.02[c]	0.0006	0.0002
Overall acceptability	3.76±0.03[c]	4.10±0.04[b]	4.30±0.06[a]	4.10±0.04[b]	3.68±0.02[d]	0.1409	0.0447

*Means±SD. Values having different superscript letter in rows are significantly different ($p \leq 0.05$)

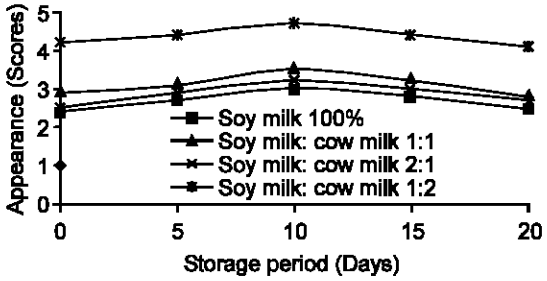

Fig. 4: Effect of storage period on appearance of soy-yoghurt

Organoleptic quality of Sudanese soy yoghurt samples

Appearance: The type of milk was significantly ($p \leq 0.05$) affected the appearance of yoghurt. Table 4 shows the appearance score of Sudanese soy yoghurt. Sample D (soymilk: cow milk 1:2) significantly ($p \leq 0.05$) secured the best appearance (4.38). Sample B and C in an intermediate position (3.37 and 2.86, respectively). The worst (2.68) recorded by sample A (soymilk 100%).

Storage period significantly ($p \leq 0.05$) affected the appearance of the soy yoghurt samples (Table 5 and Fig. 4). The best scores (3.86) were obtained at day 10 and the worst (2.28) at the end of the storage period. Pinthong *et al.* (1980a); Nielsen (1985) reported that, the fermented soybean milk was more acceptable than others and was preferred in terms of colour. Cuenca *et al.* (2005) found that, the soy yoghurt fermented with starter culture improve colour can be fortified with especially natural fruit juices to meet the requirement of consumers.

Flavour: Table 5 illustrated the flavour score of the Sudanese soy yoghurt. Sample D made with (Soymilk: cow milk 1:2) significantly ($p \leq 0.05$) secured the best flavour (4.10), followed by sample B and C. The worst flavour (3.48) was recorded by sample A (Soymilk 100%).

Storage period significantly ($p \leq 0.05$) affected the flavour of the soy yoghurt samples (Fig. 5). The best score (4.30) was obtained at day 10 and the worst (3.58) at the beginning of the storage period.

Lactic acid fermentation reduced beany flavours in soybean products (Pinthong *et al.*, 1980a,b; Buono, 1988; Buono *et al.*, 1990a).

Liu (1997b) found that, fermentation of soymilk offers not only a means of preserving soymilk but also a possibility for modifying or improving flavour. Cuenca *et al.* (2005) mentioned that, the soy yoghurt fermented with starter culture improve taste can be fortified with especially natural fruit juices to meet the requirement of consumers.

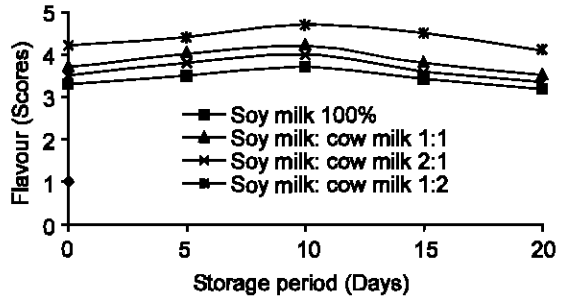

Fig. 5: Effect of storage period on flavour of soy-yoghurt

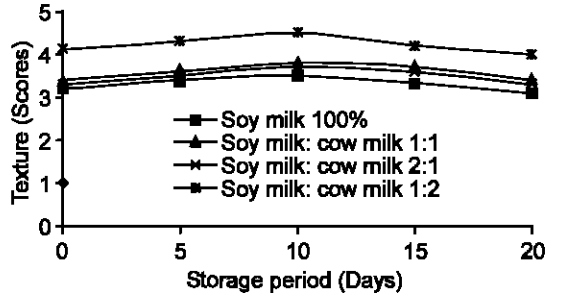

Fig. 6: Effect of storage period on texture of soy-yoghurt

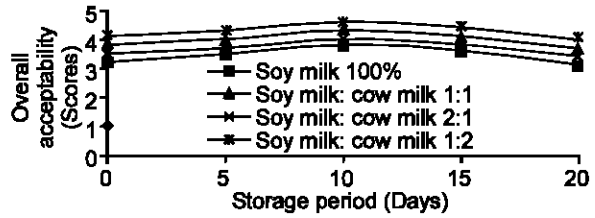

Fig. 7: Effect of storage period on over all acceptability of soy-yoghurt

Texture: The type of soy milk was significantly ($p \leq 0.05$) affected the texture of yoghurt. Sample D significantly secured the best texture (4.22). Samples B and C ranked in an intermediate position (3.56 and 3.46, respectively). The worst texture (3.36) recorded by sample A (Table 5).

Storage period significantly ($p \leq 0.05$) affected the texture of the soy yoghurt samples (Fig. 6). The best scores (4.07) were obtained at day 10 and the worst (3.62) were obtained at the end of the storage.

Liu (1997a) stated that, fermentation of soymilk offers not only a means of preserving soymilk but also a possibility for modifying or improving texture. Lucey *et al.* (2000) found that, stabilizers are commonly used in cultured products to control texture soy yoghurt.

Overall acceptability: The overall acceptability of Sudanese soy yoghurt samples prepared from soybean milk were affected by the type of milk (Table 5). Sample D made with (soymilk:cow milk 1:2) significantly secured the best acceptability (4.28), followed by sample B (3.88) and C (3.68). The worst (3.44) was recorded in sample A made with 100% soymilk.

Storage period significantly ($p \leq 0.05$) affected the overall acceptability of the soy yoghurt samples (Fig. 7). The best score (4.30) was obtained at day 10 and the worst (3.68) at the end of the storage period.

Organoleptic quality of fermented products is directly related to levels of n-pentanal and n-hexanal, the form being produced by *S. thermophilus* and the latter being naturally present in soy milk (Pinthong *et al.*, 1980a). Pinthong *et al.* (1980b) found that, fermented soy milk contained less n-hexanal than the unfermented soymilk. Beany flavours, chalkiness and astringent characteristics have been reported as the major sensory attributes found by sensory evaluation of soy yoghurt samples by trained panels (Tuitemwong *et al.*, 1993).

REFERENCES

Angeles, A.G. and E.H. Marth, 1971. Growth and activity of lactic acid bacteria in soymilk. I. Growth and acid production. J. Milk Food Technol., 34: 30.

AOAC, 1995. Official Methods of Analysis. 14th Edn., Association of official analytical chemists, Washington, DC.

Buono, M.A., 1988. An engineering microbiology and sensory study of yoghurt from soy milk. Ph.D. Dissertation. Kansas State University, Manhattan.

Buono, M.A., L.E. Erickson and D.Y.C. Fung, 1990b. Carbohydrate utilization and growth kinetics in the production of yoghurt from soymilk. Part II: Experimental and parameter estimation results. J. Food Proc. Preserv., 14: 179-204.

Buono, M.A., L.E. Erickson, D.Y. Fung and I. Jeon, 1990a. Carbohydrate utilization and growth kinetics in the production of yoghurt from soymilk. Part I: Experimental methods. J. Food. Proc. Preserv., 14: 135-153.

Burrington, K., 2000. Nutritional and beneficial ingredients. Food Product Desing, pp: 1-12.

Burton, J.W., 1985. Breeding soybean for improved protein quality and quantity. In: Proceeding of the 3rd World Soybean Research Conference, (Shibles R Ed.). West View Press, bowlder, Colorado pp: 136-165.

Chang, I.C., H.F. Shang, T. Lin, T.H. Wang and S.H. Lin, 2005. Effect of fermented soymilk in the intestinal bacteria ecosystem. World J. Gastroenterol., 11: 1225-1227.

Cuenca, M., L. Caicedo, M. Holguin and M. Quicazan, 2005. Fermentaion lactica de una mezcla de bebida de soya leche de vaca utilizando celulas libres inmovilizadas. Memorias Segundo simposio sobre biofabricas. Cited from PDF (2005).

Dashiell, K.E., S.R. Singh, O. Nakayama, H.O. Ogundipe and C.N. Akeem, 1990. Soybean research at IITA. GLIP Research Monograph No. 1Ibadan.

Deshpande, S., P. Bargale and K. Jha, 2008. Suitability of soymilk for development of shrikhand. J. Food Sci. Technol., 45: 284-286.

Friedman, M. and D.L. Brandon, 2001. Nutritional and health benefits of soy proteins. J. Agric. Food Chem., 49: 1069-1086.

Ihekoronye, N.I. and P.O. Nogddy, 1985. Integrated food, Sci. and Tech. for Tropics. Macmillan Publisher.

Lee, S.Y., V.C. Morr and A. Seo, 1990. Comparison of milk based and soy milk yoghurt. J. Food Sci., 55: 532-536.

Liu, K., 1997a. Chemistry and nutritional value of soybean components. In soybeans: Chemistry, technology and utilization. Liu K. Chapman and Hall: New York, pp: 25-113.

Liu, K., 1997b. Soybeans: Chemistry, technology and utilization. International Thompson Publisher.

Lucey, J.A., M. Tamehana, H. Singh and P.A. Munro, 2000. Rheological properties of milk gels formed by a combination of rennet and glucono-lactone. J. Dairy Res., 67: 415-427.

Mital, B.K., R.S. Schallenberger and K.H. Steinkraus, 1973.Alpha-galactosidase activity of lactobacilli. Appl. Microbiol., 26: 783-788.

Moriguchi, S., H. Ishikawa, R. Ueda and M. Hayashida, 1961. Studies on organic acids in soybeans and defatted soybeans. Hokko Kogaku Zasshi, 39: 237-243.

Newlander, J.A. and H.V. Atherton, 1964. The chemistry and testing of dairy products, 3rd Edn., (revised), Olsen-Publishing Co., Milwaukee, Wisconsin.

Nielsen, N.C., 1985. Structure of soy proteins. Ch. II. In new protein foods, vol. 5, seed storage proteins, A.M. Altschul and H.L. Wilcke (Eds.). Academic press, Inc. Orlando, FL.

Pinthong, R., R. Macrae and J. Rothwell, 1980a. The development of a soya-based yoghurt: I. Acid production by lactic acid bacteria. J. Food. Tech., 15: 647-652.

Pinthong, R., R. Macrae and J. Rothwell, 1980b. The development of a soya- based yoghurt. II. Sensory evaluation and analysis of volatiles. J. Food Technol., 15: 653-660.

SAS, 1988. Statistical Analysis System Institute, Inc., Cary, NC.

Sugimoto, H. and J.P. Van Buren, 1970. Removal of oligosaccharides from soy milk by an enzyme from *Aspergillus saitoi*. J. Food Sci., 35: 655-660.

Tamime, A.K. and R.K. Robinson, 1999. Yoghurt Science and Technology. Woodhead publishing Ltd and CRC press LLC. Boca Raton, Florida, pp: 619.

Tuitemwong, P., L.E. Erickson, D.Y. Fung, C.S. Setser and S.K. Perng, 1993. Sensory analysis of soy yoghurt and frozen soy yoghurt produced from rapid hydration hydrothermal cooked soy milk. J. Food Quality, 16: 223-239.

Wolf, W.J., 1978. Purification and properties of the proteins. Ch. 4 in soybeans: chemistry and technology. Vol. 1 protein, revised 2nd printing. A.K. Smith and S.J. Circle. (Eds.), AVI publishing Co., Inc., Westport, CN.

Pakistan Journal of Nutrition 9 (11): 1094-1099, 2010
ISSN 1680-5194

Relationship Between Malnutrition and Parasitic Infection among School Children in the Adamawa Region of Cameroon

C.M.G. Garba and C.M.F. Mbofung
National School of Agro-industrial Sciences (ENSAI), University of Ngaoundere,
P.O. Box 455, Ngaoundere, Cameroon

Abstract: Malnutrition and parasitic infections are common public health problems of children in developing countries. The prevalence of malnutrition and parasitic infections in school children in the Adamawa region of Cameroon was carried out. Anthropometric measurements consisting of height and weight were measured according to WHO guide lines (WHO, 1983, 1987). Body Mass Index (BMI) which is weight/height^2 for age was used as indicator to determine nutritional status. Examination of stool specimens was done using direct smear examination and concentration techniques and malaria parasitemia was determined microscopically from Giemsa stained blood films. The nutritional status and parasitosis was studied in 1200 (715 boys and 485 girls) school children aged 6 to 17 years. For malnutrition, severe acute malnutrition prevalence and moderate acute malnutrition was recorded respectively as 15.5% and 35.9%. 14.2% of which 64.3% were boys while 35.6% girls were found positive for various parasitic infections with malaria parasite accounting for the highest (64.9%) and hookworm the lowest (18.7%) prevalence rates. The difference in the level of parasitism was found to be statistically significant ($p<0.05$, chi square = 15.5) in the region. Severe and mild malnutrition was more prevalence in girls (12.9% and 46.7% respectively) than in boys (11% and 44.9% respectively). The relationship between the parasitic infection prevalence and nutritional status of the children showed that 98 (57.2%) of the infected children were malnourished ($p<0.05$) and there was no significant difference ($p>0.05$) of malnutrition between infected and non-infected children. The relationship between malnutrition and parasitic infection revealed that the correlation coefficient was 0.85, indicating a moderately strong relationship between the variables. The study confirmed that malnutrition and parasitosis were important child health problems. Therefore, it is recommended that lunch meals, nutrition education, sanitation education, treatment of parasitic infections be added to the school curriculum of school children in the Adamawa region of Cameroon.

Key words: Malnutrition, parasitic infections, school children, Adamawa region

INTRODUCTION

Malnutrition and parasitic infections are common public health problems of children in developing countries. An estimated global infection rate for some parasites has primarily been attributed to the appalling unhygienic and environmental conditions; poverty and over-dispersion of parasites and disease are immediate causes of malnutrition and deaths of young children. They reinforce each other synergistically (UNICEF, 1998). As children are most at risk at an age when they are both growing and learning, parasitic infections potentially threatens a child's overall physical and psychological development and may cause or aggravate malnutrition (Stephension *et al*., 2000).

Globally it is estimated that among preschool-age children in developing countries 183 million are underweight, 226 million are stunted and 67 million wasted (Mitra and Tiwari, 1997). Over 1 billion people mostly in the tropics and sub-tropics are infested with parasites mostly soil transmitted helminths, malaria parasites and about 200 millions are infected with schistosomes (Latham, 1997). Each year, an estimated 300-500 million malaria infections occur with 1.2 million deaths worldwide, 85% of these deaths occur in sub-Saharan Africa. Although malaria was successfully eliminated in many countries with temperate climates during the 1950s, It remains a major killer in Africa (WHO, 1996).

In developing countries where overall rates of infections are high, recent studies have shown that for some parasites, egg counts are likely to be higher among subjects carrying mixed infections than in subjects carrying single species infections (Booth, 1998; Needham, 1998).

Malnutrition is widespread in Cameroon, like in many other developing countries and every year in the developing world, close to 12 million children die of inevitable causes and more than 6 million (55%) of these deaths could be directly or indirectly linked with malnutrition (Louis, 1993).

Corresponding Author: C.M.G. Garba, National School of Agro-industrial Sciences (ENSAI), University of Ngaoundere, P.O. Box 455, Ngaoundere, Cameroon

Intestinal parasites are among the major diseases of public health problems in sub-Saharan Africa. Apart from causing mortality and morbidity, infection with intestinal parasites has been associated with stunting of linear growth, physical weakness and low educational achievement in schoolchildren (Nokes and Bundy, 1993).

In Cameroon, schistosomiasis and soil transmitted helminth infections are important parasitic diseases. Recent estimates indicate that more than 1.7 million people (of a total of 14 millions inhabitants) in the country are infected with either Schistosomes, 5.6 million with *Ascaris lumbricoides*, 6.5 million with *Trichuris trichiura* and 2.6 million with hookworm (Brooker, 2000). However, infections are unevenly distributed within the country. The highest transmission levels of schistosomiasis occur in the northern regions (Tchuente *et al*., 2003).

Malaria and other parasitic infections are known to be the most important causes of morbidity and mortality of pre-school children in the Adamawa province of Cameroon and the whole national territory (Carte De Sante, 1999).

However, there is no information on the prevalence of parasitic infections and nutritional status of school children in Adamawa region of Cameroon. Thus, the present study was undertaken to: 1). Assess the nutritional status of school children using anthropometric indices of height and weight with age by comparing the values obtained from the study with W.H.O recommended standards (functional indicators for malnutrition), 2). Assess the extent of parasitic infection among school children in the Adamawa region and 3) Produce data that can provide reliable information that can enable nutrition and health planners to design a good programme that can improve the health and nutrition status of Cameroonian school children.

MATERIALS AND METHODS

Study site

Sample selection: The sampling strategy used was the four-stage stratified cluster sampling method. The Adamawa region has been divided into five administrative divisions and sub-divisions. The divisions are namely, Mayo Banyo, Faro et Deo, Mbere, Djerem et Vina. The list of all schools in each administrative division and sub-division constituted the sampling frame in the first stage of sampling. Four schools were selected from the list of schools in the sub-divisions of each division using the simple random sampling method to include schools in urban, semi urban and rural areas. In all, 20 randomly selected primary schools and 1200 pupils both boys and girls ages ranging from 6-17 years were randomly selected from each class using random number table.

Study design: After explaining the objectives of the study, to the delegate of Basic Education, delegate of public health, traditional authorities and authorization obtained, parents and teachers and full informed consent obtained, a preliminary visit was made to the 20 randomly selected public primary schools.

There was questionnaire administration and collection of stool and urine samples and thick blood films slides which were transported to local field laboratories (divisional hospital laboratories) for parasitological examination (eggs of *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworms, Schistosomes and cysts of; *Entamoeba histolytica*, *Giardia lamblia* (intestinalis) and malaria parasites). The questionnaire was designed to obtain information on civil status, anthropometric measurements (height and weight), quality drinkable water in schools, sanitation, socio-economic situation of the parents and religious affiliations.

Anthropometric data: Anthropometric measurements such as height and weight were made by a trained investigator following the internationally accepted standard techniques (WHO, 1995). Height and weight measurements were recorded to the nearest 0.1 kg cm and 0.5 kg respectively. The BMI was computed following the standard formula:

$$BMI = \text{Weight (kg)}/\text{Height}^2\ (m^2)$$

BMI for age was used to evaluate the nutritional status of the subjects.

The BMI for age is the most appropriate variable for determining nutritional status for children above 5 years and adolescence (Cole *et al*., 2007).

Collection of stool and urine samples for laboratory examination: Stool samples and urine samples were collected in labeled sterile, screw capped plastic containers and transported to the nearby hospital laboratory for analysis.

From each of the stool specimen, a direct saline smear preparation was made and examined by light microscope for identification of nematode ova and for differential diagnosis of protozoan cyst, lugol's solution was added. Samples that did not reveal any intestinal parasites on direct saline smear, a further search was conducted by formol-ether concentration method. Cellophane tape feacal smear (Kato Katz technique) was used to search for Schistosoma mansoni eggs.

For the search of schistosoma haematobium in urine, visual observation was done by carefully examining the bottled urine specimen for macrohaematuria before carrying out the filtration technique.

Search for malaria parasites: Thick blood films were made from finger pricks of the subjects, stained with Giemsa stain and examined microscopically under the 100x objective. Malaria parasites were counted against 200 leucocytes in thick films to obtain the parasite density.

Statistical analyses: The data obtained was analyzed using the XLSTAT package to bring out possible relationships, dependencies, correspondences (correlations) among the variables in correspondence and multi correspondence analysis. In addition to descriptive statistics, chi-square test were performed to test the significance of the differences among children who are malnourished with or without parasitic infections and ANOVA for both simple and multiple variances. Results were graphically designed using Sigma plot 9.0 package.

RESULTS

1200 primary school children of ages 6-17 years were used in the study with 59.6% (715) made of boys and 40.5% (485) girls. Most of the children, 34.28% (409) were between the ages of 10-12 years while the least, 2.01% (24) were between the ages of 16-17 years. 56.24% (671) were Moslems and 43.75% (522) were Christians. As concerns the socio-economic situations of the parents of the children, 65.46% (781) were farmers, 31.51% (376) were functionaries and 2%(32) were businessmen. The socio-demographic characteristics of the study population is shown in Table 1.

In the region, children between the age range 6-13 that were mostly moderately malnourished. Severe malnutrition prevalence in the school children was 20.8% while moderate malnutrition was 97.1%. Severe malnutrition was higher in girls (23.8%) than in boys (22.3%), while moderate malnutrition was higher in boys (93.1%) than in girls (86.7%). There was no significant difference between sexes of the school children and malnutrition because malnutrition was recorded in both boys and girls ($p>0.05$, $x^2 = 4.0$).

As concerns parasitic infection, a total of 171 (14.2%) of the school children were infected, of which 64.3% (110) were boys while 35.6% (61) were girls. The most infected divisions were Faro et Deo (48.3%) and Djerem (40%) while the least infected was Mbere (<1%) as shown in Table 2. The difference in the level of parasitism was found to be statistically significant in the four infected divisions of the region ($p<0.05$, chi square = 15.5). Malaria parasite infection recorded the highest prevalence rate (64.9%) followed by *Entamoeba histolytica* (61.9%) and *Schistosoma mansoni* (43.8%). The least prevalence rate was recorded for Hookworm (18.7%) as shown in Table 3. Single and multiple type parasitic infections were recorded. The highest single and multiple parasitism was observed in 10-12 age group (15.2%) and (28.6%) respectively and the least in the age group 16-17 (2.9%) as seen in Table 4. Parasitism was significantly different in the different age groups ($p<0.05$; chi square = 12.5). Table 6 shows that, more girls had multiple type parasitic infection (74.1%) than boys while more boys had single type parasitic

Table 1: Socio-demographic characteristics of the study population (n = 1200)

Variable	Category	(% of sample)
Age	5-9	30.17
	10-12	34.28
	13-15	33.52
	16-17	2.01
Sex	1=male	59.6
	2=female	40.5
Profession of parents	1=famers	65.46
	2=government workers	31.51
	3=businessmen	2.00
Religion	1=christians	43.75
	2=muslems	56.24

Table 2: Percentage parasitic/malnutrition prevalences by division

Division	% parasitic prevalence	% Acute malnutrition prevalence
Faro et Deo	48.3	10.00
Djerem	40.0	8.57
Mbere	38.5	54.55
Vina	35.0	4.76
Mayo Banyo	<1%	6.38

Table 3: Percentage of parasitism among infected school children in the Adamawa region

Parasite	Number positive (%)
Schistosoma mansoni	75 (43.8)
Hookworm	32 (18.7)
Entamoeba histolytica	106 (61.9)
Malaria parasite	111 (64.9)

Table 4: Effect of age on the prevalence of parasitic infections in school children in the Adamawa region

Age group (years)	Prevalence P1(%)	Prevalence P2 (%)
5-9 years	11.6 (20)	14.0 (24)
10-12	15.2 (26)	28.6 (49)
13-15	7.6 (13)	21.0 (36)
16-17	0.0 (0)	2.9 (5)

P1 = single type parasitic infections; P2 = Multiple type parasitic infections; p = 0.016; chi square = 12.5

Table 5: Effect of age on the prevalence of malnutrition and parasitic infection in school children

Age range	(n) % BMI-1* P1	(n) % BMI-1* P2
6-9	6 (3.5)	30 (17.5)
10-12	33 (19.2)	75 (43.8)
13-15	2 (1.1)	15 (8.7)
16-18	1 (0.5)	00

BMI-1* = acute malnutrition, P1 = single type parasitic infection, P2 = Multiple type parasitic infection

Table 6: Pattern of Parasitic infection in male and female school children

Sex	% P1	% P2
Boys	30.2 (34)	69.7 (76)
Girls	25.8 (15)	74.1 (46)

P1 = Single type parasitic infection; P2 = Multiple type parasitic infection. p = 0.530; chi sq = 3.8

Table 7: Prevalence of parasites by age groups

Age	% Prevalence *Schistosoma mansoni* (n)	% Prevalence Hookworm (n)	% Prevalence *Entamoeba histolytica* (n)	% Prevalence malaria parasite (n)
6-9	12.2 (21)	9.9 (17)	14.0 (24)	10.5 (18)
10-12	21.6 (37)	12.8 (22)	18.1 (31)	21.6 (37)
13-15	11.6 (20)	12.8 (22)	14.6 (25)	19.8 (34)
16-17	2.9 (5)	2.3 (4)	1.7 (3)	2.3 (4)

infection (30.2%) than girls but the difference was found to be non significant (p>0.05; chi square = 3.8). Malaria parasite and *Shistosoma mansoni* were most prevalent parasitic infections (21.6%) in the age group 10-12 years. In the age group of 13-15 years, malaria parasite was most prevalent 19.8%) and the least parasitic prevalence was recorded in the age group 16-17 years (1.7%) as shown in Table 7. Acute malnutrition was recorded to be most prevalent (43.8%) in children of the age group of 10-12 years having multiple type parasitic infection as shown in Table 5.

The R-Squared (73,6309%) statistics indicates that the model as fitted explains of the variability in BMImal. The correlation coefficient equals 0,858084, indicating a moderately strong relationship between the variables.

Associating malnutrition and parasitic infection at the regional level, infected children of ages 8 and 9 recorded severe malnutrition while moderate malnutrition was recorded in infected girls and non- infected boys of ages 7, infected boys and girls of age group 10-14 and infected boys of ages 15. Moreover the mean Body Mass Index (BMI) for those infected was 17.8 kg/m^2±1,1 with a range of 8.3-22.4, While those non-infected had a mean of 18.3 kg/m^2±2.4 with a range of 10.3-24.4. Severe malnutrition (BMI < 15.3) and moderate malnutrition (BMI = 15.3-18) were recorded to be highest 9.8% and 27.4% in the age group 13-15 and 9.3% and 27.9% respectively in the age group 5-9 as shown in Table 8. However the difference in the presence of malnutrition in the different age groups was found to be non significant (p>0.05, chi square = 16.9) because it was recorded in all age groups. Table 9 shows malnutrition distribution in infected and non-infected children. The prevalence of severe malnutrition at the regional level was higher (11.1%) in infected children than in non-infected children (9.7%) while moderate malnutrition was higher (46.1%) in infected children than non-infected children (43.8%). With infected girls, severe malnutrition was higher (12.9%) than infected boys (11%) and moderate malnutrition also higher (46.7%) in infected girls than in infected boys (44.9%). With non- infected children, severe malnutrition was recorded as higher (10.9%) in girls than in boys (9.2%) while moderate malnutrition was found to be higher (46.2%) in boys than in girls. Table 10 shows the pattern of infection and malnutrition between male and female children. Infection prevalence was higher (64.3%) in boys than in girls while severe malnutrition and moderate malnutrition were more prevalent (11% and 46.7%) respectively in girls.

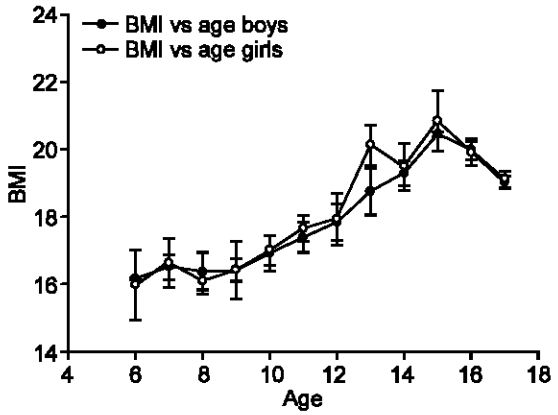

Fig. 1: Regional BMI by age of boys and girls

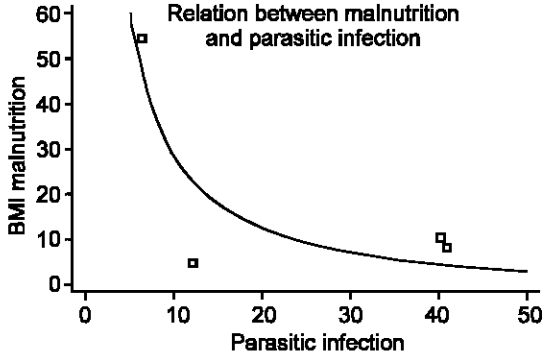

Fig. 2: Relation between malnutrition and parasitic infection

Table 8: The effect of age on the Body mass index of infected children

Age	% BMI-1*	% BMI-1	% BMI-2
5-9	9.3	27.9	62.7
10-12	7.2	26.0	66.6
13-15	9.8	27.4	62.7
16-18	2.5	12.5	62.5

P = 0.905, chi sq = 16.9; BMI -1* (<15.3) = severe malnutrition; BMI-1 (15.3-18) = moderate malnutrition;
BMI-2 (≥18.5) = Normal

DISCUSSION

Undernutrition among children and adolescents is a serious public health problem internationally, especially in developing countries (Pelletier *et al*., 2003; El-Ghannam, 2003; Staton and Harding, 2004). A recent study (Cole *et al*., 2007) has stated that undernutrition is better assessed as thinness (low body mass index for

Table 9: Malnutrition distribution between infected and non-infected children regional malnutrition distribution for boys and girls

Body mass index	No. of infected boys and %	No. of non infected boys and %	No. of infected girls and %	No. of non infected girls and %
Severe malnutrition (<15.3)	12 (11.00%)	54 (9.29%)	8 (12.90%)	48 (10.95%)
Mild malnutrition (15.3-18.5)	49 (44.95%)	269 (46.29%)	29 (46.77%)	177 (40.41%)
Normal (>18.5)	48 (44.03%)	25 (40.40%)	25 (40.32%)	213 (48.63%)

Table 10: Pattern of infection and malnutrition in male and female school children in the region

Sex	Infection (%)	Severe malnutrition (%)	Mild malnutrition (%)
Boys	64.3	11.0	44.9
Girls	35.6	12.9	46.7

age) than as wasting (low weight for height). Prior to this report there were no suitable thinness cut-offs for this age group. They have suggested that these new cut-off points should encourage direct comparison of trends in child and adolescent thinness worldwide. These cut-offs provide a classification of thinness for public health purposes. Nutrition plays a major role in maintaining health and malnutrition appears to generate vulnerability to a wide variety of disease and general ill health. The high prevalence of malnutrition in the Adamawa region (severe malnutrition records 20.8% and moderate malnutrition records 97.1%) most likely reflect the low socioeconomic status of the inhabitants which leads to low dietary intake by the school children due variability in quantity and quality of food supply and food production in the region. This further explains why malnutrition was prevalent in the children of all ages (6-15 years) and significantly different in the different age groups and different divisions of the region ($p<0.05$, chi square = 12.9). Children of the ages 15 years and below appear to be more predisposed to malnutrition than the older ones. The high prevalence of malnutrition in this region was similar to the prevalence of 58.1% reported in the south-west region of Cameroon (Nkuo-Akenji *et al*., 2008).

It has well been noted that not only malnutrition but also parasitic infections, cause serious health problems in many tropical countries. Our study revealed parasitic prevalence rate of 14.2% in this region with malaria parasitic infection being asymptomatic and recording a prevalence rate of 64.9%. this may indicate that the children in this region have acquired the ability to tolerate parasites without having fever or other symptoms. The humid climate observed in the area provides favourable environmental conditions for the development of oval, larval and cyst stages of the parasite (schistosomes, hookworm, Entamoeba and malaria parasites) and their transmission to man. However, this result is consistent with the findings of (Egwunyenga and Ataikiru, 2005) who reported parasitic infection prevalence rate of 54.7% among school children in Nigeria and of (Amuta *et al*., 2009) who reported a prevalence rate of 57.9% of intestinal parasites among school children in Makurdi, Benue state-Nigeria. Although parasitosis is endemic in the tropics, the reasons could be attributable mainly to environmental conditions and poor hygiene, causing significant morbidity such as anaemia, diarrhea and dysentery, malnutrition, mental deficits and poor growth. The mean body mass index showed remarkable difference of nutritional status between infected (11.1%) and non-infected (9.7%) children for severe malnutrition. Children infected with parasites were thinner than the non-infected ones. This could be that parasites produce significant adverse effects on weight gain, thereby inducing a significant contribution to the development of nutritional deficiencies in the group of children studied. However, the malnutrition observed among non-infected children may be due to inadequate food intake that led to poor appetite, metabolic and clinical disturbances as well as their socioeconomic status. The prevalence of infections was higher in boys (64.3%) than in girls (35.6%). This could be attributed to the fact that boys are more often involved in outdoors activities such as playing football and fishing and as such more exposed to infection. The girls showed a higher rate of malnutrition (12.9% for severe malnutrition and 46.75% for moderate malnutrition) than the boys. This could be attributed to limited food supply in many households and traditional feeding practices (scramble for served food thus leading to the phenomenon of the survival of the fittest) and customs that limit the girls consumption of certain energy or nutrient rich foods.

Conclusion: This study shows that there is high prevalence of global malnutrition and parasitic infection and a relationship of malnutrition and parasitic infections among school children in the Adamawa region. Recording malnutrition among non-infected children in the region explains that, there are other causes of malnutrition apart from parasitic infections, such as deficiencies in macronutrients intake (Protein, carbohydrates and fats) and micronutrients (Vitamins and minerals) as well as some other factors that could lead to the development of malnutrition. Therefore investment in education that is not accompanied by investment in health and nutrition of school children is a net loss for a country. If an improved health and amelioration of nutritional status in school children could be done, it will obviously contribute to high enrolments, better school attendance, low rates of dropouts and improved performance in academic work. It is recommended that all schools must have clean toilets, clean drinking water, clean school environment. Lunch

at school should be provided to the children and nutrition education should be added to their school curriculum and local health sectors should make provision for regular examination and treatment for parasitic infections among school children in the Adamawa region of Cameroon.

ACKNOWLEDGEMENT

We thank the ministry of health and the research centre of shistosomiasis and intestinal parasites for the assistance they rendered to us during the field work. The subjects and the school authorities are equally thanked for their cooperation.

REFERENCES

Amuta, E., T. Olusi and R. Houmsou, 2009. Relationship of intestinal parasitic infections and malnutrition among school children in Makurdi, Benue State-Nigeria. The Internet J. Epidemiol., Vol. 7.

Booth, 1998. Display of a positive interaction such that infection intensity of T. trichiura is higher in persons with concurrent A. lumbricoides than those without and vice versa; Available on google.com.

Brooker, 2000. Towards an atlas of human helminth infection in sub-saharan Africa; the use of geographical information systems (GIS). Parasitology today - Elsevier; 2000.

Bulletin of the World Health Organization, 1995. 73: 443-448.

Carte de santé, 1999. Institute de recherché pour le developpement- Camerun.

Cole, T.J., K.M. Flegal, D. Nicholls and A.A. Jackson, 2007. Body mass index cut offs to define thinness in children and adolescents: International survey. BMJ, 335: 194.

Egwunyenga, A. and D.P. Ataikiru, 2005. Soil-transmitted helminthiasis among school age Children in Ethiope East Local Government Area of Delta State, Nigeria. Afr. J. Biotech., 4: 938-941.

El-Ghannam, A.R., 2003. The global problems of child malnutrition and mortality in different world regions. J. Health Soc. Policy, 16: 1-26.

Louis, 1993. Problems with follow up particularly in respect of measurements of infant mortality in developing countries. Int. J., 51-52.

Latham, M., 1997. Human Nutrition in Developing Countries. Food and Nutrition Division FAO Rome, Italy.

Mitra, M. and A. Tiwari, 1997. Malnutrition in preschool children - A study of Brahmin Rawat and Teli preschool children of Raipur. Annual conference of Indian society of human genetics 16th and 18th Dec. 1997 XXIII, New Delhi.

Needham, 1998. Epidemiology of soil transmitted nematode infections in Ha Nam province, Vietmam. Trop. Med. Int. Health, 3: 904-912.

Nokes, C. and D.A.P. Bundy, 1993. Compliance and absenteeism in School children: Implications for helminth control. Trans. R Soc. Trop. Med. Hyg., 87: 148-152.

Pelletier, D.L. and E.A. Frongillo, 2003. Changes in child survival are strongly associated wit changes in malnutrition in developing countries. J Nutr., 133: 107-119.

Staton, D.M. and M.H. Harding, 2004. Protecting child health worldwide. Implementation is the biggest challenge slowing efforts to reduce childhood morbidity and mortality indeveloping countries. Pediatr. Ann., 33: 647-655.

Stephension, L.S., M.C. Latham and E.A. Ottesen, 2000. Malnutrition and parasitic infections. Parasitology, 121: 532-538.

Tchuente, L.A., J.M. Behnke, F.S. Gilbert, V.R. Southgate and J. Vercruysse, 2003. Polyparasitism with Schistosoma haematobium and soil transmitted helminth infections among school children in Loum, Cameroon. J. Trop. Med. Int. Health, 8: 975-986.

UNICEF, 1998. The state of the world's children. Oxford University Press, New York.

WHO, 1995. Physical status: The use and interpretation of anthropometry. Geneva, World Health Organization (WHO Technical Report Series, No. 854).

WHO, 1996. Management of severe malnutrition : A manual for physicians and other senior health workers, Geneva.

Pakistan Journal of Nutrition 9 (11): 1100-1106, 2010
ISSN 1680-5194

Enzyme Activities and Histology Study on High Fat Diet-induced Obese Rats by Pink Guava Puree

M.N. Norazmir[1,2], M.Y. Ayub[2] and M.M.A. Ummi[3]
[1]Department of Nutrition and Dietetics, Faculty of Health Sciences, MARA University of Technology, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia
[2]School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
[3]Department of Pathology, Faculty of Medicine, MARA University of Technology, 40450 Shah Alam, Selangor, Malaysia

Abstract: The effects of pink guava (*Psidium guajava*) puree on enzyme activities and histology on High Fat Diet (HFD)-induced obese rats were investigated. Thirty male Sprague-Dawley rats were divided into Control Negative (CN) fed with rat pellet; control positive, low, medium and high dose group (CP, LDG, MDG and HDG) were fed HFD-AIN93G, respectively. CN and CP were given distilled water; meanwhile treated group were given the aqueous puree, at concentration of 500, 1000 and 2000 mg/kg body weight, dissolved in distilled water were administered orally via a drinking bottle, respectively. Pink guava puree was supplemented with the HFD diet for six weeks. The rats were fasted overnight and euthanized under an anesthetic condition with ethyl ether and blood was collected from the posterior vena cava at the end of experiment. A significant reduction in body weight was observed in the treated groups as compared to CN and CP group. Specific activities of Glutathione Peroxidase (GPx), Glutathione Reductase (GR) and Superoxide Dismutase (SOD) of the HFD-induced obese rats were significantly increased in comparison with the CN group. Histologically, the liver and kidney cells in LDG, MDG and HDG showed no significant differences as compared to CN and CP's liver and kidney cells. Treatment with low, medium and high doses showed improved features in HFD induced-obese rat's liver and kidney cells. In conclusion, pink guava puree due to its antioxidant role was helpful in protecting organ tissues in experimental animals and has a significant impact on specific activities of HFD induced-obese rats.

Key words: Pink guava, enzyme activities, kidney, liver, histology

INTRODUCTION

Oxidative stress is thought to contribute to the development of a wide range of diseases (Nunomura *et al*., 2006), Although oxidative stress generally seems to contribute to chronic diseases *via* a lifetime accumulation of oxidative events, systems for studying the role of dietary antioxidants *in vivo* generally include imposition of oxidative stress so that responses can be studied in a reasonably short time frame. Decades of research on oxidative stress have contributed to our understanding of mechanisms that underlie the health benefits and the potential dangers of cardiovascular disease (Wiseman and Halliwell, 1996).

Reactive Oxygen Species (ROS) are continuously produced in biological system by the action of mitochondrial electron transport system and nicotinamide adenine dinucleotide phosphate oxidase (Cadenas *et al*., 1997). These ROS are cellular renegades and wreak havoc in biological system by tissues damage, altering biochemical compounds, corroding cell membranes and killing out rightly (Wiseman and Halliwell, 1996). To scavenge ROS, cells have several antioxidant enzymes including catalase, glutathione peroxidase, superoxide dismutase and glutathione-S-transferase.

Obesity and diabetes have reached epidemic proportions throughout the world (James, 2004). Epidemiological studies of cancer and cardiovascular disease suggest that consumption of fruits, vegetables, and plant-derived beverages is correlated with reduced risk of chronic disease (Appel *et al*., 1997). The benefits of plant-based foods may be a consequence of bioactive phytochemicals found in these foods. Phytochemicals include a wide variety of non-nutritive plant constituents that have diverse biochemical activities, including antioxidant properties (Norazmir *et al*., 2009b). Previous study by Asmah *et al*. (2006) showed that guava consumption could reduce oxidative stress and improve blood lipid profile.

Guava (*Psidium guajava*) is widely cultivated and Malaysia is the largest producer and exporter of pink guava puree. Guava has several carotenoids such as

Corresponding Author: M.N. Norazmir, Department of Nutrition and Dietetics, Faculty of Health Sciences, MARA University of Technology, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

phytofluene, ß-carotene, ß-cryptoxanthin, lycopene, rubixanthin and lutein (Thaipong *et al*., 2006). In our previous study, it showed anti-hypertensive (Ayub *et al*., 2010) and lipid-lowering properties (Norazmir and Ayub, 2010a). Therefore, pink guava puree thus represents a useful way to study the effects of pink guava (*Psidium guajava*) puree on oxidative stress in targeting organ tissues in High Fat Diet (HFD)-induced obese rats.

MATERIALS AND METHODS

Pink guava puree supplement: Pink guava (*Psidium guajava*) puree from mix variety of *Beaumont Sungkai* and *Beaumont Semenyih* obtained directly from Golden Hope Food and Beverages Sdn. Bhd. Ayub *et al*. (2010) has studied the contents of the pink guava puree. The puree that was packed in a metalized (aluminum) packages was stored immediately at -70°C until the study was carried out. Once opened, the puree was repackaged into container of about 5L before make the aqueous puree, at concentration of 500, 1000 and 2000 mg/kg body weight on low, medium and high dose group, dissolved in distilled water respectively; every 3 days and stored again at -70°C until used.

Experimental procedure: Thirty male Sprague-Dawley rats each weighing between 200-280 g obtained from UKM animal house were kept one per metabolic cages in a temperature-controlled room at 25±2°C with a 12:12 h light: darkness cycle with lights on at 8:00 am before starting the experiment. The rats were allowed free access to water and food during acclimatized week. The rats were divided into five groups: Control Negative (CN) fed with rat pellet; control positive, low, medium and high dose group (CP, LDG, MDG and HDG) were fed High Fat Diet-AIN93G, respectively. CN and CP were given distilled water; meanwhile treated group were given the aqueous puree, at concentration of 500, 1000 and 2000 mg/kg body weight, dissolved in distilled water were administered orally via a drinking bottle, respectively. All animals were observed daily for any clinical signs of disease. Body weight, blood chemistry and urine profile were measured through the study. After six weeks, the HFD induced-obese rats were fasted overnight (12-14 h) and euthanized under an anesthetic condition with ethyl ether. Blood was collected from the posterior vena cava for biochemical analysis on a blood haematology, enzyme activities, kidney function test and liver function test; respectively (Norazmir and Ayub, 2010b). The organs were excised, weighed and immediately frozen in liquid nitrogen and stored at -70°C until further tests. The study was approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee.

Analytical procedures: HFD induced-obese rats were fasted overnight (12-14 h) and euthanized under an anesthetic condition using ethyl ether, after six weeks of oral administration. Blood was collected from the posterior vena cava, transferred into tube containing Ethylene Diamine Tetraacetic Acid (EDTA) and centrifuged at 3500 g for 20 min to obtain the plasma fraction. The plasma samples were kept frozen at -70°C until used. Serum was obtained by collecting blood in non-EDTA tube. The serum was used for determine kidney and liver function test. Plasma and serum samples were kept at -70°C. All analysis was done using Blood Chemical Analyzer (Vitalab Selectra E, UK) to measure the following parameters: blood hematology, enzyme activities, kidney and liver function test were calculated. Urine was collected after the rats were fasted overnight and analyzed by using a Urine Analyzer (Bayer Diagnostics) to measure the following parameters: glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrates and leukocyte esterase.

Histology of kidney and liver: Histological examination was based on an earlier protocol (Humason, 1979). Slices of the liver lobe and kidney were fixed in Bouin's solution for 24 h. All samples were then dehydrated in graded ethanol series, cleared in toluene and embedded in paraffin; 5-6 µm sections were routinely stained with Trichrome Stains (Masson) (Sigma-Aldrich) and were assessed under light microscope (Nikon Eclipse E400).

Statistical analysis: Data was analyzed using SAS system. The significant differences between the control and treated groups were analyzed using Duncan's Multiple Range Test. All mean values were expressed as group means ± Standard Error of Mean (SEM). The minimal level of significance accepted was $p<0.05$.

RESULTS AND DISCUSSION

Body and organs weight: Pink guava (*Psidium guajava*) puree supplement had significantly decreased the body weight of HFD induced-obese rats. As shown in Table 1, mean body weights almost same (~300 g) in all groups at the start of the study. At the time of killing, mean body weight was significantly lowest in HDG (413.70±37.22 g), followed by LDG (439.25±30.84 g) and MDG (444.94±39.01 g) compared to CN (447.00±32.76 g) and CP (467.24±47.77 g), respectively. Pink guava puree intake had effect on bodyweight gain. HFD-induced obese rats gained positive weight, indicating good health status.

Organ's relative weight such as liver, heart, kidney, lung, spleen and testes (Table 1) were not affected by the pink guava puree supplementation. They were not significantly different compared to the CN and CP. Organ weight measurement is important to access general toxicity because any change in organ weight is a sensitive indicator of toxicity. This finding is similar to the Ayub *et al*. (2010) report. In theory, organ weight will be affected by the suppression of body weight as described by Marshall (2000). In this study, the pink guava puree

Table 1: Effects of pink guava puree on body and organ weights in High Fat Diet (HFD) induced-obese rats

Body weight (g)	CNG	CPG	LDG	MDG	HDG
Initial	303.87±29.95[a]	303.35±31.53[a]	302.51±35.94[a]	305.66±42.75[a]	305.68±48.20[a]
Final	447.00±32.76[a]	467.24±47.77[a]	439.25±30.84[b]	444.94±39.01[b]	413.70±37.22[b]
Organ weight (g)					
Liver	15.02±2.66[a]	12.66±0.54[ab]	11.86±1.74[b]	13.22±3.53[ab]	11.20±0.82[b]
Heart	1.17±0.16[a]	1.18±0.12[a]	1.10±0.09[a]	1.17±0.16[a]	1.18±0.22[a]
Kidney	2.55±0.23[a]	2.51±0.28[a]	2.44±0.22[a]	2.53±0.25[a]	2.30±0.28[a]
Lung	1.91±0.29[a]	1.97±0.04[a]	1.82±0.23[a]	2.00±0.52[a]	1.97±0.27[a]
Spleen	0.69±0.09[a]	0.61±0.07[a]	0.70±0.29[a]	0.63±0.08[a]	0.70±0.16[a]
Testes	3.47±0.13[ab]	3.08±0.21[ab]	3.18±0.25[ab]	3.75±0.80[a]	2.75±1.36[b]

Means with the same letter in same row are not significantly different ($p<0.05$); n = 6. CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink guava puree)

supplement did not give any significant changes in the organs' relative weights of HFD induced-obese rats compared to control group.

Oral administration of pink guava puree drinking solution did not induce mortality up to the highest dose, which was 2000 mg/kg body weight. No HFD induced-obese rats showed any toxic signs such as nose bleeding, vomiting, fur loss, diarrhea and death throughout the observation period. The administration of the highest dose used in the experiment does not show any toxicity effects can be considered as safe (OECD, 2006). Thus, the result may suggest the pink guava puree dosage is more than 2000 mg/kg body weight. Norazmir and Ayub (2010) also reported similar results in sub-acute studies of pink guava puree in spontaneous hypertensive rats.

Blood haematology: Blood hematology showed significant differences in HDG's red blood cell, hemoglobin and hematocrit amount (Table 2) compared to CN group. HDG's red blood cell count (9.20±0.53 $x10^{12}$/l) was significantly different compared to CN group (10.78±1.38 $x10^{12}$/l). HDG's hemoglobin (163.33±11.72 g/dL) was significantly different compared to CN group (192.17±26.42 g/dL). Hematocrit value also higher in HDG's (46.97±3.65) compared to CN group (55.55±7.87). This result was similar to Yin-Tzu (2008) study of guava extract on immune response. It showed that the action of the guava extract has increased the hematocrit value, but was not as efficient with carrying oxygen throughout the body, according to the hemoglobin value; vice-verse with this study result. The red blood cell indices suggested that the *Psidium guava* extract has no adverse effect on the HFD-induced obese rats.

Antioxidant enzyme activities: The specific activities of Glutathione Peroxidase (GPx), Glutathione Reductase (GR) and Superoxide Dismutase (SOD) and Total Antioxidant Status (TAS) concentration are given in Table 3. GPx, GR and SOD specific activities of the HFD-induced obese rats were significantly increased compared to the CN rats. Specific activity for GPx was significantly higher in HDG (2897.33±674.97 U/L), MDG (2819.50±262.04 U/L) and LDG (2787.50±266.36 U/L) compared to CN (2184.50±816.59 U/L) and CP (2610.17±61.63 U/L), respectively. Specific activity for SOD also significantly higher in HDG (418.67±35.48 U/L), MDG (409.33±55.22 U/L) and LDG (404.67±18.32 U/L) compared to CN (164.33±43.81 U/L) and CP (341.33±60.27 U/L), respectively. GR specific activity was significantly different in HDG (203.00±10.30 U/L) and MDG (181.00±30.26 U/L) compared to CN (116.17±10.76 U/L).

Administering pink guava puree to the HFD-induced obese rats significantly increased those antioxidant enzyme activities. The effect was more pronounced in the HDG supplemented group than in the CN or CP group. In a rat model of diet-induced obesity, Dobrian *et al*. (2000) reported increases in the activities of erythrocyte CuZn-SOD and GPx after 10 weeks on the diet. It attributed the increases in SOD and GPx enzymes, which are antioxidants, to their stimulation by oxidative stress. Similarly, Vincent *et al*. (1999) study of obese Zucker rats, reported increased activities of SOD and GPX. The similarity between our results and those studies of Dobrian et al. and Vincent et al. could be due to the duration of the obesity. It is likely that, in the early days of the development of obesity, antioxidant enzyme activity will be stimulated. However, once the obesity persists for a long time, as in humans, the sources of the antioxidant enzymes become depleted, leading to a low level of activity, as we found in total antioxidant status. Total antioxidant status of the treated groups did not show significant differences compared to the CN and CP group. Prince and Menon (1999) study showed that oral administration of aqueous *Tinospora cordifolia* root extract, an indigenous plant used as medicine in India, resulted in an increase in the levels of glutathione, which is similar to this study.

Kidney function tests: Kidney function tests of urea concentration were significantly decreased in HFD-induced obese rats; LDG (4.28±0.69 mmol/L), MDG (4.35±0.87 mmol/L) and HDG (3.85±0.71 mmol/L) as compared to CN (7.02±1.81 mmol/L) respectively as shown in Table 4. Creatinine and uric acid concentrations did not show any significant differences between supplemented pink guava-treated rats

Table 2: Blood hematology of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

	CNG	CPG	LDG	MDG	HDG
RBC	10.78±1.38^{a}	9.36±1.39^{ab}	9.40±0.68^{ab}	9.55±1.28^{ab}	9.20±0.53^{b}
WBC	7.42±1.69^{a}	4.70±1.60^{b}	4.42±1.19^{b}	5.58±1.48^{ab}	5.53±3.21^{ab}
PLT	1350.8±163.8^{a}	1089.5±127.0^{b}	1092.6±213.3^{b}	1190.3±251.1^{ab}	1260.0±138.7^{ab}
Hb	192.17±26.42^{a}	166.83±20.51^{ab}	167.00±11.08^{ab}	168.00±24.22^{ab}	163.33±11.72^{b}
HCT	55.55±7.87^{a}	48.95±6.57^{ab}	48.55±2.86^{ab}	47.87±7.71^{ab}	46.97±3.65^{b}

Means with the same letter in same row are not significantly different ($p<0.05$); n = 6. RBC : Red Blood Cell (10^{12}/L); WBC : White Blood Cell (10^{9}/L); PLT : Platelet (10^{9}/L); Hb : Haemoglobin (g/dL); HCT : Hematocrit (%).
CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink guava puree)

Table 3: Effect of pink guava puree on enzyme activities in High Fat Diet (HFD) induce-obese rats

	CNG	CPG	LDG	MDG	HDG
GPx	2184.5±816.6^{b}	2610.2±61.6^{b}	2787.5±266.4^{a}	2819.5±262.0^{a}	2897.3±674.9^{a}
SOD	164.33±43.81^{c}	341.33±60.27^{b}	404.67±18.32^{a}	409.33±55.22^{a}	418.67±35.48^{a}
GR	116.17±10.76^{c}	132.50±19.41^{bc}	137.33±9.69^{bc}	181.00±30.26^{ab}	203.00±10.30^{a}
TAS	1.33±0.19^{a}	1.35±0.14^{a}	1.44±0.22^{a}	1.56±0.29^{a}	1.63±0.63^{a}

Superscripts with different letters are significantly different at $p<0.05$ within the same row; n = 6. GPx : Glutathione Peroxidase (U/L); SOD: Superoxide Dismutase (U/L); GR : Glutathione Reductase (U/L); TAS : Total Antioxidant Status (mmol/L).
CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink guava puree)

Table 4: Kidney function test of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

	CNG	CPG	LDG	MDG	HDG
Creatinine (µmol/L)	75.01±3.83^{a}	75.17±6.35^{a}	77.38±7.78^{a}	78.90±12.20^{a}	71.98±7.91^{a}
Urea (mmol/L)	7.02±1.81^{a}	3.92±0.49^{b}	4.28±0.69^{b}	4.35±0.87^{b}	3.85±0.71^{b}
Uric acid (mmol/L)	0.38±0.24^{a}	0.41±0.19^{a}	0.37±0.15^{a}	0.34±0.17^{a}	0.33±0.06^{a}

Means with the same letter in same row are not significantly different ($p<0.05$); n = 6.
CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink guava puree)

compared to CN and CP group. Creatinine value for treated group ranged from 71.98-78.90 µmol/L compared to control group ranged from 75.01-75.17 µmol/L; meanwhile uric acid value ranged from 0.33-0.41 mmol/L.

Kidney is the second organ most frequently affected by any compound (Marshall, 2000). Therefore, renal functions can be assessed by measuring the concentration of creatinine and urea in plasma (Moshi *et al*., 2001). Previous report showed that some herbal preparations used in long period are associated with kidney injury (Kadiri *et al*., 1999). Plasma urea and creatinine concentrations are often used as an index of renal glomerular function and will be increased in renal injuries (Hughes and Jefferson, 2008). Urea is synthesized in the liver, primarily as by-product of the deamination of amino acids. Creatinine is a by-product from muscle mass will affect its concentration in blood (Vaughn, 1999). Creatinine is a nitrogenous waste product produced from creatinine in muscle and excreted by the kidneys. The majority of creatinine is excreted by glomerular filtration, but a small portion (~10%) is secreted into the proximal tubular lumen. The normal serum concentration of creatinine varies considerably between 60-120 µmol/L, depending on muscle mass and can be used to estimate renal function. Nzi *et al*. (2007) found that based on biochemical analysis of renal and hepatobiliary functions, such as the level of urea, creatinine and alkaline phosphate value, the fruit extract/juices generally tolerated by rats. These findings were similar with this study.

Liver function tests: The activities of total protein, albumin, globulin, AG ratio, total bilirubin, ALP, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) level of treated group and control group are given in Table 5. Pink guava supplement showed significantly decreased levels of total protein, globulin and ALT for treated group as compared to CN group. Total protein of MDG (72.67±3.65 g/L) and HDG (76.00±2.49 g/L) were significantly lower compared to CN (80.11±1.98 g/L). Globulin value for LDG (34.17±3.43 g/L), MDG (32.17±1.83 g/L) and HDG (35.00±3.41 g/L) were significantly lower compared to CN (39.67±0.82 g/L). AG ratio for LDG (1.22±0.16), MDG (1.28±0.07) and HDG (1.19±0.14) significantly different compared to CN (1.03±0.08). ALT value were also significantly lower for LDG (55.83±15.12 U/L), MDG (50.67±22.65 U/L) and HDG (57.50±8.48 U/L) compared to CN (77.00±16.26 U/L), respectively.

Liver is the target organ because most toxicants enter the body via the gastrointestinal tract and after absorption, the toxicants are carried by the hepatic portal vein to the liver. These parameters are commonly used to evaluate the status of liver function (Norazmir and Ayub, 2010b). Liver function test is crucial because liver

Table 5: Liver function test of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

	CNG	CPG	LDG	MDG	HDG
Protein (g/L)	80.11±1.98[a]	75.02±3.88[b]	76.26±3.86[ab]	72.67±3.65[b]	76.00±2.49[b]
Albumin (g/L)	40.60±2.58[a]	40.90±2.22[a]	41.78±2.63[a]	40.85±2.61[a]	41.15±1.52[a]
Globulin (g/L)	39.67±0.82[a]	34.17±2.32[b]	34.17±3.43[b]	32.17±1.83[b]	35.00±3.41[b]
AG ratio	1.03±0.08[b]	1.20±0.08[a]	1.22±0.16[a]	1.28±0.07[a]	1.19±0.14[a]
Bilirubin (µmol/L)	6.18±1.05[a]	4.67±2.12[a]	4.99±2.01[a]	4.63±0.86[a]	4.65±0.76[a]
ALP (U/L)	122.00±30.62[a]	123.50±39.83[a]	96.50±43.13[a]	114.17±35.27[a]	104.83±29.44[a]
ALT (U/L)	77.00±16.26[a]	53.67±11.09[b]	55.83±15.12[b]	50.67±22.65[b]	57.50±8.48[b]
AST (U/L)	132.67±29.62[a]	93.33±21.80[a]	102.33±12.55[a]	132.33±39.00[a]	104.33±16.18[a]

Means with the same letter in same row are not significantly different ($p<0.05$); n=6. ALP : Alkaline Phosphate; ALT : Alanine Aminotransferase; AST : Aspartate Aminotransferase.

CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink guava puree)

Table 6: Urine profile of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

Urine profile components	CNG	CPG	LDG	MDG	HDG
Specific gravity	1.005[a]	1.015[a]	1.015[a]	1.015[a]	1.005[a]
pH	6.9[a]	6.8[a]	7.3[a]	7.3[a]	7.7[a]
Ketones	-ve	Trace	Trace	Trace	-ve
Blood	1.5	-ve	-ve	-ve	-ve
Protein	Trace	1.7	2	1.7	1.3
Nitrates	-ve	Trace	-ve	-ve	-ve
Glucose	-ve	-ve	-ve	-ve	-ve
Urobilinogen	Normal	Normal	Normal	Normal	Normal
Leukocyte	-ve	-ve	-ve	-ve	-ve

Means with the same letter in same row are not significantly different ($p<0.05$); n = 6. -ve : negative.

CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink Guava puree)

is the central organ in detoxification of compounds. In general, enzymes provide an excellent marker of tissue damage. Organ or tissue damage causes the release of increased amounts of many enzymes into the blood stream (Marshall, 2000). Vaughn (1999) reported that the activities of most enzymes normally detectable in blood remain constant in healthy and normal person.

The result of total protein, globulin and ALT concentrations were not affected by the pink guava puree in treated group compared to CN. This shows that the synthesis of protein in the HFD induced-obese rat's liver is not influenced by the supplementation. Similar results were also obtained in the studies of *Psidium guajava* on spontaneous hypertensive rats (Norazmir *et al*., 2009b). A healthy liver is so crucial for protein metabolism since liver disease is frequently associated with alterations in proteins and disturbances of protein metabolism (Marshall, 2000). Total protein and albumin concentrations will be decreased by inadequate synthesis due to liver disease (Datta *et al*., 1999).

Urine profile: Table 6 showed the urine profile between dosage groups on the last day of experiment. No glucose and blood were found in the urine of supplemented groups. The values of urobilinogen, bilirubin, nitrates and leukocyte esterase in HDG, MDG and LDG same as the CP and CN values. Glucose and bilirubin can be found in urine when the kidneys are damaged or diseased (Hughes and Jefferson, 2008). Nitrites were present in the CP group. Bacteria that cause a Urinary Tract Infection (UTI) have an enzyme that can convert urinary nitrates to nitrites (Lahlou *et al*., 2006). Therefore, nitrites in urine are an indication of UTI. Human trial was also conducted with *Hibiscus sabdariffa* calyces (Herrera-Arellano *et al*., 2004). Hypertensive patients were recruited and aged between 30 and 80 years of age. *Hibiscus sabdariffa* (10 g) was consumed with 0.5 L of water at breakfast for 4 weeks. Results showed that pH values showed no significant differences by *Hibiscus sabdariffa, which* is similar with Norazmir *et al*. (2009a) study using pink guava puree on spontaneous hypertensive rats.

Histology analysis: Liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and are especially vulnerable to damage (Brzoska *et al*., 2003). Photomicrograph of rat kidney in control group and treated group were closely observed (Fig. 1). Normal structure of the kidney was observed in the kidney of control rats. Renal corpuscles, proximal and distal convoluted tubules can be seen in the sections. No significant changes were observed in the kidney histology of rats of the treated groups. Figure 2 showed photomicrograph of rat liver in control group and treated groups. Microscopic observations showed a normal liver histomorphology in control rats. In treated rats, liver showed normal structure of liver tissue composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and

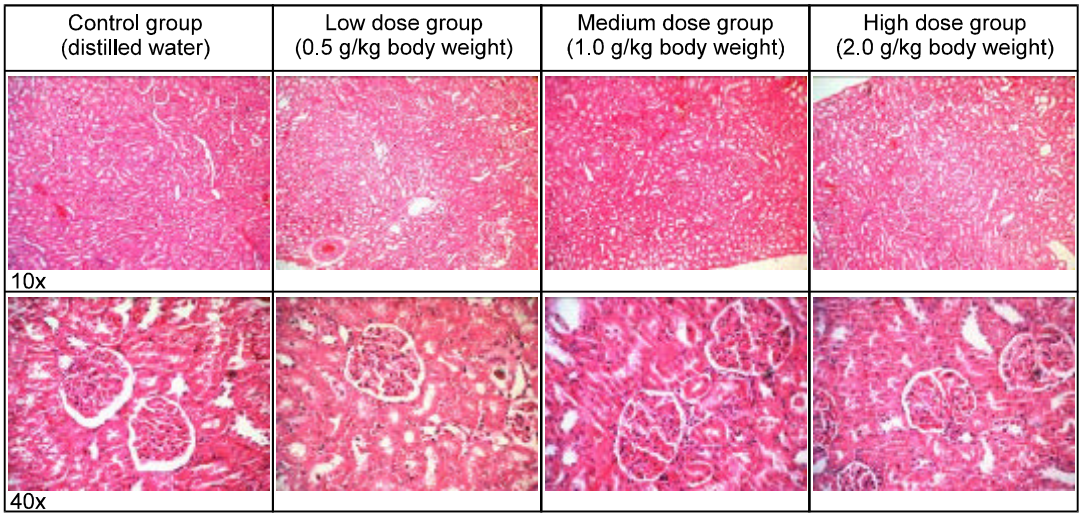

Fig. 1: Photomicrograph of kidney from the control group and treated groups (10x and 40x magnifications). No significant damage was detected in any treatment group. Trichrome stain (a) x 10 (b) x 40

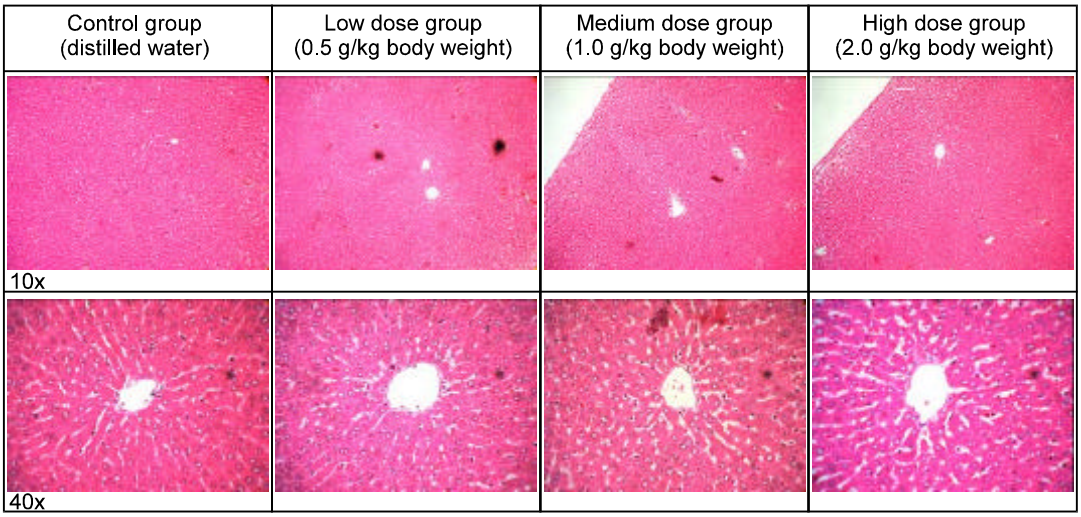

Fig. 2: Photomicrograph of liver from the control group and treated groups (10x and 40x magnifications). No significant damage was detected in any treatment group. Trichrome stain (a) x 10 (b) x 40

are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Histopathological examinations of the liver revealed no pathological abnormality of the low, medium and high dose treatment groups as compared with the control groups.

Conclusion: Pink guava puree supplements seem to be beneficial for increased antioxidant enzyme activities with significant result to reduce body weight. Blood haematology, kidney and liver function test showed extensively differences in treated groups as compared to control groups. Histology analysis of the liver and kidney revealed no pathological abnormality as compared to with the control groups. These results suggest that the guava puree did not cause toxicities in rats. In conclusion, pink guava puree due to its antioxidant role was helpful in protecting tissues in experimental animals from oxidative stress and has a significant impact on health status of HFD induced-obese rats.

ACKNOWLEDGEMENT

This research was supported financially by the Universiti Kebangsaan Malaysia through the grant UKM-ABI-NBD00011-2007 and UKM-GUP-BTK-08-14-307.

REFERENCES

Appel, L.J., T.J. Moore and E. Obarzanek, 1997. A clinical trial of the effects of dietary patterns on blood pressure. New Eng. J. Med., 336: 1117-1124.

Asmah, R., F.A.B. Mohd and H. Zarida, 2006. The effects of guava (*Psidium guava*) consumption on total antioxidant and lipid profile in normal male youth. Afr. J. Food. Agric. Nutr. Dev., 6: 1-12.

Ayub, M.Y., M.N. Norazmir, S. Mamot, K. Jeeven and H. Hadijah, 2010. Anti-hypertensive effect of pink guava (*Psidium guajava*) puree on Spontaneous Hypertensive Rats. Int. Food Res. J., 17: 89-96.

Brzoska, M.M., J.M. Jakoniuk, B.P. Marcinkiewicz and B. Sawicki, 2003. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol and Alcoholism., 38: 2-10.

Cadenas, E., A. Boveris and C.I. Ragan, 1997. Production of superoxide radical and hydrogen peroxide by NADH-ubiqunine reductase and ubiquinol-cytochrome c reductase from beef heart mitochondria. Arch. Biochem. Biophys., 180: 248-257.

Datta, S., S. Sinha and P. Bhattacharyya, 1999. Effect of an herbal protein, CI-I, purified from *Cajanus indicus*, in models of liver failure in mice. Drug Dev. Res., 48: 76-83.

Dobrian, A.D., M.J. Davies, R.L. Prewitt and T.J. Lauterio, 2000. Development of hypertension in a rat model of diet-induced obesity. Hypertension, 35: 1009-1015.

Herrera-Arellano, A., S. Flores-Romero, M. Chavez-Soto and J. Tortoriello, 2004. Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: A controlled and randomized clinical trial. Phytomedicine, 11: 375-382.

Hughes, J. and A. Jefferson, 2008. Clinical chemistry. Churchill Livingstone. Elsevier.

Humason, G.P., 1979. Animal tissue techniques. San Fransisco: W.H. Freeman and Company.

James, P.T., 2004. Obesity: the worldwide epidemic. Clin. Dermatol., 22: 276-280.

Kadiri, S., A. Arije and B.L. Salako, 1999. Tradititional herbal preparations and acute renal failure in South West Nigeria. Trop. Doc., 29: 244-246.

Lahlou, S., A. Tahraoui, Z. Israili and B. Lyoussi, 2006. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. J. Ethnopharm., 110: 458-463.

Marshall, W.J., 2000. Clinical chemistry. Edinburgh, Mosby.

Moshi, M.J., J.J.K. Lutale, G.H. Rimoy, Z.G. Abbas, R.M. Josiah and B.M. Andrew, 2001. The effect of *Phyllanthus amarus* aqueous extract on blood glucose in non-insulin dependent diabetic patients. Phytochem. Res., 15: 577-580.

Norazmir, M.N., M.Y. Ayub and S. Mamot, 2009a. Blood and urine profiles of Spontaneous Hypertensive Rats supplemented with pink guava (*Psidium guajava*) puree. Sains Malaysiana, 38: 929-934.

Norazmir, M.N., M.Y. Ayub, S. Mamot, H. Hadijah and S. Ahmad Tarmizi, 2009b. Effects of pink guava (*Psidium guajava*) puree supplementation on Spontaneous Hypertensive Rat's enzyme activities. Proceedings of the Seminar UKM-Universitas Indonesia ke-2. Bangi: Universiti Kebangsaan Malaysia, pp: 75.

Norazmir, M.N. and M.Y. Ayub, 2010a. Beneficial lipid-lowering effects of pink guava puree in high fat diet induced-obese rats. Malaysian J. Nutr., 16: 171-185.

Norazmir, M.N. and Ayub, M.Y. 2010b. Effects of pink guava (Psidium guajava) puree supplementation on antioxidant enzymes activities and organs functions of spontaneous hypertensive rat. Sains Malaysiana., In press

Nunomura, A., R. Castellani, X. Zhu, P. Moreira, G. Perry and M. Smith, 2006. Involvement of oxidative stress in Alzheimer disease. J. Neuropathol. Exp. Neurol., 65: 631-641.

Nzi, A.K., M.B. Elfriede, L. Nilton, D. Soraya and E.A. Varela, 2007. Acute, sub-acute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (*Anacardium occidentale* L.). J. Ethnopharm., 110: 30-38.

O.E.C.D., 2006. Draft Final Report of the Validation of the Updated Test Guideline 407 Repeat Dose 28-day Oral Toxicity Study in Laboratory Rats.

Prince, P.S. and V.P. Menon, 1999. Antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes. J. Ethnopharm., 65: 277-281.

Thaipong, K., B. Unaroj, C. Kevin, C.Z. Luis and H.B. David, 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Comp. Analy., 19: 669-675.

Vaughn, G., 1999. Understanding and evaluating common laboratory test. Stamford, Appleton and Lange.

Vincent, H.K., S.K. Powers, D.J. Stewart, R.A. Shanely, H. Demirel and H. Nalto, 1999. Obesity is associated with increased myocardial oxidative stress. Int. J. Ob. Rel. Met. Dis., 23: 67-74.

Wiseman, H. and B. Halliwell, 1996. Damage to DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer. Biochem. J., 313: 17-29.

Yin, T.L., 2008. Influence of guava (*Psidium guajava*) extract on immune response of *Puntius altus*. Dissertation, Mahidol University International College Thailand.

Pakistan Journal of Nutrition 9 (11): 1107-1112, 2010
ISSN 1680-5194

Estimation of Carcass Composition of Sheep, Goats and Cattle by the Urea Dilution Technique

Samir S. Hanna
Department of Animal Production, College of Agriculture, University of Basra, Basra, Iraq

Abstract: The study involved 30 local Iraqi sheep and goats (15 each) and 10 Friesian x local dry cows. Urea Space Volume (USV) was calculated from 2 collection periods of blood samples following infusion of urea at 12 (US 12 kg) and 30 (US 30 kg) min after infusion and then as a proportion of live weight (BW) or Empty Body Weight (EBW). All animals were slaughtered within 2 d of the USV trials. Sheep recorded the highest percentage (80.57%) of carcass soft tissue followed by goat (79.10%) and cattle (77.77%). Cattle bone/meat ratio was significantly lower than that of sheep or goats. Water content of cattle soft tissue was higher (74.78%) than that of sheep (64.86%) and goats (69.76%); however, fat% showed reverse percentages (15.39% for sheep and 9.025% for cattle). The pooled regression between either BW or EBW and carcass soft tissue composition was similar. Water content indicated positive slope; whereas protein and fat showed negative slope with either BW or EBW. BW or EBW effectively predicted the amount of fat and protein in the carcass soft tissue rather than their percentages when pooled data were used. Initial Plasma Urea Concentration (PUC) mean did not differ significantly among the three studied species. All correlation coefficients between urea concentration at zero time and soft tissue composition were negative and significant except that of protein% and fat%. However, positive and significant correlation coefficients between US12 and kg of water, protein, fat and ash in carcass soft tissue (ranged 0.921-0.948) were found. US expressed as kg or % to either BW or EBW after 12 min of infusion showed higher correlation and regression coefficients than that of urea concentration at zero time with reverse direction. Correlation coefficients within each species between soft tissue compositions with urea concentration at zero time were non-significant except for protein amount of goats and ash% of cattle ($p<0.05$). For sheep and goats, correlation coefficients using water, fat and protein of sheep only (expressed in kilograms) and US at 12 min (kg) or as percentages to BW and EBW were highly significant ($p<0.01$). Cattle resulted in significant correlation values ($p<0.05$ and $p<0.01$) for water% with US at 12 min as percentages to body weight or empty body weight.

Key words: Body composition, sheep, goat, cattle, prediction, urea space

INTRODUCTION

Body energy reserves, mainly represented by fat and muscle body content, as well as body composition, are important determinants of carcass quality in livestock (Mora *et al*., 2007). Although Body Condition Score (BCS) has been demonstrated to be an adequate estimator of these body variables under most practical conditions (Villaquiran *et al*., 2005), the qualitative and subjective character of this measurement must be considered when precise and repeatable data are required.

A number of approaches to predict the body composition of live animals are tried including use of the urea dilution technique. Agnew *et al*. (2005) reported that urea seemed to meet all the requirements of a satisfactory tracer. It is nontoxic, non foreign to the body and shows an even and rapid distribution throughout total body water without any physiological effect. The urea dilution procedure has no detrimental effects on performance characteristics of feedlot steer cattle (Wells and Preston, 1998). For these reasons, in addition to being an easy and accurate measurement, urea is an ideal candidate tracer to estimate Empty Body (EB) water *in vivo*. Total body water volume can be estimated by dividing the total amount of urea infused by the increase in plasma urea concentration before and after infusion. Many studies have examined the relationships between urea space and body composition in sheep, beef cows and dry cows. Bartle *et al*. (1987) evaluated some of these equations and concluded that urea dilution was a valid estimator of body composition in growing-finishing cattle. The urea dilution technique could be a valuable research tool if multiple estimates of body composition over time are needed when the slaughtering of the animal is not desired (Wells and Preston, 1998). Therefore, the objective of this study was to evaluate the usefulness of this technique in estimating carcass soft tissue composition in Iraqi Arabi sheep, local goats and Friesian x Local cattle.

MATERIALS AND METHODS

Animals and management: Mature Arabi ewes (no = 15), Iraqi local nannies (no = 15) and ten Friesian x local

Iraqi cross dry cows were randomly selected for slaughter at the commencement of the trail. They were selected from the herd at the Animal Farm, College of Agriculture, University of Basra. Animals remained at the same management and feeding regimens during the last two years before this study. They offered mixed diets of local grass and concentrate supplements, with forage proportions in diets ranging proportionately from 0.30-0.60 (DM basis). Concentrates used included some of the following ingredients: barley, wheat bran, corn and soybean meal in addition to a vitamin and mineral supplement.

Urea space determination: Feed was withheld but water was available for 24 h before the urea dilution was performed. Urea dilution was performed 1 d before slaughter. Animal weight was determined 10 min before the urea was administered. The technique used was described in detail by Preston and Kock (1973). A 12-gauge needle was inserted into the jugular vein and a catheter was introduced through the needle. The needle was then removed and the catheter was firmly taped to the neck and closed with a three-way stopcock. A 15-mL blood sample was taken and put into a plastic tube containing 30 mg of sodium oxalate. The catheter was then flushed with heparin solution (100 heparin units/mL, 0.1% benzyl alcohol and 0.9% sodium chloride). A solution containing 20% urea dissolved in 0.9% saline was infused through the catheter over a 2-min period. The volume injected was calculated to provide 130 mg urea/kg body weight. The catheter was flushed with 5 mL of heparin solution after the infusion and after each sample. Zero time was defined as the end of the 2-min period. Samples were obtained at 0, 12 and 30 min after the infusion. Thirty seconds before each sampling, 10 mL of blood was taken and discarded to ensure that the heparin solution contained in the catheter did not dilute the sample. The blood was kept at room temperature before centrifugation for 20 min at 2,460 * g and plasma was removed and frozen at -20°C until analysis. Plasma Urea Concentration (PUC) was determined by using a kit provided by Biochemeca and Diagnostica mbH. The coefficient of variation between determinations of the same sample was less than 1%, as recommended by Preston and Kock (1973). The solution used for infusion was also analyzed for urea concentration.

The US was calculated by the precise quantity of urea infused by the difference in plasma urea concentration before and after infusion at 12 (US12, kg) or 30 (US30, kg) min. The US was also expressed as a proportion of LW (US12/BW, or US30/BW, kg/kg) and empty BW (EBW; US12/EBW or US30/EBW, kg/kg), respectively.

$$\text{Urea space (\%)} = V\ (ml) * C\ (mg/dl)/D\text{-}PUC\ (mg/dl) * BW\ (kg) * 10$$

(Bartle *et al*., 1983)

Where, V is the volume infused, C is the concentration of the urea solution, D-PUC is the difference in PUC before and after the infusion and BW is body weight or empty BW. Omitting BW in the formula resulted in calculation of urea space volume. A density factor (0.99299 L/kg, density of water at body temperature) was used to convert liters to kilograms. Urea space was calculated at 0, 12 and 30 min after infusion.

Carcass measurements: Animals were slaughtered at the Animal Farm, College of Agriculture, University of Basra. Animals body was divided into carcass and non-carcass fractions; weight of these parts were recorded. Contents of gastrointestinal tract and bladders that were not a part of the empty body were determined by the difference between weights before and after washing. Carcasses were split longitudinally and chilled at 4°C. Cooler shrinkage was assumed to be water loss. The right side of the carcass was physically separated into soft tissue and bone. Soft tissue was ground and mixed for 3 min and then frozen at -20°C until analysis. Proximate analysis (protein, fat and ash) of the carcass soft tissue was determined as described by AOAC (1990).

Statistical methods: Correlation coefficients between urea space (kg, % EBW and % BW) and carcass soft tissue composition (protein, fat, moisture and ash, expressed in percentage of the total matter and in kilograms) were calculated for the three species groups of animals (pooled data) (SPSS, 1999). Partial correlation coefficients between US and carcass Soft Tissue Composition (STC) were calculated for each species group by excluding species and weight (SPSS, 1999).

Simple and multiple regression analyses were performed by SPSS (1999) to develop equations for prediction of STC from US expressed in kilograms, percentage of EBW and percentage of BW. Independent variables in the model were BW (24 h feed deprived), EBW, US % BW (calculated for each time-sample), US% EBW and US in kilograms. Dependent variables studied were carcass protein, fat, water and ash, expressed both as a percentage of EBW and in kilograms. The STEPWISE procedure (SPSS, 1999) was then used to study the effect of additional variables in the model on the coefficient of determination (R^2).

RESULTS AND DISCUSSION

Carcass soft tissue composition: Since there are species differences in body and carcass weight, our

Table 1: Means of body weight, empty body weight, carcass weight, carcass proximal components and initial Plasma Urea Concentration (PUC) of sheep, goats and cattle (±standard error)

	Species		
Item	Sheep	Goats	Cattle
No. animals	15	15	10
Body weight (kg)	53.34±3.11	40.56±2.66	192.74±14.81
Empty body weight (kg)	49.25±1.24	35.38±1.98	169.97±7.83
Cold carcass weight (kg)	26.27±2.10	20.19±2.23	103.56±9.23
Soft tissue (%)	80.57^{a}±1.80	79.12^{b}±1.23	77.77^{c}±1.05
Bone/meat ratio	41.46^{a}±0.03	40.75^{a}±0.02	34.31^{b}±0.02
Soft tissue weight (kg)	42.98±1.22	32.22±1.16	148.15±2.27
Water (%)	64.88^{c}±0.89	69.76^{b}±0.82	74.78^{a}±1.23
Protein (%)	18.73±1.77	18.14±1.78	16.33±1.12
Fat (%)	15.39^{a}±0.56	11.18^{b}±0.46	9.02^{c}±0.57
Ash (%)	1.00^{b}±0.001	0.92^{c}±0.01	1.13^{a}±0.02
PUC (mmol/L)	4.97±0.14	5.20±0.15	4.24±0.15

Means within each row with different letter significantly differ at 5%

Table 2: Linear equations between either Body Weight (BW) or Empty Body Weight (EBW) and percentages (%) or weight (kg) of carcass soft tissue components for the pooled data

Independent	Item	Intercept (a)	Slope (B)	SE	R^2 (%)
BW	Water (%)	64.77	0.0521	0.005	68.84
	Fat (%)	14.67	-0.0286	0.005	47.38
	Protein (%)	19.89	-0.0168	0.004	34.67
	Ash (%)	0.90	0.0012	0.0007	87.65
EBW	Water (%)	64.70	0.059	0.006	67.72
	Fat (%)	14.71	-0.032	0.005	47.09
	Protein (%)	20.00	-0.020	0.004	38.39
	Ash (%)	0.988	0.0014	0.00006	90.66
BW	Water (kg)	-3.053	0.599	0.004	99.80
	Fat (kg)	2.409	0.057	0.003	91.90
	Protein (kg)	1.439	0.122	0.003	98.00
	Ash (kg)	-0.066	0.009	0.000	99.80
EBW	Water (kg)	-2.887	0.667	0.020	96.30
	Fat (kg)	2.442	0.064	0.004	88.10
	Protein (kg)	1.581	0.135	0.006	92.80
	Ash (kg)	-0.066	0.010	0.000	96.80

comparison here was concentrated on percentages only. There were significant ($p<0.05$) differences in carcass soft tissue due to different species (Table, 1). Sheep recorded the highest percentage (80.57%) followed by goat (79.12%), whereas cattle had the lowest (77.77%). However, bone to meat ratio was lowest ($p<0.05$) in cattle in comparison to sheep and goats. This explains that cattle had more meat and less fat than both sheep and goats. Water content was higher ($p<0.05$) in cattle soft tissue (74.78%) than sheep (64.88%) and goats (69.76%). Fat% behaved completely in reverse to water%, since sheep got the highest value (15.39%) and cattle the lowest one (9.02%). Protein% did not show statistically differences among species. These results were in agreement with previous studies on the same genotypes of sheep, goats and cattle (Tahir *et al*., 1986, 1987, 1992; Mohammed, 1988; Al-Saigh and Al-Jassim, 1998).

Relationship of body weight and empty body weight with carcass soft tissue composition: Linear equations expressed as a percentage of soft tissue for estimating the carcass soft tissue components from BW are presented in Table 2. Equations developed with the pooled data produced higher coefficients of determination (R^2) for ash% followed by water% and generally smaller model SE than protein% and fat%. The pooled models to predict contents of water, protein and ash resulted were nearly similar when prediction was either BW or EBW, as all coefficients, SE and R^2 were very close. These results confirmed the above findings, that cattle were heavier than sheep and goats, however, they recorded low protein% and fat% but high level of water%.

Positive slopes in the equations for percentage of water indicated the tendency of the carcass soft tissue to have proportionally more water in cattle than sheep or goats, as body weight of cattle bigger than that of sheep or

goats. However, negative slopes for protein and fat indicated that cattle soft tissue protein and fat were less than that of sheep and goats. Body weight or EBW was accurately estimating the ash content in soft tissue as determinant coefficient reached 90%. Body weight or empty body weight was effectively predicted the amount of fat and protein in the carcass soft tissue rather than their percentages when the pooled data were used (R^2 ranged 91.90-99.80 or 88.10-96.80% for body weight and empty body weight respectively). Swartz *et al.* (1991), Velazco *et al.* (1997), Wuliji *et al.* (2003) and Ngwa *et al.* (2006) found different results in predicting whole-carcass protein and fat in young Holstein calves, Holstein steers and goats respectively.

In general, equations obtained for BW and composition were similar to those for EBW either for the amount or the percentages. Gut fill has been reported to introduce substantial variability in regression models for prediction of body composition (Preston and Kock, 1973). Perhaps the period of feed deprivation and the low level of roughage of the diets accounted for this effect.

Urea space and carcass soft tissue composition: Initial Plasma Urea Concentrations (PUC) mean did not differ significantly among species. Their values for sheep, goats and cattle were 4.97±0.14, 5.20±0.15 and 4.24±0.15 mmol/L, respectively (Table, 1). Carcass soft tissue composition data were used to estimate correlation coefficients (r) with US at 0, 12 and 30 min after infusion.

For the pooled data (Table 3), all correlation coefficients between urea concentration at zero time and soft tissue composition were negatively significant ($p<0.01$) except those of protein% and fat%, which were positive and significant ($p<0.01$ and 0.05 respectively). However, significant correlation coefficients ($p<0.01$) between US and kilograms of water, protein, fat and ash in carcass soft tissue ranged from 0.921-0.948 at 12 min. Kock and Preston (1979) reported that correlation coefficients between US and body water were highest at 12 min. The correlation coefficients in this study did not show any particular pattern after the infusion. Urea space had higher and positive correlation coefficients with protein and fat when expressed in kilograms than when calculated as a percentage of the total carcass soft tissue (Table 3). Urea space expressed as kg or as percentages to either BW or EBW after 12 min of infusion showed higher correlation coefficients and regression (Fig. 1) with soft tissue compositions than that of urea concentration at zero time with reverse sign. Correlation coefficients for US at zero, 12 min (kg, % to BW and EMB) and kilograms or % of fat in carcass soft tissue got the least value (0.342, -0.515, -0.535 and -0.373 respectively). Calculating US as a percentage of BW or EBW did not increase correlation coefficients for any of the carcass soft tissue components.

Correlation coefficients within each species were presented in Table 4. Correlations between soft tissue compositions with urea concentration at zero time were non-significant except for protein amount of goats and ash% of cattle ($p<0.05$). When STC was expressed as a percentage, r values were low and not significant for all components. For sheep and goats, correlation coefficients using water, fat and protein of sheep only (expressed in kilograms) and US at 12 min (kg) or as percentages to BW and EBW were highly significant ($p<0.01$).

Cattle resulted in significant correlation values ($p<0.05$ and $p<0.01$) for water% with US at 12 min as percentages to body weight or empty body weight (Table 4). It is possible that in the leaner animals, urea equilibrated faster (mostly at 12 min) with body water than in fatter animals (Agnew *et al.*, 2005), explaining the appearance of maximum correlations values at different times after infusion for different species.

Urea showed fast disappearance from the blood in this study, which can be due to kidney excretion of both ammonia and urea, transference of urea to salivary glands and to the rumen via saliva, or diffusion of urea through the rumen wall (Swartz *et al.*, 1991). Therefore, Velazco *et al.* (1997) suggested that the amount of urea being excreted should be established for young, lean cattle, as the metabolism of urea could be different in fast-growing calves than in older ruminants, because D-PUC and protein deposition decreased as animals became older.

Table 3: Pooled data correlation coefficients between urea concentration at zero time and urea space after 12 min of infusion (kg) with soft tissue composition amounts and percentages

Soft tissue composition	Urea zero time	Urea 12 (kg)	Urea 12/BW	Urea 12/EBW
Water (kg)	-0.601**	0.927**	0.946**	0.861**
Protein (kg)	-0.577**	0.921**	0.942**	0.896**
Fat (kg)	-0.594**	0.948**	0.960**	0.920**
Ash (kg)	-0.596**	0.936**	0.954**	0.868**
Water (%)	-0.469**	0.689**	0.696**	0.553**
Protein (%)	0.512**	-0.533**	-0.539**	-0.364*
Fat (%)	0.342*	-0.515**	-0.535**	-0.373*
Ash (%)	-0.561**	0.968**	0.966**	0.897**

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed)

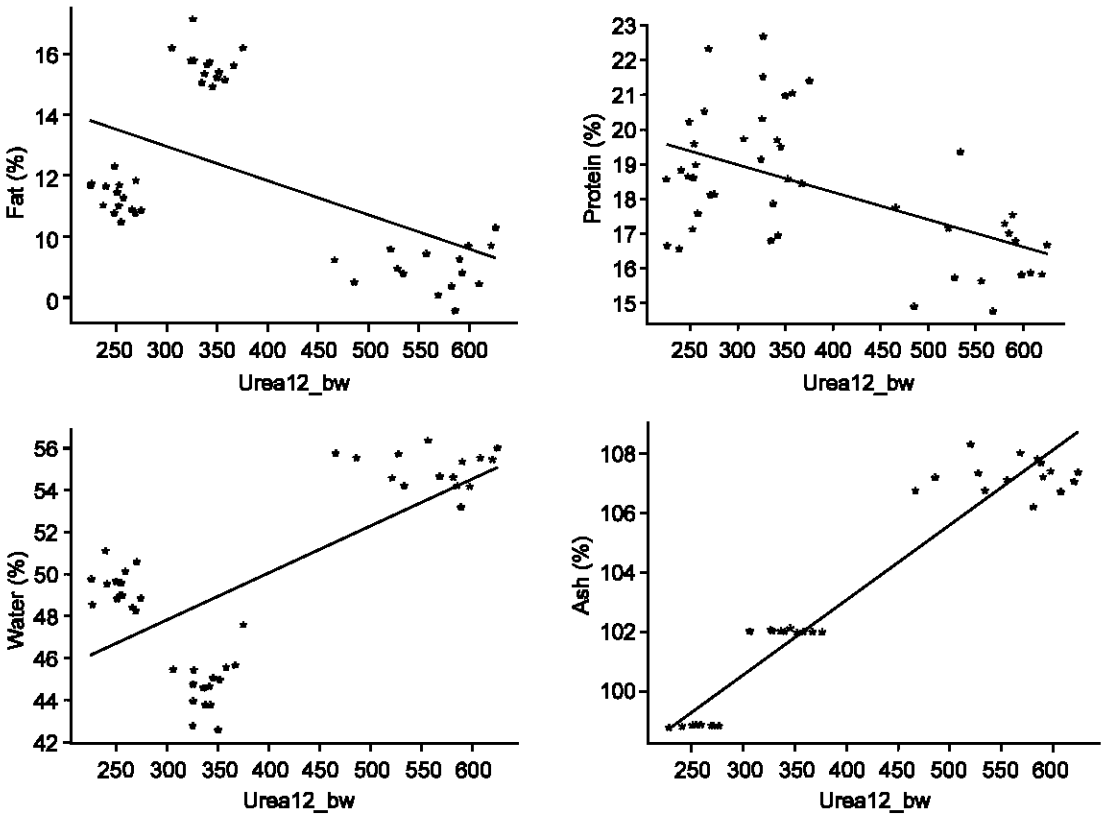

Fig. 1: Relationship between soft tissue component (%) and urea space at 12 min/body weight

Table 4: Correlation coefficients between urea concentration at zero time and urea space after 12 min of infusion (kg) with soft tissue composition amounts and percentages of different species

Species	Soft tissue composition	Urea zero time	Urea 12 (kg)	Urea 12/BW	Urea 12/EBW
Sheep	Water (kg)	-0.005	0.961**	0.910**	0.902**
	Protein (kg)	0.258	0.473	0.488	0.446
	Fat (kg)	-0.087	0.730**	0.729**	0.723**
	Ash (kg)	-0.022	0.895**	0.900**	0.867**
	Water (%)	0.039	0.406	0.411	0.400
	Protein (%)	0.325	-0.013	-0.012	-0.013
	Fat (%)	-0.105	-0.308	-0.307	-0.310
	Ash (%)	0.121	-0.211	-0.201	-0.209
Goats	Water (kg)	0.483	0.926**	0.922**	0.930**
	Protein (kg)	0.551*	0.769**	0.739**	0.770**
	Fat (kg)	0.342	0.655**	0.665**	0.656**
	Ash (kg)	0.470	0.964**	0.960**	0.961**
	Water (%)	0.072	-0.202	-0.200	-0.200
	Protein (%)	0.463	0.429	0.430	0.425
	Fat (%)	-0.159	-0.370	-0.375	-0.373
	Ash (%)	0.209	0.498	0.490	0.491
Cattle	Water (kg)	-0.357	-0.209	0.056	0.234
	Protein (kg)	-0.187	-0.232	-0.026	0.471
	Fat (kg)	-0.199	0.058	0.153	0.264
	Ash (kg)	-0.233	-0.168	0.079	0.151
	Water (%)	0.026	0.708*	0.577*	0.815**
	Protein (%)	0.031	-0.138	-0.127	0.545*
	Fat (%)	0.055	0.345	0.138	0.108
	Ash (%)	0.549*	0.481	0.012	-0.644**

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed)

It can be concluded that urea space at 12 min is feasible to calculate and is effectively related to soft tissue composition of live animals.

REFERENCES

Agnew, R.E., T. Yan, W.J. McCaughey, J.D. McEvoy, D.C. Patterson, M.G. Porter and R.W.J. Steen, 2005. Relationships between urea dilution measurements and body Weight and composition of lactating dairy cows. J. Dairy Sci., 88: 2478-2486.

Al-Saigh, M.N.R. and A.F.H. Al-Jassim, 1998. Some aspect of postnatal growth of Arabi sheep. 1. Carcass muscle, bone, fat and wholesale joints. In. J. Anim. Sci., 68: 965-968.

AOAC, 1990. Official Methods of Analysis (15th Edn.). Association of Official Agricultural Chemists, Arlington, VA.

Bartle, S.J., J.R. Males and R.L. Preston, 1983. Evaluation of urea dilution as an estimator of body composition in mature cows. J. Anim. Sci., 56: 410.

Bartle, S.J., S.W. Kock, R.L. Preston, T.L. Wheeler and G.W. Davis, 1987. Validation of urea dilution to estimate *in vivo* body composition in cattle. J. Anim. Sci., 64: 1024.

Kock, S.W. and R.L. Preston, 1979. Estimation of bovine carcass composition by the urea dilution technique. J. Anim. Sci., 48: 319.

Mohammed, S.T., 1988. Carcass characteristics of native goat slaughtered at different weights. M.Sc. Thesis, College of Agriculture, University of Basra. (In Arabic).

Mora, O., H. Vera-Avila and A. Shimada, 2007. Mecanismos celulares y endocrinos afectados por la subnutrición en pequeños rumiantes. Ciencia Veterinaria, 10: 107-135.

Ngwa, A.T., L.J. Dawson, R. Puchala, G. Detweiler, R.C. Merckel, I. Tovar-Luna, T. Saúl, C.L. Ferrel and A.L. Goetsch, 2007. Urea space and body condition score to predict body composition of meat goats. Small Rumin. Res., 73: 13-26.

Preston, R.L. and S.W. Kock, 1973. *In vivo* prediction of body composition in cattle from urea space measurement. Proc. Soc. Exp. Biol. Med., 143: 1057.

SPSS, 1999. SPSS User's Guide: Statistics. (Version 10 Ed.). SPSS Inc. USA.

Swartz, L.A., A.J. Heinrichs, G.A. Varga and L.D. Muller, 1991. Effects of varying dietary undegradable protein on dry matter intake, growth and carcass composition of Holstein calves. J. Dairy Sci., 74: 3884.

Tahir, M.A., S.K. Al-Amin, A.M. Al-Maraashi and S.S. Hanna, 1986. A comparison between carcass characteristics of yearling rams lambs and old rams and ewes of Arabi sheep. World Rev. Anim. Prod., 22: 91-95.

Tahir, M.A., S.K. Al-Amin and S.S. Hanna, 1987. Carcass characteristics of Janoubi, Friesian and Friesian x Janoubi bulls slaughtered at 18 months. World Rev. Anim. Prod., 23: 51-54.

Tahir, M.A., S.S. Hanna, S.K. Al-Amin and I.T. Kadim, 1992. The effect of slaughter age on carcass traits and chemical composition of buffalo meat. Arab gulf J. Scient. Res., 10: 133-139.

Velazco, J., J.L. Morrill, D.H. Kropf, R.T. Brandt Jr., D.L. Harmon, R.L. Preston and R. Clarenburg, 1997. The use of urea dilution for estimation of carcass composition of Holstein steers at 3, 6, 9 and 12 months of age. J. Anim. Sci., 75: 139-147.

Villaquiran, M., T.A. Gipson, R.C. Merkel, A.L. Goetsch and T. Sahlu, 2005. Body condition score for improved management. In: Proceedings of the Annual Goat Field Day, Langston University, Langston, OK, USA, pp: 111-117.

Wells, R.S. and R.L. Preston, 1998. Effects of repeated urea dilution measurement on feedlot performance and consistency of estimated body composition in steers of different breed types. J. Anim. Sci., 76: 2799-2804.

Wuliji, T., A. Goetsch, R. Puchala, T. Sahlu, R. Merkel, G. Detweiler, S. Soto-Navarro, J. Luo and T. Shenkoru, 2003. Relationships between body composition and shrunk body weight and urea space in growing goats. J. Applied Anim. Res., 23: 1-24.

Pakistan Journal of Nutrition 9 (11): 1113-1117, 2010
ISSN 1680-5194

Chemical and Nutritional Properties of Some Maize (*Zea mays* L.) Varieties Grown in NWFP, Pakistan

Ikram Ullah[1], Muhammad Ali[2] and Arifa Farooqi[1]
[1]Department of Biotechnology, University of Malakand, NWFP, Pakistan
[2]National Center of Excellence in Molecular Biology (CEMB), University of the Punjab, Lahore, Pakistan

Abstract: Grains of ten maize varieties grown in NWFP, Pakistan were obtained and investigated for physicochemical characteristics, proximate composition, energy content and mineral composition. Mean electrical conductivity and thousand seed mass were determined in the range of 9.26-33.83 µS and 319-230.4 g, respectively. Proximate composition shows moisture content in the range of 9.201-10.908%, ash (0.7-1.3%), fats (3.21-7.71%), protein (7.71-14.60%), crude fiber (0.80-2.32%) and carbohydrates (69.659-74.549%). The data indicate that seeds of these varieties vary greatly in term of protein, fats and crude fiber contents. Pahari, Jalal 2003, WD- 2*8 and Azam varieties were determined to contain high protein content (>10% protein) while WD-2*8, Pop. 2004B, PSEV 3-2 and Sarhad (W) contain high fats content of >6%. The energy value of the grains of these varieties was determined in the range of 307.047-394.066 kcal/100 g which shows that the grains of these varieties are rich source of energy. In minerals the level of sodium is 540.30-620.41ppm, K (2915-3471 ppm), Ca (410-590 ppm), Fe (38.02-56.14 ppm), Zn (37.05-52.4 ppm), Mg (985.2-1125.3 ppm) and Cu (11.02-14.25 ppm).

Key words: Proximate composition, minerals, maize varieties, energy value, maize

INTRODUCTION

In Pakistan, maize is the third most important cereal crop after wheat and rice and is used as a staple food for humans, as feed for livestock and as raw material for industry. During 2006, it was planted on 1030 thousand hectares in Pakistan, with total production of 3560 thousand tons and having an average yield of 3.458 tons ha-1 (Ministry of Food, Agriculture and Livestock, 2006; Khan *et al*., 2009). Maize accounts for 4.8% of the total cropped area and 3.5% of the value of agricultural output of Pakistan. It is planted on an estimated area of 0.9 million hectare with an annual production of 1.3 million tones. The bulk (97%) of the total production come from two major provinces (NWFP and Punjab), NWFP, accounting for 57% of the total area and 68% of total production (PARC, 2007).

World collections of maize comprise about 12,000 accessions that are represented in 256 races, of which about 30 are in the process of extermination. Genetic erosion and habitat destruction by modern agriculture has increased the importance of germplasm characterization of plant materials (Carvalho *et al*., 2004). Maize is a multipurpose crop, providing food and fuel for human beings, feed for animals, poultry and livestock. Its grains have great nutritional value and are used as raw material for manufacturing many industrial products (Afzal *et al*., 2009). Its grains are important for the production of oil, starch and glucose (Krishnamurthi, 1969; Niaz and Dawar, 2009). Moreover, Food composition data is important in nutritional planning and provides data for epidemiological studies (Bruce and Bergstrom, 1983; Ali *et al*., 2008). However, there is limited information about the nutritional composition of the different maize varieties growing in Pakistan. The present study aims to investigate physicochemical characteristics, proximate composition and mineral composition of the different maize varieties grown in NWFP province of Pakistan.

MATERIALS AND METHODS

Plant material and physicochemical characteristics: Seeds of 10 maize varieties grown at the Cereal Crop Research Institute (CCRI), NWFP Pakistan were collected and studied for their morphological characters, chemical and nutritional properties. This collection includes the dent and flat kernel types with yellow and white endosperm. Seeds were analyzed for their kernel color and kernel texture as described by Carvalho *et al*. (2004).

Electrical conductivity: Then electrical conductivity of the whole imbibed seeds was determined on eight replications of 10 seeds for each variety, placed in conical flasks with 75 ml deionised water at equal interval of 10, 20, 30, 40, 50, 60, 70 and 80 min. Total of eight reading were taken for each variety. Electrical conductivity was determined by the following formula:

Electrical Conductivity of Seeds = Conductivity of Seeds + D.W - Conductivity of D.W

Corresponding Author: Muhammad Ali, National Center of Excellence in Molecular Biology (CEMB), University of the Punjab, Lahore, Pakistan

Thousand Seeds Mass (TSM): Thousand seeds weight (TSM) is very important parameter to study the net productivity of *Zea mays*. Hundred seeds were counted and their average mass was measured with help of Shimadzu electronic balance. The method is reported in work of Zeb *et al.* (2006).

Proximate composition: Fats contents were determined by using AOAC (1990) 22.034 and protein content AOAC, PN-75/A-04018. Crude fiber was determined by treating oil-free sample by sulphuric acid (0.26 N) and potassium hydroxide (0.23 N) solution in refluxing systems, followed by oven drying and muffle furnace incineration (AOAC, 1984).

For the determination of ash content 3 g of grinded *Zea mays* seeds were taken in desiccated china dishes. The samples were then charred and ashed by using muffle-furnace in two shocks first at 550°C for 30 min and then at 850°C for 30 min. The dishes were removed and when cool to room temperature each dish was reweighed containing white appearing ash. By difference the weight of ash was calculated.

$$\text{Ash}\,(\%) = \frac{(w2 - w)\,x\,100}{w1 - w}$$

Where

W2 = Weight in gm of the dish with the ash
W = Weight in gm of empty dish
W1 = Weight in gm of the dish with the dried material taken for test

Moisture Contents were determined according to the method used by Khan and Kulachi (2002). For this purpose 10 seeds were taken and their fresh weight was determined. Then they were placed in oven at 72°C for 48 h and were again weighted. The moisture content was determined according to the formula:

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh seeds-weight of dry seeds x 100}}{\text{Weight of fresh}}$$

The digestible carbohydrates were calculated by difference.

Total energy values were calculated by multiplying the amounts of protein and carbohydrate by the factor of 4 kcal/g and lipid by the factor of 9 kcal/g as described by Bazi Yabani *et al.* (2009).

Mineral composition: The mineral composition (Na+ and K+) was determined with the help of flame photometer (Jenway PFP7) by the method describe recently (Khan and Zeb, 2007). Heavy metals like Ca, Fe, Mg, Zn and Cu were determined with help of Atomic Adsorption Spectrometer (Perkin Elmer, model Analyst 700) with air/acetylene flame at 2200-2400K (photo multiplier tube detector), against the standard (Hanlon, 1992).

All the parameters were determined at least in triplicate and the results were presented in mean±standard deviation (SD) by using SPSS version 12.0 software package.

RESULTS AND DISCUSSION

Electrical conductivity and 1000 Seeds Mass (TSM): The physicochemical composition investigated maize varieties are shown in Table 1. Maize varieties used in the present study shows significant differences for Electrical Conductivity (EC). The lowest mean EC of Seeds of 9.26 µS was determined for the PSEV 3-2 variety give while the highest (33.83 µS) were obtained for Pahari. The variety WD-2*8 have mean EC of 11.14 µS. The EC of the Jalal 2003 was 13.86 µS, Pop.2006 (13.3 µS) and Pop. 2004B (13.1 µS), Sarhad-W (16.31 µS) and Azam (17.75 µS), WD-3*6 (23.2 µS) and Gh-3*Tam1 (27.38 µS). Munamava *et al.* (2004) also found the mean EC in the same range for different maize varieties.

1000 seeds mass was obtained in the range of 319 g (Jalal 2003 variety) to 230.4 g (WD-2*8). This notion is in agreement with the results obtained by (Kipkech and Kipserem, 2001; Jayan and Kumar, 2004) for different maize varieties.

Proximate composition: Proximate composition and calculated energy values for the maize varieties are shown in (Table 2).

Seed moisture content: Data regarding moisture contents of different maize varieties is given in Table 2. The highest value of moisture content was found for Sarhad (W) (10.908%) and the lowest was found for WD-2*8 varieties (9.201%). Samir *et al.* (1998) measured the moisture content in the range of 9-19%, which is in close consistency with our results. Aisha and El-Tinay (2004) found the moisture value in 12 corn genotypes in the range of 4.3-6.7% which is also in close agreement with our results. Dorsey-Redding *et al.* (1990) found out the moisture content for 10 different hybrids maize varieties in the ranges of 8.43-22.77%. This notion is not in agreement with the results of study.

% Ash value: Ash is defined as the quantity of mineral matter which, after application of the described working methods, remains as incombustible residue of the tested substance. Percent ash content of different maize varieties were found in the range of 0.7% (Jalal, 2003) to 1.3% (WD-3*6) the highest. Peplinski *et al.* (1989) reported values of ash between 1.3 and 1.5%. This notion is in agreement with the results of the present study. Maziya-Dixon *et al.* (2000) found results in the range of 1.4-3.3%, which are higher than the values determined in the present study. Aisha and El-Tinay (2004) investigated the ash value in the range of 1.0-2.0% which is in close consistency with present results of our study.

Table 1: Physicochemical properties of the maize varieties

S/N	Varieties	Variety Type	Kernel colour	Kernel texture	EC Value (µS)	TSW (g)
1	Jalal 2003	Composite	White	Flint grain	13.86	319.0
2	Azam	Composite	White	Flint grain	17.75	278.8
3	WD-3*6	Candidate	White	Flint grain	23.2	272.5
4	PSEV 3-2	Candidate	Yellow	Dented	9.26	307.4
5	Gh-3*Tam1	Candidate	Yellow	Dented	27.38	279.0
6	Pahari	Composite	Yellow	Flint grain	33.83	286.3
7	WD- 2*8	Candidate	Yellow	Flinted	11.14	230.4
8	Sarhad (W)	Composite	Yellow	Dent grain	16.31	252.1
9	Pop. 2006	Composite	Yellow	Dented	13.3	247.0
10	Pop. 2004 B	Candidate	White	Dented	13.1	233.1

Table 2: Proximate composition and calculated energy values of the maize varieties grown in Pakistan

S/N	Varieties	Moisture content (%)	% Ash Value (g)	% Fats	Protein (%)	% Crude Fiber	Carbohydrates (%)	Energy (kcal/100 g)
1	Jalal 2003	9.843±0.1	0.70±0.05	5.30±0.15	12.58±0.05	1.04±0.01	70.615±2.5	380.488±5.01
2	Azam	9.684±0.2	0.93±0.051	4.62±0.24	11.14±0.07	1.00±0.01	72.626±2.3	376.644±4.01
3	WD-3*6	10.311±0.15	1.3±0.38	4.32±0.14	8.72±0.024	0.80±0.02	74.549±3.07	371.95±3.18
4	PSEV 3-2	9.954±0.12	1.06±0.40	6.75±0.31	8.49±0.22	2.11±0.02	71.636±3.17	381.254±4.25
5	Gh-3*Tam1	10.501±0.22	0.91±0.09	5.73±0.5	7.71±0.32	2.30±0.01	72.849±5.10	373.806±3.27
6	Pahari	10.164±0.09	0.93±0.071	3.21±0.34	14.60±0.21	2.9±0.01	70.196±3.70	307.047±2.91
7	WD- 2*8	9.201±0.081	1.03±0.51	7.71±0.12	11.51±0.05	0.89±0.00	69.659±4.15	394.066±5.41
8	Sarhad (W)	10.908±0.12	1.16±0.23	6.30±0.47	8.50±0.12	1.15±0.04	71.982±5.10	378.592±5.04
9	Pop. 2006	10.607±0.21	1.20±0.35	5.20±0.25	9.31±0.07	1.27±0.14	72.36±4.37	373.692±4.71
10	Pop. 2004 B	10.853±0.13	0.86±0.12	7.14±0.47	8.67±0.04	2.32±0.03	70.157±5.09	379.568±3.95

*Mean±standard deviation

The grains with high ash contained a greater proportion of non-endosperm material. Ash Values are determined in order to indicate the level to which non-endosperm components are present (Evers, 2001).

% Fats: Percent Fats were determined in the range of 3.21% (Pahari variety) to 7.71% (WD-2*8 variety). Ijabadeniyi and Adebolu (2005) determined the %fat content of three maize varieties grown in Nigeria in the range of 4.77-5.00% for the maize grains, which is in an agreement with the present study. The results of the present study show that WD-2*8, Pop. 2004B, PSEV 3-2 and Sarhad (W) contain high fats content of >6%.

% Protein: The second largest chemical component of the kernel is protein. Percent protein content was found in the range of 7.71-14.60%. Ijabadeniyi and Adebolu, (2005) found the % protein content of three maize varieties grown in Nigeria in the range of 10.67-11.27% for the maize grains. This notion is in agreement with the results of the present study.

Maize kernels contain albumins, globulins, prolamins, and glutelins. However, two types of storage proteins predominate in the seed: the embryo contains globulin and the endosperm, the major site of storage protein accumulation, contains predominantly prolamins, the so-called zein fraction (Woo *et al*., 2001). These proteins are used as genetic markers for the identification of a variety, for the determination of some important characteristics and traits and for the determination of genetic constitution of the variety. Sampling and assays have been successfully used to study the geographic variation of seed storage protein (Bogyo *et al*., 1980; Erskine and Muehlbauer, 1991). The present study shows that Pahari, Jalal 2003, WD- 2*8 and Azam varieties contain high protein content of 14.60%, 12.58%, 8.72% and 11.51% (>10% protein).

% Crude fiber: Crude fiber was found fourth largest chemical present in the maize grains after carbohydrates, protein, fats and moisture content. Percent crude fiber was found in the range of 0.80-2.32%. Ijabadeniyi and Adebolu (2005) reported slightly higher values (2.07-2.77%) of the fiber content for the maize varieties grown in Nigeria.

% Carbohydrates: Carbohydrates are the major chemical component of the maize grains. It was found in the range of 69.659 (WD-2*8 variety)-74.549% (WD-3*6 variety). Ijabadeniyi and Adebolu (2005) reported slightly lower values (65.63-70.23%) of the carbohydrate content for the maize varieties grown in Nigeria.

Energy value (kcal/100 g): Calculated energy values of maize varieties grown in Pakistan varied from 307.047 kcal/100g (Pahari variety) to 394.066 kcal/100g (WD-2*8 variety) in dry matter basis (Table 2). Kouakou *et al.* (2008) showed the energy level of maize grains as 387.7 kcal/100 g. This notion is in agreement with the results of the present study. In another study Ejigue *et al.* (2005) found the energy value of 447kcal/100 g for yellow maize, which is higher than the values determined in this study. The difference in the energy level is due to differences in the proximate composition of the varieties. The results of the present study show that these maize varieties are rich source of energy.

Table 3: Mineral composition of the maize grains

S/N	Varieties	Na	K	Ca	Fe	Zn	Mg	Cu
1	Jalal 2003	550.52±21	3210.3±20	430±15	42.3±2.1	39.2±2.5	985.2±11.2	14.25±0.5
2	Azam	590.05±35	3458.7±15	410±25	42.5±1.5	45.2±2.3	1625±10.5	14.03±0.6
3	WD-3*6	540.30±32	2980±12	564±10	43.15±1.04	37.05±1.5	1125±11.5	14.12±0.5
4	PSEV 3-2	620.41±10.5	2915±4.1	390±10	56.14±0.05	52.4±0.08	986.2±9.3	11.02±0.2
5	Gh-3*Tam1	550.45±5.3	3471±5	425±15	45.5±0.84	38.6±4.1	1150±5.5	14.01±1.3
6	Pahari	587.02±15	3211±25	590±14	39.2±1.25	45.06±8.7	1125.3±1.15	13.25±0.5
7	WD- 2*8	596.65±12	3245±14	520±12	38.02±1.02	48.2±5.3	1065±12.3	12.48±0.14
8	Sarhad (W)	560.61±10.2	3105±11	540±5.2	45.5±2.3	52.15±2.40	1024±2.5	12.62±0.58
9	Pop. 2006	559.22±8.2	3314±9.2	525±6.5	45.3±0.87	50.04±2.3	1087±3.7	13.08±0.30
10	Pop. 2004 B	573.80±7.9	2948±3.2	510±23	50.06±0.75	49.87±1.04	1125±5.4	12.05±1.20

*Mean±standard deviation

Mineral composition: The result of the mineral composition grains of different maize varieties is shown in Table 3. The analysis shows the level of Na (540.30-620.41 ppm), K (2915-3471 ppm), Ca (410-590 ppm), Fe (38.02-56.14 ppm), Zn (37.05-52.4 ppm), Mg (985.2-1125.3 ppm) and Cu (11.02-14.25 ppm). Hassan *et al.* (2009) determined the mineral content of two maize varieties grown in Sudan and showed the level of Na in the range of 15-18 ppm, K (93-108 ppm), Ca (216-162 ppm), Fe (18 ppm) and Zn (5 ppm). These results are not in agreement with the results of the present study. Hussaini *et al.* (2008), determined mineral content of K in the range of 3400-3600 ppm, Ca (350-360) and Mg (1060-1120 ppm). This notion is in agreement with the results of the present study. Feil *et al.* (2005) showed the concentration of K in the range of 3930-3710 ppm, Mg (1120-1130 ppm), Ca (82-137 ppm), Zn (23.1-25 ppm) and Cu (2.21-2.36 ppm). The concentration of K and Mg are in agreement with the results of the present study while, that of Ca, Zn and Cu are less than it. Hussaini *et al.* (2008) showed that nitrogen fertilizer application up to 60 kg N ha^{-1} significantly increased the concentrations of N, P, Ca and Mg in maize grain. Hence, the differences in the mineral composition may be due to genetic factors or environmental factors like irrigation frequency, soil composition and fertilizer used.

Conclusion: The data indicate that seeds of these varieties vary greatly in term of protein, fats and crude fiber contents. The variability observed in carbohydrates, protein, fats, ash content, crude fiber and moisture content is both genetic and environmental which may influence the individual chemical composition and weight distribution of the endosperm and hull of the kernels. Moreover, Pahari, Jalal 2003, WD- 2*8 and Azam varieties were determined to contain high protein content (>10% protein). The varieties WD-2*8, Pop. 2004B, PSEV 3-2 and Sarhad (W) contain high (>6%) fats content of 7.71, 7.14, 6.75 and 6.30, respectively. These results will be useful to know about the nutritional properties of the local maize varieties and may guide us in designing strategies that maximize the utility of maize germplasm.

ACKNOWLEDGEMENTS

We are deeply grateful to Mr. Alam Zeb, Dr. Ayaz Ali khan, (Lecturer, Department of Biotechnology, University of Malakand (UOM)) and Dr. Muhammad Ali khan (Chairman, Department of Biotechnology, UOM) for the critical evaluation of this manuscript. Authors are also thankful to Mr. Hazrat Ali and Mr. Waqar Ali for their help during this study.

REFERENCES

A.O.A.C., 1984. Official methods of the analysis of the ASSOC. of office. Analy, Chem. 14th Edn., published y the A.O.A.C, Inc. North 19th street, suite 210, Arlington, Virginia 22209, USA.

Afzal, M., Z. Nazir, M.H. Bashir and B.S. Khan, 2009. Analysis of host plant resistance in some genotypes of maize against Chilo partellus (swinhoe) (pyralidae: lepidoptera). Pak. J. Bot., 41: 421-428.

Aisha, S.M.F. and A.H. El-Tinay, 2004. Effect of genotype, malt pretreatment and cooking on *in vitro* protein digestibility and protein fractions of corn. Food Chem., 84: 613-619.

Ali, M., W. Ali, S. Ahmed and I. Ullah, 2008. Mineral Composition, Quality and Physico-chemical Parameters of the Local Tallow of Pakistan. Pak. J. Nut., 7: 717-720.

AOAC, 1990. American Official Chemist Methods, Washington, USA.

Bazi Yabani, Yenilenebilir Mantarlarin and Besinsel Içerigi, 2009. Nutritional Composition of Some Wild Edible Mushrooms. Turk J. Biochem., 34: 25-31.

Bogyo, T.P., E. Porceddu and P. Perrino, 1980. Analyses of sampling strategies for collecting genetic material. Economic Botany, 34: 160-174.

Bruce, A. and L. Bergstrom, 1983. User requirement for food data bases and applications in nutritional research. Food Nutr. Bull., 5: 24-29.

Carvalho, V.P., C.F. Ruas, J.M. Ferreira, R.M.P. Moreira and P.M. Ruas, 2004. Genetic diversity among maize (*Zea mays* L.) landraces assessed by RAPD markers. Genet. Mol. Biol., 27: 228-236.

Dorsey-Redding, C., C.R. Hurburgh, L.A. Johnson and S.R. Fox, 1990. Adjustment of Maize Quality Data for Moisture Content. Cereal Chem., 67: 292-295.

Ejigue, J., L. Savoie, J. Marin and T. Desrosier, 2005. Benificial changes and drawbacks of traditional fermentation process on chemical composition and antinutritional factors of yellow maize (Zea mays). J. Biol. Sci., 5: 590-596.

Erskine, W. and F.J. Muehlbauer, 1991. Allozyme and morphological variability.

Evers, A.D., 2001. Ash determination-a useful standard or a flash in the pan. <http://www.satake.co.uk/>.

Feil, B., S.B. Moser, S. Jampatong and P. Stamp, 2005. Mineral Composition of the Grains of Tropical Maize Varieties as Affected by Pre-Anthesis Drought and Rate of Nitrogen Fertilization. Crop Sci., 45: 516-523.

Hanlon, E.A., 1992. Determination of potassium, calcium and magnesium in plants by Atomic Absorption Techniques. In: Plant analysis reference procedures for the southern region of the United States. Southern Cooperative Series Bulletin #368, C.O. Plank (Ed). Athens, pp: 30-33.

Hassan, A.B., G.A.M. Osman and M.A.H. Rushdi, 2009. Effect of Gamma Irradiation on the Nutritional Quality of Maize Cultivars (Zea mays) and Sorghum (Sorghum bicolor) Grains. Pak. J. Nutr., 8: 167-171.

Hussaini, M.A., V.B. Ogunlela, A.A. Ramalan and A.M. Falaki, 2008. Mineral Composition of Dry Season Maize (*Zea mays* L.) in Response to Varying Levels of Nitrogen, Phosphorus and Irrigation at Kadawa, Nigeria. World J. Agri. Sci., 4: 775-780.

Ijabadeniyi, A.O. and T.T. Adebolu, 2005. The effect of processing methods on the nutritional properties of ogi produced from three maize varieties. J. Food, Agric. and Environ., 3: 108-109.

Jayan, P.R. and V.J.F. Kumar, 2004. Planter design in relation to the physical properties of seeds. J. Trop. Agri. 42: 69-71.

Khan, A., M.T. Jan, K.B. Marwat and M. Arif, 2009. Organic and inorganic nitrogen treatments effects on plant and yield attributes of maize in a different tillage systems. Pak. J. Bot., 41: 99-108.

Khan, I. and A. Zeb, 2007. Nutritional composition of Pakistani wheat varieties. J. Zhejiang Univ. Sci. B., 8: 555-559.

Khan, S.M. and I.R. Kulachi, 2002. Assessment of post harvest wheat losses in D.I. Khan. Asian J. Plant Sci., 1: 103-106.

Kipkech, F.C. and L.K. Kipserem, 2001. Use of tied riding and soil fertilization to improve maize yield in Rft valley, Kenya. 7th Eastern and South Africa regional maize conference, pp: 292-294.

Kouakou, B., G. Albarin1, O.A. Louise, D.N. Theodore, K. Youssouf and G. Dago, 2008. Assessment of Some Chemical and Nutritional Properties of Maize, Rice and Millet Grains and Their Weaning Mushes. Pak. J. Nutr., 7: 721-725.

Krishnamurthi, A., 1969. The wealth of India. A dictionary of Indian., raw materials Vol. VIII. Publication Information Directorate CSIR. New Dehli, pp: 349.

Maziya-Dixon, B.B., J.G. Kling and A.E. Okoruwa, 2000. Physical, Chemical and Water Absorption Characteristics of Tropical Maize Hybrids. Afr. Crop Sci. J., 8: 419-428.

Ministry of Food, Agriculture and Livestock, Islamabad, Pakistan, 2006. Agricultural Statistics of Pakistan 2005-2006. Ministry of Food, Agriculture and Livestock, Islamabad, Pakistan.

Munamava, M.R., A.S. Goggi and L. Pollak, 2004. Seed Quality of Maize Inbred Lines with Different Composition and Genetic Backgrounds. Crop Sci., 44: 542-548.

Niaz, I. and S. Dawar, 2009. Detection of seed borne mycoflora in maize (Zea mays L.). Pak. J. Bot., 41: 443-451.

PARC, 2007. Status paper on maize, sorghum and millet. www.parc.gov.pk/1SubDivisions/NARCCSI/CSI/StatusPaper.doc <http://www.parc.gov.pk/1SubDivisions/NARCCSI/CSI/StatusPaper.doc>.

Peplinski, A.J., M.R. Paulsen, R.A. Anderson and W.F. Kwolek, 1989. Physical, chemical and dry-milling characteristics of corn hybrids from various genotypes. J. Cereal Chem., 66: 117-120.

Samir, T., A.W. Kraszewski and S.O. Nelson, 1998. Nondestructive microwave characterization for determining the bulk density and moisture content of shelled corn. Sci. Technol., 9: 1548-1556.

Woo, Y., D. Hu, B.A. Larkins and R. Jung, 2001. Genomics Analysis of Genes Expressed in Maize Endosperm Identifies Novel Seed Proteins and Clarifies Patterns of Zein Gene Expression. Plant Cell, 13: 2297-2318.

Zeb, A., Z. Ali, T. Ahmad and A. Abdumanon, 2006. Physicochemical characteristics of wheat varieties growing in the same and different ecological regions of Pakistan. Pak. J. Biol. Sci., 9: 1823-1828.

Pakistan Journal of Nutrition 9 (11): 1118-1121, 2010
ISSN 1680-5194

Seasonal Variation of Metal Concentrations in Catfish, Blue Crab and Crayfish from Warri Coastal Water of Delta State, Nigeria

D.N. Olowoyo[1], O.O. Ajayi[2], I.A. Amoo[2] and A.F. Ayeisanmi[2]
[1]Department of Industrial Safety and Environmental Technology,
Petroleum Training Institute, Effurun-Warri, Delta State, Nigeria
[2]Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria

Abstract: The concentrations of Pb, Ni, Fe and Cd were determined using Atomic Absorption Spectrophotometer-Varian Spectral AA220 in catfish, blue crab and crayfish from Warri coastal water of Delta state. Of all the heavy metals examined, the line charts presented in Fig. 2, 3 and 4 indicate that Cd and Pb have the highest concentration while Fe and Ni have the least concentration in the three fauna considered for the experiment at both dry and wet seasons of 2008. The mean concentration values for catfish are: 0.002-2.52 mg/kg, 0.22-3.50 mg/kg, 0.002-1.48 mg/kg and 0.009-18.13 mg/kg; blue crab: 0.82-3.40 mg/kg, 0.35-2.50 mg/kg, 0.06-1.61 mg/kg and 1.62-12.88 mg/kg and crayfish: 0.22-8.00 mg/kg, 0.01-2.25 mg/kg, 0.004-0.55 mg/kg and 0.038-18.13 mg/kg for heavy metals of Pb, Ni, Fe and Cd respectively. The concentrations of the metals determined varied from one location to another and from one specie to another. Hence, Egbokodo location has the highest Cd content in catfish while Jeddo and Ughoton locations have highest Cd content in crayfish and blue crab respectively. Lastly, the order of increasing in concentration of the metals is Fe<Ni<Pb<Cd.

Key words: Heavy metals, coastal water, fauna, dry and wet seasons, atomic absorption spectrophotometer

INTRODUCTION

Studies on the pollution status of catfish, blue crab and crayfish in some parts of Nigeria and around Niger Delta area have been reported (Chindah and Braide, 2003; Davies *et al*., 2006).

Several factors such as size, nature of the environment, seasonal variation and variability in species have been identified as important independent variable influencing metal levels in marine organisms (Lueng and Furness, 1999). The variation in metal levels within biota (fauna) is part of an indicative of the degree to which particular specie picks up particulate matter from the surrounding water and in particular sediment while feeding.

This type of critical information is good in making accurate risk assessment for seafood safety purposes. Also, the increase in metal content at some locations can be informative sub lethal response indicating increased metal availability and potential mental stress on the biota. Thus the ability to regulate the internal metal levels is impaired. This might be sufficient to cause chronic toxicity or mortality during early development (Ololade *et al*., 2007).

Finally, in the natural aquatic ecosystems, metals occur normally in nanogram and microgram levels. However, some of these metals occurring at low concentrations in surface water are found in high concentrations in the corresponding sediments and fishes in the aquatic environments (Kakulu and Osibanjo, 1986; Asaolu *et al*., 1997) Therefore, this present work is aimed at providing information on the levels of some heavy metal concentration in some biota (fauna) from warri coastal water Delta axis of Niger Delta region of Nigeria.

MATERIALS AND METHODS

Sampling: Three different types of biota namely Catfish, crayfish and blue crab used for the analysis were bought from fishermen fishing along the Warri coastal river in ten (10) different locations. The samples were thoroughly washed with the sea water, placed in labeled cellophane bags and preserved in ice-cooled box. The samples were later transferred into the laboratory and stored in the freezer at -4°C prior to laboratory analysis.

Sample treatment: The soft parts of the crayfish were obtained by removing the shells. The mean soft body weight for each sample was determined after drying at 80°C for 24 h for catfish and crayfish and at 80°C for 48 h for crab based on literature (Leung and Furness, 1999).

Some metals namely Pb, Ni, Fe and Cd were determined with 2.0 g of finely ground tissue samples homogenized with 25 ml of de-ionized water after 10 ml of concentrated HCL and 2 ml of HNO_3 in succession. The mixture was heated and boiled off to near dryness, given a thick yellow liquid. However, for samples of blue crab, additional 25 ml of 95% H_2O_2 was added before digestion was completed. This was probably due to the

Corresponding Author: D.N. Olowoyo, Department of Industrial Safety and Environmental Technology, Petroleum Training Institute, Effurun-Warri, Delta State, Nigeria

physiological nature of the hard shell cover, Metals concentrations were analyzed using flame atomic absorption spectrophotometer-Varian model spectral AA 220. The results obtained from this analysis were the average of duplicate determinations and the analysis was carried out during dry and wet seasons.

RESULTS AND DISCUSSION

Figure 1 presents the map of sample locations while the line charts in Fig. 2, 3 and 4 present heavy metal concentrations in Catfish, Blue crab and Crayfish respectively at various locations along Warri coastal water for both dry and wet seasons.

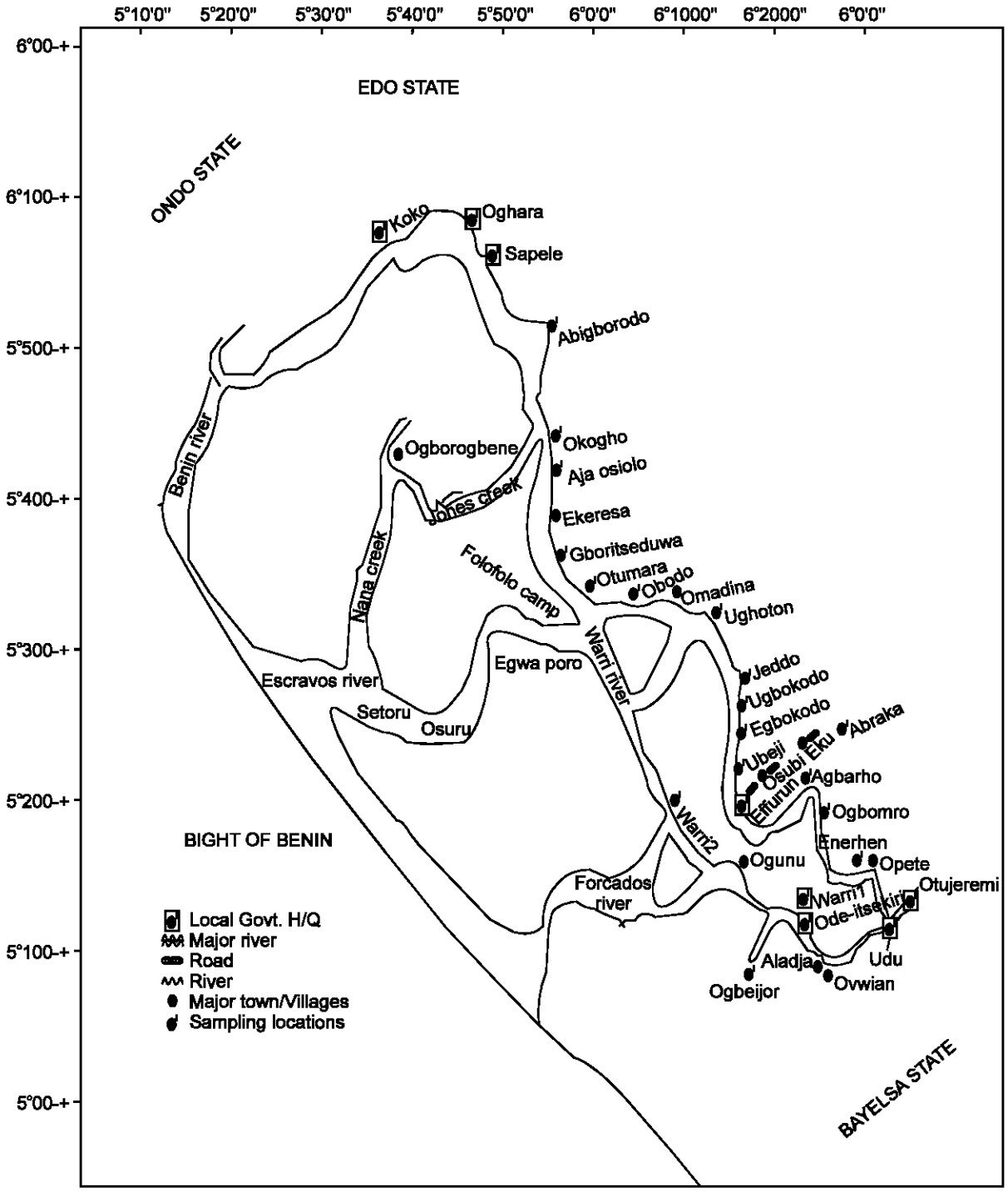

Fig. 1: Map of Delta State showing sampling locations along warri-sapele-koko coastal river route

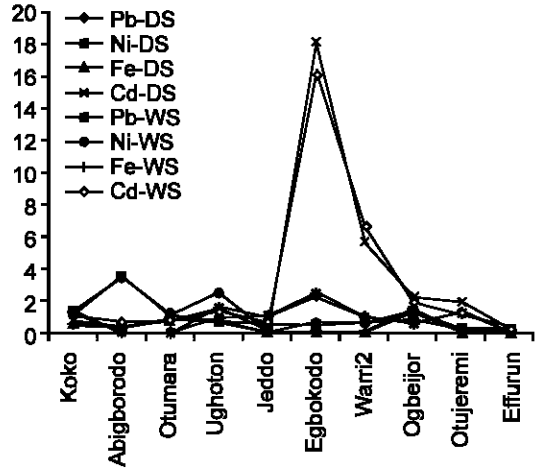

Fig. 2: Heavy metal concentration (mg/kg) in catfish during dry and wet seasons

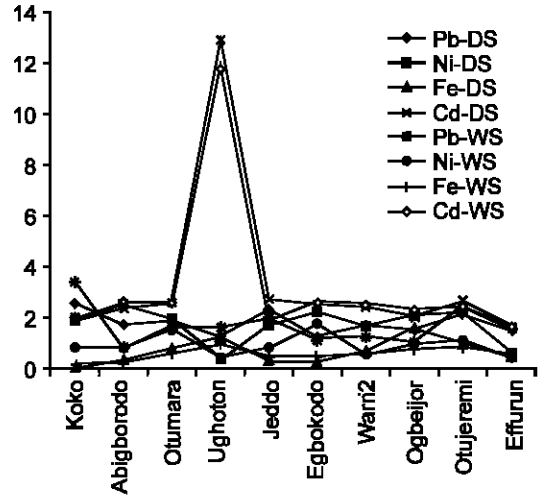

Fig. 3: Heavy metal concentration (mg/kg) in blue crab during dry and wet seasons

The concentrations of heavy metals determined in fauna fluctuated from one location to another location and from one season to another season. In catfish, the heavy metal concentrations ranged between 0.002-2.52 mg/kg, 0.22-3.50 mg/kg, 0.002-1.48 mg/kg and 0.009-18.13 mg/kg for Pb, Ni, Fe and Cd respectively at both dry and wet seasons (Fig. 2), while concentrations of heavy metals in blue crab ranged between 0.82-3.4 mg/kg, 0.35-2.5 mg/kg, 0.06-1.61 mg/kg and 1.62-12.88 mg/kg for the same heavy metals above and in the same order (Fig. 3). The heavy metal concentrations in crayfish also ranged between 0.22-8.00 mg/kg, 0.01-2.25 mg/kg, 0.004-0.55 mg/kg and 0.038-18.13 mg/kg for Pb, Ni, Fe and Cd respectively (Fig. 4). In all heavy metals investigated, Cd has the highest concentration especially in catfish and crayfish while that of blue crab was slightly lower in concentration. This may be due to

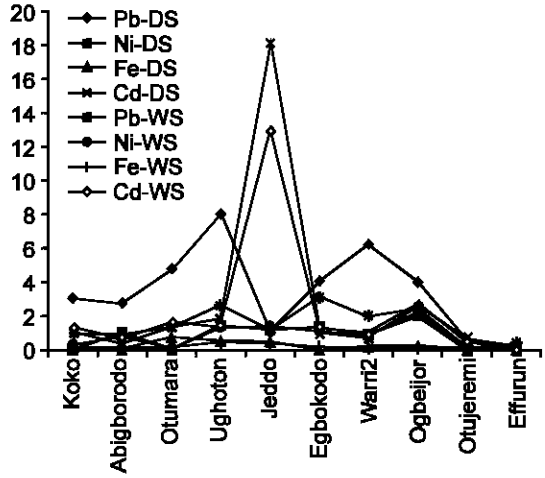

Fig. 4: Heavy metal concentration (mg/kg) in crayfish during dry and wet seasons

logging and milling activities along the coast of Warri river since trace metals such as Cd is known to be found in woody and herbaceous materials which will settle down to be part of sediments (Clarke and Sloss, 1992). Also, the level of bio-concentration of Cd into catfish, blue crab and crayfish could be due to their being bottom feeders (Kakulu *et al*., 1987). However, the concentrations of Ni and Fe are the least in the three fauna determined while crayfish seems to contain higher concentrations of Pb compared to catfish and blue crab. Egbokodo location has the highest concentration of Cd in catfish while Jeddo and Ughoton locations had the highest in crayfish and blue crab respectively.

Conclusion: The high values of Cd and Pb may be due to logging, milling and oil operational activities that are common in the area.

ACKNOWLEDGEMENT

The authors warmly appreciate Tudaka Environmental Consultants, Effurun-Warri, Delta state for allowing us to use their laboratory and equipment.

REFERENCES

Asaolu, S.S., K.O. Ipinmoroti, O. Olaofe and C.O. Adeeyinwo, 1997. Interrelationship of heavy metal concentration in water, sediment and fish samples from Ondo State coastal area, Nigeria. Afr. J. Sci., 1: 55-61.

Chindah, A.C. and S.A. Braide, 2003. Cadmium and lead concentrations in fish species of a brackish wetland/upper Bonny Estuary, Niger Delta. J. Nig. Environ. Soc., 1: 399-405.

Clarke, L.K. and L.L. Sloss, 1992. Trace Element Emissions from coal Combustion and Gasification, IEA coal research, Gemini House, London.

Davies, O.A, W.E. Allison and H.S. Uyi, 2006. Bioaccumulation of heavy metals in water, sediment and periwinkle from the Elechi creek, Niger-Delta, Nig. Afr. J. Biotechnol., 5: 968-973.

Kakulu, S.E. and O. Osibanjo, 1986. Trace metal analysis. Environ. Pollut. Ser., 8: 315-322.

Kakulu, S.E., O. Osibanjo and S.O. Ajayi, 1987. Trace metal content of fish and shellfishes of the Niger delta area of Nig. Environ. Int., 13: 247-251.

Leung, K.M.Y. and R.W. Furness, 1999. Effects of animal size on concentrations of metallothionein and metals in periwinkles (*lithrina lithorea*) collected from fifth of clyde, Scotland, Marine Pollut. Bull., 39: 126-136.

Ololade, I.A., L. Laide and I.A. Amoo, 2007. Accumulation of heavy metals by fish, crab and periwinkles. Sci. Res. Ann., 3: 16-23.

Pakistan Journal of Nutrition 9 (11): 1122-1123, 2010
ISSN 1680-5194

Monograph of *Withania somnifera* (L.) Dunal

M. Akram[1], S.M. Ali Shah[4], E. Mohiuddin[2], Abdul Hannan[3] and Khan Usmanghani[3]
[1]Department of Basic Medical Sciences, [2]Department of Surgery and Allied Sciences
[3]Department of Pre-Clinical Sciences,
Faculty of Eastern Medicine, Hamdard University, Karachi, Pakistan
[4]Department of Conventional Medicine, Islamia University, Bahawalpur, Pakistan

***Withania somnifera* (L.) Dunal**

Tibbi name	:	Asgand
English name	:	Winter Cherry
Botanical name	:	*Withania somnifera* Dunal
Family	:	Solanaceae
Parts used	:	Root, tubers, seeds, leaves, fruit

Fig. 1: *Withania somnifera* fruit

Fig. 2: *Withania somnifera* root

Description: The plant is cultivated as an annual crop and this herb is found growing wild in Pakistan. Asgandh plant is erect, 30-150 cm high with fleshy roots which is whitish brown in color. Leaves are simple and ovate. Flowers look like lucid-yellow or greenish and it contains small berries which is orange-red in color. Fruits are orange-red, containing many seeds. Florescence occurs in fall and spring (Davis and Kuttan, 2000).

Active constituents: Withaferin A and withanolide D (Bhattacharya *et al*., 1997). Anaferine (alkaloid), anahygrine (alkaloid), beta-sisterol, chlorogenic acid (in leaf only), cysteine (in fruit), cuscohygrine (alkaloid), iron, pseudotropine (alkaloid), scopoletin, somniferinine (alkaloid), somniferiene (alkaloid), tropanol (alkaloid), withanine (alkaloid), withananine (alkaloid) and withanolides A-Y(steroidal lactones), somniferine, somnine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropane, cuscohygrine, isopelletierine, anahydrine, sitoindoside VII and sitoindoside VIII (Chaurasia *et al*., 2000). Pseudo-withanine, tropino, choline, cuscohygrine, isolettetierine, anahydrine, 3-alpha-gloyloxy tropane (Arieh and David, 1975).

Medicinal use: Abortifacient; Antiinflammatory, Adaptogen; Antibiotic; Aphrodisiac; Astringent; Deobstruent; Diuretic; Sedative; Tonic. According to Unani system of medicine, the root is bitter, tonic, aphrodisiac (Lumbreras *et al*., 2005), emmenagogue, anti-inflammatory (Kulkarni *et al*., 1991). Studies have shown asgandh to be effective in stimulating the immune system. It also appears to inhibit swelling and aid memory (Choudhary *et al*., 1995). It is especially beneficial in stress related disorders such as arthritis and premature aging (Archana and Namasivayam, 1999). Withanolides possess remarkable antitumour, antiarthritic, anti-inflammatory and immunosupressive properties (Bhattacharya *et al*., 1997). Asgandh is used for treatment of rheumatism, hyperuricemia, and as sex stimulant (Al-Hindawi *et al*., 1989). Asgandh is one of the most widespread tranquillisers used in India and Pakistan, where it holds a position of importance similar to ginseng in China and Korea (Bown, 1995). It is an anti-inflammatory agent (Tyler Varro, 1994). It is used to improve vitality and aid recovery after chronic illness. The

Corresponding Author: Muhammad Akram, Department of Basic Clinical and Medical Sciences, Faculty of Eastern Medicine, Hamdard University, Karachi, Madinat-al-Hikmah, Muhammad Bin Qasim Avenue, Karachi, Pakistan

leaves and the root bark, are deobstruent, diuretic, narcotic, strongly sedative and tonic (Uma and Akagi, 1996). It is also used to treat, debility, insomnia, impotence, infertility, multiple sclerosis etc. The seed is diuretic and hypnotic (Chopra *et al*., 1986).

Pharmacological activity: The effect of *Withania somnifera* root powder on paw volume and serum lysosomal enzyme activities was investigated in monosodium urate crystal-induced rats. The levels of β-glucuronidase and lactate dehydrogenase were also measured in monosodium urate crystal incubated Polymorphonuclear Leucocytes (PMNL). A significant increase in the level of paw volume and serum lysosomal enzymes was observed in monosodium urate crystal-induced rats. The increased β-glucuronidase and lactate dehydrogenase level were observed in untreated monosodium urate crystal incubated polymorphonuclear leucocytes. On treatment with the *Withania somnifera* root powder (500/1000 mg/kg body weight), the above changes were reverted back to near normal levels. *Withania somnifera* also showed potent analgesic and antipyretic effect with the absence of gastric damage at different dose levels in experimental rats. For comparison purpose, Non-Steroidal Anti-Inflammatory Drug (NSAID) indomethacin was used as a standard. These results provide evidence for the suppressive effect of *Withania somnifera* root powder by retarding amplification and propagation of the inflammatory response without causing any gastric damage (American Botanical Council, 1996).

REFERENCES

Al-Hindawi, M.K., I.H. Al-Deen, M.H. Nabi and M.H. Ismail, 1989. Anti-inflammatory activity of some Iraqi plants using intact rats. J. Ethnopharmacol., 26: 163-168.

American Botanical Council (trans.), 1996. The German Commission E Monographs. Austin, TX: American Botanical Council.

Archana, R. and A. Namasivayam, 1999. Anti-stressor effect of Withania somnifera, J. Ethnopharmacol., 64: 91.

Arieh Abraham Isaac Kirson and David Lavie Erwin Glotte, 1975. The withanolides of *Withania somnifera* chemotypes I and II. Phytochemistry, 14: 189-194.

Bhattacharya, S.K., K.S. Satyan and S. Ghosal, 1997. Antioxidant activity of glycowithanolides from *Withania somnifera*. Int. J. Exp. Biol., 35: 236-239.

Bown, D., 1995. Encyclopaedia of Herbs and their Uses. Dorling Kindersley, London, ISBN 0-7513-020-31

Chaurasia, S.S., S. Panda and A. Kar, 2000. *Withania somnifera* root extract in the regulation of lead-induced oxidative damage in the male mouse. Pharmacol. Res., 41: 663-666.

Chopra, R.N., S.L. Nayar and I.C. Chopra, 1986. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi, India.

Choudhary, M.I., Z. Dur-e-Shahwar, A. Parveen, I. Jabbar and Ali Atta-Ur-Rahman, 1995. Antifungal steroidal lactones from Withania coagulance. Phytochemistry, 40: 1243-1246.

Davis, L. and G. Kuttan, 2000. Immunomodulatory activity of *Withania somnifera*. J. Ethnopharmacol., 71: 193-200.

Kulkarni, R.R., P.S. Patki, V.P. Jog, S.G. Gandage and B. Patwardhan, 1991. Treatment of osteoarthritis with a herbomineral formulation: a double-blind, placebo-controlled, cross-over study. J. Ethnopharmacol., 33: 91-95.

Lumbreras, B., E. Pascual and J. Frasquet, 2005. Analysis for crystals in synovial fluid: Training of the analysts results in high consistency. Ann. Rheum Dis., 64: 612-615.

Tyler Varro,1994. Herbs of Choice: The Therapeutic Use of Phytomedicinals. Haworth Press, New York.

Uma, D.P. and K. Akagi, V. Ostapenko, Y. Tanaka and T. Sugihara, 1996. Withaferin-A: A new radiosensitizer from the Indian medicinal plant *Withania somnifera*. Int. J. Radiation Biol., 69: 193-197.