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Adsorption, Metabolism and Degradation of Erythromycin in Giant Freshwater Prawn and Tilapia Aquaculture in Mekong River Delta

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Abstract: Adsorption, metabolism and degradation of erythromycin in freshwater prawn and tilapia aquaculture in Mekong River Delta were monitored and evaluated. They were fed practical diets medicated with erythromycin (50 and 100 mg/kg body weight for 7 days). Erythromycin residues in their muscle were determined by the Liquid Chromatography - Mass Spectrometry/Mass Spectrometry (LC-MS/MS) method. Our study provided preliminary data for a more prudent use of erythromycin in giant freshwater prawn and tilapia, suggesting a possible withdrawal time after treatment as well as clearing away the awareness of forming and accumulating a harmful over-threshold level of derived products from parental drug during veterinary usage in aquaculture.

Key words: Giant freshwater prawn, tilapia, erythromycin, metabolism, degradation, LC-MS/MS

INTRODUCTION

Erythromycin is a macrolide antibiotic that is produced by the actinomycete species, *Streptomyces erythreus*. The chemical structures of Erythromycin A (EA), which is the major component of erythromycin base and its related substances are depicted in Fig. 1. Erythromycin is a polyhydroxylactone that contains two sugars. The aglycone portion of the molecule, erythranolide, is a 14-membered lactone ring. An amino sugar, desosamine, is attached through a β-glycosidic linkage to the C-5 position of the lactone ring. The tertiary amine of desosamine confers a basic character to erythromycin (pK_a 8.8). Through this group, a number of acid salts of the antibiotic have been prepared. A second sugar, cladinose, which is unique to erythromycin, is attached via a β-glycosidic linkage to the C-3 position of the lactone ring.

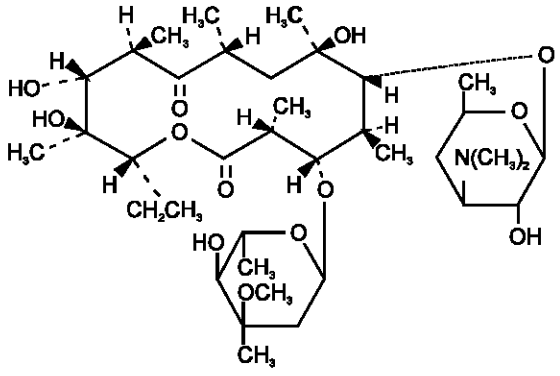

Fig. 1: Chemical structures of erythromycin A

The fermentation process that produces commercial grade erythromycin is not entirely selective. It results in the production of small quantities of Erythromycin B (EB), C(EC), D(ED), E(EE) and F(EF), in addition to EA, which is the major component. EB, EC and EE are the most important impurities found in commercial samples of erythromycin (Table 1).

In addition to the related substances, the metabolite, demethylerythromycin(dMeE) and acidic and basic degradation products are also present in small quantities in commercial samples of erythromycin. These include Erythromycin Enol Ether (EEE), Anhydroerythromycin (AE), Erythrolosamine (ESM), pseudoerythromycin A Hemiketal (psEAHK), pseudoerythromycin A Enol Ether (psEAEE) and Dehydroerythromycin (DE). Other related substances such as erythromycin A N-oxide (EANO), Erythromycin Oxime (EOXM) and erythromycylamine also exist and are structurally very similar, differing by only hydrogen, hydroxyl and/or methoxy groups.

Morover, erythromycin also exists in forms of Erythromycin Stearate (ES), Erythromycin Ethylsuccinate (EESC), Propionyl Erythromycin (PE), Erythromycin Estolate (EES), Erythromycin Lactobionate (EL), Erythromycin Glucoheptonate (EG), Erythromycin Ethyl Carbonate (EEC) and erythromycin acistrate.

Giant freshwater prawn (*Macrobrachium rosenbergii*) and Nile tilapia (*Oreochromis niloticus)* have been considered two of the most important species of freshwater aquaculture in Viet Nam, especially in the Mekong River Delta. Bacterial necrosis is a common disease observed in adult prawns (Winton and Jiam-

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Table 1: Formula of erythromycin A and related substances

Erythromycin	Formula	Molecular mass	R_1	R_2	R_3	R_4	R_5
A	$C_{37}H_{57}NO_{13}$	734	OH	H	H	OCH_3	CH_3
B	$C_{37}H_{57}NO_{12}$	718	H	H	H	OCH_3	CH_3
C	$C_{38}H_{55}NO_{13}$	720	OH	H	H	OH	CH_3
D	$C_{36}H_{65}NO_{12}$	704	H	H	H	OH	CH_3
E	$C_{37}H_{67}NO_{13}$	748	OH	O	O	OCH_3	CH_3
F	$C_{37}H_{67}NO_{14}$	750	OH	OH	H	CH_3	CH_3

Chu, 1998). Bacterial necrosis has variously been termed as 'black spot', 'brown spot', 'shell disease' or chitinolytic bacterial disease. It is caused by the invasion of chitinolytic bacteria, which break down the chitin of the exoskeleton. *Aeromonas hydrophila, Aeromonas caviea, A. sorbia* and *Aeromonas sp.* were bacterial flora isolated from necrosis prawns (Dat, 2002). *Pseudomonas fluorescens, Aeromonas* sp., *Lactococcus garvieae* and *Edwardsiella tarda* were bacteria flora isolated from adult prawns (Ahmed, 2003; Be, 2002; Lalitha and Surendran, 2006; Shih-Chu *et al*., 2001; Tran *et al*., 2002). Meanwhile, the most significant diseases in Nile tilapia (*Oreochromis niloticus)* culture are caused by *Streptococcus iniae, Aeromonas hydrophila*, *Trichodina* sp., Flexibacter, *Edwardsiella* spp. (Nagla *et al*., 2005).

The macrolide antibiotic erythromycin has long been the chemotherapeutant of choice to prevent and tackle these pathogenic bacteria. However, there are limited studies being published relating to adsorption, metabolism and degradation of erythromycin in aquatic species. Purpose of this study was to survey the adsorption, metabolism and degradation of erythromycin in giant freshwater prawn muscle (*Macrobrachium rosenbergii*) and Nile tilapia (*Oreochromis niloticus)* after oral administration of the drug given by medicated feed. From that, we can interpolate appropriately tentative withdrawal times. In addition, an evidence of bio-transformative forms of erythromycin, not only in fermentation process but in endo-enzymatic aquaculture pathway as well can be obviously seen.

MATERIALS AND METHODS

Erythromycin base in white powder and purity 96.5% was purchased from DHG Pharma (Can Tho, Vietnam). Feed and coating agent (squid liver oil) were supplied from Grobest Ltd.

Animals and diet: 750 adult giant freshwater prawns (*Macrobrachium rosenbergii*), with an average weight 40±2 g and 120 adult Nile tilapias (*Oreochromis niloticus)* with an average weight 500±5 g were used for the investigation.

750 adult giant freshwater prawns (*Macrobrachium rosenbergii*) were separated into two groups: group A (375 prawns) and group B (375 prawns). Two different diets were prepared for the experimental trial. Group A was treated with 50 mg/kg prawn body weight / day for 7 days through medicated feed (water temperature, 28°C). Group B was treated with 100 mg/kg prawn body weight/day for 7 days through medicated feed (water temperature, 28°C).

120 adult Nile tilapias (*Oreochromis niloticus*) were divided into group A (60 tilapias) and group B (60 tilapias). Two different diets were prepared for the experimental trial. Group A was treated with 50 mg/kg tilapia body weight/day for 7 days through medicated feed (water temperature, 28°C) while group B was treated with 100 mg/kg tilapia body weight/day for 7 days through medicated feed (water temperature, 28°C).

Two groups of medicated feed were conditioned by weighing and mixing feed with erythromycin base at appropriate dosages. Combination between drug and feed was adhesively guaranteed by a coating agent (squid liver oil).

Temperature monitoring: Freshwater prawn and tilapia were poikilothermic species. The optimum metabolic temperature range for them is between 26 and 32°C. Temperature could strongly affect to their survival and enzymatic metabolism, including drug biotransformation. So influence of temperature fluctuation at sampling time was recorded and mentioned in drug metabolism calculation.

Sample collection: Erythromycin base material had been estimated previously to screen and confirm whether other derivatives of erythromycin A, such as erythromycin B, C, D, E and F have been available or not. Sampling times for the prawn and fish in group A and group B were 1, 3, 6, 9 and 23 days after 7 days of the pharmacological treatment. At each sampling time, individuals in each group were sacrificed to confirm erythromycin A residue. Meanwhile, bio-transformation of erythromycin in prawn and tilapia was monitored by screening and confirming derivative forms of erythromycin at the beginning and the end of sampling stage.

Muscle samples in natural proportion were collected, and placed into polyethylene bags, coded and transferred to the laboratory on dry ice, stored at -40°C before analysis.

Analytical procedures: The methodology used for the determination of erythromycin A as well as derivatives of erythromycin in erythromycin base material, in prawn and fish muscle was based on LC-MS/MS. Parameters of measurements: methanol as the extraction solvent; a temperature of 80°C; a pressure of 1500 psi; an extraction time of 15 min; 2 cycles; a flush volume of 150% and a purge time of 300 s.

Table 2: Erythromycin depletion at different times in giant prawn muscles treated with 50 mg/kg prawn body weight/day for 7 days

Time		Erythromycin residue in prawn muscle (µg/kg)$^{\alpha}$	
Day	°C-Day	Group A	Group B
1	28	15.4±3.3	632.4±74.1
3	84	10.6±2.1	199.0±31.2
6	168	5.9±3.1	141.8±3.1
9	252	5.5±4.1	54.2±9.0
23	644	2.8±0.8	31.4±7.5

α Values shown are concentration means±standard deviations from 5 prawn samples

Table 3: Erythromycin A depletion at different times in tilapia fillet samples treated with 50 mg/kg and 100 mg/kg fish body weight/day for 7 days

Time		Erythromycin A residue in tilapia fillet (µg/kg)$^{\alpha}$	
Day	°C-Day	Group A	Group B
1	28	22216.0±22023.0	46960.0±9054.7
3	84	13590.0±14415.9	14328.0±18336.1
6	168	940.8±460.3	6382.0±5582.5
9	252	131.4±31.9	379.7±99.3
23	644	34.7±9.6	42.9±17.4

α Values shown are concentration means ± standard deviations from 5 tilapia fillet samples

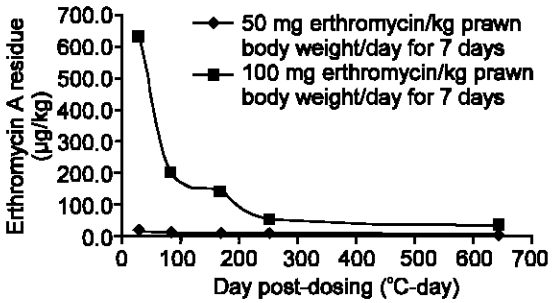

Fig. 2: Erythromycin A depletion at different times in giant prawn muscles treated with 50 and 100 mg/kg prawn body weight/day for 7 day

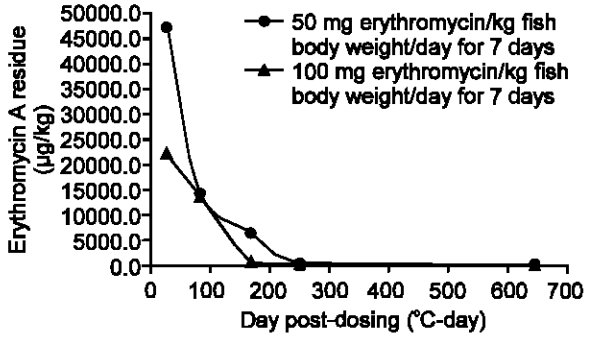

Fig. 3: Erythromycin A depletion in tilapia fillet samples treated with 50 and 100 mg/kg fish body weight/ day for 7 days

RESULTS

Adsorption and depletion of erythromycin A: The influence of water temperature on fish metabolism and consequently, on the drug pharmacokinetics, the time parameter was also expressed as °C-day. Degree-days were calculated by multiplying the mean daily water temperature by the total number of days at which the temperature was measured to that point.

Prawn: Results of erythromycin depletion at different times in prawn muscle samples treated with 50 mg/kg (group A) and 100 mg/kg (group B) prawn body weight/ day for 7 days were shown in Table 2 and Fig. 2.

Tilapia: Results of erythromycin A depletion at different times in tilapia samples treated with 50 and 100 mg/kg fish body weight/day for 7 days were shown in Table 3 and Fig. 3.

Determination of withdrawal time: The MRL value for erythromycin was set at 30 µg/kg, as reported by CFIA (Canadian Food Inspection Agency), date 17/11/2009. The regression line and the upper, one-sided tolerance limit (95%) regression line with a confidence of 95% were also traced. This graph had been obtained using the statistical program recommended by the European Agency for the Evaluation of Medicinal Products (EMEA) and was downloadable from the same EMEA web site (European Agency for the Evaluation of Medicinal Products, 2009).

Prawn: A withdrawal time of 976°C-days was interpolated for giant freshwater prawn treatment - group B (Fig. 4).

Tilapia: A withdrawal time interpolated for tilapia treatment was 908°C-days (group A) and 1150°C-days (group B) (Fig. 5 and 6).

DISCUSSION

Our research was designed in conditions that were quite close to actual aquaculture. The minimum inhibited concentration of erythromycins A, B, C and D and some of their derivatives were determined against 21 gram-positive and 15 gram-negative microorganisms. Antibacterial activity was confined to gram-positive and very few gram-negative bacteria. Erythromycin B was somewhat less active than erythromycin A and erythromycin C and D showed about half that activity or even less. Most other derivatives had negligible activity (Isaac *et al*., 1985).

Metabolism and degradation of parental drug

Prawn: The mean concentration of erythromycin in group A was lower in comparison with that in group B. However, the eliminating slope of erythromycin residue in group B was faster than in group A.

Salmon *Oncorhynchus mykiss*, after its erythromycin administration at 100 mg/kg trout body weight/day for 21 days through medicated feed (water temperature,

11.5°C) gave a withdrawal time of 255°C-days (Annarita *et al*., 2007). Salmon *Oncorhynchus tshawytscha* through intraperitoneal injection (William *et al*., 2006) as well as orally administered erythromycin (Fairgrieve *et al*., 2005), the mechanism of its retention and depletion was also investigated.

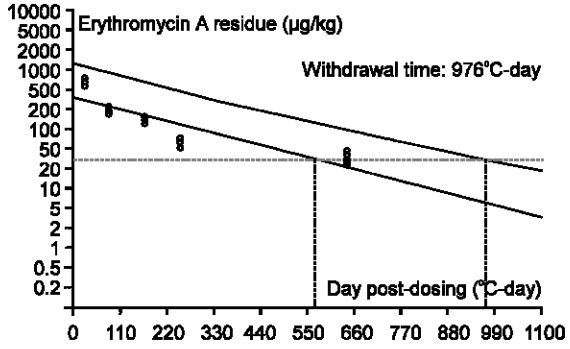

Fig. 4: Withdrawal time in muscle prawn treated with erythromycin - group B

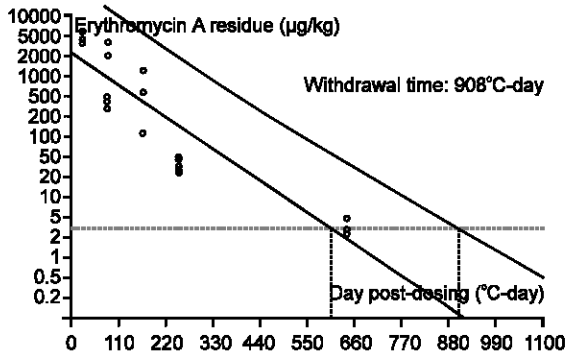

Fig. 5: Withdrawal time in muscle tilapia treated with erythromycin - group A

The digestive enzymes of tryptase, pepsin, cellulase, amylase and metabolic enzymes of Alkaline Phosphatase (AKP), Acid Phosphatase (ACP), Superoxide Dismutase (SOD) and Glutathione-S-Transferase (GST) were dominated in the hepatopancreas of *M. rosenbergii* (Na *et al*., 2008). Only erythromycin F (5 µg/kg) presented in erythromycin base. During medication at dose 100 mg/kg prawn body weight/day for 7 days via feed, erythromycin has slightly changed to erythromycin E (2.09 µg/kg) after ceasing drug one day. At day 23 of post-treatment, erythromycin E (5.81 µg/kg) and erythromycin F (3.52 µg/kg) was detected and fortunately was not significant to our concern (Table 4). Considering the fact that biotransformation has been kept erythromycin derivatives at safe residue if we tightly obey recommendation of withdrawal time and drug dosage.

Drug residue levels dropped quickly during the first 3 days after treatment termination, then slowly and steadily until a residue level of <100 µg/kg, considered a safe limit by requirements of FDA and the European Community, was attained at day 9 of erythromycin

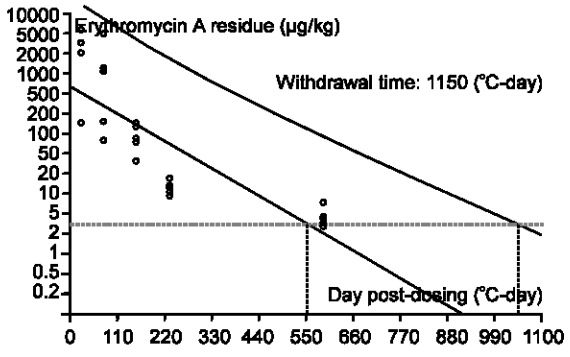

Fig. 6: Withdrawal time in muscle tilapia treated with erythromycin - group B

Table 4: Degradation of erythromycin at different times in giant prawn muscle samples treated with 50 and 100 mg/kg prawn body weight/day for 7 days

				Result (µg/kg)	
Name of sample	Identification	Test parameter	MDL (µg/kg)	Group A	Group B
Erythromycin base	EBS/0901-RC-002	Erythromycin B	1.0	ND	ND
		Erythromycin C	1.0	ND	ND
		Erythromycin D	1.0	ND	ND
		Erythromycin E	1.0	ND	ND
		Erythromycin F	1.0	5.00	5.00
Giant prawn muscle	GP - S1	Erythromycin B	10.0	ND	ND
		Erythromycin C	10.0	ND	ND
		Erythromycin D	10.0	ND	ND
		Erythromycin E	10.0	ND	2.09
		Erythromycin F	10.0	ND	ND
Giant prawn muscle	GP - S5	Erythromycin B	10.0	ND	ND
		Erythromycin C	10.0	ND	ND
		Erythromycin D	10.0	ND	ND
		Erythromycin E	10.0	ND	5.81
		Erythromycin F	10.0	ND	3.52

*MDL: Method Detection Limit; ** ND: Not Detected

Table 5: Degradation of erythromycin at different times in tilapia muscle samples treated with 50 and 100 mg/kg fish body weight/day for 7 days

				Result (µg/kg)	
Name of sample	Identification	Test parameter	MDL (µg/kg)	Group A	Group B
Erythromycin base	EBS/0901-RC-002	Erythromycin B	1.0	ND	ND
		Erythromycin C	1.0	ND	ND
		Erythromycin D	1.0	ND	ND
		Erythromycin E	1.0	ND	ND
		Erythromycin F	1.0	5.00	5.00
Tilapia fillet	TL - S1	Erythromycin B	10.0	ND	ND
		Erythromycin C	10.0	ND	131.49
		Erythromycin D	10.0	ND	ND
		Erythromycin E	10.0	ND	258.28
		Erythromycin F	10.0	ND	ND
Tilapia fillet	TL - S5	Erythromycin B	10.0	ND	ND
		Erythromycin C	10.0	ND	ND
		Erythromycin D	10.0	ND	ND
		Erythromycin E	10.0	0.30	6.94
		Erythromycin F	10.0	1.37	5.90

*MDL: Method Detection Limit; **ND: Not Detected

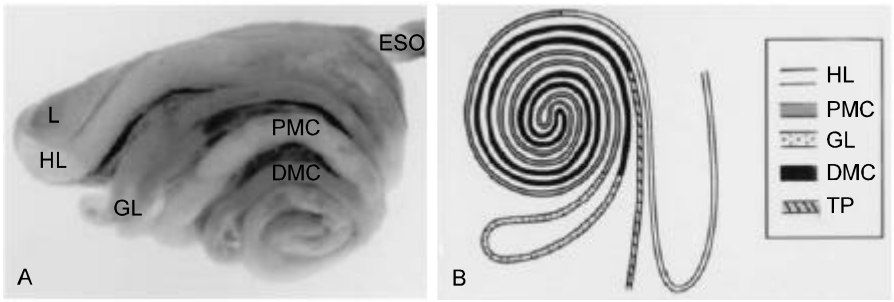

Fig. 7: Photomicrograph (right view) (A) and schematic drawing (ventral view) (B) of five intestinal segments of tilapia. HL, Hepatic Loop; PMC, Proximal Major Coil; GL, Gastric Loop; DMC, Distal Major Coil; TP, Terminal Portion of the Intestine

withdrawal. However, a longer withdrawal period (35 days of post-treatment) was recommended to ensure complete drug depletion to satisfy CFIA's concern.

Tilapia: The high metabolic rate of furazolidone, AOZ in Nile tilapia was 22 days at least (Weihai *et al*., 2006). Meanwhile, a research of accumulation and clearance of florfenicol in tilapia didn't rule out the withdrawal times (Bowser *et al*., 2009).

When tilapias were medicated with erythromycin base at low dose (group A), none of derivatives of erythromycin was detected in tilapia muscle at day 1 of post-treatment. At day 23 of post-treatment, erythromycin E (0.30 µg/kg) and erythromycin F (1.37 µg/kg) was not significant to our concern (Table 5).

In case tilapias were fed with erythromycin at higher dose (group B), two derivatives erythromycin C (131.49 µg/kg) and erythromycin E (258.28 µg/kg) appeared right after ceasing drug treatment. At day 23 of post-treatment, erythromycin E (6.94 µg/kg) and erythromycin F (5.90 µg/kg) was detected and fortunately was also not significant to our concern (Table 5). This phenomenon could be explained by intestinal and hepatic enzymes. Maltase, leucine aminopeptidase, dipeptidyl aminopeptidase IV, lipase, non-specific esterases, and alkaline phosphatase were their intestinal enzymes participated in erythromycin metabolism.

Maltase, leucine aminopeptidase, dipeptidyl aminopeptidase IV, lipase, non-specific esterases and alkaline phosphatase were present at specific sites along the first four intestinal segments (Bundit *et al*., 2000). Strong reaction for maltase was present in the third intestinal segment, while aminopeptidases and alkaline phosphatase were detected in the first three parts. The most intense activity for lipase was present in the first two parts, while non-specific esterases were observed in the first four portions. Activities of all these enzymes were demonstrated in the brush border. Non-specific esterases were also present in the cytoplasm of the enterocytes. In addition to its brush border localization in the cranial segments, dipeptidylamino-peptidase IV was also observed in the basal lamina of all segments, including the terminal segment. The first four regions played the most important role in both digestion and absorption of erythromycin (Fig. 7).

Parallel with intestinal enzymes, hepatic biotransformation enzymes in tilapia such as CYP1A protein, 7-ethoxyresorufin O-deethylase (EROD), Glutathione S-Transferase (GST), UDP-Glucuronasyl Transferase (UDP-GT) and lipogenic enzyme were also dominated and highly correlated with erythromycin demethylase (Bernard *et al*., 1996). They eliminated erythromycin derivatives to minor level at date 23 post-dosing.

Our study provides preliminary data for a prudent use of the antimicrobial drug erythromycin in Nile tilapia, in order to guarantee safety in foods for the consumers and to improve fish farming management. The withdrawal time of erythromycin in Nile tilapia was recommended 33 days or 42 days at least depend on dosage of chemotherapy.

Conclusion: There were lots of researches about toxicity and carcinogenicity of erythromycin A and erythromycin derivatives on mice, rat, dog and even on human. However, studies about toxicity and carcinogenicity of erythromycin A and erythromycin derivatives in aquatic animals were scarcely investigated. Bio-transformative forms of erythromycin B, C, D, E, F were always in mind of human whether they could be transformed from parental drugs in aquaculture or not. Whether could they create harmful risks to human health. So this research confirmed an obvious evidence that bio-transformative forms of erythromycin appeared through endo-enzymatic mechanism and quickly decomposed to minor level. This would set a basic foundation that there will be no risk of toxicity and carcinogenicity of erythromycin B, C, D, E, F in aquaculture if farmers strictly follow the recommended veterinary dose.

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Effect of Supplementing Different Levels of Chromium Yeast to Diet on Broiler Chickens on Some Physiological Traits

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Abstract: The experiment was conducted at the faculty of agriculture University of Ain Shams-Egypt, from January to March 2008, to study the effect of different levels of chromium yeast (cr-yeast) on broiler chickens on some physiological traits. A total of 450, one-day old unsexed chickens (Cobb) strain were used. The birds were randomly allocated to five treatments with 3 replicates each. The treatments were control (T_1), without supplementation, T_2, T_3, T_4 and T_5 which were supplemented with 0.5, 1, 1.5 and 2 mg cr-yeast/kg diet respectively. Chromium yeast supplementation treatments caused a significant ($p \leq 0.05$) increase in plasma glucose levels, while supplemented Cr-yeast at levels of 1 (T_3), 1.5 (T_4), 2 (T_5) mg/kg diet resulted in a significant ($p \leq 0.05$) increase in total protein and globulin as compared to control group. Also supplemented 0.5 (T_2) or 2 (T_5) mg Cr-yeast resulted in a significant ($p \leq 0.05$) reduction in total lipid in plasma, whereas cholesterol levels which were significantly ($p \leq 0.05$) decreased when Cr-yeast was supplemented at levels of 1 (T_3), 1.5 (T_4) and 2 (T_5) mg/kg diet. Although LDL was significantly ($p \leq 0.05$) decreased when 1.5 (T_4) or 2 (T_5) mg Cr-yeast was supplemented to the diet. Lymphoid organs percentage which was not affected by dietary supplementation of Cr-yeast except spleen percentage that was significantly ($p \leq 0.05$) higher in the group that was supplemented with Cr yeast .Chromium level increased in liver, muscle and plasma as levels of supplementation was increased. Protein percentage in the breast and thigh increased significantly ($p \leq 0.05$) in all chromium supplementation groups as compared to the control group, while fat percentage in the breast and thigh decrease significantly ($p \leq 0.05$) when chromium level increased from 1-2 mg/kg diet. It can be concluded that Cr-yeast had a beneficial effect on some of the physiological measurements of broilers under such experimental conditions.

Key words: Broiler chicken, chromium, chromium yeast, broiler performance

INTRODUCTION

The first suggestion that chromium participates in carbohydrate metabolism in animals was mentioned by Schwartz and Mertz (1957). They observed that glucose tolerance factor GTF, which was shown later to contain chromium, was deficient in animals with impaired glucose tolerance and that supplemental chromium improved glucose tolerance (Mertz, 1993).

Chromium is a co-factor of insulin, promoting insulin activity (McCary *et al*., 1988), enhancing amino acid uptake, promoting lipogenesis from glucose and lipid storage in the liver and adipose tissues (Steele and Rosebrough, 1979) dietary chromium can increase lipoprotein lipase activity and eventually decrease the contents of triglycerides rich lipoproteins (Garfinkel *et al*., 1976; Howard *et al*., 1993) also can increase liver LDL receptors there by reducing the LDL content and concomitantly the HDL proportion is increased (Brindley and Salter, 1991; Lien *et al*., 1999). The rate limiting enzyme in cholesterol synthesis is 3-hydroxyl-3-methylglutaryl Co enzyme A HMG-CoA reductase (Merlin, 1998). The mechanism whereby chromium alters cholesterol levels and fractions is not fairly understood.

Chromium is also considered as antistress factor (Kegley and Spears, 1995) and increases immune capability (Uyanik *et al*., 2002).Chromium excretion may increase 10 to 300 fold in stress situations. This also becomes nutritionally important because in such condition it is necessary to increase the trace element concentration in the diet. However, an appropriate recommendation on the chromium requirement for poultry has not been made NRC (1994) and most poultry diets are basically composed of plant origin ingredients which have usually a low content of chromium (Giri *et al*., 1990).

Inorganic chromium such as chromic chloride and Cr oxide are poorly absorbed in animals, absorption ranges from 0.4 to 3% or less, regardless of dose and dietary Cr status (Anderson, 1987; Underwood and Suttle, 1999). There are six known sources of organic Cr compounds (Zinpro, 2003). Chromium-L-methionine, Chromium Nicotinate, Chromium Chelate, Chromium Proteinate, chromium picolinate (Cr Pic) and Cr yeast. Researches on animals have confirmed that Cr from organic complex is absorbed more efficiently, about 25-30% more than inorganic compounds (Mowat, 1997; Olin *et al*., 1994).

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Chromium yeast (Cr yeast): Brewer's yeast is an example of natural chromium yeast. Typically it contains approximately 2 ppm of organic chromium. The actual chemical structure of chromium compound in brewer's yeast is unknown, because of its low chromium content, manufacturers often raise the content of chromium in yeast by the following methods: Yeast-bound chromium is produced by introducing an inorganic chromium source such as chromic chloride into live yeast culture. As the brewer's yeast cells grow and multiply chromium is taken up into the yeast cells, increasing the chromium content of the yeast. Some yeast-bound chromium products also contain the culture from which the live yeast cells were grown. It is difficult to know the actual amount of organic chromium in the yeast cells in these products (Zinpro, 2003). Chromium Fortified yeast: Brewer's yeast or yeast culture is blended with inorganic chromium salt, such as chromic chloride or organic chromium to form a mixture. This mixture is sold as chromium yeast (Zinpro, 2003).

The objective of the present work was to investigate the effects different levels of dietary chromium yeast supplementation to broiler chickens on some physiological traits.

MATERIALS AND METHODS

The experiment were conducted at Broiler Nutrition Unit, Faculty of Agriculture Ain Shams University during the period from January to March 2008. This study aimed to investigate the effects of adding different levels chromium yeast (Cr yeast), to the broiler chickens diets on, blood constituents and physiological characters.

Birds were raised from day-old to 5 weeks of age. Cobb broiler chickens were randomly allocated to floor pens. Electrical heaters were used to maintain room temperature at 34°C during the first week of age and then the temperature was being decreased gradually to 26°C during the 3rd week of age. Artificial lighting was provided constantly during the experimental period. Water and mash feed were provided *ad lib* through the 35 days experimental period. All chickens were vaccinated against avian influenza at one day old and Newcastle disease at 6, 18 days old.

Four hundred and fifty, one-day old Cobb broiler chickens were allocated randomly into five treatment groups of 90 birds and divided into three replicates with 30 birds each.

The chickens were received starter diet from one to 21 day of age and then switched to grower diet from 22 to 35 days of age, as shown in Table 1. The diets were formulated according to NRC (1994).

In this experiment, five different dietary treatments were used as follows:

Treatment 1 (Control) T_1: The diet without chromium yeast supplementation, treatment 2 (T_2): The diet + 0.5 mg Cr yeast/kg diet, treatment 3 (T_3): The diet + 1 mg Cr yeast/kg diet, Treatment 4 (T_4): The diet + 1.5 mg Cr yeast/kg diet, Treatment 5 (T_5): The diet + 2 mg Cr yeast/kg diet.

Table 1: Composition and calculated analysis of the experimental diets

Ingredient (%)	Starter (0-3 wks)	Grower (3-5 wks)
Yellow corn	55.80	59.71
Soybean meal (44%)	34.32	30.00
Corn gluten	3.33	2.80
Vegetable oil	2.79	4.00
Dicalcium phosphate	1.94	1.67
Limestone	1.14	1.14
Common salt	0.25	0.25
Vit and min. premix*	0.25	0.25
DL. methionine	0.18	0.18
Total	100.00	100.00
Calculated composition**		
Crude protein (%)	22.00	20.00
ME. kcal/kg kcal ME/kg	3000.00	3100.00
Calcium (%)	0.97	0.91
Available Phosphorus (%)	0.50	0.45
Methionine + Cystein (%)	0.91	0.78
Lysine (%)	1.10	1.10

*Composition of vitamin and minerals premix. Each 3 kg of vitamin and minerals mixture contatin: 12000000 IU vitamin A; 2000000 IU D3; 10 gE; 1gk; 1 g Bl; 5 g B2 1500 mg B6; 10 mg B12; 10 g pantothenic acid; 20 g Nicotinic acid, 1 g Folic acid; 50 mg Biotin, 500 g choline chloride; 4 g copper; 300 mg iodine; 30 g iron; 60 g manganese; 50 g zinc and 100 mg selenium.
**According to NRC (1994)

Blood samples were collected at 35 days of age, 45 chickens where the number of chickens per treatment was 9. These chickens were slaughtered and blood samples were collected in centrifuge tubes with EDTA. The tubes were stoppered and centrifuged immediately (4000 rpm) for 15 min to separate plasma which was decanted into sterilized glass vials which were stoppered tightly and stored in a deep freezer until test. Plasma glucose was determined according to Trinder (1969) using commercial kits of (spectrum Co.). Plasma total proteins were determined by using colorimetric method according to Henery (1964) using commercial kits of (Biodiagnostic Co.). Plasma Albumin was determined using colorimetric method according to Doumas *et al.* (1971) using commercial kits of (Biodiagnostic Co.). The concentration of plasma globulins was obtained by subtracting the albumin value from the value of total protein for each plasma sample. Plasma total lipids were determined according to Knight *et al.* (1972) using commercial kits of (Biocon Co.) Plasma triglycerides were determined by triglycerides kits according Sidney and Barnard (1973) using commercial kits of (Biocon Co.). Plasma total cholesterol and HDL cholesterol was determined by cholesterol kits according to Richmond (1973) using commercial kits of (Biocon CO.). LDL cholesterol was determined according to Bergmenyer (1985) by calculation LDL = total cholesterol - HDL - TG/5 Plasma calcium and phosphorus were determined by using commercial kits of (Giesse Diagnostics company). According to Gindler and King (1972). T3 and T4 were analyzed by using radio immune assay kits as described by Sharp *et al.* (1987).

Uric acid was determined by enzymatic colorimetric test to Arliss and Entvistle (1981), using commercial kits (Biodiagnostic CO.). Plasma creatinine was determined by using spectrophotometer to Husdan and Rapaport (1968), using commercial kits (Biodiagnostic CO.). AST was determined according to Reitman and Frankel, (1957) using commercial kits (Biodiagnostic CO.). ALT was determined according to Reitman and Frankel, (1957) using commercial kits (Biodiagnostic CO.).

At the end of the experiment (5 weeks), three chickens from each replicate were chosen randomly and slaughtered, then internal organs (liver, Gizzard and heart) and lymphois organs (bursa, thymus and spleen) were removed, weighted and calculated as percentage of carcass weight.

Plasma chromium levels were analyzed by using atomic absorption spectrophotometer as described by Perkin, (1982). Five gram add justly weighed of selected tissue (liver, muscles, plasma) which were ashes in a muffle at 600^{0}C for 3 h. The ash was solved in concentrated hydrochloric acid at first, the washed in a limit quantity of hydrochloric acid 1 molar concentration and filtrate using ashless filtering papers. The filtrate was diluted properly and flam photometer was used for chromium determining (AOAC, 1980). The Cr was measured using Atomic Absorption (Spectrophotometer).

Meat samples were taken from breast and thigh to measure the biochemical analysis Ether Extract (EE), protein, Moisture and Ash, according to AOAC (1980).

Completely Randomized Design (CRD) was used to study the effect of difference treatment in all traits. Duncan (1955) multiple range test was used to compare the significant differences between means. Data were analyzed using statistical analysis system (SAS, 2001) by assuming the following model.

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = The value of observation of traits
μ = The overall mean of traits
T_i = The effect of treatments, control (T_1), (T_2), (T_3), (T_4) and (T_5).
e_{ij} = Random error assumed to be mean equal to zero and variance is σ^2e (N ~ 0, σ^2e)

RESULTS AND DISCUSSION

Figure 1 shows the effect of chromium yeast supplementation on glucose of chickens in different experimental groups. Analysis of data on plasma glucose concentration showed a significant ($p \leq 0.05$) difference between control and all Cr yeast supplemented groups. However there was no difference among Cr yeast groups. Glucose concentration in plasma was reduced as a result of feeding dietary Cr yeast at level 0.5, 1, 1.5, 2 mg, the values were 204.5, 202.4, 210, 201 mg/dl, respectively compared to control group 246.33 mg/dl.

Plasma total protein (Table 2) increased significantly ($p \leq 0.05$) due to inclusion Cr yeast into chickens diet in T_3, T_4 and T_5 and values were 2.55, 2.93, 3.13, 3.27, 3.54 g/dl for control T_2, T_3, T_4, T_5 groups, respectively. Plasma total protein value in 0.5 mg Cr yeast group (T_2) did not differ from the value of control (T_1), T_3 and T_4 group. Simultaneously plasma albumin values were not affected by treatments so that the elevation in plasma total protein was reflected as an increment in plasma globulin values which were affected significantly ($p \leq 0.05$) by treatments and were parallel to the total protein and the values were 1.16, 1.42, 1.94, 1.79, 2.07 g/dl for control, T_1, T_2, T_3, T_4, T_5 respectively.

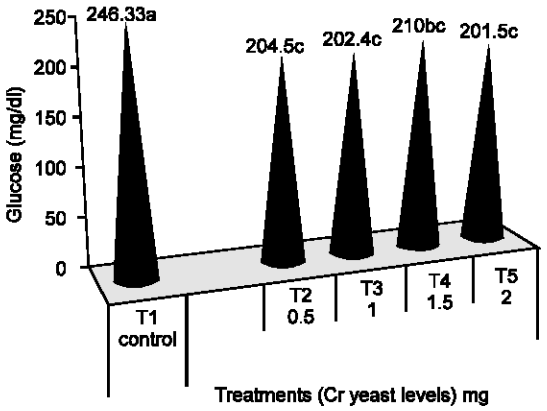

Fig. 1: Effect of Cr yeast levels on plasma glucose concentration in broiler chickens at 5 weeks. Means having different letters are significantly different ($p \leq 0.05$)

Table 2: Effect of Cr yeast levels on plasma total protein, albumin, globulin, total lipid, triglycerides and HDL in broiler chickens at 5 weeks

	Treatments (Cr yeast levels) mg				
Characters	T_1 (Control)	T_2 (0.5)	T_3 (1)	T_4 (1.5)	T_5 (2)
Total protein g/dl	2.55^{c}	2.93^{bc}	3.13^{ab}	3.27^{ab}	3.54^{a}
Albumin g/dl	1.39	1.51	1.19	1.48	1.48
Globulin g/dl	1.16^{c}	1.42^{bc}	1.94^{ab}	1.79^{ab}	2.07^{a}
Total lipid (g/dl)	4.86^{a}	3.86^{b}	4.30^{ab}	4.24^{ab}	3.72^{b}
Triglycerides (mg/dl)	165.33	158.00	138.00	157.33	143.00
HDL (mg/dl)	97.07	89.00	78.00	78.50	87.00

Means having different letters at the same row are significantly different ($p \leq 0.05$)

Table 2 shows the effect of chromium supplementation on lipid derivatives of broiler chickens. Plasma total lipid was significantly ($p \leq 0.05$) lower in T_2 and T_5 group ($p \leq 0.05$) as compared to the control group (T_1), T_2 and T_5 while there were no significant differences between T_1, T_5 and T_4. Significantly ($p \leq 0.05$) lowest total lipid levels of 3.72 and 3.86 g/dl were recorded for chickens supplemented 0.5, 2 mg chromium yeast/kg diet, respectively. The control group recorded the higher plasma total lipid 4.86 g/dl. while the other groups. T_3, T_4 recorded intermediate in total lipid level 4.3, 4.24 g/dl respectively with no significant ($p \leq 0.05$) difference compared to control as well as T_2, T_5 groups. There were reductions in plasma triglycerides due to feeding chromium yeast. This reduction lacked significance, triglyceride values 165.33, 158, 138, 157.3, 143 mg/dl for control, T_1, T_2, T_3, T_4 and T_5 respectively. The data indicated non significant ($p \leq 0.05$) difference between chromium yeast supplemented groups and the control group in plasma HDL.

Analysis of data on plasma total cholesterol showed significant ($p \leq 0.05$) differences between control (T_1) and chromium supplemented groups T_3, T_4 and T_5. However, there were no differences among chromium supplemented groups at all levels. Plasma total cholesterol was reduced as a result of adding chromium yeast into chick's diets. Furthermore, the reduction was significant at levels of 1, 1.5 and 2 mg Cr/kg diet and values were 128.3 130.3, 130.7, 146.66 mg/dl respectively compared to control group 168.06 mg/dl, but the cholesterol value in 0.5 mg Cr yeast group did not differ from the value observed in control group (Fig. 2).

The T_5 and T_4 group showed a significant decrease in LDL 14.7, 18.3 mg/dl respectively compared to control group 39.79 mg/dl. The LDL of chick fed T_2 and T_3 diet did not differ from the value recorded in control group T_1 as well as in T_4 and T_5 (Fig. 3).

The effect of chromium yeast on plasma calcium, phosphorus, T_3 and T_4 hormones are illustrated in Table 3. Blood calcium concentration was not affected significantly ($p \leq 0.05$) by adding different levels of chromium yeast into broiler diets. The calcium concentration were 4.25, 4.38, 4.23, 3.43, 4.67 mg/dl for control, T_1, T_2, T_3, T_4, T_5 group respectively. Also the plasma phosphorus concentration did not significantly ($p \leq 0.05$) differ among chromium yeast supplementation groups and control group. It is obvious that the phosphorus of chickens feed diet supplemented with chromium yeast was slightly lower than those fed control diet, the values were 5.94, 5, 5.46, 5.55, 5.5 mg/dl for control T_1, T_2, T_3, T_4, T_5 groups, respectively. Analysis of variance showed a non significant ($p \leq 0.05$) effect of the different levels of chromium yeast supplementation on the level of T_3, T_4 hormones.

Table 4 shows the effect of Cr yeast on some kidney function test of chickens in different experimental groups. The results showed that the dietary control and diet with 0.5, 1.5, 2 mg Cr yeast supplemented had no effect on plasma creatinine level and values were 0.82, 0.83, 0.77, 0.74, 0.64 mg/dl respectively. Plasma uric acid levels were not affected by feeding diet with Cr yeast and values were 3.45, 4, 3.79, 3.35, 3.55 mg/dl for control, T_1, T_2, T_3, T_4, T_5 groups respectively. The same Table 4 shows the effect of chromium yeast on some liver enzymes activity of chickens fed experimental diets. The present data showed that the blood concentration of ALT and AST enzyme was not affected significantly ($p \leq 0.05$) by adding Cr yeast into diets.

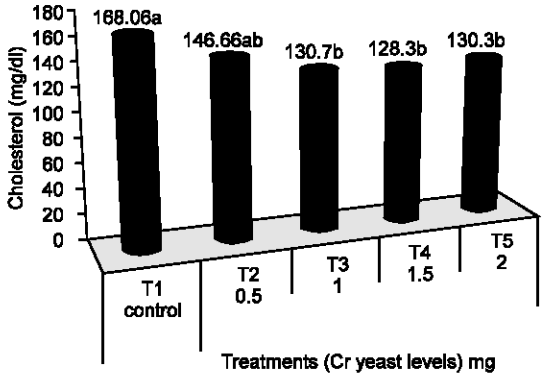

Fig. 2: Effect of Cr yeast levels on plasma cholesterol in broiler chickens at 5 weeks. Means having different letters are significantly different ($p \leq 0.05$)

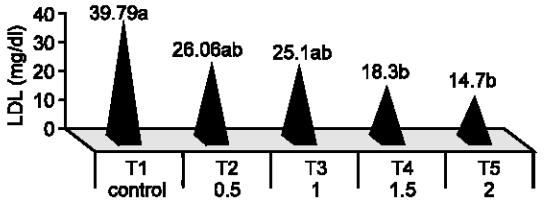

Fig. 3: Effect of Cr yeast levels on plasma LDL in broiler chickens at 5 weeks. Means having different letters are significantly different ($p \leq 0.05$)

Table 3: Effect of Cr yeast levels on plasma calcium, phosphorus, T3 and T4 level in blood plasma of broiler chickens at 5 weeks

	Treatments (Cr yeast levels) mg				
Characters	T_1 (Control)	T_2 (0.5)	T_3 (1)	T_4 (1.5)	T_5 (2)
Calcium (mg/dl)	4.25	4.38	4.23	3.43	4.67
Phosphorus (mg/dl)	5.94	5.00	5.46	5.55	5.50
T_3 (ng/ml)	3.95	3.77	3.42	3.78	3.91
T_4 (ng/ml)	12.95	12.52	13.05	12.73	13.55

Table 4: Effect of Cr yeast levels on plasma creatinine uric acid, ALT and AST in broiler chickens at 5 weeks of age

	Treatments (Cr yeast levels) mg				
Characters	T_1 (Control)	T_2 (0.5)	T_3 (1)	T_4 (1.5)	T_5 (2)
Creatinine (mg/dl)	0.82	0.83	0.77	0.74	0.64
Uric acid (mg/dl)	3.45	4.00	3.79	3.35	3.55
ALT (µ/L)	72.8	74.35	82.56	69.10	80.2
AST (µ/L)	10.15	10.89	11.19	9.89	11.29

Table 5: Effect of Cr yeast on internal organs percentage in broiler chickens at 5 weeks of age

	Treatments (Cr yeast levels) mg				
Characters (%)	T_1 (Control)	T_2 (0.5)	T_3 (1)	T_4 (1.5)	T_5 (2)
Liver	2.11	2.31	2.61	2.12	2.21
Gizzard	2.71	2.48	2.27	2.43	2.61
Heart	0.64	0.62	0.58	0.59	0.74

Table 6: Effect of Cr yeast levels on lymphoid organs percentage in broiler chickens at 5 weeks of age

	Treatments (Cr yeast levels) mg				
Characters (%)	T_1 (Control)	T_2 (0.5)	T_3 (1)	T_4 (1.5)	T_5 (2)
Bursa	0.264	0.192	0.191	0.170	0.186
Thymus	0.499	0.531	0.486	0.276	0.455
Spleen	0.110^{b}	0.115^{b}	0.158^{ab}	0.140^{ab}	0.173^{a}

Means having different letters in the same row are significantly different ($p \leq 0.05$)

The relative weights of the internal organs of chickens fed different experimental diets are shown in Table 5. Liver weight % was not affected by supplementing different levels of chromium yeast in the diet of chickens. The values of gizzard weight percentage were 2.71, 2.48, 2.27, 2.43 and 2.61 for control T_1, T_2, T_3, T_4, T_5 respectively which were not affected by dietary treatments. Heart weight percentage was not affected by using chromium yeast into diets. And average values were 0.64, 0.62, 0.58, 0.59 and 0.74 for treatment T_4, T_2, T_3, T_1 and T_5 respectively.

The relative weights of lymphoid organs are shown in Table 6. Neither bursa nor thymus percentage was affected by supplemented chromium yeast to chickens diets, whereas spleen relative weight significantly ($p \leq 0.05$) increased due to feeding Cr yeast (T_5). The spleen relative weight was significantly ($p \leq 0.05$) higher in chickens which received 2 mg Cr yeast (T_5) 0.173% as compared to control group (T_1) (0.11%) and T_2 group (0.115%). Both 1 mg Cr yeast (T_3) and 1.5 mg Cr yeast (T_4) groups (0.158%, 0.14%, respectively) did not differ from the 2 mg Cr yeast T_5 group, as well as from control and 0.5 mg cr yeast (T_2) groups.

The effects of chromium levels on tissue concentration is presented Table 7. Chromium levels for T_3, T_4 and T_5 were significantly ($p \leq 0.05$) higher than those the control group, while supplementing 0.5 mg chromium group did not differ significantly from all other supplemented groups and the control.

The values for liver chromium were 18.62, 20.70, 23.50, 25.12 and 25.53 (µg/100 mg) for the treatment T_1, T_2, T_3, T_4 and T_5 respectively.

Muscle chromium were significantly ($p \leq 0.05$) higher for T_4 and T_5 (1.5 mg and 2 mg chromium/kg diet), while chromium levels for T_3 (1 mg/kg diet) did not differ significantly than T_4, T_5 and the control group. Average values for muscle chromium level were 14.80, 16.32, 20.33, 22.21 and 22.00 for the T_1, T_2, T_3, T_4 and T_5 respectively.

Plasma chromium levels followed a similar trend as muscle chromium (Table 7). Plasma chromium levels for the groups that were supplemented with 1.5 mg and 2 mg/kg diet had significantly ($p \leq 0.05$) higher plasma chromium levels as compared to the control group while plasma chromium levels for T_3 (1 mg/kg diet) did not differ significantly than T_4 and T_5 (1.5 mg and 2 mg/kg diet) and the control group. Average values for plasma chromium were 4.05, 4.38, 5.25. 5.79 and 5.82 treatments T_1, T_2, T_3, T_4 and T_5 respectively.

Chemical composition of breast muscles is presented in Table 8. Protein percentage of the breast muscle was significantly ($p \leq 0.05$) higher in chickens which received 1, 1.5 and 2 mg Cr yeast (T_3, T_4 and T_5) followed by chickens which received 0.5 mg Cr yeast (T_2) compared to control group. The values were 25.9, 25.53, 25.52, 23.59 and 22.84% for treatments T_5, T_4, T_3, T_2 and T_1 respectively. The protein percentage in thigh muscle depicted a similar trend as the breast muscle the average values were 21.53, 21.47, 21.34, 20.28 and 19.4% for T_5, T_4, T_3, T_2 and control T_1 groups respectively. The use of Cr yeast in feeding of chickens caused the significant ($p \leq 0.05$) reduction in the breast muscles fat content in and the highest reduction was observed in T_3 group (6.52%) followed by T_5, T_4, T_2 group compared to control (T_1) group, 7.04, 7.1, 7.4 and 8.2% respectively. The thigh muscle fat percentage was significantly ($p \leq 0.05$) lower in chickens which received 2, 1.5, 1 mg Cr yeast compared to 0.5 cr yeast group and control group the values were 10, 10.9, 11.08, 12.5 and 13.09 respectively. The moisture and Ash content of breast and thigh muscle did not differ significantly ($p \leq 0.05$) between treatment groups.

Table 7: Effect of Cr yeast levels on liver, muscle, plasma, chromium level in broiler chickens at 5 weeks

Characters (chromium level)	Treatments (Cr yeast levels) mg				
	T_1 (Control)	T_2 (0.5)	T_3 (1)	T_4 (1.5)	T_5 (2)
Liver (µg/100 g)	18.62^{b}	20.70^{ab}	23.50^{a}	25.12^{a}	25.53^{a}
Muscle (µg/100 g)	14.80^{b}	16.32^{b}	20.33^{ab}	22.21^{a}	22.00^{a}
Plasma (µg/dl)	4.05^{b}	4.38^{b}	5.25^{ab}	5.79^{a}	5.82^{a}

Means having different letters in the same row are significantly different ($p \leq 0.05$)

Table 8: Effect of Cr yeast levels on chemical analysis of broiler breast and thigh muscles

Characters (%)	Treatments (Cr yeast levels) mg				
	T_1 (Control)	T_2 (0.5)	T_3 (1)	T_4 (1.5)	T_5 (2)
Muscle protein					
Breast	22.84^{c}	23.59^{b}	25.53^{a}	25.52^{a}	25.9^{a}
Thigh	19.40^{c}	20.28^{b}	21.34^{a}	21.47^{a}	21.53^{a}
Muscle fat					
Breast	8.20^{a}	7.40^{b}	6.52^{c}	7.10^{bc}	7.04^{bc}
Thigh	13.09^{a}	12.50^{a}	11.08^{bc}	10.90^{bc}	10.00^{c}
Muscle moisture					
Breast	67.71	67.79	66.59	66.00	65.63
Thigh	66.32	65.93	66.42	66.34	67.14
Muscle ash					
Breast	1.25	1.22	1.36	1.38	1.33
thigh	1.19	1.29	1.29	1.29	1.23

Means having different letters in the same row are significantly different ($p \leq 0.05$)

In this study there was a significant reduction in blood glucose level (Fig. 1) in chickens fed dietary Cr yeast. Ali (2006) found that organic Cr supplementation markedly decreased blood glucose level and this could be explained by supplementing chromium may increase glucose clearance rate which resulted in decrease plasma glucose and cholesterol in chickens fed 800 or 1.600 ug/kg of diet (Kim *et al*., 1996).

Plasma total protein (Table 2) increased significantly due to adding Cr yeast. This result confirmed the findings of Eshra (2005) who observed a linear increase in blood total proteins levels with increasing dietary supplementation of Cr yeast in chicken. In contrary to the present results. Chen *et al*. (2001) reported that dietary Cr at 1 to 3 mg/kg diet to male turkey diet did not significantly influence serum total proteins. Blood content of albumin was not affected significantly by Cr yeast levels. The same results were recorded by Ibrahim (2005). Plasma globulin values were affected significantly by Cr yeast supplementation.

The increase in plasma protein and Globulin in the chickens that were supplemented with 1, 1.5 and 2 mg/kg (T_3, T_4 and T_5) increased protein synrhesis in the supplemented group over the control group, which resulted in highly live body weight and weight gain, these group as compared to the control group. These results are in agreement with the findings of Roginiski and Mertz (1969) who reported that chromium supplementation increased amino acid incorporation into heart protein and amino acid uptake into tissues of rats.

There were a significant reduction in plasma total lipids, cholesterol, LDL due to feeding Cr yeast, this results confirms the finding of Abraham *et al*. (1982 a,b) that, Cr is essential for lipid metabolism. Also Uyanik *et al*. (2002), Ali (2006) observed a reduction in plasma total lipid, cholesterol by adding Cr and illustrated that, the decrease in lipid parameters could results from the increasing activity of insulin that depressed the fatty acid synthesis by increasing glycogen build up. Triglycerides and HDL cholesterol were did not affect these results is similar to finding of El-Afifi (2008).

Blood calcium and phosphorus (Table 3) were not affected significantly by adding Cr yeast into diets. This result is similar to that findings of Kalaycioglu *et al*. (1999).

The data (Table 3) showed a non significant effect of the Cr yeast supplementation on the level of T_3, T_4 hormones. The same result were recorded by Mostafa (2007).

The results (Table 4) showed that the dietary Cr yeast supplementation had no effect on plasma ALT, AST enzymes and indicated that Cr yeast had no deleterious effect on liver function. These results are in agreement with there obtained by (Karam *et al*., 2007; Mostafa 2007).

The results showed that the dietary Cr yeast had no effect on plasma uric acid plasma creatinine (Table 4) which may reveal that the Cr yeast levels used in the present study were safe for birds and had non deleterious effects on kidney function of treated chickens Mostafa (2007).

Bursa and Thymus percentage Table 6. were not affected by dietary supplementation of chromium yeast, while spleen percentage was only significantly ($p<0.05$)

in T_5 (2 mg Cr yeast) this could be an indicator to supplement Cr in levels lower than 2 mg/kg due the negative effect of this levels. These results are not in agreement with Mostafa (2007) who used Cr yeast a level of 4 mg/kg.

The Liver, muscle and Plasma chromium level (Table 7) increased significantly due to adding chromium yeast. This result confirmed the findings of Hossain *et al.* (1998), who observed a linear increase in blood, Muscle and liver, chromium levels with increasing dietary supplementation of Cr yeast.

This study showed that altering the dietary concentration of Cr yeast in broiler diets affected deposition of fat and protein (Table 8). The breast muscle protein percentage was significantly high in broiler received diet with 1, 1.5 and 2 mg Cr yeast. The protein percentage in thigh muscle depicted a similar trend as that of the breast muscle. These are in agreement with Anandhi *et al.* (2006) who concluded that the breast and thigh muscle protein levels significantly increased in 500 and 750 µg Cr yeast supplemented of broiler. But, Amatya *et al.* (2004) observed a non significant increase in protein accretion in broiler meat when Cr level was 0.2 mg/kg diet. Adding Cr yeast decreased carcass fat deposition in breast and thigh muscle. The same results were obtained by (Lien *et al.*, 1999; Choct *et al.*, 2000) who had recorded less fat content in muscle of broiler fed diet with Cr at different levels. Significant increase in muscle protein content may be attributed to the stimulating effect of protein synthesis by a supplement of Cr (Lien *et al.*, 1999). Chromium supplementation increased amino acids uptake into tissue (Chen *et al.*, 2001; Roginiski and Mertz, 1969).

IT can be concluded that using Cr yeast to broiler feeding had beneficial effects on some physiological traits.

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Manipulation of Yeast Fermented Cassava Chip Supplementation in Dairy Heifer Raised under Tropical Condition

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Abstract: Four, one-year old of dairy heifers, weighing at 200±10 kg were selected. Cows were randomly assigned according to a 4 x 4 Latin square design to study supplementation levels of Yeast Fermented Cassava Chip (YFCC) replaced concentrate on rumen ecology, cost production and average daily gain. The dietary treatments were as follows: T1 = supplementation of concentrate: YFCC ratio at 100:0; T2 = supplementation of concentrate:YFCC ratio at 75:25; T3 = supplementation of concentrate:YFCC ratio at 50:50; T4 = supplementation of concentrate:YFCC ratio at 25:75, respectively. The animals were offered the treatment concentrate at 1.5 %BW and rice straw was fed *ad libitum*. The results have revealed that feed intake and average daily gain cost productions were significantly different among treatments especially affected the rice straw intake and average daily gain were higher in dairy heifers receiving T3 than T4, T2 and T1. In contrast, the cost productions was lower in dairy heifers receiving T3 than T4, T2 and T1. However, the rumen fermentation and blood metabolites were similar for all treatments. The populations of protozoa and fungal zoospores were significantly different as affected by levels of yeast fermented cassava chip supplementation. These results suggest that supplementation of yeast fermented cassava chip could highest replace at 75% of concentrate in dairy heifers.

Key words: *Saccharomyces cerevisiae*, cassava chip, rumen ecology, dairy heifer

INTRODUCTION

The rumen has been well recognized as an essential fermentation that is capable of preparing end-products particularly Volatile Fatty Acids (VFAs) and microbial protein synthesis as major energy and protein for the ruminant host, hence, the more efficient the rumen is, the optimum the fermentation end-products are being synthesized. In recent years, there have been increasing interests, researches conducted as well as reviews in relation to rumen studies, rumen ecology and rumen manipulation (Martin *et al*., 1999; Khampa *et al*., 2009). Cassava (*Manihot esculenta*, Crantz) production in tropical areas has a potential use in ruminant livestock nutrition and feeding. Cassava root contains high levels of energy and has been used as a source of readily fermentable energy in ruminant rations (Wanapat, 2003; Kiyothong and Wanapat, 2004; Promkot and Wanapat, 2005). One strategy for using high degradable carbohydrates is to use in combination with readily available NPN sources such as urea. Urea is commonly used as N source when highly soluble carbohydrates are fed and maintained (Wohlt *et al*., 1978). However, efficient utilization of protein and Non-Protein Nitrogen (NPN) in ruminants depends upon knowledge of the basic principles underlying ruminal microbial N metabolism (Fernandez *et al*., 1997). Moreover, ruminal pH has great impact on rumen fermentation efficiency (Wanapat, 2003).

Some strictly anaerobic bacteria use a reductive or reverse citric acid cycle known as the succinate-propionate pathway to synthesize succinate and (or) propionate. Both malate and fumalate are key intermediates in the succinate propionate pathway and *S. ruminantium* uses this pathway (Gottschalk, 1986). The fact dicarboxylic acids, especially malate and fumalate, stimulate lactate utilization is consistent with the presence of this pathway in this ruminal anaerobe (Callaway and Martin, 1996). Previous studies by Sanson and Stallcup (1984) reported that supplementation of malate in ruminant diets has been shown to increase nitrogen retention in sheep and steers and to improve average daily gain and feed efficiency in bull calves. In addition, supplementing diets with yeast *(Saccharomyces cerevisiae)* increases milk

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production of dairy cows and weight gain of growing cattle (Brossard *et al*., 2006). Production responses attributed to yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion and increased flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-grain diets (Guedes *et al*., 2007). However, the use of malate and yeast in cassava based-diets has not yet been investigated. Therefore, the objective of this experiment was to investigate the supplementation levels of yeast fermented cassava chip with rice straw as a basal roughage on rumen ecology in dairy heifers.

MATERIALS AND METHODS

Animals, diets and experimental design: Four, one-year old of dairy heifers weighing at 200±10 kg. Cows were randomly assigned according to a 4 x 4 Latin square design to study supplementation levels of Yeast Fermented Cassava Chip (YFCC) replaced concentrate on rumen ecology, cost production and average daily gain. The dietary treatments were as follows: T1 = supplementation of concentrate:YFCC ratio at 100:0 (control); T2 = supplementation of concentrate:YFCC ratio at 75:25; T3 = supplementation of concentrate: YFCC ratio at 50:50; T4 = supplementation of concentrate:YFCC ratio at 25:75, respectively. The composition of dietary treatments and rice straw are shown in Table 1, 2.

Table 1: Ingredients of concentrate used in the experiment (% of DM basis)

Ingredient (DM%)	Concentrates
Cassava chip	65
Rice bran	6
Palm meal	10
Brewer's gain	10
Urea	2
Molasses	5
Sulfer	0.5
Salt	0.5
Mineral mix	1
Total	100

Table 2: Chemical composition of concentrates yeast fermented cassava chip and rice straw used in the experiment

Chemical compositions (%)	Concentrate	Yeast fermented cassava chip	Rice straw
DM	91.5	89.1	91.2
OM	90.3	89.4	86.2
CP	14.2	36.1	3.0
Ash	9.7	10.5	13.8
ADF	35.7	7.5	76.5
NDF	14.6	6.1	54.6
ME (Mcal/kg)	3.1	3.3	1.5
Feed cost (baht/kg)	8.0	6.0	1.0

DM = Dry Matter, CP = Crude Protein, OM = Organic Matter, NDF = Neutral-Detergent Fiber, ADF = Acid-Detergent Fiber

Cows were housed in individual pens and individually fed concentrate at 1.5 %BW. All cows were fed *ad libitum* of rice straw with water and a mineral-salt block. Feed intake of concentrate and roughage were measured separately and refusals recorded. The experiment was run in four periods, each experimental period lasted for 21 days, the first 14 days for treatment adaptation and for feed intake measurements whist the last 7 days were for sample collections of rumen fluid and faeces. Body weights were measured daily during the sampling period prior to feeding.

Data collection and sampling procedures: Concentrate and rice straw were sampled daily during the collection period and were composted by period prior to analyses. Composites samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for DM, ether extract, ash and CP content (AOAC, 1985), NDF, ADF and ADL (Goering and Van Soest, 1970).

Rumen fluid samples were collected at 0, 2 and 4 h post-feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by a stomach tube connected with a vacuum pump at each time at the end of each period. Rumen fluid was immediately measured for pH and temperature using (HANNA instruments HI 8424 microcomputer) after withdrawal. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into two portions. One portion was used for NH_3-N analyses where 5 ml of H_2SO_4 solution (1M) was added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000 g for 15 min and the supernatant stored at -20°C prior to NH_3-N analysis using the micro Kjeldahl methods (AOAC, 1985) and Volatile Fatty Acids (VFAs) analyses using a HPLC according to Zinn and Owen (1986). Another portion was fixed with 10% formalin solution in normal saline (Galyean, 1989).

The total count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haematocytometer (Boeco). A blood sample (about 10 ml) was drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 5,000 g for 10 min and stored at -20°C until analysis of Blood Urea Nitrogen (BUN) according to the method of Crocker (1967).

Statistical analysis: All data obtained from the experiment were subjected to ANOVA for a 4 x 4 Latin square design of treatments using the General Linear Models (GLM) procedures of the Statistical Analysis System Institute (SAS, 1998). Treatment means were compared by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition of diets, feed-intake average daily gain: The chemical compositions of rice straw and concentrate diets fed in dairy heifers are presented in Table 2. Concentrate diets contained similar concentrations of DM, OM, CP, NDF, ADF and TDN. Diets containing high levels of cassava chip based diets had a slightly higher Non-Structural Carbohydrate (NSC) and lower NDF due to increased level of cassava chip in the diets. Furthermore, the chemical composition of ruzi grass is presented in Table 2.

The effects supplementation levels of yeast *(Saccharomyces cerevisiae)* fermented cassava chip replace concentrate on feed-intake of dairy heifers are presented in Table 3. Feed intake and Average Daily Gain (ADG) were significantly different among treatments especially affected to rice straw intake which higher in dairy heifers receiving T3 than T4, T2, T1 (1.7, 1.6, 1.5 and 1.4 %BW) and (420, 390, 380 and 375 g/day), respectively. Most importantly, the cost productions was significantly differently and lower in dairy heifers receiving T3 than T4, T2, T1 (29.4, 27.4, 30.7 and 32.3 baht/day). This data indicated that yeast fermented cassava chip supplementation replace concentrate had no effect on feed-intake in dairy heifers. This result was in agreement with earlier work by Khampa *et al*. (2009) which reported that inclusion of cassava chip in diets resulted in satisfactory animal performance and had no negative effects on animal health in finishing beef cattle and lactating dairy cows.

Characteristics of ruminal fermentation and blood metabolism: Rumen ecology parameters were measured for temperature, pH and NH_3-N, VFA (Table 3). In addition, BUN was determined to investigate their relationships with rumen NH_3-N and protein utilization. Rumen pH at 0, 2 and 4 h post-feeding were unchanged by dietary treatments and the values were quite stable at 6.6-6.7, but all treatment means were within the normal range which has been reported as optimal for microbial digestion of fiber and also digestion of protein (6.0-7.0) (Hoover, 1986). Ruminal NH_3-N and BUN concentrations were non-significantly different among treatments by levels supplementation of yeast fermented cassava chip replaced concentrate. As NH_3-N is regarded as the most important nitrogen source for microbial protein synthesis in the rumen. In addition, the result obtained was closer to optimal ruminal NH_3-N between at 15-30 mg% (Wanapat and Pimpa, 1999; Chanjula *et al*., 2004) for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminant fed on low-quality roughages.

Rumen microorganisms populations: Table 4 presents rumen microorganism populations. The populations of fungal zoospores, protozoa and total bacteria direct

Table 3: Effects supplementation levels of Yeast Fermented Cassava Chip (YFCC) on feed-intake and rumen fermentation in dairy heifers

	Treatments[1]					Contrast[2]		
Item	T1	T2	T3	T4	SEM	L	Q	C
DM intake (%BW)								
Concentrate	1.5[a]	1.1[b]	0.7[c]	0.3[d]	0.06	*	NS	NS
YFCC	0[a]	0.3[b]	0.7[c]	1.1[d]	0.07	*	NS	NS
Rice straw	1.4[a]	1.5[ab]	1.7[b]	1.6[ab]	0.07	*	NS	NS
Total	2.9	2.9	3.2	3.1	0.13	NS	NS	NS
ADG (g/day)	375[a]	380[a]	420[b]	390[c]	2.75	*	*	NS
Cost productions (baht/day)	32.3[a]	30.7[ab]	29.4[ab]	27.4[b]	0.65	*	NS	NS
Ruminal pH	6.7	6.6	6.6	6.6	0.09	NS	NS	NS
NH_3-N (mg%)	17.8	18.1	20.4	18.4	1.35	NS	NS	NS
BUN (mg%)	9.2	10.6	11.4	10.2	0.89	NS	NS	NS

[a,b,c]Values on the same row with different superscripts differ ($p<0.05$).
[1]T1 = Supplementation of concentrate:YFCC ratio at 100:0 T2 = Supplementation of concentrate:YFCC ratio at 75:25
T3 = Supplementation of concentrate:YFCC ratio at 50:50 T4 = supplementation of concentrate:YFCC ratio at 25:75
L = linear, Q = quadratic, C = cubic. * = $p<0.05$, NS = $p>0.05$

Table 4: Effects supplementation levels of Yeast Fermented Cassava Chip (YFCC) on rumen microorganisms in dairy heifers

	Treatments[1]					Contrast[2]		
Total direct counts (cell/ml)	T1	T2	T3	T4	SEM	M	Y	M x Y
Bacteria ($x10^{12}$)	4.7[a]	6.2[b]	9.7[c]	7.2[b]	0.37	*	NS	*
Protozoa								
Holotric ($x10^3$)	8.2[a]	5.7[b]	4.4[c]	6.1[b]	0.35	*	NS	NS
Entodiniomorph ($x10^6$)	8.0[a]	6.7[b]	5.8[b]	6.4[b]	0.27	*	NS	NS
Fungal zoospores ($x10^6$)	5.0[a]	6.6[b]	8.7[c]	7.3[d]	0.21	*	NS	*

[a,b,c]Values on the same row with different superscripts differ ($p<0.05$).
[1]T1 = Supplementation of concentrate:YFCC ratio at 100:0 T2 = Supplementation of concentrate:YFCC ratio at 75:25
T3 = Supplementation of concentrate:YFCC ratio at 50:50 T4 = Supplementation of concentrate:YFCC ratio at 25:75
L = linear, Q = quadratic, C = cubic. * = $p<0.05$, NS = $p>0.05$

counts were significantly different and populations of bacteria had higher numbers in heifer receiving diets T3 than T4, T2 and T1. In contrast, the present number of protozoa in the rumen was decreased by yeast fermented cassava chip supplementation replace concentrate diets. In the experiment by Guedes *et al.* (2007) reported that yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion and increased flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-grain diets. As cassava chip can be readily degraded in the rumen and ruminal pH was decreased, malate could stimulate lactate utilization by *S. ruminantium* and could improve pH in the rumen. It is possible that supplementation of malate with yeast may play an important role in increasing bacterial populations.

Conclusion: Based on this experiment, it could be concluded that supplementation of yeast *(Saccharomyces cerevisiae)* fermented cassava chip replace concentrate could improve ruminal fermentation efficiency, average daily gain and reduced cost production in daily heifers. Moreover, supplementation of yeast fermented cassava chip in diet resulted increase populations of bacteria, but decreased protozoal populations. These results suggest that supplementation of yeast fermented cassava chip could highest replace at 75% of concentrate in dairy heifers.

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Banana Frozen Yoghurt from Camel Milk

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Abstract: This study is conducted in the dairy laboratory of the faculty of Agricultural Technology and Fish Sciences, Al-Neelain University. The objective of this study was to investigate the possibility of manufacture frozen yoghurt from camel milk with banana flavor and investigate the suitable levels of banana which were 10, 12 and 14%. pH value, fat%, total solids, solids not fat, specific gravity, overrun, sensory properties and acceptability were studied. The result of chemical analysis showed no significant differences between the three levels of banana on pH value, fat %, total solids, solids not fat at $p>0.05$, while the differences were significant in specific gravity and overrun. The panel test showed that there was no significant differences between three levels of banana on sensory evaluations at $p>0.05$. 14% obtained the best score.

Key words: Frozen, yoghurt, camel, milk, banana

INTRODUCTION

Camel milk is very important source of nutrient for human in several arid and semi arid zones (Schwartz, 1992), it is complex mixture of fat, protein, lactose, minerals and vitamins (Schwartz and Dioli, 1992) and miscellaneous constituents dispersed in water (Ibrahim, 1998). In the traditional pastoral communities, camel milk is consumed fresh or fermented (Farah, 1996). Frozen yoghurt is a yoghurt product, with or without flavor, it is freeze in ice cream freezers to obtain 50% overrun (Abu Lehia and Abu Tarboush, 1995). The food value of frozen yoghurt obviously depends upon the food value of ingredients involved, the ingredients which go into the mix contain the same constituents of ice cream and ordinary yoghurt, but in different amounts (Rea, 1983).

The manufacture of butter, ghee, cheese and ice cream from camel milk is still not well developed and accepted (Farah, 1996). The objective of this study was to investigate the possibility of manufacture of frozen yoghurt from camel milk with banana flavor and investigate the suitable levels of banana which will be added to the product.

MATERIALS AND METHODS

Ingredients: Camel milk was obtained from west Omdurman camel farms. Skim milk powder, sugar, banana, gelatin, color and cream were obtained from the local market.

Preparing of banana: According to Arbuckle (1977) banana washed, peeled and sliced, then mixed with pasteurized sucrose (50%) of the required amount and held at 40° F for 24 h, it was mashed and added to plain mix prior to freezing.

Method of manufacture frozen yoghurt: The method used in preparing frozen yoghurt was mentioned by Nadia (2007).

- The required amount of milk solids not fat (140 g), gelatin (5 g) and half the amount of granulated sucrose (65 g) needed were dry blended, then mixed with milk.
- The mixture was pasteurized at 80°C for 30 min.
- Cooled to 40°C.
- Inoculate with active yoghurt culture starter at the rate of (1-3%).
- Incubate for 4 h at 40°C.
- The yoghurt cooled to 5°C again for 24 h. The other half of the amount of sucrose was mixed with 100 ml milk which was subtracted from the needed amount of milk and pasteurized at 80°C for 25 sec (Arbuckle, 1977) then cooled to 5°C.
- Sugar solution and banana were added to yoghurt mix, prior to freezing.
- The product was packaged in cups 45 ml in volume.
- Hardening held at -25°C for 24 h.

Chemical analysis: The pH of frozen yoghurt was determined by using pH meter (HANNA-instrument. model 209 Bench meter). Fat content, total solids and specific gravity was determined according to AOAC (1990).

Sensory evaluations: Frozen yoghurt was scored by a regular score panel from the staff members and students of the faculty of agricultural technology and fish sciences, Al-neelain University, scoring was carried out for appearance, texture, color, flavor and overall acceptability.

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Table 1: Proximate chemical composition of frozen yoghurt from camel milk with different levels of banana

Composition	10% Banana	12% Banana	14% Banana	Sig. level
Fat	1.83±0.30	1.66±0.25	1.80±0.30	NS
pH	4.26±0.11	4.06±0.78	4.26±0.15	NS
Total solids	38.03±1.00	37.5±1.2	37.9±0.7	NS
Solids not fat	36.2±1.27	35.8±1.4	36.1±0.7	NS
Specific gravity	0.940±0.35	0.955±0.18	0.977±0.23	*
Overrun	47.4±5.2	34±7.2	29.7±6.4	*

NS = Non Sig.; *Sig. ($p<0.05$)

Table 2: Effect of banana percent on properties of frozen yoghurt from camel milk

Properties studied	10% Banana	12% Banana	14% Banana	Control	Sig. level
Appearance (9 point)	7.46±0.61	7.06±0.80	7.93±0.23	7.33±0.83	NS
Texture (9 point)	8.13±0.23	7.40±1.11	7.73±0.30	7.60±0.20	NS
Color (9 point)	7.33±0	7.46±0.75	7.80±0.20	7.06±1.10	NS
Flavor (9 point)	7.53±0.80	6.80±1.92	7.86±0.41	7.26±1.00	NS
Overall acceptability (9 point)	7.40±0.86	7.43±0.90	7.73±0.25	7.23±0.81	NS

NS = Non Sig.; ($p<0.05$)

Statistical analysis: The collected data was subjected to statistical analysis program, SPSS. Analysis of Variance (ANOVA) was used to find out the significant difference between the three level of banana used in the manufacture the frozen yogurt from camel milk and to test out the sensory characteristics of the product.

RESULTS AND DISCUSSION

Table 1 presents the proximate chemical composition of frozen yoghurt from camel milk with levels of 10%, 12%, 14% banana. The averages fat % of the resultant frozen yoghurt were (1.83±0.30), (1.66±0.25), (1.80±0.30) respectively. pH values were (4.26±0.11), (4.06±0.78), (4.26±0.15) respectively, total solids were (38.03±1.00), (37.5±1.2), (37.9±0.7) respectively, solids not fat were (36.2±1.27), (35.8±1.4), (36.1±0.7) respectively, specific gravity were (0.940±0.35), (0.955±0.18), (0.977±0.23) respectively.

The results showed that there is no significant differences between 10%, 12%, 14% banana at $p>0.05$ in fat%, pH, total solids, solids not fat values, while the differences were significant in specific gravity and overrun %. The high value of specific gravity obtained by 14% banana with mean 0.977±0.27, high percent of overrun obtained by 10% banana with mean 47.4±5.2, this result might be due to banana composition which contain high starch percent, that decrease the ability of whipping air into the mix.

Regarding sensory evaluations of banana frozen yoghurt from camel milk the data are given in Table 2 declared that the best score of appearance (7.93±0.23), color, (7.80±0.20), flavor, (7.86±0.41) were obtained by 14% banana and the best score of texture (8.13±0.23) was obtained by 10% banana. In general observation, 14% banana frozen yoghurt was more acceptable and palatable among consumers.

Conclusion and recommendation: According to the results of this study, it could be concluded that it is possible to manufacture banana frozen yoghurt from camel milk as fallows 5% fat, 13% sugar, 0.5% gelatin, 14% SNF and 14% banana, it was more suitable and obtained the best score, more study recommended.

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Trace Metals Distribution in Some Common Tuber Crops and Leafy Vegetables Grown in the Niger Delta Region of Nigeria

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Abstract: The main sources of trace metals to plants are the air and soil media from which trace elements are taken up by the root or foliage. Understanding the distribution of some trace metals in some common leafy vegetables and tuber cops is important for establishing baseline concentrations from which anthropometric effects can be measured. The trace metal distribution in some selected leafy vegetables and tuber crops in our study area were determined in samples that were dried, milled and digested. All the minerals investigated were found present in all the components of the selected vegetables and tuber crops. Iron was the most abundant mineral in the vegetables with concentrations ranging between 0.32mg/kg in *Ocimum gratissum* root to 9.7 mg/kg in *Telfaria occidentalis* roots. In the root tubers, zinc was the most abundant mineral ranging from 0.62 mg/kg in *Manihot esculenta* stem to 1.97 mg/kg in *Manihot esculenta* leaf. The bioconcentration factor indicates that the roots of the food crops concentrate most of the metals than the stems and leaves.

Key words: Trace metals, leafy vegetables, tuber crops, Niger Delta Region

INTRODUCTION

Trace metals analysis are an important part of environmental pollution studies (Loska *et al*., 2000; Chibowski, 2000; Solecki and Chibowski, 2000; Narin *et al*., 1998; Czarrnowska and Milewska, 2000). Some trace metals are essential in plant nutrition, but plants growing in a polluted environment can accumulate trace elements at high concentrations, causing a serious risk to human health (Vousta *et al*. 1996; Sharma *et al*., 2004). The main sources of trace metals to plants are the air and soil from which metals are taken up by the root or foliage. The uptake of metal concentration by roots depends on speciation of metal and soil characteristics and type of plant species etc. Consequently, metal mobility and plant availability are very important when assessing the effect of soil contamination on plant metal uptake, as well as translocation and toxicity or ultra structural alterations (Chandra Sekhar *et al*., 2001).

Atmospheric metals are deposited on plant surfaces by rain and dust. Several authors have shown a relationship between atmospheric element deposition and elevated element concentrations in plants and top soils, especially in cities and in the vicinity of emitting factories (Andersen *et al*., 1978; Pilegaard, 1978; Larsen *et al*., 1992; Sunchez *et al*., 1994; Srinivas *et al*., 2002). Airborne submicron particles are also filtered out on plant surfaces, constituting a substantial, but unknown contribution to the atmospheric supply. Indirect effects of air pollutants through the soil are also great interference, because of the large scale sustained exposure of soil to both wet and dry depositions of trace elements.

Widespread interest in trace metal contamination in plant systems has emerged only over the last three decades and several research articles reported concentrations of a number of trace elements in the local crops and other plants as a consequence of anthropogenic emissions (Bernard *et al*., 2004a; Bernard *et al*., 2004b). The consequence of trace metals in foods such as vegetables and tubers have been a considerable interest because of their toxicity effect which are important in human beings (Asaolu, 1995). Also, information on the distribution of some of these metals in the various components of the vegetables and tubers contains the highest level of minerals and in assessing the nutritional and medicinal value of the various components for appropriate application. Equally, any component could serve as pollution indicators for some metals. The data will be useful for assessing trace metals contamination and determining the need for remediation. The results obtained from the study would also provide information for background levels of metals in the plants in the study area.

MATERIALS AND METHODS

Study area: Amai, a semi urban area in Delta State, Nigeria and the host community of a campus of Novena University was selected as the study area. Amai is surrounded by other urban and semi urban towns some of where human activities that introduce pollutants into the ecosystem takes place.

Sample collection: Four common and widely consumed Nigerian Leafy vegetable and two common and widely

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consumed tuber crops were selected for analysis. The varieties of the leafy vegetables are: *Telfaria occidentalis, Verona amygdalina, Ocimum gratissum* and *Talinum triangulare*. The varieties of the tuber crops are: *Discorea alata* and *Manihot esculenta*. The vegetables and tuber crops were obtained from local farmers at Amai, host community of Novena University. Soil sample was also collected from the farm.

Sample preparation: The roots, stems and leaves were separated in each case and the components were cut into pieces, washed, air dried for one week and then dried in the Oven (Gallen Kamp, England) at 80°C for 6 h. About 10 g of the dried materials of each component were powdered in a hammered mill. The powdered sample from each component was packaged in glass bottles and stored a 4°C in a refrigerator. About 1.0 g of dried and ground soil samples were placed inside a crucible and ignited in a muffle furnace at 500°C for 3 h. The ignited mass was cooled inside desiccators and transferred into a 100 ml Borosil beaker and kept in a desiccator until analysis.

Analysis of trace metal concentration: Trace metals concentrations were determined according to AOAC methods (AOAC, 1990). A quantity, 1.0 g of each powdered plant components were weighed and put in a Pyrex crucible and 10 ml of pure HNO_3 was added. This was incinerated in a GallenKamp Oven at 250°C for 18 h and then diluted to volume of 25 ml with water. Samples were filtered through a Filter paper. The digest were analyzed for Trace metals content by atomic absorption spectrophotometer (Buck Scientific Model-210). The soil inside the beaker was added 10ml of concentrated HCl and the suspension was swirled. The suspension was kept inside a thermostat controlled water bath in a temperature range of 70-80°C for 1 h. The supernatant was decanted and kept inside a 100 ml volumetric flask. This contains mostly alkaline earth metal. To the residue in the beaker, 10 ml each of the HCl (concentrated) and $HClO_4$ (concentrated 70% Pure) and few porous beads were added and were evaporated to complete dryness over a hot plate. This procedure was repeated when necessary. The dried residue was dissolved completely by using minimum amount of concentrated HCl. This solution was then transferred to the same volumetric flask where previous extract containing alkaline earth metal extract was stored. The flask were then made up to the mark by distilled water and stored inside a refrigerator. This extracts were analyzed for trace metals concentration by atomic absorption spectrophotometer (Buck Scientific Model-210).

Statistical analysis: Data were presented as mean of duplicate determinations.

Table 1: Concentrations of trace metal in (mg/kg) soil sample

	Elements				
Sample	Fe	Zn	Ni	Cd	Pb
Soil	20.0	3.20	0.40	0.10	1.20

Table 1 shows the trace metal concentration in soil sample from the study area. The result obtained showed that the trace metals concentration were decreased in the sequence Fe>Zn>Pb>Ni>Cd

RESULTS AND DISCUSSION

Trace metal contamination is of concern due to its effects as a carcinogen. Understanding the distribution of some trace metals in some common leafy vegetables and tuber crops is important for establishing baseline concentrations from which anthropogenic effects can be measured. All the trace metals analyzed were found present in the soil sample with Iron having the highest concentration. It has been reported that Iron occurs at high concentration in most Nigerian soils (Adefemi *et al*., 2008). The trace metals concentrations of lead, iron, zinc, cadmium and nickel in the present study are presented in Table 1 and are within the permissible limits for agricultural soils (Alloway, 1990; Aswatharayana, 1999). This indicates that, despite the close proximity of the cultivated land to high pollution sources, agricultural soils does not seem to have been contaminated by atmospheric deposition. This may be due to low deposition rate resulting from the dispersion of atmospheric pollutants and variations in soil physico-chemical characteristics (Srinivas *et al*., 2009).

All the minerals examined were found to be present in the various components of the selected vegetable and tuber crops. On the average, iron is the most abundant mineral; lead was the least in the various components of the vegetables and tuber crops. Analysis of the data in the present study showed that the leaves of *Ocimum gratissum* and *Verona amygdalina* accumulated more iron from the soil than the root and stem as presented in Table 2 and further confirmed by the data on the bioconcentration factor presented in Table 3. The high concentration of iron in *V. Amygdalina* leaves, followed by *O. Gratissum* leaves might be due to the participation of the green vegetables in the synthesis of ferrodoxin, an attribute which makes them useful sources of iron (Hart *et al*., 2005). This result are not totally consistent with literature reports which indicate that the highest concentration of heavy metals in food crops occur in lateral roots than main roots, rhizomes, leaves and the lowest in shoots (Romera *et al*., 2005). The environmental pollution of zinc greatly influences the concentrations of this metal in plants. In ecosystems where zinc is an airborne pollutant, the tops of plants are likely to concentrate more zinc; on the other hand, plants grown in zinc contaminated soils accumulate a great proportion of the metal in roots (Kabata-Pendias and Pendias, 1992). In this study, zinc concentration reported for the plant components were within the permissible

Table 2: Trace metal distribution in (mg/kg) vegetable dry matter

	Fe			Zn			Ni			Cd			Pb		
Sample	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
V1	9.77	6.44	1.17	0.46	0.21	0.53	0.51	0.34	0.37	0.60	0.32	0.56	0.82	0.01	<0.01
V2	0.39	0.42	6.37	0.01	0.22	1.99	0.24	0.19	0.21	0.78	0.82	0.90	0.03	0.02	<0.01
V3	3.22	3.01	3.79	1.32	1.29	1.44	0.30	0.28	0.31	0.06	0.03	0.07	0.08	0.07	0.06
V4	0.65	0.55	0.50	2.63	2.10	1.92	0.41	0.29	0.20	0.60	0.52	0.46	0.02	<0.01	<0.01

V1 = *Telfaria occidentalis*, V2 = *Veronia amygdalina*, V3 = *Ocimum gratissum*, V4 = *Talinum triangulare*.

Table 2 shows the distribution of trace metals in the various parts of the leafy vegetables. The result obtained showed that the leaves of *Veronia amygdalina* and *Ocimum gratissum* accumulated more iron and zinc than the stem and root.

Table 3: Bioconcentration factor in vegetable samples

	Fe			Zn			Ni			Cd			Pb		
Sample	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
V1	0.48	0.32	0.05	0.14	0.06	0.16	1.29	0.87	0.94	6.04	3.21	5.66	0.68	0.01	ND
V2	0.01	0.02	0.31	0.01	0.07	0.62	0.60	0.49	0.52	7.88	8.22	9.08	0.02	0.01	ND
V3	0.16	0.15	0.18	0.41	0.40	0.45	0.75	0.70	0.77	0.60	0.30	0.76	0.06	0.06	0.05
V4	0.03	0.02	0.02	0.82	0.65	0.60	1.04	0.74	0.50	6.06	5.20	4.62	0.01	ND	ND

V1 = *Telfaria occidentalis*, V2 = *Veronia amygdalina*, V3 = *Ocimum gratissum*, V4 = *Talinum triangulare*. ND = Not Determined.

$$\text{Bioconcentration factor} = \frac{\text{Concentration in plant tissue}}{\text{Concentration in soil sample}}$$

Table 3 shows the bioconcentration factor in the various parts of the leafy vegetables. The result obtained showed that the roots concentrated more of the trace metals than the stems and leaves.

Table 4: Trace metal distribution in (mg/kg) tuber crops dry matter

	Fe			Zn			Ni			Cd			Pb		
Sample	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
T1	0.60	0.81	1.30	1.21	1.01	0.62	0.16	0.21	0.30	0.33	0.22	0.48	<0.01	<0.01	<0.01
T2	1.34	1.32	1.30	0.71	0.62	1.79	0.60	0.54	0.45	0.89	0.69	0.62	0.10	0.08	0.02

T1 = Discorea alata, T2= Manihot esculenta. Table 4 shows the distribution of trace metals in the various parts of the tuber crops. The result obtained showed that the leaves of *Discorea alata* concentrated more iron than the stem and root while the leaves of *Manihot esculenta* concentrated more zinc than the stem and root

Table 5: Bioconcentration factor in tuber crops

	Fe			Zn			Ni			Cd			Pb		
Sample	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
T1	0.03	0.04	0.06	0.37	0.31	0.19	0.40	0.52	0.76	3.32	2.24	4.88	ND	ND	ND
T2	0.06	0.06	0.06	0.22	0.19	0.55	1.50	1.35	1.14	8.93	6.90	6.20	0.08	0.06	0.01

T1 = *Discorea alata*, T2 = *Manihot esculenta*

$$\text{Bioconcentration factor} = \frac{\text{Concentration in plant tissue}}{\text{Concentration in soil sample}}$$

Table 5 shows the bioconcentration factor in the parts of the tuber crops. The result obtained showed that the roots concentrated more of the trace metals than the stems and leaves

limits (10-50 mg/kg) for human consumption (Samara *et al*., 1992). The leaves of *T. Occidentalis*, *T. gratissum and V. amygdalina* accumulated more zinc as indicated by the data on bioconcentration factors presented in Table 3. While the essential elements iron and zinc are desirable in the nutrition of man, animals and plants, their presence could reduce the bioavailability of lead; their undue presence in food could be harmful (Davidson *et al*., 1979; Udosen *et al*., 1990). The high storage of iron and zinc in the leafy part of some of the vegetables might be advantageous for their useful biochemical functions in human nutrition (Asaolu and Asaolu, 2010). Studies have shown that the excessive intake of zinc and iron results to vomiting, dehydration, electrolyte imbalance and lack of muscular co-ordination (WHO, 1984).

In general, lead concentration in food crops has increased in recent decades owing to human activities (Srinivas *et al*., 2009). For all the trace elements analyzed and presented in Table 2 and 4, lead was the least abundant in vegetables and tuber crops. The permissible limit of lead in vegetables for human

consumption is 2.0-2.5 mg/kg dry weight (Samara *et al*., 1992). The concentration of lead in the vegetables and tuber crops were found to be below the permissible limits. Plants are known to have more nickel than animal products (Kabata-Pendias and Pendias, 1992). The concentration of nickel on plants generally ranges from 0.05-5.0 mg/kg dry weight. According to WHO (1984), the nickel concentration in vegetables and fruits should be within the range of 0.02-2.7 mg/kg. The concentration of nickel in vegetables and tuber crops are presented in Table 2 and Table 3 were found to be within the permissible limit. The concentration of cadmium in the various plant components in the present study is low and is below the FAO/WHO limit for cadmium toxicity (5-30 mg/kg) (Awofulu, 2005).

Conclusion: This study has revealed low concentration of heavy metal lead, with correspondingly high levels of iron and zinc in the various food crops harvested at the study areas. The high storage of iron and zinc as indicated by the bioconcentration factor in the leaf of some of the vegetables might be advantageous for their useful biochemical function in human system. Although the essential elements are beneficial to man and plants, when found in food can prove detrimental to health. This is more so when they exist in commonly consumed food crops, particularly the green vegetables which are generously consumed by all households especially during the season of abundance.

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Micro Nutrient Content of Selected Indigenous Soups in Nigeria

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Abstract: The micronutrient content of selected standardized indigenous soups from the six geopolitical zones of Nigeria was analyzed with reference to iron, calcium, phosphorus, sodium and potassium contents of the soups. Results revealed a high iron level in ogbono soup; Egusi + vegetable soup showed a very low level of calcium, while afang and soko soup had a relatively high level; Turkey stew and ogbono soup had a very high level of phosphorus, while the other soups had adequate levels except for egusi + vegetable soup which was extremely low; while egusi + vegetable, onugbu, edikang-ikong and miyan kuka soups had high levels of potassium. This study has established that the selected soups from the six geopolitical zones of Nigeria are good sources of micronutrients.

Key words: Micronutrients, indigenous soups, Nigeria

INTRODUCTION

There is growing interest in the role of the micronutrients (essential trace elements and vitamins) in optimizing health and in prevention or treatment of disease (Field *et al*., 2002). This stems partly from the increase in knowledge and understanding of the biochemical functions of these nutrients (Shenkin, 2006). Micronutrients have been reported to play an important role in mounting immune response and deficiency of single micronutrients alone, or in combination with other micronutrients, substantially increase the risk of having a poor immune response to infection (Walker, 2000; Black, 2001). They also influence adult and child productivity as well as educational achievement, child survival and maternal health (FSAU, 2005).

Dietary intakes in developing countries usually consist of plant-based staple foods which are monotonous with little variation. Methods of preparation and storage of these foods often leads to large loss of micro nutrients thus creating a deficiency risk of micro nutrient. Owing to the importance of micronutrients, efforts have been geared towards the study of the mineral composition of prepared Nigerian foods (Akindahunsi and Oboh, 1999; Elemo *et al*., 2010a).

Nigeria is multi-cultural society with different traditional soups which are indigenous to the different ethnic and cultural society. This study therefore aims at determining the iron, calcium, phosphorus, sodium and potassium contents of selected standardized indigenous soups from the six geopolitical zones of Nigeria. This is to promote consumption of soups which are rich naturally in micronutrient within different ethnic groups so as to reduce occurrences of some micronutrient deficiencies.

MATERIALS AND METHODS

Ingredients: Ingredients used were purchased from local markets at Mushin, Oto and Oshodi all in Lagos, Nigeria.

Preparation of soups: The selected soups from the six geopolitical zones are presented in Table 1. The dishes were prepared in the kitchen facilities of the department of food and analytical services of the Federal Institute of Industrial Research, Oshodi, Nigeria. The preparation methods used for the selected recipes are those earlier established by cookery and recipe books (Food specialties, 1988; FIIRO, 2006), with some modifications by the indigents of the geopolitical zones familiar with such soups. Each dish was prepared in triplicates and analysis carried out on fresh weight basis.

Sample collection and preparation: Each soup was cooled to room temperature and equal portion of the dishes were homogenized with a warring blender. Samples of the meals were analyzed in-situ for moisture and ash contents at 105 and 550 degrees Celsius in air-oven and muffle furnace respectively.

Determination of moisture content: This was carried out according to methods described by AOAC (1999).

Mineral analysis: 1 gram of each soup sample was transferred to acid washed crucibles and dry-ashed in a muffle furnace at 550^{o}C initially for 2 h until well ashed. The ash was dissolved and made up into 100ml solution prior analysis.

The iron, phosphorus, calcium, sodium and potassium contents of all the food samples were determined on aliquots of the solutions of the ash by UV/Visible and atomic absorption spectrophotometers (AOAC, 1999). Replicates of soup composites were analyzed to check the homogeneity of the portions sampled from the soup and the reproducibility of the method. The accuracy of the method was studied by analyzing Orchard Leaves Standard, Reference Material No. 1571, National Bureau of Standards (NBS). Phosphorus was determined by the molybdo-vanadate solution method (AOAC, 1999).

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Table 1: Selected indigenous soups from the six geopolitical zones of Nigeria

South-South	South-East	South-West	*North	General
Afang	Oha	Tete	Miyan kuka	Chicken stew
Edikang Ikong	Ogbono	Soko	Groundnut	Turkey stew
Nsala	Onugbu	Ewedu	§G. nut + vegetable	Beef stew
Banga	Egusi + ugu	Gbegiri	Beans + vegetable	Fish stew

*North: this consists of North Central, North East and North West geopolitical zones. §G. nut: Groundnut

Statistical analysis: Data were reported as mean±standard deviation. Statistical analyses were carried out using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, IL.USA).

RESULTS

The micronutrient composition of selected soups consumed in South-South geopolitical zone of Nigeria is shown in Table 2. Edikang-Ikong had the highest concentration of Iron (6.30±0.25 mg/100 g); this was followed by afang soup (4.20±0.40 mg/100 g), while nsala soup was observed to have the lowest concentration (1.40±0.40 mg/100 g). Afang soup was observed to have the highest concentration of calcium (850±5.00 mg/100 g) with edikang-ikong having the lowest concentration (120±2.52 mg/100 g). The phosphorus concentration of afang and banga soups were observed to be high, while a very high concentration was observed in nsala soup (790±1.15 mg/100 g). Both afang and banga soups had the same concentration of sodium (470±10.00 mg/100 g); edikang-ikong was observed to have the lowest concentration (230±4.58 mg/100 g). Edikang-Ikong was observed to have a very high concentration of calcium (710±4.16 mg/100 g).

Table 3 shows the micronutrient composition of selected soups consumed in South-East geopolitical zone of Nigeria. Ogbono was observed to have the highest concentration of Iron (11.00±2.08 mg/100 g), with onugbu having the lowest concentration (2.00±0.25 mg/100 g). A very low concentration of calcium was observed in Egusi + ugu (4.00±0.31 mg/100 g), while onugbu had the highest concentration (320±1.15 mg/100 g). Phosphorus was observed to be very low in Egusi + ugu (46.0±1.32 mg/100 g) compared to ogbono which had a rather very high concentration (1840±2.65 mg/100 g). Onugbu was observed a very high concentration of sodium (274.4±5.03 mg/100 g) with egusi + ugu having the lowest (160±1.50 mg/100 g). A high concentration of potassium was observed in egusi + ugu with onugbu having the highest concentration (840±1.15 mg/100 g).

The micronutrient composition of selected soups consumed in the South-West geopolitical zone of Nigeria is presented in Table 4. Ewedu was observed to have the highest concentration of iron (4.60±0.17 mg/100 g), this was followed by tete (3.20±0.12 mg/100 g); while a rather low concentration was observed in gbegiri. Soko was observed to have the highest concentration of calcium (500±1.15 mg/100 g), while gbegiri had the lowest (75.0±1.52 mg/100 g). Ewedu had the lowest concentration of phosphorus (170±0.58 mg/100 g) compared to soko (650±2.08 mg/100 g) which was highest. Gbegiri, soko and tete had higher concentrations of sodium, with gbegiri having the highest concentration (490±2.31 mg/100 g). A concentration of potassium was observed in ewedu (570±1.73 mg/100 g), while tete had the lowest concentration (128±1.15 mg/100 g).

The micronutrient composition of soups consumed in northern Nigeria is presented in Table 5. Miyan kuka was observed to have the highest concentration of iron (3.10±0.26 mg/100 g), while groundnut + vegetable had the lowest concentration (1.20±0.20 mg/100 g). Groundnut + vegetable was observed to have the highest concentration of calcium (190±2.64 mg/100 g), while beans + vegetable and groundnut had the same concentration (90.0±1.15 mg/100 g). Groundnut + vegetable had the highest concentration of phosphorus (540±1.52 mg/100 g), while miyan kuka had the lowest concentration (180±1.65 mg/100 g). All soups were observed to have close range of sodium concentration except groundnut + vegetable (165±1.15 mg/100 g). Miyan kuka was observed to have the highest concentration of potassium (840±1.15 mg/100 g).

Table 6 shows the micronutrient composition of selected soups general consumed in Nigeria. Both turkey and beef stews were observed to have the same level of iron concentration (5.00 mg/100 g), while a close range was also observed in chicken and fish stews (11.00 and 10.00 mg/100 g). Turkey stew had the highest concentration of calcium (200±1.15 mg/100 g), while beef stew had the lowest concentration (110±1.52 mg/100 g). A rather very high concentration of phosphorus was observed in turkey stew (1830±1.27 mg/100 g), while chicken stew had the lowest concentration (100±1.04 mg/100 g). Chicken stew was observed to have the highest concentration of sodium (190±2.89 mg/100 g), with the others had concentrations of close ranges. Potassium concentration was observed to be low (140±1.15 mg/100 g) compared to that of fish stew (430±1.78 mg/100 g) which was highest.

Table 2: Micronutrient composition of soups from South-South geopolitical zone of Nigeria

Soups	Moisture	Ash	Iron	Calcium	Phosphorus	Sodium	Potassium
Afang	76.92±0.88	2.10±0.30	4.20±0.40	850±5.00	550±4.04	470±10.00	110±3.61
Banga	77.30±1.59	3.18±0.25	3.00±0.66	180±7.64	510±6.81	470±11.53	70±3.61
Edikang Ikong	76.25±0.52	4.50±0.76	6.30±0.25	120±2.52	700±6.81	230±4.58	710±4.16
Nsala	78.1±0.30	3.28±0.25	1.40±0.40	310±2.52	790±1.15	300±1.53	100±1.53

Data = mean±SD; n = 3. Values of minerals are in mg/100 g

Table 3: Micronutrient composition of soups from South-East geopolitical zone of Nigeria

Soups	Moisture	Ash	Iron	Calcium	Phosphorus	Sodium	Potassium
Egusi + ugu	63.11±0.25	2.94±0.05	3.00±0.11	4.00±0.31	46.0±1.32	160±1.50	260±4.04
Oha	77.30±1.59	2.84±0.05	4.00±0.30	250±1.15	480±1.53	430±2.52	30±1.15
Ogbono	70.44±0.27	6.05±0.13	11.00±2.08	120±2.08	1840±2.65	250±0.58	82.8±0.40
Onugbu	74.23±0.52	4.67±0.28	2.00±0.25	320±1.15	770±1.15	2744±5.03	840±1.15

Data = mean±SD; n = 3. Values of minerals are in mg/100 g

Table 4: Micronutrient composition of soups from South-West geopolitical zone of Nigeria

Soups	Moisture	Ash	Iron	Calcium	Phosphorus	Sodium	Potassium
Ewedu	90.92±0.06	2.07±0.03	4.60±0.17	120±1.25	170±0.58	240±1.00	570±1.73
Gbegiri	73.84±0.21	1.54±0.05	0.80±0.11	75.0±1.52	218±1.15	467±1.53	475±1.53
Soko	69.35±0.08	2.97±0.05	1.40±0.06	500±1.15	650±2.08	490±2.31	180±3.06
Tete	76.86±0.05	2.94±0.03	3.20±0.12	160±1.53	480±1.26	420±1.70	128±1.15

Data = mean±SD; n = 3. Values of minerals are in mg/100g.

Table 5: Micronutrient composition of soups from *Northern Nigeria

Soups	Moisture	Ash	Iron	Calcium	Phosphorus	Sodium	Potassium
Beans + Vegetable	74.67±0.63	1.36±0.03	1.80±0.13	90.0±1.15	304±1.53	467±1.15	480±1.53
Groundnut	60.58±0.12	1.76±0.05	1.90±0.30	90±1.15	470±1.53	479±4.04	483±1.15
Groundnut + Vegetable	60.67±0.05	1.87±0.05	1.20±0.20	190±2.64	540±1.52	165±1.15	500±1.78
Miyan Kuka	78.33±0.09	1.99±0.06	3.10±0.26	120±1.53	180±1.65	480±2.08	840±1.15

*Northern Nigeria: this consists of North Central, North East and North West geopolitical zones.
Data = mean±SD; n = 3. Values of minerals are in mg/100 g

Table 6: Micronutrient composition of soups generally consumed in all the six geopolitical zones of Nigeria

Soups	Moisture	Ash	Iron	Calcium	Phosphorus	Sodium	Potassium
Beef stew	40.16±0.03	2.18±0.11	5.00±0.29	110±1.52	270±1.15	150±2.87	320±1.53
Chicken stew	57.78±0.05	4.69±0.10	1.10±0.10	190±2.51	100±1.04	190±2.89	140±1.15
Fish stew	40.30±0.25	3.95±0.10	1.00±0.12	130±2.65	500±1.53	140±1.73	430±1.78
Turkey stew	41.96±0.05	6.47±0.05	5.00±0.50	200±1.15	1830±1.27	160±1.00	200±1.73

Data = mean±SD; n = 3. Values of minerals are in mg/100 g

DISCUSSION

Dietary studies in developing countries have consistently shown that multiple micronutrient deficiencies, rather than single deficiencies, are common and that low dietary intake and poor bioavailability of micronutrients account for the high prevalence of these multiple deficiencies (MI, 2000). This paper reports the mineral contents of selected indigenous soups from the six geopolitical zones of Nigeria.

The metabolic roles of minerals and the amounts of them in the body vary considerably (Wardlaw, 1999). Ogbono appeared to have an exceptionally high level of iron probably due to the ingredients and inclusion of meat which is a source of iron (Onabanjo and Oguntona, 2003). Chicken and fish stews, edikang-ikong and ewedu soups had appreciable levels of iron content too. The adult RDA for iron is 10 mg/day for men and 15 mg/day for women indicating that the selected stews and soups will be able to meet the daily dietary iron requirements (Wardlaw, 1999). This corresponds to earlier reports that most Nigerian natural foods are rich in iron (Latunde-Dada, 1997). Iron deficiency has been reported to be extremely common in the developing world, with >50% of the world's population having some degree of deficient iron status based on a wide variety of tests (Openheimer, 2000). This corresponds to studies by Elemo *et al.* (2010b) on the iron status of premenopausal women in a Nigerian university. They reported that these women were at a very high risk of nutrition anaemia. This could be attributed to their regular diet, socioeconomic status and consumption pattern. However, the presence of anti-nutrients such as phytate in food could reduce iron absorption and utilization in humans.

Calcium has been reported to be the most abundant mineral in the human body, with 99% of it contained within bones and teeth. The other 1% found in the cellular and extracellular fluid is important for biological functions (Wardlaw, 1999). Egusi + vegetable soup showed a very low level of calcium, while afang and soko soup had relatively high levels but not sufficient to meet the Adequate Intake (AI) of calcium for adults (1000-1200 mg/day) and adolescence (1300 mg/day). Calcium deficiency is certainly a risk factor for osteoporosis in later life (Allen, 2001). This makes supplementation very important. Flesh and sea foods are often included in these soups and also consumed with tuber or cereal-based dishes such as cooked cassava, yam, plantain, rice or maize- based dishes thus improving the calcium level.

Although no disease is currently associated with an inadequate phosphorus intake, its deficiency may contribute to bone loss in elderly women (Wardlaw, 1999). Turkey stew and ogbono soup had a very high level of phosphorus, while the other soups had adequate levels except for egusi + vegetable soup which was extremely low. This indicates that the soups can meet the daily requirements of phosphorus (RDA for adults is $\geq$700 mg/day).

Sodium is the major positive ion in the extracellular fluid and a key factor in retaining body water. All the soups analysed had values within the RDA. Under the FDA food-labeling rules, the Daily Value for sodium is 2400 mg (Greely, 1997). High sodium content has been shown to contribute to hypertension in susceptible individuals, leading to increased calcium loss in urine (Wardlaw, 1999).

Potassium plays a similar role with sodium in the biological system, but it is located in the intracellular fluid. Unlike sodium it associated with lower rather than higher blood pressure values (Wardlaw, 1999). Potassium level was high in egusi + vegetable, onugbu, edikang-ikong and miyan kuka soups but this is far below the RDA (2000 mg/day). Deficiency in potassium leads to an irregular heartbeat, loss of appetite and muscle cramps. But as earlier stated these soups are often not consumed alone but with other food types which could improve the potassium level.

Conclusion: This study has established that the selected soups from the six geopolitical zones of Nigeria are good sources of micronutrients. The general stews are usually combined with some of the soups in the different geo-political zones thereby increasing the concentration of these micronutrients in the diets especially, beef, fish and poultry foods which are rich in Iron and calcium.

However, the presence of anti nutrients such as phytate especially in cereals such as maize and millet could affect the utilization of some of these micronutrients. Consumption with other food type with caution is recommended so as to improve the micronutrient level.

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Identification of Essential Oil Components from *Nigella sativa* Seed by Gas Chromatography-mass Spectroscopy

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Abstract: The volatile oils from the seed of *Nigella sativa* were obtained by steam distillation. Gas chromatography-mass spectrometry was used to identify the components. Nine volatile oils were identified and 2-methyl-5(1-methyl ethyl)-Bicyclo[3.1.0]hex-2-ene was the major constituent (62.28%) while alpha-pinene was the minor (2.28%).

Key words: Volatile oils, *Nigella sativa*, steam distillation, extraction

INTRODUCTION

Essential oils are oily liquids which are entirely, or almost entirely, volatile without decomposition, such as oil of anise, are solid at 15.5°C, but melt to form liquid at slightly higher temperature, Denston (1939). The essential oils have characteristic fragrances and tastes and are mixtures of known and unknown compounds. They may contain hydrocarbons, terpene alcohols, aldehydes, ketones, phenols and esters.

Nigella sativa belongs to the family *Ranunculaceae* and is called *Habbatus sauda* in Hausa. It is an annual herbaceous plant and is believed to be indigenous to the Mediterranean region but now it has been cultivated into other parts of the world including Africa (Mozoffarin, 1998; Zargari, 1990). According to Amin (1991), the seeds are believed to have galactagogue, carminative, laxative and antiparasitic properties. An antibacterial effect of the phenolics fraction of the seed oil was first reported by Topozada *et al*. (1965). Latter, the diethyl ether extract of *Nigella sativa* was also reported by Hanafi and Hatem (1991) to inhibit the growth of bacteria. Aqueous suspension of *Nigella sativa* seeds was reported to have an analgesic effect comparable to aspirin test on rat (Randhawa and Al-Ghandhi, 2002).

The antischisommicidal effect against *Schistosoma mansoni* and antimalarial activities have been studied (Azza *et al*., 2005; Abdulelah and Zainal-Abidin, 2007).

The chemical constituents of the fixed and volatile oils of *Nigella sativa* obtained from Iran have been reported by Nickavar *et al*. (2003). The study indicates the total fatty acid composition and some volatile components.

This study is aimed to describe the chemical composition of volatile oil of *Nigella sativa* seeds obtained from Maiduguri extracted by steam distillation method.

MATERIALS AND METHODS

Collection of seeds: The seeds of *Nigella sativa* were obtained from Monday market in Maiduguri, Borno state and authenticated by malam H.U. Dukku of Biological sciences programmed, Abubakar Tafawa Balewa University, Bauchi. The seeds were crushed and ground to fine powder. The powdered sample was stored in clean and dry place until used.

Extraction of the volatile oils: Steam distillation method was employed in the extraction process. 400 g of finely powdered sample was placed in a distillation flask connected to a steam distiller, condenser and a receiver and was hydro distilled for four hours. The distillate was extracted with dichloromethane. The dichloromethane layer was separated and dried over anhydrous magnesium sulphate and filtered. The filtrate was concentrated by distilling off the solvent in a rotary evaporator. The percentage yield was calculated and the oil was subjected to Thin Layer Chromatography (TLC), Infra Red (IR) and Gas-mass Spectrometry (GC-MS) analysis.

Analysis of the volatile oil: The GC-MS analysis was performed with a quadruple GC-MS system, Agilent GC model 6890N and Agilent mass selective detector, 5973 series; capillary column (30 m x 0.25 mm; 0.25 μm film thickness). The carrier gas was helium and column head pressure of 15 psi yielding a linear flow rate of 0.8 m/min. The split ratio was 1: 10 and the initial column temperature was held at 200°C for 15 min and then raised at 10°C/min and maintained at 260°C until all components had eluted. The components were identified by matching their mass spectra in the Wiley 275 library and their retention indices were compared with literature values.

RESULTS AND DISCUSSION

The seed of *Nigella sativa* was subjected to steam distillation and extracted with dichloromethane, dried over magnesium sulphate and the yield was 0.4%. The thin layer chromatogram of the components shows the

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Table 1: Infra Red (IR) spectral data of volatile oil from *Nigella sativa*

Group	Intensity	Description	Wave number cm^{-1}	Extract
-OH (hydrogen bonded)	Medium	Broad absorption	3460	Volatile oil extract
C-H stretching	Strong	Sharp absorption	2900	
C=O stretching	Strong	Sharp absorption	1650	
C=C	Weak	Sharp absorption	1598	
C-O	Strong	Sharp absorption	1210	

Table 2: Chemical composition of the volatile oil of *Nigella sativa*

Compound	P	RT (min)	MW	M/Z (% intensity of fragments
2-methyl-5(1-methyl ethyl)-Bicyclo[3.1.0]hex-2-ene	62.28	5.305	136	136(20), 121(10), 105(10), 93(100), 91(60), 79(40), 65(5), 39(10)
Alpha-pinene	2.28	5.000	136	136(13), 121(20), 93(100), 91(42), 77(25), 67(10), 53(10),41(10)
Beta-pinene	2.49	6.527	136	136(14), 121(15), 93(100), 91(27), 79(25), 69(26), 53(8), 41(24),
1-methyl-2-(1-methyl ethyl)benzene	45.70	7.725	134	134(27),119(100), 93(4), 91(22), 63(5), 39(4)
4-methyl -1-(1-methyl ethyl)-3- cyclohexen-1-ol	4.80	10.89	154	154(41), 136(25), 139(5), 111(80), 93(79), 121(5), 95(10), 69(4), 43(30), 41(24)
2-methyl-5-(1-methyl ethyl)-2,5-cyclohexadiene-1,4-dione	30.8	12.302	164	164(68), 136(48), 121(54), 108(8), 93(40)
2-methyl-5-(1-methyl ethyl)phenol	4.45	13.175	150	150(35), 135(98), 91(10),65(3), 39(30)
2,6,6,9-tetramethyl tricyclo[5.4.0.$0^{(2,9)}$]undec-9-ene	2.49	13.709	204	204(25), 189(8), 133(48), 119(100), 105(50),91(30), 69(6), 41(10)
Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	4.20	9.163	204	204(40), 189(50), 175(20), 161(100), 147(30), 133(50), 119(50), 105(55), 91(60), 79(50), 41(55)

P = Percentage; RT = Retention time; MW = Molecular weight

following Rf values: 0.07, 0.21, 0.33, 0.42, 0.60, 0.80 and 0.90 by using dichloromethane and petroleum ether of ratio 1:1.

The IR values are shown in Table 1. The absorption band at 3460 cm^{-1} revealed the presence of OH group. The stretching absorption band at 1650 cm^{-1} may be due to carbonyl absorption, while the absorption band at 2900 cm^{-1} revealed C-H stretching.

The mass spectral data for the volatile oil from *Nigella sativa* is presented in Table 2. Nine components were identified as seen in Table 2.

The oil consists of three monoterpenoid hydrocarbons (2-methyl-5-(1-methyl ethyl) bicyclo[3.1.0]hex-2-ene, alpha-pinene and beta-pinene), one phenyl hydrocarbon (1-methyl-2-(1-methylethyl)benzene), one phenol hydrocarbon (2-methyl-5-(1-methyl ethyl)phenol), one monoterpenoid alcohol (4-methyl -1-(1-methyl ethyl)-3-cyclohexen-1-ol), two sesquiterpenoid hydrocarbons (2,6,6,9-tetramethyl tricyclo[5.4.0.$0^{(2,8)}$]undec-9-ene and Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene) and one monoterpenoid ketones (thymoquinone or 2-methyl-5-(1-methyl ethyl)-2,5-cyclohexadiene-1,4-dione).

Volatile oils have been found to exhibit antioxidant activity for example, the volatile oils studied by Lado *et al.* (2004) showed that the reducing capacities of the components were lower than the values obtained for volatile oils.

Conclusion: The ingredients obtained from this study indicate that the oil can be fully utilized for the manufacture of perfumery products, antimicrobial and antiseptic agents.

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Determination and Comparison of Total Polyphenol and Vitamin C Contents of Natural Fresh and Commercial Fruit Juices

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Abstract: Owing to interest in the role of antioxidants in human health which has prompted research to assess fruit and their products antioxidants, such as vitamin C and phenolics and also because of increment in commercial fruit juices consumption, the objectives of present study were to determine and compare the total polyphenol and vitamin C contents of natural fresh and commercial fruit juices. Folin-Ciocalteu assay and Spectrophotometric method were used to measure the total polyphenol and vitamin C. The concentrations of total polyphenol were ranging from 23.75±1.6 to 420.69±4.9 mg/100 ml for fresh and 18.57±3 to 381.9±3.4 mg/100 ml for commercial juices with lowest amount in apricot and the highest amount in pomegranate juices in both sources. Significant differences ($p<0.05$) were observed in the polyphenol contents of peach, pomegranate, grapefruit and orange between fresh and commercial juices. The vitamin C levels of fresh juices were ranging from 14.31 for apricot to 24.51 mg/100 ml for orange juices and those of commercial were from 13 for mango to 17.29 mg/100 ml for pomegranate juices. The mean vitamin C contents of fresh juices were higher than those of commercial but these differences were not significant. Although various juices differed markedly in the quantity of polyphenol and vitamin C, they can be considered as a good source of these components.

Key words: Fruit juice, total polyphenol, vitamin C, health

INTRODUCTION

Current recommendation of health experts is to increase the consumption of fruits since there is convincing evidences linking a diet rich in fruits with reduced incidence of coronary heart disease, cancer and various age relating chronic diseases (Margetts and Buttriss, 2003). These protective effects are hypothesized to owe, at least in part, to antioxidant and anti-proliferative effects of various polyphenols and vitamins such as vitamin C that present in fruits and their products (Collins, 1999; Strain and Benzie, 1999).

Polyphenols are a group of secondary metabolites widely distributed in the medicinal plants, vegetables, fruit and a variety of beverages such as tea, wine and fruit juices. These metabolites are the most abundant antioxidants in human diets (Scalbert *et al*., 2005) and recently receiving increasing interest from consumers, manufacturers and food marketing for their health benefits (Scalbert and Williamson, 2000).

Vitamin C is an essential phytonutrients for the metabolism of living cells that occurs in different concentrations in natural foods especially fruits and their products. It is considered as the major antioxidant in the diet. Gardner *et al*. (2000) have revealed that Vitamin C accounted for 65-100% of antioxidant capacity of citrus juices.

Juices besides fruits are suitable food products in term of ingestion of health protective phytochemicals (Netzel *et al*., 2002). The bioactive components may even be better absorbed from juices than from plant tissues (Bitscha *et al*., 2001) and also the consumption of fruit juices help fulfil the recommended fruit servings (Dennison, 1996). Currently the American Academy of Pediatrics issued recommendations for natural fresh fruit juice consumption for children and adolescents (American Academy of Pediatrics, 2001) for achieving recommended intakes of important nutrients such as vitamin C, folate and magnesium (Ballew *et al*., 2000). In recent years the consumption of fruit juices increased at very quick rates (Kabasakalis *et al*., 2000) but rapid growth in the commercial fruit juices and fruit drinks production propel public to consume these types of juices instead of fresh and natural juices. Rampersaud *et al*. (2003) showed that at around age seven, children's consumption of natural real juice flat-lines, while intake of fruit-flavoured beverages increases.

Due to the great importance of polyphenols and vitamin C in human health and also because of the growth in commercial fruit juices production and consumption, this study was carried out with aim of determination and comparison of the polyphenol and vitamin C contents of natural fresh and commercial fruit juices.

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MATERIALS AND METHODS

Sample preparation: In Iran, fruit juices are generally consumed in two types of natural fresh and commercial packaged fruit juices. To achieve the objectives of present study, three bottles of 33 (total = 99) commercial fruit juices from 3 different brands and 11 different type of fruit juices were collected from local stores in Tabriz-Iran between July and October 2008. Three different samples of 11 different types of fresh fruits (total = 33) were prepared by squeezing juice out of the fruits. All fruits were thoroughly washed with tap water and any fruit with signs of defect and immaturity sorted out in the laboratory. The juices were extracted from fruits by food processor. Fruit juices having fibre content were filtered before the measurements.

All reagents and solvents used were of analytical grade and were used without further purification. Doubly distilled water was used throughout. Terbium (III) chloride hexa-hydrate ($TbCl_3$-$6H_2O$) was from Acros Organics, USA. Quercetin was obtained from Sigma. All other materials purchased from Merck.

Total polyphenol assay: The total polyphenol contents of fruit juices were determined by using Folin-Ciocalteu method (Singleton *et al*., 1965). Briefly 1 ml of extract or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l) was decanted in 25 ml volumetric flask which containing 9 ml of distilled deionized water. 1 ml of Folin-Ciocalteu reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added and the solution was dilute to volume with distilled deionized H_2O and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank (distilled deionized H_2O) was measures at wavelength of 750 nm. Total polyphenol contents of fruit juices were reported as mg Gallic Acid Equivalent (GAE)/100 ml.

Vitamin C assay: Vitamin C contents of fruit juices were determined spectrophotometrically by metaphosphoric acid extraction of 2,6-dichlorophenol indophenol dye (Robinson and Stotz, 1945) using a Cecil spectrophotometer (model 8000) in wavelength of 500 nm with a 1 cm quartz cell. The vitamin C contents of fruit juices were reported as mg/100 ml.

Statistical analysis: Parameters were expressed as mean and standard deviation. Significant differences between the group of commercial and natural fresh fruit juices were calculated by Independent t-test. The ANOVA test with Tukey post Hoc test was used to compare the total polyphenol and vitamin C contents of different natural fresh and commercial fruit juices. P-values of less than 0.05 were considered statistically significant.

RESULTS

Total polyphenol contents of fresh fruit juices presented in Table 1. The mean polyphenol contents of fresh juices (90.49±0.2 mg GAE/100 ml) were significantly higher than commercial fruit juices (79.82±0.3 mg GAE/100 ml) ($p<0.001$).

The total polyphenol contents of fresh fruit juices were ranging from 23.75±0.1 (apricot juices) to 421.42±0.5 (pomegranate juice) mg GAE/100 ml while those of commercial fruit juices were ranging from 18.57±0.4 (apricot juice) to 381.91±0.4 mg GAE/100 ml (pomegranate juice). Considering the wide variation in the total polyphenol contents of both fresh and commercial fruit juices, they were divided into two groups of high polyphenol contents (>100 mg GAE 100 ml) including pomegranate and red grape juices and low polyphenol contents (<100 mg GAE/100 ml) including all other fruit juices.

Table 2 shows the vitamin C contents of different natural fresh and commercial fruit juices. The mean vitamin C contents of fresh fruit juices (17.44±1.06 mg/100 ml) were reasonably higher than those of commercial (15.01±1.2 mg/100 ml) ones ($p<0.05$). The vitamin C contents of natural fresh fruit juices were higher than those of commercials except for peach and apricot juices however the differences between the vitamin C contents of peach and pineapple juices from natural and commercial sources were not statistically significant. It has been suggested that pasteurization can decrease the vitamin C content of juices. Klopotek *et al*. showed that the vitamin C contents of strawberry juices decrease 35% by pasteurization. In another study, Taoukis et al. revealed that in the case of pineapple juices, the loss of vitamin C in 45° C, was up to 25%.

In the case of fresh juices, the vitamin C contents were ranging from 14.31±0.2 (apricot juice) to 24.51±0.15 (orange juice) mg/100 ml. The statistical analysis indicated that the vitamin C contents of orange juice was significantly higher than all other fruit juices ($p = 0.000$).

DISCUSSION

All fresh fruit juices had higher polyphenol content than commercial ones and only in the case of pineapple the difference were not statistically significant. Different factors such as processing techniques, clarification and pasteurization can affect polyphenol contents of commercial juices. Hertog *et al*. (1992) and Shadidi and Nazck (1995) had shown that polyphenol contents could be affected by different processing techniques. According to Ritter *et al*. (1992) and Karadeniz and Eksi (2001) reports, clarification also could decrease the polyphenolic contents of commercial fruit juices, furthermore klopotek and collegeuse revealed that pasteurization had influence (-27% decrease) on the polyphenol contents.

Table 1: Mean±SD of polyphenol contents of different types of natural fresh and commercial fruit juices

	PC (mg GAE. 100 ml^{-1})		
Fruit juice	Natural fresh juices	Commercial juices	p-value
Total mean	90.49±0.2	79.82±0.3	<0.001
Pomegranate	421.42±0.51	381.73±0.43	<0.001
Red grape	144.56±0.64	135.2±0.36	<0.001
Sour cherry	67.29±0.26	64.28±0.51	0.001
Peach	58.29±0.39	50.05±0.32	<0.001
Mango	56.72±0.25	28.57±0.23	<0.001
Orange	54.28±0.33	42.85±0.14	<0.001
White grape	37.69±0.38	33.71±0.18	<0.001
Pineapple	36.16±0.50	35.74±0.32	0.28
Red grape fruit	49.4±0.33	41.81±0.34	<0.001
Apple	45.38±0.41	42.81±0.25	<0.001
Apricot	23.75±0.11	18.57±0.41	<0.001

All analytical data are the mean of triplicate measurements of three independent samples ±SD. *p-value of independent t-test.
PC = Polyphenol content (mg GAE.100 ml^{-1})

Table 2: Mean±SD of vitamin C contents of different types of natural fresh and commercial fruit juices

	Vitamin C content (mg. 100 ml^{-1})		
Fruit juice	Natural fresh juices	Commercial juices	p-value
Total mean	17.44±1.06	15.01±1.2	0.05
Orange	24.51±0.15	15.86±0.62	<0.001
Red grape fruit	23.2±0.23	14.81±0.31	<0.001
Pomegranate	19.01±0.15	17.34±0.23	<0.001
Apple	17.45±0.15	13.4±0.77	<0.001
Sour cherry	17.36±0.13	16.44±0.42	0.005
Red grape	16.75±0.12	15.18±0.76	0.005
Pineapple	15.46±0.17	13.6±0.21	<0.001
White grape	14.79±0.06	14.13±1.31	0.63
Mango	14.65±0.15	12.57±0.02	<0.001
Peach	14.42±0.33	15.63±0.56	0.005
Apricot	14.31±0.21	16.2±0.15	<0.001

All analytical data are the mean of triplicate measurements of three independent samples ±SD. *p-value of independent t-test

In the case of polyphenol contents of fresh fruit juices, our findings were partly similar to the results of Loots *et al.* (2006) who showed that the total polyphenol contents of orange and grape juices were 36.4±9 and 26.9±1.4 mg GAE/100 ml respectively. As reported by other researchers, the polyphenol contents of red grape was higher than those of white grapes that may be due to high concentration of anthocyanin in dark fruits (93,97). Comparison of our results with Gaedener *et al.* (2000) report had revealed that the polyphenol contents of orange and grapefruit juices in our study were lower while in the case of apple and pineapple juices, it was higher than mentioned study's results. The observed variations between the total polyphenol contents of different fruit juices from different studies may be due to differences in varieties, climate, ripeness, extraction method, analytical procedure employed, etc (Vasco *et al.*, 2008). For example in white coloured fruits the polyphenol contents decreases constantly with the progress of the ripening, while in red coloured varieties it increases during the last ripening stage due to the maximal accumulation of anthocyanidines and flavonols (Marinova *et al.*, 2005).

Comparison of total polyphenol contents of commercial fruit juices in present study with the results of Lugasi and Hovari (2003) from hungry showed that the polyphenol content of red grape juice in present study was higher (135.2±0.3 vs 68-98 mg/l00ml) while for pineapple juice it was lower (35.74±0.3 vs. 67.4 mg GAE/100 ml).

The polyphenol contents of commercial fruit juices in the case of pineapple, orange and mango juices in present study were higher than those of Thai beverages, reported by Abdullakasim *et al.* (2007).

The variations between the polyphenol contents of commercial fruit juices in different studies may be due to various factors such as different variety of fruits that used for juice production, the percent of pure juices in final product, squeezing and pasteurization techniques. Various processing techniques in commercial field and the methods of analysis as well may affect the polyphenol content.

Because of the lake of comprehensive data on vitamin C contents of fresh fruit juices, we compared our results with the vitamin C contents of fresh fruits. Comparison of our results with United States Department of Agriculture (USDA) database for standard references (USDA, 1998) clarify that the vitamin C contents of natural fresh fruit juices in our study were less than fruits from USA.

The vitamin C contents of our fruit juices in the case of pomegranate and grapefruit were more than and in the case of orange was less than Pakistani fruits (Iqbal *et al.*, 2006). The observed differences in the contents of vitamin C may be as a result of differences in maturity stage and regional varieties of fruits. It has been reported that the amount of vitamin could even vary between different samples of the same species (Davidson, 1979). Different techniques of measuring and squeezing process may also affect the vitamin C contents of fruit juices (Gil-Izquierdo *et al.*, 2002).

The vitamin C contents of commercial fruit juices were ranging from 12.57±0.02 mg/100 ml to 17.34±0.2 mg/100 ml. The lowest amount of vitamin C was in mango and the highest amount was in pomegranate juices.

Kabasakalis *et al.* (2000) reported that the ascorbic acid contents of orange juice were 42.4 mg/100 ml and grapefruit juice was 43.4 mg/100 ml. The vitamin C contents of commercial pure orange juices immediately after production in Klimczak *et al.* (2007) study was in the range of 36.15-40.85 mg/100 m. Massaioli and Haddad (1981) also have reported the vitamin C contents of commercial orange juices in the range of 27.5-73.11

mg/100 ml. Comparison of our data with these studies indicated that the vitamin C contents of orange juice in these studies were higher than those of our commercial juices. it seems that synthetically added vitamin C to the commercial fruit juices is the major determinant of this vitamin in these juices however it must imply that natural vitamin C is superior to synthetic one.

Conclusion: Although the mean total polyphenol and vitamin C contents of fresh fruit juices were higher than those of commercial ones, as a good source of biologically active compounds such as fibre and total polyphenol compounds, both fresh and commercial fruit juices are so effective in enhancing health status and could be considered as a good replacement to carbonated soft drinks. It seems that until the active components of fruits and their products are clearly established, measuring their total polyphenol and vitamin C contents may be useful in planning diets for health promotion.

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Functional, Particle Size and Sorption Isotherm of Cocoyam Cormel Flours

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Abstract: The functional, particle size distribution and sorption kinetics of cocoyam cormels were investigated to reveal their suitability in food systems and storage stability. Four cultivars of cocoyam cormel were harvested processed into flour and the resultant flours investigated for the functional, particle size distribution and sorption isotherm. The *Ede ofe* of the *Colocasia* spp. had highest crude protein content (9.72%), followed by *Ede ocha*, *Xanthosoma* spp, (8.13%) while *Ede cocoindia* had the least, 7.93%. These values are higher than are obtainable in other root crops such as yam or cassava. *Ede uhie* had proportional distribution than the rest of the cultivers. *Ede uhie* had the highest value for viscosity, 0.246cp while *Ede cocoindia* had the least, 0.089 cp. *Ede cocoindia* had the highest water absorption capacity, 2.410g/g, followed by *Ede ofe*, 2.195 g/g while *Ede uhie* scored the least, 2.082 g/g. The bulk density of *Ede ofe* was highest, 0.95 g/cm^3 while *Ede uhie* had the least score, 0.76 g/cm^3. The sorption isotherm study revealed that relative humidity in the neighborhoods of 65-70% would be ideal for storage of the flours in moisture tight package materials. Monolayer values ranged from 0.0367-0.0787 gH_2O/g solid which suggest better storage stability of the flour when store at ambient temperature, 30^{0}C. Going by the data obtained cormel flours from the *Xanthosoma* species can be used as a composite in bread making while their Colocasia counterparts would perform better in emulsion food system.

Key words: Cocoyam cormel, protein content, viscosity, bulk density, equilibrium moisture, monolayer moisture

INTRODUCTION

Cocoyam is regarded as the third most important root crop after yam and cassava in West Africa (Obomegheive *et al*., 1998). It is a staple food for millions of people living in the tropics. Onwueme and Simha (1991) reported that cocoyam cultivars have yield potentials for 37-75 tonnes /hectare and the corms and cormels are rich in minerals, vitamins and digestive starch grains. Despite these nutritional benefits, cocoyam is less valued in areas like Eastern Nigeria where it is produced in abundance (IITA, 1992; FAO, 2006). According to Kordylas (1990) about 30-40 specie of cocoyam have been identified but only 5-6 specie produce edible parts. Two genera of cocoyam are widely cultivated in Africa-these are namely taro (*Colocasia esculenta*) and tannia (*Xanthosoma sagittifolium*). Cocoyam is one of the under exploited tropical plants though with promising quality. However research and development on cocoyam have been meager in Nigeria when compared with other tropical root crops like yam and cassava (Onwuka and Eneh, 1998). Among the reasons advanced for the under utilization of cocoyam is due to the presence of calcium oxalate raphide-the irritant which causes itching effect felt through out the throat when consumed (Purseglove, 1983). Another reason is that cocoyam is prone to pre harvest and post harvest diseases, which reduce storage stability and quality of the tubers (Hahn *et al*., 1987). According to Iwuoha and Kalu (1995) proper cooking eliminates the harsh and sharp irritation in the throat and mouth while the post harvest losses would be obviated by prompt processing of the harvested tubers into cocoyam flour. The present study investigates the physical, chemical and sorption isotherm of flour from selected cormels of cocoyam cultivars. According to Enwere (1998) the cormels of cocoyam are used traditional as soup thickeners. Some skeletal works have been reported on some proximate and functional properties as well as their industrial application (Osisiogwu *et al*., 1974; Olaofe *et al*., 1998). However no work has been done exclusively on the cormels and the utilization of the flour in food formulation and preparation. None also has been reported more especially on their sorption isotherm. The physical, chemical and sorption isotherm provide indexes for food material characterization and storage stability in varying humidity. This work is expected to draw more attention on research and development activity on cocoyam toward provision of food security for people living in the tropics.

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MATERIALS AND METHODS

Sample preparation: Wholesome cocoyam cormels (*Colocasia esculenta cv ede ofe, Colocasia esculenta cv ede cocoindia, Xanthosoma sagitifo litium cv ede ocha* and *Xanthosoma sagittifolium cv edeuhuie)* used in this study were harvested on the month of November, 2008 from an experimental farm at Imo State University Owerri, Nigeria. The cornels were cleaned, peeled and sliced with stainless kitchen knife and washed with tap water. The slices were treated with 20 ppm solution of sodium metabisulphate in water for 20 min. The slices were subsequently treated with hot water for 5 min and then oven dried at 70°C. The dried samples were milled into flour and stored in air-tight containers.

Chemical composition: Moisture content crude protein, fibre, fat, ash and carbohydrate were determined according to AOAC (1990).

Moisture content determination: The moisture cans were washed and dried in the oven and weighed using analytical weighing balance. Five grams of the sample were put into previously weighed moisture can. The sample in the moisture can was put into the oven (Gallenkamp Hot box size 1, air- dried type) at 105°C for 3.0 h. The sample was removed and placed in the desiccators to cool and weighing was carried out afterwards. The sample was reheated and cooled intermittently until constant mass was obtained. The difference in mass as percent moisture was calculated as the % moisture content.

Crude protein determination: The Kjeldahl apparatus was used for the determination of crude protein. One half grams (0.5 g) of each dry sample was weighed and put into a Kjeldahl digestion flask. One tablet of Selenium catalyst was added into each of the flask moistened with distilled water and mixed with 10 ml of concentrated H_2SO_4. The mixture was heated to red-hot temperature under a fume cupboard for 2 h to obtain a clear solution. The digest was transferred quantitatively to 100 ml volume flask and diluted to mark with distilled water. An aliquot of the digest (10 ml) was mixed with equal volume of 45% NaOH solution in a semi-micro kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 10 ml of 4% boric solution containing 3 drops of mixed indicator (methyl red and bromocressol green). A total of 50 ml distillate was collected and titrated against 0.02N H_2SO_4 solution. A blank experiment was also set involving digestion of all the materials except the sample. The distillation was also carried out on the blank. The titre value of the blank was subtracted from that of the sample and the difference obtained was used to calculate the crude protein.

The percent nitrogen content was calculated as

$$\text{Crude protein (\%)} = \text{\% N} \times 16.25$$

Crude fibre determination: Two (2 g) grams of each sample were digested with 200 ml of 1.25% H_2SO_4 solution under reflux for 30 min boiling. The digest was allowed to cool and than filtered with Buckner funnel equipped with muslin cloth. The residue was washed thrice with hot water, scooped into a conical flask and digested with 200 ml of 1.25% NaOH solution under reflux for 30 min boiling. The digest was cooled, filtered and washed thrice with distilled water. The residue was drained and scooped into a previously dried and weighed crucible and then put into the oven to dry at 105°C to a constant mass. The dish with its content was reweighed after drying and then placed in the muffle furnace to ash at temperature of 550°C for 3 h. The ash was withdrawn at the end and put in a bell jar and reweighed. The difference in mass of the sample was calculated as crude fibre and expressed as a percent of the initial mass.

Ash determination: Two grams of the sample was weighed into previously cleaned, dried crucible of known mass. The crucible with the content was weighed and the mass recorded. The crucible with the content was placed into a muffle furnace at 550°C for 3 h until the sample turned white and free from carbon. At the end of incineration, the ash substance was withdrawn and cooled in a bell jar and reweighed. The mass of the residual incinerate was calculated as % ash content.

$$\text{Ash (\%)} = \frac{\text{Mass of ash x 100}}{\text{Mass of sample}}$$

Carbohydrate determination: The carbohydrate content was determined by deference that is by deducting the mean values of other parameters that were determined from 100. Therefore

$$\text{Carbohydrate (\%)} = 100 - (\text{\%mc} + \text{\%CP} + \text{\%fat} + \text{crude fibre} + \text{\%Ash})$$

Where:
mc = Moisture content
CP = Crude protein

Physical properties

Particle size: The method of Idowu *et al.* (1996) was used. Test sieves of various apertures (90 μm, 75 μm and 50 μm) were arranged in ascending order and mounted on the test sieve shaker. 20 g of the flour was put in the top sieve and covered with the lid. The shaker was switched on and operated for 30 min after which the sieves were removed and the retained amount was determined by weighing. The percent retention of each

sieve was calculated. Means values were calculated after four determinations.

Viscosity: The brook field synchrolectric viscometer was used to determine the viscosity of slurry made from the flour. Twenty (20) g of the flour was put in 250 ml beaker and 200 ml of tap water added to form slurry. The slurry was heated at 100°C for 15 min to gelatinize. The brook field synchrolectric viscometer was set at zero and 60 rev per minute. The viscosity of the pap was determined when the spindle revolves 60 time/minute.

Blue value index (BVI): The method of Atkins (1982) was followed. Three (3) g of the flour was weighed into 50 ml beaker and 30 ml dispersion made and allowed to stand for 30 min 30°C, which was filtered afterward with whatman (No 42) filter paper. 10ml of the filtrate was measured into 25 ml conical flask and titrated with O.I.N iodine solution using phenophtalein as indicator. The titre value was recorded at the blue colour end point. Percent blue value index was calculated as:

$$BVI\ (\%) = \frac{VD}{VA} \times \frac{Vt}{Mf} \times \frac{N}{100} \times 100$$

Where:
VD = Total volume of dispersion
VA = Volume of aliquot used for filtration
Vt = Titre value
Mf = Mass of flour used
N = Normality of iodine

Water absorption capacity: The method of Abbey and Ibeh (1988) was followed to determine water absorption capacity. One (1) gram of the flour was mixed with tap water in a centrifuge tube and made up to 10 ml dispersion and allowed to rest at room temperature for 30 mins. The sample was centrifuge at 3000 rpm with Heltich model centrifuge. The volume of the supernatant was measured using 10 ml graduated cylinder. The density value 1000 kg/m^3 was assumed and mean water absorption capacity obtained after four determinations in (g/g).

Gelatinization temperature: The method of Narayana and Narasanya-Rao (1982) was adopted with slight modification. Twenty five (25) grams of the flour was dissolved in tap water in a beaker and made up to 100ml dispersion. The dispersion was placed on heating mantle and stirred as heating progresses. The gelling temperature was recorded at the gelling point of the flour in °C.

Bulk density: The method of Milson and Kirk (1980) was followed to determine the bulk density. Fifty (50) grams of the sample was weighed into 100 ml graduated cylinder and the initial volume recorded. The cylinder was tapped repeatedly for 100 times to a constant volume and the final volume recorded. The bulk density was calculated as the mass of the sample divided by the volume at the end of tapping.

Porosity: The porosity was calculated using the values obtained for the case of bulk density: thus

$$Porosity = \frac{Initial\ volume - Final\ volume}{Initial\ volume}$$

Sorption isotherm: The method of Greenspan (1977) was followed; inorganic salt solutions ($LiCl.H_2O$, $MgCl_2.6H_2O$, Na $Br.2H_2O$, $NaNO_3$, NaCl, KCl, BaCl and K_2SO_4) were prepared to create varying humidity (11, 33, 56, 65, 75, 85, 90 and 97%) respectively in desiccators. One gram flour was placed in the desiccator above the saturated solution. Weighing was carried out twelve hourly until a constant weight is achieved, according to Zurith *et al.* (1979).

Monolayer value: The Caurie's (1981) modified Brunauer-Emmett and Teller (BET) (1938) equation was followed for the determination of monolayer adsorption of water at 30°C, expressed as:

$$\frac{a_w}{Xm\,(1-a_w)} = \frac{1}{MoC} + \frac{1}{Xm} \quad (1)$$

Where:
Xm = Equilibrium moisture content at a given water activity
Mo = Monolayer value
C = Constant for a given system

RESULTS AND DISCUSSION

Chemical properties: The crude proteins of the various cultivars were found to differ significantly at $p<0.05$ as shown in Table 1. The *Xanthosoma* spp performed better than the *Colocasia* spp. and *Ede ocha* had the highest while cocoindia had the least crude protein. High protein content is desirable not only on nutritional ground but for the functionality of the flour in food system. Thus, functional properties such as absorption capacity, gelling and rheology are influenced by the type and nature of proteins found in the flour. From this, *Ede ocha* might offer better advantage to processors who may wish to use the flour as composite in bread making.

The carbohydrate value did not differ among the cultivars at $p<0.05$, thus signifying the importance of cocoyam as energy giving food nutrient. According to Srilakshmi (2008) cocoyam contains starch molecules that are not very easily digestibly - Slowly Digestible Starch (SDS) - thus making cocoyam a low glycaemic food. Hence cocoyam has become a favorable food for diabetes (Eneh, 1992).

Table 1: Chemical properties cocoyam cormel flours

	Colocasia esculenta		*Xanthosoma sagittifolium*		
Chemical properties (%)	*Ede cocoindia*	*Ede ofe*	*Ede Uhie*	*Ede ocha*	LSD
Ash	2.00^{a}±1.37	1.38^{a}±0.70	0.63^{a}±0.23	0.68^{a}±0.23	Nil
Fat	0.88^{a}±0.03	0.51^{b}±0.05	0.83^{a}±0.02	0.80^{a}±0.08	0.13
Crude protein	4.64^{c}±0.46	5.08^{b}±0.61	5.69^{c}±0.10	5.70^{a}±1.08	0.27
Moisture content	7.93^{a}±1.33	9.72^{a}±1.41	8.04^{a}±1.20	8.13^{a}±1.44	Nil
Fibre	0.36^{c}±0.02	0.48^{b}±0.07	0.20^{d}±0.82	0.62^{a}±0.52	0.12
Carbohydrate	84.19^{a}±4.2	82.83^{a}±3.7	84.61^{a}±0.1	84.075^{a}±7.5	Nil

Mean ± standard deviation of quadruplet determination on dry weight basis. Means with similar alphabets across the row are not significant different at $p<0.05$

The fibre of *Ede ocha* was highest among the cormel's flour while *Ede uhie* was the least. Fibre plays more important role in human nutrition than its functionality in food system. It contributes to bowl movement and cocoyam has been a health food for people with gastro-intestinal disorders (Onwueme, 1978). With the exception of *Ede ofe*, the rest of the cultivars did no differ in fat content. More over, no significant difference was observed in the ash and moisture content.

Particle size: The particle size of the cormel's flours revealed that majority of the particles was retained on 90 µm sieve signifying that the average size of the flour might be 90 µm or more (Table 2). However the retention on the sieve differed significantly at $p<0.05$ and at 90 µm *Ede ofe* had the highest retention of flour and *Ede ocha* the least. Large particle size might play a much greater role than has been suggested in terms of bonding forces on particulate surfaces which influences functional properties such as water absorption capacity, viscosity and gelling temperature. According to Ayernor (1983) particle size highly correlates with the extent of damaged starch as measured by the blue value index. The suitability of cocoyam flour in infant based products would depend on the smoothness of the reconstituted end product which is directly influence by the particle size.

Physical properties: The result in Table 3 shows that the blue value index as measured did not vary in their means ($p<0.05$) for the flours from the various cultivars. However among the means, *Ede ofe* scored the highest while *Ede cocoindia* had the least. Blue value index is influenced by the degree of damaged starch during milling (Greer and Stewart, 1959).

The viscosity of the cultivars from the *Xanthosoma* spp. were higher than those of their Colocasia counterparts, thus *Ede ocha* had the highest (0.24 cp) while *Ede ofe* had the least. High viscosity flour is required for baking purpose, hence the *Xanthosoma* spp. could be used as composites in bread making rather than the *Colocasia* spp.

The water absorption capacity of the cormel's flours differed significantly ($p<0.05$) with *Ede ofe* having the highest value and *Ede ocha* the least. The low water absorption capacity of *Ede ocha* may be associated with large amount of small particles on 50 µm mesh size. Again, it is revealed that large particle size favors high water absorption capacity. Ayernor (1983) stated that the degree of disintegration of the native starch granule influences the water binding ability of starchy system. The ability of the starchy system to incorporate water molecules enables addition of water during food preparation (Akobundu *et al*., 1982). Absorption of water ensures improved handling of flour, maintenance of freshness of baked goods and useful in sausage production.

Ede uhie flour exhibited the highest gelling temperature while *Ede cocoindia* had the least. Low gelling temperature is desirable in terms of energy cost for cooking. Onwueme (1978) associated gelling ability to the percentage of damaged amylose fraction to amylopectin ratio of the starch. Thus *Ede cocoindia* is expected to have high ratio of amylose than amylopectin. The bulk density and the porosity of the flours were significantly different at $p<0.05$. The *Xanthosoma* spp. scored higher than the *Colocasia* spp. with *Ede uhie* having the highest value and *Ede cocoindia* the least. Porosity is the reverse of bulk density as shown by the result in Table 4. It is possible the particle size of the granules would affect the bulk density. These physical properties are considered during packaging and transportation in the industry (Haung and Clayton, 1987). With the exception of *Ede ofe* the rest of the cultivars did not differ at $p<0.05$ in which the study was conducted for the solid density. Solid density is another important physical property of food that is considered in separation processes such as sedimentation, centrifugation and pneumatic and as well as hydraulic transport of powders and particulate foods (Lewis, 1987). It is also measured in quality assessment of flour.

The equilibrium moisture content of the flours the various cocoyam cultivars is shown in Table 4. At water activity range 0.0-0.30, *Ede cocoindia* absorbed the lowest moisture 0.01-0.02 and *Ede ocha* of the *Xanthosoma* species had the highest moisture 0.03-0.08. These levels of moisture absorption typify monomolecular absorptivity in which water is firmly

Table 2: Particle size distribution of cocoyam cormel flours

	Colocasia esculenta		*Xanthosoma sagittifolium*		
Mesh size	*Ede ofe*	*Ede cocoindia*	*Ede ocha*	*Ede uhie*	LSD
90 µm (%)	$94.73^{a}\pm0.25$	$87.63^{b}\pm0.06$	$81.63^{d}\pm0.09$	$81.90^{c}\pm0.03$	0.50
75 µm (%)	$1.66^{b}\pm0.02$	$1.46^{c}\pm0.01$	$3.50^{a}\pm0.02$	$1.50^{c}\pm0.02$	0.04
50 µm (%)	$2.93^{d}\pm0.09$	$2.80^{c}\pm0.04$	$17.10^{a}\pm0.03$	$12.60^{b}\pm0.02$	0.13

Mean quadruplet samples of cocoyam cormel flours. Means of samples with similar alphabets across the row are not significant at $p<0.05$

Table 3: Physical properties of cocoyam cormel flours

	Colocasia esculanta		*Xanthosoma sagittifolium*		
Physical properties	*Ede ofe*	*Cocoindia*	*Ede ocha*	*Ede uhie*	LSD
Blue value index	$1.43x10^{-3a}\pm2x10^{-4}$	$1.00^{a}x10^{-3}\pm7x10^{-6}$	$1.02x10^{-3a}\pm5x10^{-6}$	$1.30x10^{-3a}\pm3x10^{-4}$	Nil
Viscosity (cp)	$0.153^{c}\pm1.0x10^{-3}$	$0.089^{d}\pm2x10^{-3}$	$0.213^{b}\pm6x10^{-3}$	$0.246^{a}\pm2x10^{-3}$	0.01
Bulk density (g/cm^3)	$0.95^{a}\pm0.01$	$0.80^{b}\pm5x10^{-3}$	$0.82^{a}\pm5x10^{-3}$	$0.76^{b}\pm5x10^{-3}$	0.09
Porosity (g/cm^3)	$0.323^{b}\pm8x10^{-3}$	$0.318^{b}\pm7x10^{-4}$	$0.340^{a}\pm1.4x10^{-3}$	$0.350^{a}\pm2.1x10^{-3}$	0.01
Solid density (kg/m^3)	$0.244^{b}\pm3.64$	$0.280^{a}\pm0.37$	$0.281^{a}\pm0.61$	$0.284^{a}\pm0.42$	0.10
Water absorption (g/g)	$2.195^{b}\pm9.6x10^{-4}$	$2.410^{a}\pm9.6x10^{-4}$	$2.178^{c}\pm9.6x10^{-4}$	$2.082^{d}\pm9.6x10^{-4}$	0.00143
Gelling temperature (°C)	$63.75^{c}\pm0.43$	$69.75^{b}\pm0.50$	$65^{c}\pm0.0$	$73.8^{b}\pm1.3$	1.28

Mean quadruplet samples of Cocoyam cormel's flours. Means of samples with similar alphabets across the row are not significant at $p<0.05$

Table 4: Equilibrium moisture content of cocoyam cormel flours (g H_2O/g flour)

Water activity (a_w)	Relative humidity (RH%)	*Ede ofe*	*Cocoindia*	*Ede ocha*	*Ede uhie*
0.11	11	0.02	0.01	0.04	0.03
0.33	33	0.05	0.02	0.07	0.08
0.56	56	0.07	0.05	0.10	0.10
0.65	65	0.10	0.08	0.12	0.13
0.75	75	0.12	0.13	0.18	0.20
0.80	80	0.18	0.19	0.25	0.25
0.90	90	0.26	0.29	0.30	0.33
0.97	97	0.39	0.46	0.41	0.47

bound and is difficult to remove by physical means such as drying. At the water activity range 0.30-0.70, shows a moderate absorption and ede had the highest moisture while *Ede ofe* had the least. This region is referred to as zone of multilayer deposition. However the amount of moisture absorbed by the cultivars is within the range that is safe for safe storage using moderate moisture impervious packaging material. Furthermore the region 0.70-1.0, witnessed steep rise in equilibrium moisture content with a small increase in water activity. Dincer and Esin (1996) reported that the region is characterized by capillary condensation, which may be due to the degree of amorphism reached by the starchy samples. The condition promotes hygroscopy which makes the flour susceptible to caking (Maia and Cal-Vidal, 1994; Nwanekezi, 2007).

The monolayer moisture content ranged from 0.0367-0.0787 gH_2O/g solid with *Ede ocha* having the highest value, 0.0471 gH_2O/g solid as presented in Table 5. Onwuka (2003) listed monolayer range, 0.0320-0.160 g H_2O/g solid for some dry starchy flours. Monolayer moisture content is a measure of sorption ability of starchy food (Kiranoudis *et al*., 1993). It is the minimum amount of water bound to active sites and guaranties the stability of flour during storage (Iglesias and Chirife, 1975). The higher the monolayer values the lower the stability of the flour. Thus suggesting that *Ede ocha* provides more binding sites for water molecules and

Table 5: Monolayer moisture content of cocoyam cormel flours

Cultiver	Monolayer (Mo)gH_2O/g solid
Ede uhie	0.0520
Ede ocha	0.0787
Cocoindia	0.0661
Ede ofe	0.0367

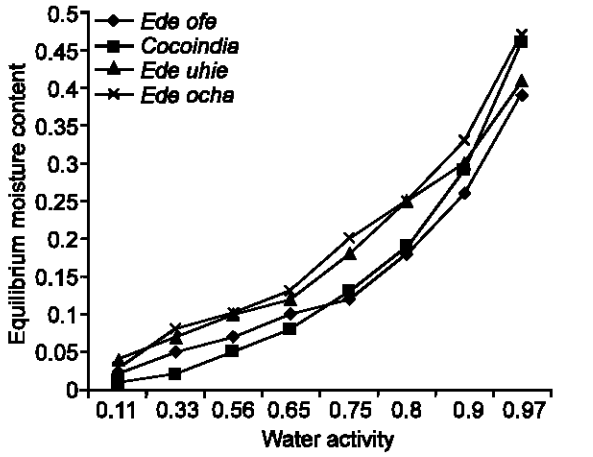

Fig. 1: The sorption isotherm of cocoyam cormel's flours at 30°C

would least stable in storage, while *Ede ofe* with the lowest monolayer value store for longer time than the rest.

Conclusion: The study has revealed that both cormels of the *Xanthosoma* spp. and the *Colocasia* spp. are comparably richer in crude protein than other tropical root crops. They also contribute quality carbohydrate and dietary fibre to human nutrition. The crude protein and the nature of the carbohydrate play important roles in the functionality of the flour in food system. It is worthy to note that particle sizes of these cultivars influences functional properties such as the water absorption capacity and gelling characteristics of the flours.

The high viscosity of the *Xanthosoma* spp (*Ede uhie* and *Ede ocha*) makes it the preferred choice for baking into bread and different types of cookies. In Nigeria flour millers have been compelled by legislation to incorporate 5% of cassava starch in bread making flours, such commensurate legislation is yet to be extended to cocoyam which is also produced in abundance. Going by this work, it is expected that government should through appropriate legislation compel millers to incorporate cocoyam flour in baking flour.

The results of equilibrium moisture content and monolayer moisture content revealed the drying and storage abilities of the cormel flours. The flours could be stored properly in the environment with relative humidity range 0.60-0.70.

Considering the quality attributes of the cocoyam cormels' flour it is expected that more research work is carried out in the area of storage stability using various packaging materials and at different storage environment and also more comparative study on particle size as it affects the behaviour of the flour in food system is need.

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Cyanide Reduction, Functional and Sensory Quality of Gari as Affected by pH, Temperature and Fermentation Time

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Abstract: The effects of control pH, temperature and fermentation time on the cyanide reduction, functional and sensory properties of gari were investigated. Freshly harvested cassava roots (local variety) were peeled, washed and grated into a mash. The meal was divided into five equal portions and mixed thoroughly with already prepared buffer solutions from citric acid, sodium orthophosphate, -analytical grade (10% by weight buffer and kept in stainless containers to ferment at room temperature (30°C). Samples were withdrawn at intervals of 12, 24, 36, 48 and 72 h, dewatered, sifted and toasted into gari and packaged in cellophane bags. The process was again carried out at 35 and 40°C. The product gari was subjected to functional analysis (swelling index, pH, titrable acidity, water absorption capacity and residual cyanide) and sensory evaluation (appearance, taste and general acceptability) for the uncooked gari. The results obtained show that the buffer treated samples had high pH than the control sample. The highest mean pH was recorded for the BS = 8.0, (7.19), followed by BS = 7.0 (6.55) and BS = 6.0 (5.97), while the control had the lowest 4.5. The highest Swelling Index (SI) (17.45 ml/ml) was obtained for BS = 5.0 and closely followed by BS = 6.0 (17.14 ml/ml) while BS = 8.0 recorded the least 16.91 ml/ml. The buffer at pH 7.0 reduced the cyanide content to 7.69 mg HCN/kg, which is lower than the safe level of 10 mg HCN/kg. Moreover the gari from BF = 5.0 (5.4) and BF = 6.0 were the preferred in terms of general acceptability while the gari from the control BF = 0.0 (4.8) was rated the least. The buffer treated samples also performed better than the control in bulk density and general acceptability as rated by the panelists. Therefore controlling the process variables (pH, temperature and fermentation time) while fermenting cassava mash for gari production is sure way to enhance product quality and safety.

Key words: Cassava, buffer, titrable acidity, hydrogen cyanide, sensory property

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is a major root crop in the tropics and its starchy roots are significant source of calories for more than 500 million people world-wild (FAO, 2000). Cassava is diversified into different food products and these products are available year round thus making cassava an important staple food for many rural households in Nigeria (Onabolu, 2001).

Gari is the most popular of the cassava products in Africa (Oluwole *et al*., 2004). To prepare gari, fresh cassava roots are peeled, washed and grated. The resultant pulp is put in a porous sack (polypropylene bag) and weighed down with heavy object or with hydraulic press while it is fermenting. The dewatered and fermented lump of pulp is pulverized, sifted and the resulting semi dried mash is toasted in a pan (Nweke *et al*., 2002). The resultant granulated product which is preferred because it can be consumed dry or with cold water and or reconstituted with hot water to form "dough" which is eaten with soup (Oluwole *et al*., 2004).

A safety concern among gari consumers arises from the presence of cyanogenic glucoside which upon hydrolysis produces cyanohydrin that further breaks down to release hydrogen cyanide - a known plant toxin (Bokanga, 1994; Ernesto *et al*., 2000). The traditional developed methods of processing cassava products have been found to be grossly inadequate in the removal of cyanogens, irrespective of whether the roots are from low or high cyanide variety (Koch *et al*., 1994; Achinewhu and Owuamanam, 2001).

Presence of cyanide above the safe level of 10mg HCN/kg cassava flour by FAO/WHO (1999), may pose health risk to the consumers. Some of the health conditions associated with cassava meals include: Tropical Ataxic Neuropathy (TAN); (Oshuntokun *et al*., 1968; Akintonwa *et al*., 1994); Konso - a spastic paraparesis of the leg attributable by consumption of insufficiently processed cassava (Cliff, 1994; Lambien *et al*., 2004).

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Bradbury (2004) had proposed that processing is the suitable strategy to reduce cyanide in cassava products. However, grating remains the critical process step in gari processing in that hydrolysis is initiated by intimate contact between naturally compartmentalized linamarin and the degrading enzyme linamarase (Vasconcelos *et al*., 1990).

Researchers have observed that the extent of hydrolysis and liberation of hydrogen cyanide is pH dependant (Sokari and Karibo, 1992; Vasconcelos *et al*., 1990). In the current study, traditional method of processing gari is modified by introduction of buffer solution in grated pulp at varying temperature and time. This study is aimed at optimizing hydrolysis and removal of cyanide in the resultant product while monitoring the implications of the treatments on the functional and sensory qualities of gari.

MATERIALS AND METHODS

The raw cassava roots (local variety,) were harvested in the month on January 2009 from the farm the farm of Federal University of Technology Owerri, Nigeria. The chemicals used were procured from a store in Owerri, Imo State Nigeria.

Preparation of buffer solution: Buffer solutions were prepared from the following: citric acid and sodium orthophosphate - analytical grade. 0.1 M citric acid solution was prepared by dissolving 10.5 g of citric acid in 500 cm^3 of distilled water. Similarly, 0.1 M sodium orthophosphate solution was prepared by dissolving 17.8 g in 1000 cm^3 of distilled water. The two solutions were used in preparing acetate buffers by appropriate combination following the ratio below. pH meter was used to confirm the reliability of buffer strength as were prepared.

Table 1: Preparation scheme for buffer

$NaHPO_4$ (cm^3)	Citric acid (cm^3)	pH
51.60	48.50	5.0
63.15	36.85	6.0
32.50	17.65	7.0
97.25	2.76	8.0

The freshly harvested cassava roots were peeled, washed and grated. 1000 kg of the cassava mash was divided into five equal portions. Each portion of the mash was thoroughly mixed with 10% by weight of the buffer solution and put into stainless containers. They were left to ferment at room temperature (30°C). Samples were withdrawn at intervals of 12, 24, 36, 48 and 72 h, dewatered, sifted and toasted into gari. The process was again carried out at 35 and 40°C for the various duration of fermentation. All the determinations were replicated thrice. The (gari) samples were packaged in cellophane bags and used for analysis.

Analyses: The samples were analyzed for the functional properties (swelling index, pH, titrable acidity, water absorption capacity and residual cyanide). Sensory evaluation was performed for the appearance, taste and general acceptability for the uncooked gari.

pH determination: The pH of the sample was determined using the method of Association of Official Analytical Chemists (AOAC, 1990). Ten (10) grammes of the sample were put into a 100 ml beaker and was added 100 ml of distilled water. The pH was analyzed using a standardized pH meter (Prazisions pH meta ES10 model). Triplicate values were obtained and the mean value taken as the pH value.

Total titrable acidity (TTA): The percent titrable acidity was determined following the method of FAO (1970). Five (5 g) grammes of the sample was dissolved in a beaker and made up to 100 ml with distilled water and allowed to stand for 30 min. The solution was filtered with whatman filter paper. 25 ml of the filtrate was titrated against 0.1 M NaOH, using phenolphthalein as indicator. The end point was obtained when the colour became colourless. The mean (TTA) was obtained from triplicate determination. The percent titrable acidity (TTA %) was calculated using the formula:

$$TTA\ (\%) = 0.01X$$

Where X = mean titre value

Swelling index: The method of Ukpabi and Ndimele (1990) was followed with slight modification. Ten (10 g) grammes of the sample was transferred into a clean, dried, calibrated measuring cylinder. The gari was gently leveled by tapping and the initial volume recorded. 50 ml of distilled water was poured into the measuring cylinder containing the sample and allowed to stand for 4 h. The value for Swelling Index (SI) was taken as the multiples of the original volume.

Water absorption capacity (WAC): The method of Sosulski (1962) as described by Abbey and Ibeh (1988) was followed. One (1.0 g) gramme of the sample was weighed out and transferred into clean dried centrifuge tube, which the weight has previously been determined. 20 ml of distilled water was poured into the centrifuge tube and stirred properly. The centrifuge tube with the sample was placed inside and operated at a speed 3500 rpm for 45 min. The supernatant was discarded and the tube and its content reweighed. The gain in mass was taken as the water absorbed. Mean value of water absorption was obtained from triplicate determinations.

Bulk density: The method of Akpapunam and Markakis (1981) was followed. Ten (10 g) grammes of the sample were transferred into 50 ml measuring cylinder. The cylinder was tapped repeatedly for 5 min. The bulk density of gari was calculated as the mass of gari over the volume at the end of tapping. The mean value was recorded from triplicate determinations.

Determination of residual hydrogen cyanide: The residual cyanide content of gari was determined using the method of Esser *et al.* (1993).

Preparation of enzyme linamarase from freshly harvested roots: Freshly harvested cassava roots were peeled to remove the cortex. The cortex was shredded to small pieces of about 1 cm in size and refrigerated after wards. 25 g of the shredded cortex was homogenized with 250 ml of 0.1 M acetate buffer pH 5.5. The homogenate was filtered through cheese cloth. The filtrate was used to homogenize another batch of 25 g of cortex and again or the third batch. A total of one litre of the extract was prepared and stored in the refrigerator.

Preparation of KCN standard: A stock solution was prepared by dissolving 50 mg of KCN in 0.2 M NaOH. The stock solution was diluted 1:50 with 0.2 M NaOH. The automatic pipette was used to pipette into marked tubes: 0.025, 0.050, 0.075 and 0.100 ml of the diluted KCN stock, the volume was made up to 0.100 ml corresponding to 5, 10, 15 and 20 µg/ml with 0.2 M NaOH. 0.5 ml of 0.1 m phosphate buffer pH 6.0 was added followed by addition of 0.6 ml, chloramines -T and 0.8 ml of the colour reagent. The absorbance reading was obtained using (visible) spectrophotometer against blank at 605 nm wavelength.

Determination of cyanide in gari: Thirty (30 g) grammes of gari was milled and homogenized with 250 ml of 0.1 M orthophosphoric acid. The homogenate was centrifuged. The supernatant was taken as the extract; 0.1 ml of the enzyme was added into 0.6 ml of the extract. 3.4 ml of the acetate buffer (pH 4.5) was added and stirred to mix well. After which 0.2 ml of 0.5% chloramin-T and 0.6 ml of colour reagent were added and allowed to stand for 15 min for colour development. The absorbance value was obtained at 605 nm against a blank similarly prepared containing all reactants but 0.1 ml phosphate buffer added instead of KCN.

Calculation: The data from the standard were used to obtain a standard curve and its slope (b) by plotting absorbance values (Y-axis) against standard concentrations (X-axis). The unknown mean absorbance (A) and the weight of the sample (g) "w" were used to calculate the residual cyanide content using the formula:

$$\text{Residual cyanide} = A \times 250 \times 0.4151b \times w$$

The unit of cyanide content = mg HCN equivalent per kg sample.

Sensory evaluation of gari: Sensory evaluation was conducted to determine consumer preferences and acceptability of the samples, using a 9-point hedonic scale as described by Watt *et al.* (1985) for the degree of likeness. In scaling, 9 represents "like extremely", mid point 5 represents "neither like nor dislike" and runs down to one which represents "dislike extremely". The quality parameters assessed include; appearance, taste and general acceptability. Twenty (20) panelists were used, 10 males and 10 females of ages ranging from 23 to 44. These panelists were usual consumers of gari. The samples were presented to them in clean dried plates and the panelists recorded their responses on the form provided.

Statistical analysis: The mean values of physicochemical properties were addressed as functions of Buffer solution (five), fermentation temperature (three) and fermentation duration (five) which fitted into 5 buffer solutions x 3 fermentation temperature x 5 fermentation duration. The 3-way analysis of variance procedure as described by Steel and Torrie (1980) was followed. The means were separated using Fisher's (LSD) least significant difference as described by Roessler (1984).

RESULTS

Functional properties of Gari as affected buffer strength (BS): Table 2 shows the functional properties of gari as affected by the Buffer Strength (BS). The mean pH differed significantly ($p<0.05$) under the condition of the study. The highest mean pH was recorded for the BS = 8.0, (7.19), followed by BS = 7.0 (6.55) and BS = 6.0 (5.97), while the control had the lowest 4.5. BS = 0.0. Buffer solution used in the treatment of cassava mash was found to leave high pH in the product up to a pH 7.18 at BS = 8.0.

The control sample (BS = 0.0) performed better in TTA% than the rest of the samples treated with buffer. It was also observed that all the samples that were treated with the buffer did not significantly ($p<0.05$).

The highest Swelling Index (SI) (17.45 ml/ml) was obtained for BS = 5.0 and closely followed by BS = 6.0 (17.14 ml/ml) while BS = 8.0 recorded the least 16.91 ml/ml. However, no significant difference ($p<0.05$) occurred for BS = 0.0 (17.04 ml/ml), BS = 6.0 (17.14 ml/ml) and BS = 7.0 (17.04 ml/ml).

The Bulk Density (BD) increased as the buffer strength increased from BS = 0.0 to BS = 7.0 and declined afterward. The values obtained for BD differed significantly at $p<0.05$. Highest mean Water Absorption Capacity (WAC), 1.33 ml/g solid was recorded for BS = 6.0. The WAC increased from the control (BS = 0.0) and reached the peak at BS = 6.0 and declined marginally to BS = 8.0.

Table 2: Mean values of functional properties of gari as affected by Buffer Strength (BS)

Buffer strength	pH	S.I. (ml)	TTA (%)	BD (g/cm^3)	WAC (ml/g)	HCN (mg/kg)
0	4.5±0.46^c	17.04±3.24^{ab}	0.0056±0.002^b	0.9993±0.23^c	1.05±0.19^b	25.52±4.81^a
5.0	5.02±1.05^b	17.45±3.19^a	0.0064±0.001^a	1.0686±0.41^{ab}	1.31±0.33^a	15.92±4.43^b
6.0	5.07±1.07^{ab}	17.14±2.86^{ab}	0.0065±0.002^a	1.1633±0.50	1.33±0.31^a	11.78±4.46^c
7.0	5.15±1.10^{ab}	17.04±2.85^{ab}	0.0064±0.002^a	1.1700±0.51^a	1.32±0.31^a	7.69±3.72^d
8.0	5.19±10.9^a	16.91±2.68^b	0.062±0.002^a	1.687±0.531^{ab}	1.32±0.37^a	9.26±3.39^c
LSD	0.167	0.4817	0.0004	0.09991	0.0977	0.6739

Means are values of triplicate determination. Means not followed by similar letter of alphabet differed significantly at p≤0.05

The residual cyanide obtained for the samples treated with buffer solution differed among their means at (p<0.05). The residual cyanide, HCN decreased with increasing buffer strength. The lowest HCN (7.69 mg HCN/kg) was obtained from BF = 7.0. While the control (BS = 0.0) had the highest 25.52 mgHCN/kg.

Functional properties of gari as affected by temperature of fermentation in buffer: The result of functional properties of gari as affected by Fermentation Temperature (FT) is shown in Table 3. The mean pH obtained for the samples differed at p<0.05. Highest mean pH was recorded for FT = 30°C (5.71). However, the pH obtained for FT = 35°C (4.61) and FT = 40°C (4.67) did not differ under the condition of the study (p<0.05).

The data for Swelling Index (S.I.) shows that the values differed significantly (p<0.05). Incidentally, the mean S.I. gari from 30°C (17.73 ml/ml) did not differ from that recorded for FT = 35°C, (17.72 ml/ml). The samples obtained from FT = 35°C and FT 40°C were found to be higher in titrable acidity, 0.0069 and 0.0075 respectively while the FT = 30°C had the least TTA% (0.0042). The SI of the control sample compared well with the sample from 35°C fermentation temperature, while it declined at 40°C. This might suggest that it would be technologically and economically advantageous to limit fermentation of cassava mash at a maximum of 35°C when S.I. as quality factor is desired.

For the Bulk Density (BD) of the sample from FT = 35°C had the highest mean BD (1.4860 g/cm^3) while that from FT = 30°C had the least BD (0.5804 g/cm^3). On the other hand the means obtained for WAC of the showed that they differed significantly (p<0.05). The highest WAC (1.48 ml/g solid) was obtained from FT = 35°C. However, the mean WAC for FT = 30°C (1.13 ml/g solid) did not differ from the score for FT = 40°C (1.18 ml/g solid) under the condition of study (p<0.05).

The least mean HCN (12.78 mgHCN/Kg) was recorded by gari from FT = 40°C while the highest residual HCN was obtained for FT = 30°C (15.30 mgHCN/Kg) which shows that more cyanide was liberated at 40°C.

Functional properties of gari as affected by duration of fermentation in buffer: The results of functional properties of gari as affected by Duration of Fermentation (DF) are shown in Table 4. The pH were high for the samples from DF = 12 (h) (5.7) and DF = 24 (h) (5.3) and declined gradually below pH = 5.0 from the DF = 36 (h). The least mean pH was recorded for DF = 72 (h) (4.37). However, the mean pH for DF = 36 (h) (4.68) did not differ from DF = 48 (h) (4.61) at p>0.05.

Similarly, the mean TTA differed significantly (p<0.05). The DF = 72 h recorded the highest mean TTA% (0.008). The least TTA% was scored by DF = 12 h (0.0049) and DF = 24 (0.0049) respectively. The pH was found to decrease with increases in process time for cassava mash, while the TTA increased in response to increase in acidity.

The Swelling Index (S.I) recorded for the samples increased from DF = 12 h (13.40ml/ml) to a peak at DF = 24 h (20.36 ml/ml) and declined afterward to DF = 72 h (15.15 ml/ml).

The S.I. and BD had optimum performance at 24 and 48 h respectively. Optimum WAC was obtained at 24 h and decline after word to 72 h.

The bulk density also differed significantly at p<0.05. The highest BD g/cm^3 was obtained from DF = 48h (1.2773). The results for DF = 24 h (1.1470) and DF = 36 h (1.1320), also DF = 72 h (1.1520) did not differ (p>0.05). However, the least BD was obtained for DF, 12 h (0.9140).

On the other hand, the mean WAC (ml/g solid) obtained for the samples differed significantly at p<0.05. The increased from DF = 12 (h) (0.91) to 1.49 ml/g solid DF = 24 (h) gradually declined to 1.26 (ml/g solid) for DF = 72 (h) sample.

The mean HCN (mg HCN kg-1) differed significantly at p<0.05. However the least residual HCN was obtained for DF = 72 (h) (9.28), while the highest residual HCN was recorded for DF = 12 h (19.39).

Sensory properties of gari as affected by buffer strength: The result of sensory properties of gari as affected by buffer strength is as shown in Table 5. The panelists preferred the appearance of the control sample (6.39) to the rest, followed by the gari from BF = 7.0 (6.26), while the sample from BF = 5.0, was rated lowest (6.0).

The taste of the gari differed significantly at the condition of study p<0.05. The panelists rated the sample from BF = 7.0 (5.3) as the best, followed by BF = 8.0 (5.2), while the control sample (BF = 0.0) was the least preferred in taste (4.6).

Moreover the gari from BF = 5.0 (5.4) and BF = 6.0 were the preferred in terms of general acceptability while the gari from the control BF = 0.0 (4.8) was rated the least.

Table 3: Mean values of physiochemical properties of gari as affected by Temperature of Fermentation (TF)

Temperature	pH	S.I. (ml)	TTA (%)	BD (g/cm^3)	WAC (ml/g)	HCN (mg)
30	5.71±1.43^a	17.73±3.23^a	0.0042±0.002^b	0.5804±0.01^c	1.13±0.31^b	15.30±7.99^a
35	4.61±0.10^b	7.72±2.46^a	0.0069±0.002^a	1.4860±030^a	1.48±0.32^a	14.02±7.53^b
40	4.6±0.25^b	15.89±2.67	0.0075±0.001^a	1.2876±0.28^b	1.18±0.20^b	12.78±7.42^c
LSD	0.1293	0.3731	0.0003	0.0767	0.0757	0.522

Means are value of triplicate determination. Means not followed by similar letter of alphabet differed significantly at $p \leq 0.05$

Table 4: Means values of physicochemical properties of gari as affected by the Duration of Fermentation (DF)

Ferm. time (h)	pH	S.I. (ml)	TTA (%)	BD (g/cm^3)	WAC (mlg)	HCN (mg/kg)
12	5.77±1.26^a	13.4±0.99^e	0.0049±0.002^a	0.9140±0.25^c	0.91±0.24^c	19.39±8.17^d
24	5.53±1.36^b	20.36±0.65^a	0.0049±0.002^d	1.1147±0.46^b	1.49±0.27^a	16.36±6.19^b
36	4.06±0.18^c	19.05±1.34^b	0.005±0.001^c	1.1320±0.46^b	1.35±0.26^b	14.89±6.26^c
48	4.61±0.14^c	17.61±2.18^c	0.0069±0.001^b	1.2773±0.55^a	1.131±0.28^b	10.32±6.65^d
72	4.37±0.18^d	15.15±1.14^d	0.008±0.001^a	1.1520±0.43^b	1.26±0.25^b	9.28±6.46^e
LSD	0.167	0.4817	0.0004	0.0991	0.0977	0.6739

Means are values of triplicate determination. Means not followed by similar letter of alphabet differed significantly at $p \leq 0.05$.
Ferm. = Fermentation

Table 5: Mean Value of sensory properties of gari as affected by buffer strength

Buffer strength	Appearance	Taste	General acceptability
0	6.39±0.75^a	4.6±0.84^d	4.8±0.99^c
5.0	6.0±0.69^b	5.0±0.92^{bc}	5.4±1.05^a
6.0	6.21±0.99^{ab}	4.9±1.02^c	5.4±0.74^a
7.0	6.26±0.74^{ab}	5.3±0.99^a	5.2±0.71^{ab}
8.0	6.27±0.69^{ab}	5.2±0.92^{ab}	5.0±0.66^{ab}
LSD	0.3824	0.3009	0.3206

Means are values of triplicate determination means with similar letters of alphabets did not differ at <0.05

Table 6: Mean Value of sensory properties of gari as affected by temperature of fermentation

Temp. of ferment	Appearance	Taste	General acceptability
30	5.80±1.00^b	4.3±0.74^b	4.5±0.79^b
35	6.52±0.35^a	5.3±0.83^a	5.5±0.64^a
40	6.35±0.79^a	5.4±0.78^a	5.6±0.64^a
LSD	0.3824	0.3009	0.3206

Means are values of triplicate determination, means followed by similar letters of alphabets did differ significantly p<0.05.
Temp. = Temperature

Sensory properties of gari as affected by temperature of fermentation in buffer: The results of sensory evaluation of gari fermented at 30, 35 and 40°C are shown in Table 6. The gari from 35°C (6.52) fermentation temperature was preferred to that from 30°C (5.80) and 40°C (6.35) in terms of appearance. The performance of gari from 40°C was adjudged second by the panelist while the 30°C (5.8) was the least preferred. The means of gari from 35°C (6.52) and 40°C (6.35) did not differ at p>0.05.

The gari fermented at 40°C (5.5) was rated best by the panelists in terms of taste. There were no significant difference for samples from 35°C (5.3) and 40°C (5.4) at p>0.05. Incidentally the gari from 30°C fermentation temperature had the lowest score. Of all the samples fermented at varying fermentation temperature, the 40°C (5.6) was preferred by the panelists to the rest. The data also show that score for 35°C (5.5) did not differ from that of 40°C in general acceptability at p>0.05.

Table 7: Mean values sensory properties of gari as affected by duration of fermentation (h)

Ferm. tim (h)	Appearance	Taste	General acceptability
12	5.80±1.14^c	4.0±0.84^d	4.5±0.78^d
24	6.13±0.87^{bc}	4.8±0.68^c	5.0±0.94^c
36	6.21±0.63^{ab}	5.1±0.69^b	5.4±0.93^b
48	6.45±0.56^{ab}	5.6±0.82^a	5.8±0.53^a
72	6.55±0.62^a	5.5±0.64^a	5.09±0.43^{cd}
LSD	0.3824	0.3009	0.3206

Means are values of triplicate determination means not followed letter of alphabet did not differ at p<0.05. Ferm. = Fermentation

Sensory properties of gari as affected by duration of fermentation in buffer: The data in Table 7 shows that the rating of appearance gari differed significantly (p<0.05) as the fermentation time increases. The sample from 72 h fermentation had the highest score, 6.55, which is followed by 48 h (6.45) while sample from 12 h had the least score (5.80). The panelists rated sample from 48 h fermentation duration as the best in gari taste (5.6), followed by 72 h (5.6) while the 12 h had the lowest score (4.0).

Moreover the 24 h fermented gari was most preferred in general acceptability (5.8), followed by 36 h (5.4) fermented sample while the 12 h was poorly rated (4.5).

DISCUSSION

Buffer solution used in the treatment of cassava mash was found to leave high pH in the product up to a pH 5.19 when used at BF = 8.0 level. The final pH of the gari was above the recommendation (pH 3.9-4.3) by Achinewhu (1994). High pH is rather undesirable in gari as it might predispose the product to bacterial spoilage. On the other hand, the tritable acidity improved marginally when the buffer treated samples were compared with the control (BF = 0), but very low when compared with 0.75-0.95% obtained by Achinewhu *et al.* 1998) and Achinewhu and Owuamanam (2001). However TTA range of 0.85-1.20% has been judged as satisfactory to good for gari quality (Achinewhu, 1994). It might be suggested, that the low TTA resulted from the

unfavorable growth medium for certain organisms in the natural flora of cassava mash. Gari is cherished for its sour taste, which is due to organic acids such as lactic, acetic, propionic, succinic, pyruvic acid and other flavor compounds: esters, aldehydes etc. produced by fermentative organisms (Collard and Levi, 1959).

The Swelling Indexes (SI) of the buffer treated samples were superior to that of the control. The swelling of gari when soaked either hot or cold water is due to partial dextrinization starch component during roasting such that when the product is rehydrated it swells considerably (Onwueme, 1978). Volume increase ranging from 300-500% has been reported as acceptable for quality gari (Achinewhu, 1994). It is an important quality index as consumers of gari would expect maximum swelling when the product is reconstituted in hot or cold water.

The performances of bulk density of the buffer treated samples were better than the control. However bulk density of the sample are found to be influence by grain size, which is affected by the agglomeration of partially gelatinized product during roasting stage (Achinewhu and Owuamanam, 2001). Intermittent scrubbing between the walls of the roasting pan is needed to disintegrate the lumpy portions of the mash in order to control agglomeration (Onwueme, 1978). It might be suggested that the perceived increase in BD and SI with increasing buffer strength, could be due to poor starch conversion by microorganisms to organic acid.

The lowest residual cyanide recorded for buffer at pH = 7.0 is in agreement with the works of Hahn (1989); Sokari and Karibo (1992) which suggested that maintaining the pH of cassava mash at 5.0-6.0 facilitate the liberation of cyanogenic glucoside. High residual cyanide has been implicated in some degenerative diseases such as tropical ataxic neuropathy, spastic paraparesis, "Konso" etc. (Rosling, 1988).

When cassava mash is fermented in buffer solution, the pH of the resulted product was found to reduce and the TTA marginally increased. Thus, suggesting that activity of microorganism may have been promoted by fermentation temperatures.

The optimum BD and WAC obtained at 35°C could mean that it is the technical temperature condition for fermentation of cassava mash.

On the other hand, residual cyanide was liberated more at 40°C, which supports the finding by Sokari and Karibo (1992) on the role of elevated temperature in bound cyanide reduction.

The pH was found to decrease with increases in process time for cassava mash, while the TTA increased substantially. The gradual decline in pH might be as a result of activities of which managed to thrive in the buffer environment to produce acid far beyond what the buffer can cope. The S.I. and BD where found to reach the optimum at 24 and 48 h respectively.

The residual HCN decreased with process time of cassava mash. The residual cyanide 9.28 mg HCN/kg obtained from sample from 72 h fermentation, which is below the safe level recommended by FAO/WHO (1999) reinforced the need to ferment cassava up to 72 h. Adindu and Aprioku (2006) obtained cyanide content up to 26.1 ppm in commercial gari sold in Rivers South - South Nigeria. Therefore keeping the pH of fermenting mash a little longer after grating cassava might have considerable reduction on cyanide content of gari.

The appearance of gari fermented in buffer solution compared well with the control.

The appearance of gari to a great extent depends on the level of hygiene exhibited by the processor, also depends on the amount wash water, neatness of utensils that come in contact mash before and after roasting (Achinewhu and Owuamanam, 2001).

The taste of the buffer treated sample was judged by the panelists to be superior to the control. Moreover, the samples from BF = 5.0 and BF = 6.0 were preferred to the rest. Thus, it might be possible to improve sensory quality by adjusting the pH of cassava mash up to pH (6.0) for better sensory quality.

The panelists preferred the taste and appearance of gari from cassava mash fermented at 35 and 40°C to the control. While the gari from cassava mash fermented at 40°C was preferred to the rest by the panelists in terms of general acceptability. The preferences of the 35 and 40°C samples over the control might suggest that the fermentation temperature enhanced activities microbes for the production of useful metabolites.

Conclusion: The study has shown that treatment of cassava mash with buffer solution was able to reduce the residual cyanide in gari below the level, 10 mgHCN/kg that is considered safe (FAO/WHO, 1999). With the exception of pH, which was high for the buffer treated samples; other physicochemical properties (SI, BD, WAC etc.) were improved for the treated samples than the control. Buffer treatment is a sure way of guaranteeing cassava food safety and will work well in a mechanized continuous processing; which is yet to be established in gari consuming countries.

The work has revealed that maintaining the temperature condition above the ambient, contributed to volatilization of the residual cyanide. More so, processing at 35°C gave better performance in the physicochemical properties than the control.

On the other hand leaving the mash to ferment for a period up to 72 h further enhanced the reduction of residual cyanide.

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Taurocholate Binding Capacity and Water Holding Capacity of Some Wild Leafy Vegetables of Northern Nigeria

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Abstract: Taurocholate binding capacity (TBC) and water holding capacity (WHC) of twelve wild leafy vegetables commonly consumed in northern Nigeria were studied. Taurocholate binding capacity varied between 17.36±2.58 to 31.02±1.21mg/g. Results obtained showed that Celtis africana had the highest taurocholate binding value of 31.02±1.21 mg/g followed by Hibiscus cannabimis (30.02±1.23) and Balanite aegyptiaca (30.12±3.23mg/g). The lowest taurocholate binding capacity value was observed in Apuim gravealens (21.73±0.25mg/g). While the highest water holding capacity value was observed in Celtis africana (2.24±0.28mg/g), closely followed by Balanite aegyptiaca (2.08±0.11mg/g) and Moringa oleifera (1.88±0.13mg/g). Corchorus oloitoms, Solanium nigrum and Mormodica basalmina had the lowest water holding capacity value (1.02±0.04, 1.21±0.01 and 1.27±0.05mg/g respectively). Thus indicating that these wild vegetables contain high fiber and have the potential to reduce cholesterol considerably from the body and other related diseases.

Key words: Taurocholate binding capacity, water holding capacity, wild vegetables, cholesterol, fiber

INTRODUCTION

The end product of cholesterol metabolism is a primary bile acid known as taurocholate. It is excreted from the body through feaces as neutral steroid of bile acid (Murray *et al*., 1988). Synthesis of bile acid is one of the predominant mechanisms for the excretion of excess cholesterol from the body. However the excretion of cholesterol in the form of bile acids is insufficient to compensate for an excess dietary intake of cholesterol (Michael, 2006). Elevated level of cholesterol in the body causes hypercholesterolemia, which occurs jointly with other disorders associated with lipid metabolism (Murray *et al*., 1988). These diseases or disorders affect middle age sedentary people and tend to occur in combination of two or three in the same individual (Umaru *et al*., 2003). These include arteriosclerosis, colorectal cancer, xanthomatosis, angina, coronary artery diseases etc (Ruales and Nair ,1994). Efforts have been directed to reduce the levels of cholesterol in the hope of reducing the degree of vascular cholesterol deposition. However, some of the drugs used to reduce cholesterol to a minimum level are harmful and have some complications (Lopez, 2002) some cholesterol lowering drugs have their side effects ranging from liver damage, muscle tenderness, weakness, to intestinal problem (Tracy, 2004).

Fiber has the ability to hold water and bind with cholesterol. Absorption of cholesterol is interrupted by dietary fiber in the small intestine, thus minimizing the rate of cholesterol absorption thereby decreasing the rate of lipid absorption (Umaru *et al*., 2003). The product of cholesterol metabolism (bile acid salts) bind with fiber complexes that are not absorbed and are excreted with feaces (Johnson, 1990). Short chain fatty acids, which are products of fermentation from soluble fiber in the gut, may inhibit synthesis of cholesterol by the liver thereby reducing the concentration of blood cholesterol (Truswell, 1990). Dietary fiber also removes health harm full factors such as artificial food, aluminium and mutagens from the body and improves the flora of intestinal bacteria (Lopez, 2002). The high viscosity of soluble fiber may also slow the rate of digestion and absorption of carbohydrate, affecting insulin activity, which is implicated in the removal of LDL-cholesterol (Truswell, 1999).

Wild vegetables are commonly consumed in northern Nigeria. Little is however known about their fiber content. This study was therefore undertaken to determine the taurocholate and water holding capacity of twelve wild leafy vegetables commonly consumed in northern Nigeria.

MATERIALS AND METHODS

Collection and treatment of samples Wild vegetables were obtained around the Federal University of Technology Yola in May, 2005. Plant materials were dried under room temperature and ground to powder using mortar and pestle. Ground materials were sieved using 1mm sieve.

Taurocholate binding capacity determination: To different weight of the samples (2.0, 1.0, and 0.5g),

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Table 1: Taurocholate binding capacities of varieties of wild g leafy vegetables (mg/g)

Vegetable	2g	1g	0.5g
Colocasia esculentus (Taro)	29.24±3.21	23.50±0.51	17.33±0.70[f]
Balanite aegyptiaca (Addua)	30.12±3.25[a]	22.51±0.05	18.64±1.67[f]
Moringa oleifera (Zogale)	29.45±0.50	25.43±2.01	23.22±0.48
Celtis africana (Zuwo)	31.02±1.21[a]	29.16±1.81[c]	6.10±0.13[e]
Apium gravealens (Karkashi)	21.73±0.25[b]	19.48±0.03[d]	17.36±2.58[f]
Hibiscus cannabimis (Rama)	30.60±1.23[a]	23.45±2.43	18.83±0.46[f]
Corchorus olitorius (Lalo)	29.67±0.85	27.39±1.42	25.08±0.72[e]
Mormodica basalmina (Grahuna)	26.95±2.26[b]	24.23±0.80	20.67±1.03
Hibiscus esculentus-bush (Kubewa)	29.63±0.09	27.72±1.37	22.37±1.53
Solanum nigrum (Kumbi)	27.33±0.06[b]	26.90±0.12	22.19±0.07
Cassia tora (tafassa)	29.15±1.38	26.49±3.05	21.80±0.20
Leptadenia histata (Yadiya)	23.01±3.0[b]	21.82±0.05	20.76±2.21
Pectin (Standard)	32.08±0.12	31.30±0.01	31.20±0.08

Results are mean ± SD for three (3) determinations.

a = significantly higher compared with Apium gravealens, Mormodica basalmina, Solanium nigrum and Leptadenia histata under 2g ($p < 0.05$). b = significantly lower compared with other vegetables under 2g ($p < 0.05$). c = significantly higher compared with other vegetables under 1g ($p < 0.05$). d = significantly lower compared with other vegetables under 1g ($p < 0.05$). e = significantly higher compared with other vegetables under 0.5g ($p < 0.05$). f = significantly lower compared with other vegetables under 0.5g ($p < 0.05$)

Table 2: Water holding capacities of varieties of wild leafy vegetables (mg/g)

Vegetables	2g	1g	0.5g
Colocasia esculentus (Taro)	1.66±0.04	0.64±0.13	0.47±0.08
Balanite aegyptiaca (Addua)	2.08±0.11[a]	1.05±0.15	0.97±0.01[c]
Moringa oleifera (Zogale)	1.88±0.13	1.15±0.19	0.57±0.07
Celtis africana (Zuwo)	2.24±0.28[a]	2.06±0.05[b]	0.50±0.05
Apium gravealens (Karkashi)	1.32±0.02	0.91±0.14	0.62±0.06
Hibiscus cannabimis (Rama)	1.67±0.03	0.97±0.07	0.65±0.03
Corchorus olitorius (Lalo)	1.02±0.04	1.05±0.21	0.65±0.08
Mormodica basalmina (Grahuna)	1.27±0.05	0.56±0.07	0.44±0.02
Hibiscus esculentus-bush (Kubewa)	1.61±0.03	0.67±0.09	0.40±0.08
Solanum nigrum (Kumbi)	1.21±0.01	0.77±0.05	0.54±0.03
Cassia tora (tafassa)	1.49±0.02	1.17±0.10	0.56±0.07
Leptadenia histata (Yadiya)	1.57±0.09	0.79±0.09	0.58±0.02
Pectin (Standard)	1.98±0.04	1.89±0.02	0.82±0.01

Results are mean ± SD for three (3) determinations.

a = significantly higher compared with other vegetables under 2g ($p < 0.05$). b = significantly higher compared with other vegetables under 1g ($p < 0.05$). c = significantly higher compared with other vegetables under 0.5g ($p < 0.05$)

10mls of water was added and allowed to boil for 5 minutes. The suspension was then filtered through Whatman (No. 1) filter paper. Samples were then washed thrice with 10ml ethanol each time followed by centrifugation at 2400rpm, the supernatant was discarded and the residue air-dried for subsequent use. Samples were then put into a dialyzing tube and kept in a refrigerator at 4° over night. Twelve test tubes were used for the test. To the test tube labelled blank, 1ml of water, 0.5ml of sucrose solution and 1ml of conc. H_2SO_4 was added drop wise from the biuret. To the eleven test tubes, 1ml of taurocholate, 0.5ml water and 0.5ml sucrose was added to each followed by drop wise addition of 1ml H_2SO_4 from the biuret. Taurocholate concentration was calculated using Lambert-Beers equation.

Water holding capacity determination

Procedure: Water holding capacity was determined according to the method of McConnell and Eastwood (1974). Different weight (2.0, 1.0 and 0.5g) of the powdered sample was soaked in water for 24hours. It was then centrifuged at 2400rpm to remove the interstitial water. The water holding capacity was calculated from the difference in weight of material before and after centrifugation.

RESULTS AND DISCUSSION

Results of taurocholate binding capacity are as shown in Table 1. Results obtained showed that Celtis africana had the highest taurocholate value of 31.0±1.2 mg/g, closely followed by Hibiscus cannabimis (30.60±1.23mg/g) and Balanite aegyptiaca (30.12±3.25mg/g). The high values indicate that these plants have the potential to reduce cholesterol. Hence they can be consumed freely especially by individuals with cholesterol related diseases since fibers are known to bind cholesterol in the body thereby increasing feacal weight, reduce transit time, decrease glucose and cholesterol level in the blood and reduce the risk of colon cancer (Truswell 1990; Kritchevsky, 1997). However it was shown that the degree of bile acids adsorption depends on the kind of raw material and the type of bile acids. According to (Umaru *et al*., 2003), there is a correlation between the taurocholate binding capacity and water holding capacity. Several

epidemiological studies have shown that the faecal bile acids especially the secondary bile acids are higher in population that consume higher-fat and lower fiber diet (Owen *et al*., 1986). If there is sufficient fiber in the diet, bile acids moving along the intestine may be broken back down into cholesterol and reabsorbed into the blood (Anderson *et al*., 1990). Fiber may also block the absorption of fat from the digestive tract and reduce cholesterol synthesis in the liver (Kesanniemi *et al*., 1990; Anderson, 1987). Results obtained in Table 1 showed that some of these wild plants could compute favorably with standard pectin used. Pectin has been shown to reduce cholesterol level (AHS, 1998 and LSRO, 1987). The water holding capacity values ranged from 2.24±0.28mg/g in Celtis africana to 1.02±0.04mg/g in Corchorus oloitoms. The water holding capacity of dietary fiber is thought to be an important factor of faecal bulking and intestinal transit which influence gastrointestinal disease (Suzuki *et al*., 1996; Biekett *et al*., 1997). This suggests that hydrogen bond and hydrophobic interaction mediated in the binding of taurocholate to alcohol insoluble solids. Variations in taurocholate binding capacity values were based on the variation in water holding capacity values. However other physiochemical factors other than water holding capacity may be responsible for the variation in taurocholate binding capacity values. Values obtained for taurocholate binding capacity and water holding capacity of the leafy vegetables for 2g, 1g, and 0.5g were significant at $p< 0.05$. However binding increases with increase in concentration of food sample in the order of 0.5g> 1.0g> 2.0g. All the wild leafy vegetables had the ability to hold water and bind to bile acids *in vitro* and the capacity depends on the tendency to imbibe water. It therefore shows that if 450g of Celtis africana is consumed for a period of three months based on the average consumption of 5g/day/individual, it will be able to extract 45g of taurocholate from enterohepatic circulation, which will bind to equal amount of cholesterol and pass out in faeces, leading to a hypocholesterolemic effect. If intended as a drug, the natural intake of 5g could be doubled to 10g and the duration of application in turn reduced since these plants are consumed freely in the study area with no cultural restriction.

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Ziziphus mauritiana Fruit Extract Inhibits Carbon Tetrachloride-induced Hepatotoxicity in Male Rats

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Abstract: The aqueous extract of *Ziziphus mauritiana* fruit (Zm) was evaluated for its protective activity against CCl_4-induced liver damage. 250, 500 mg/kg bw of Zm fruit extract or 100 mg/kg silymarin (standard) were administered to different groups of rats prior to CCl_4 administration. Both 250 and 500 mg/kg bw of Zm fruit extract significantly ($p<0.05$) reduced (dose dependently) the levels of enzymes and non-enzymes markers of tissue damage when compared to rats given CCl_4 only. These findings were supported by liver histology and suggest that Zm fruit possessed reach hepatoprotective principles that inhibited the toxicity of CCl_4 against the liver.

Key words: *Ziziphus mauritiana* fruit, CCl_4, hepatotoxicity, protection

INTRODUCTION

CCl_4 is an injury agent for animal experiment, which induced reactive oxygen formation and depletion of glutathione. It may reduce antioxidant enzymes and antioxidant substrates to induce oxidative stress. CCl_4 requires bioactivation by cytochrome p450 system of phase I in liver and yields the reactive metabolic trichloromethyl radical ($CCl_3^{\bullet}$) and proxy trichloromethyl radical ($^{\bullet}OOCCl_3$). These free radicals can bind with Polyunsaturated Fatty Acid (PUFA), forming alkoxy ($R^{\bullet}$) and peroxy radicals ($ROO^{\bullet}$), that can generate lipid peroxidation, cause damage in cell membrane, change enzyme activity and finally induce hepatic injury or necrosis (Weber *et al*., 2003). Lipid peroxidation is considered to be of fundamental importance in cell ageing and damage (Popovic *et al*., 2006). At present, in spite of an increasing need for agents to protect the liver from damage, modern medicine lacks a reliable liver protective drug. Therefore a number of natural substances have been studied to evaluate their protective activities (Willet, 1994).

Ziziphus mauritiana Lam belongs to the family *Rhamnaceae* (Michel, 2002). The fruit of *Ziziphus mauritiana* fruits (Indian jujube) is rich in vitamin C, phenolic compounds, with good mineral contents. This study was designed to evaluate the effect of administering aqueous fruits extract of *Ziziphus mauritiana* in carbon tetrachloride induced liver damage.

MATERIALS AND METHODS

Plant: *Zizphus mauritiana* fruits were purchased from Yola market Adamawa State in April, 2007 and authenticated in Botany Department of Federal University of Technology, Yola with voucher specimen number BC/DD07/01. The fruit was further dried at room temperature and made into powder using mortar and pestle.

Animals: Thirty male albino rats weighing 120-150 g were purchased from the animal house of Biochemistry Department University of Jos, Plateau State. The animals were housed in stainless steel cages and kept at room temperature 28±2°C under 12/12 h light/dark and were fed with pelleted standard laboratory feed (Vital Feed; Grand Cereal and Oil Mills, Jos) and water *ad libitum*.

Experimental design: Each group consisted of six (6) animals treated as follows:

Group I (control): Rats were given isotonic solution p.o. (0.5 ml of saline/animal)

Group II: Single dose of CCl_4 + diet/water

Group III treated (250 mg/kg bw): Zm fruit + CCl_4 + diet/water

Group IV treated (500 mg/kg bw): Zm fruit + CCl_4 + diet/water

Group V Silymarin (100 mg/kg bw): Silymarin + CCl_4 + diet/water

Group III, IV and V were pre-treated with aqueous extract of *Ziziphus mauritiana* fruit or silymarin for seven days prior to CCl_4 administration. CCl_4 was administered in olive oil (1:1) 2 ml/kg bw to induce liver damage.

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Preparation of aqueous extract: The fleshy part of the fruit was dried and pulverized in to fine powder with laboratory mortar and pestle. The powder was then sieved using 0.33 mm Endicott test sieve (Endicott, London). 100 g of the powder was mixed with 600 ml of distilled water and allowed to stand for 6 h with continuous shaking. The mixture was then filtered using Whatman No. 4 filter paper. The filtrate was evaporated using rotary evaporator at reduced temperature >50°C to obtain 22.54±2.1 g/100 g.

Collection of serum/liver for analysis: Rats from all groups were sacrificed 48 h after CCl_4 administration. Blood was collected via the ocular vein and allowed to stand for 10 min before been centrifuged at 3000 rpm for 15 min to collect the serum. Serum was separated for the estimation Alanine Aminotransferase (AST), Aspartate Aminotransferase (ALT) and Alkaline Phosphatase (ALP), Bilirubin and cholesterol.

Liver tissues were collected for histopathological analysis; a portion of the liver was fixed in 10% formalin, processed using routine histology procedures, embedded in paraffin, cut in 5 micro mole sections and mounted on a slide. The samples were stained with hematoxylin and eosin for histopathological examination.

Statistical analysis: Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS). Student's 't' test was used to determined statistical difference between two means when $p<0.05$.

RESULTS

Table 1 represents the effect of pretreatment with *Ziziphus mauritiana* aqueous fruit extract on Aspartate Amino Transaminase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) levels in CCl_4 induced liver damage. All the level of AST, ALT and ALP significantly ($p<0.05$) increased in group II (i.e. CCl_4 alone) compared to control (group I). However, pretreatment with 250 and 500 mg/kg of *Ziziphus mauritiana* aqueous fruit extract for seven days prior to CCl_4 administration significantly ($p<0.05$) decreased the levels AST, ALT and ALP (groups III and IV) compared to group administered CCl_4 alone (group II). Pretreatment with 500 mg/kg significantly ($p<0.05$) decreased indices of tissue injury compared to group treated with 250 mg/kg i.e. group III). Pretreatment with silymarin also significantly ($p<0.05$) reduced the level of AST, ALT and ALP compared to CCl_4 group alone. Pretreatment with extracts alone without CCl_4 did not significantly ($p<0.05$) change the levels of markers of tissue injury as analyzed compared to normal group. This shows that at this dose level its consumption was safe.

The levels of both bilirubin and cholesterol were significantly ($p<0.05$) increased in group II compared to group I. Pretreatment with 250 and 500 mg/kg of *Ziziphus mauritiana* aqueous fruit extract prior to CCl_4 administration resulted in significantly ($p<0.05$) decreased levels of both bilirubin and cholesterol compared to group administered CCl_4 alone. Pretreatment with the extract alone without CCl_4 did not result to any significant change in the levels of both bilirubin and cholesterol compared to control.

CCl_4 administration produced general liver morphological changes and necroses, severe centrilobular vacuolar degeneration and mononuclear cell aggregation as shown in Fig. 2 compared to normal liver morphology in Fig. 1. Pretreatment of rats with *Ziziphus mauritiana* revealed moderate and apparently normal organ with very few hepatocytes with tiny cytoplasmic vacuoles (Fig. 3 and 4). Thus *Ziziphus mauritiana* pretreatment greatly inhibited liver morphological changes and necrosis due to CCl_4 hepatotoxicity.

DISCUSSION

CCl_4-induced hepatic injury is an experimental model widely used for hepatoprotective drugs screening. CCl_4 undergoes a biotransformation by hepatic microsomal cytochrome p450, to produce trichloromethyl free radicals. These hepatotoxic metabolites can react with protein and lipid in the membrane of cells or organelles leading to necrosis of hepatocytes (Brent and Rumack, 1993). As a result of hepatic injury, the altered permeability of the membrane causes the enzymes

Table 1: Effect of CCl_4 toxicity and *Ziziphus mauritiana* aqueous fruit extract on enzyme markers and non-enzyme markers of liver damage

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB (mg/dl)	CHOL (mg/dl)
Control	25.20±2.28	13.30±1.67	576.81±10.7	10.46±2.54	1.72±0.22
CCl_4	93.60±3.51*	21.90±1.74*	1043.40±6.5*	26.56±2.90*	3.66±0.13*
Zm 250 mg/kg + CCl_4	43.92±2.89**	15.90±2.70	626.23±14.1**	17.70±3.49**	3.10±0.10
Zm 500 mg/kg + CCl_4	38.90±4.74	17.90±11.74	499.26±30.6	17.26±2.56	2.22±0.08**
Silymarin 100 mg/kg + CCl_4	23.83±2.50	11.90±2.10	558.50±29.4	10.15±1.25	1.70±0.24

Results are Mean±SD, (n = 6). *Significantly higher than normal group ($p<0.05$). **Significantly lower than group administered CCl_4 only. Significantly lower than group pre-treated with 200 mg/kg aqueous extract of *Ziziphus mauritiana* ($p<0.05$)

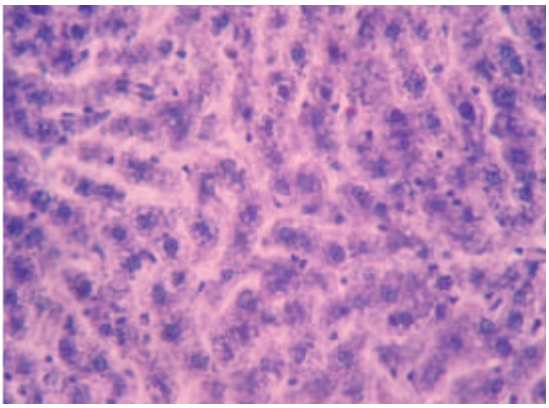

Fig. 1: Histology of normal liver tissue from control rat liver (H and E; x650)

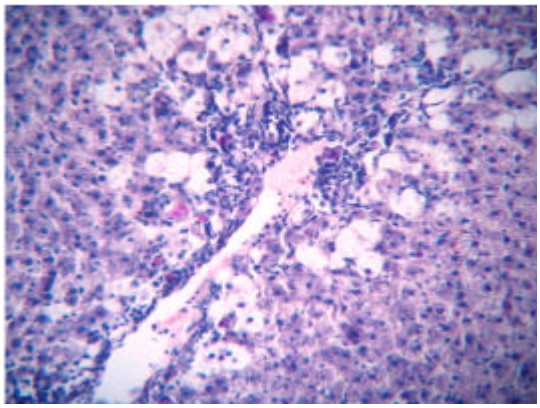

Fig. 2: CCl_4 alone showing severe centrilobular vacuolar degeneration, hepatocellular necrosis and mononuclear cell aggregations (H and E; x650)

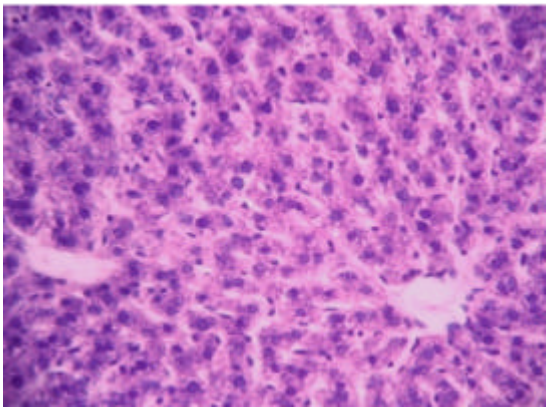

Fig. 3: Liver tissue of rat pre-treated 250 mg/kg Zm plus CCl_4, showing an apparently normal organ with very few hepatocytes with tiny cytoplasmic vacuoles (arrows) (H and E; x650)

from the cells to be released into the circulation (Drotman and Lawhorn, 1978). The magnitude of

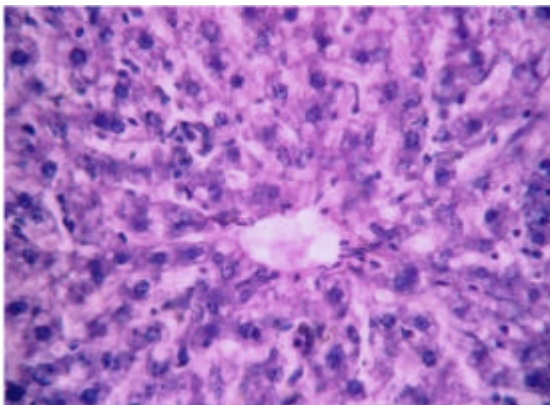

Fig. 4: 500 mg/kg Zm plus CCl_4, showing an apparently normal organ with very few hepatocytes with tiny cytoplasmic vacuoles (H and E; x650)

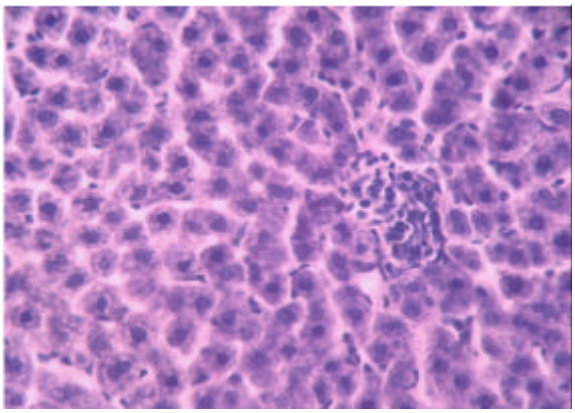

Fig. 5: Liver tissue of rat pre-treated with 100 mg/kg bw silymarin showing an apparently normal liver with a focal area of mild mononuclear periportal cell aggregate (H and E; x650)

hepatic damage is usually assessed by measuring the level of released cytosolic transaminases including ALT and AST in the circulation (Guti'errez and Solis, 2009). The rise in the serum levels of ALP, AST and ALT as observed in the present study could be attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage (Recknagel *et al*., 1989), other researchers had reported increased level of AST, ALT, ALP and bilirubin due to CCl_4 hepatotoxicity (Dahiru *et al*., 2005; Galati *et al*., 2005; Guti'errez and Solis, 2009). The increase in the level of serum bilirubin is an index of the degree of jaundice. This could possibly be as a result of increased production, decreased uptake by the liver, decreased conjugation, decreased secretion from the liver or blockage of bile ducts (Bun *et al*., 2006). Increase cholesterol level due to CCl_4 could be as a result of its peroxidative degradation in the

adipose tissues resulting in fatty in filtration in to the circulation. The result of this study demonstrated that pretreatment of rats with *Ziziphus mauritiana* aqueous fruit extract effectively protected rats against CCl_4 induced hepatotoxicity in a dose dependent manner as evidenced by the decreased serum ALT, AST, ALP, bilirubin and cholesterol.

The histopathological findings in this study agrees with earlier reports that CCl_4 causes necrosis, mononuclear cell infiltration, steatosis foamy degeneration of hepatocytes (Nan *et al*., 2003; Hung *et al*., 2006; Liu *et al*., 2006). *Ziziphus mauritiana* fruit extract protected the liver against CCl_4 induced liver morphological changes, fatty liver development and cellular degeneration. It is possible that the extract might have blocked adipogenesis. Reduced serum cholesterol as observed in the result due to pretreatment with *Ziziphus mauritiana* was an indication of anti-atherosclerotic activity, hence possible modulation of lipid metabolism.

Conclusion: The results of this study were a clear demonstration that *Ziziphus mauritiana* aqueous fruit extract possessed potent hepatoprotective action against CCl_4 induced liver damage in rats. The protective effects observed could be as a result of the rich phenolic compounds; caffeic acid, p-hydroxybenzoic acid and vitamin C (Muchuweti *et al*., 2005).

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Detection of a Toxic Phenolic Compound in Cottonseed Extract and its Products

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Abstract: Cottonseed is a good source of high quality meal and edible oil. However, it contains a toxic phenolic compound called gossypol. Gossypol is an anti-nutritious factor that limits the use of cottonseed and its products due to its toxicity associated to its reactions with amino acids and minerals. This is the first study on gossypol ever conducted in Pakistan. The purpose of this study was to estimate the level of gossypol in oil and ghee (Hydrogenated oil) samples from local markets so that awareness can be created regarding maintenance of minimum gossypol level in edible oils. During this study gossypol was extracted from cottonseeds and cotton seed cake using different organic solvents. The compound was detected in extracts by applying Chromatographic technique as well as chemical tests with $SbCl_3$, $Pb(CH_3COO)_2$ and $SnCl_3$. Spectrophotometric techniques were also employed for quantitative analysis by measuring absorbance of samples at wavelength of 290 nm. It was found that overall the contents of gossypol were higher in cottonseed oils as compared to Ghee (hydrogenated oil).

Key words: Gossypol, cottonseed extract, phenolic compounds, edible oils, ghee, Pakistan

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) seed is majorly used as a source of edible oil and ghee in Pakistan. Cotton and related species all contain gossypol, a polyphenolic compound that is an integral part of the cotton plant's self-defense system against insect pests and possibly some diseases (Jodi and Gabriela, 2008). Some amount of gossypol tends to react with many natural substances in cottonseed and forms the bound gossypol that is non-harmful. However the unreacted gossypol known as "free gossypol" is toxic. Thus free gossypol is an anti-nutritional factor that limits the use of cottonseed and its products (Hron *et al*., 1987).

Gossypol [2, 2′-Bi (8-formyl-1, 6, 7, trihydroxy 5-isopropyl-3-methyl naphthalene)] is a crystalline compound (Fig. 1). The molecular formula of gossypol is $C_{30}H_{30}O_8$. The inclusion compound formation by gossypol has been studied at different thermodynamic conditions. Most of the investigated molecules form more than one inclusion compounds with gossypol. Polymorphism exhibited by gossypol inclusion compounds is dimorphism and trimorphism.

The toxicity of gossypol is associated to the reaction of its phenolic groups to amino acids and minerals. Hydrogen bonding and oxidation of the carbonyl groups result in easily reactive quinones that bind with proteins. Heating of cottonseeds during oil extraction binds gossypol to proteins. Thus reduces protein availability from cottonseed meal.

Gossypol also reacts with phosphatide-phosphatidylethanol amine present in cottonseed. This reaction product is safer. However gossypol is again converted to its harmful free form in stomach. This conversion of the bound gossypol to free gossypol is called "gossypol reversibility".

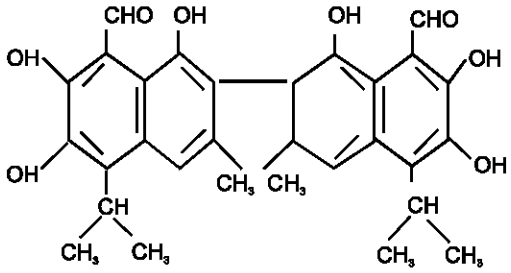

Fig. 1: Structure of gossypol

The main cumulative toxicological effect of gossypol are loss of appetite with consequent weight loss, liver and lung lesions like pulmonary edema preceded by difficult breathing, cardiac irregularity and failure which sometimes become lethal, anemia due to iron complexation and induction of male sterility. At low levels, gossypol blocks spermatogenesis and reduces sperm motility. Reversible antispermatogenic effect of gossypol in langur monkeys (*Presbytis entellus*) has also been studied (Sharma *et al*., 1999).

Randel and co-workers in 1992 studied the effects of gossypol and cottonseed products on reproduction of mammals and came to the conclusion that non-ruminant animals are particularly sensitive to the toxic effects of gossypol, whereas ruminants are somewhat more resistant. Gossypol seems to disturb estrous cycle, pregnancy and early embryo development in females of all non-ruminant species (Randel *et al*., 1992).

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Stephens and fellows in 1983 studied the mechanism of glycolysis inhibition by gossypol. It was found that the only site of glycolytic inhibition was isozyme X of Lactate dehydrogenase enzyme. Gossypol also decreases the concentration of adenine nucleotides, ATP, ADP and AMP and this is most probably the basis for its toxic effect on spermatozoa.

Gossypol usually renders harmless on crushing or heating but may retain minute amounts to which pigs and chickens are sensitive. Gossypol iron-binding properties cause olive green yolks in eggs. It also decreases the hatchability of eggs.

The toxic effect of gossypol can be used against the cancerous cells. It has been found to have antiproliferative activity on tumor cells and is thought to be a potential anticancer drug. Gossypol may provide a potential therapeutic benefit for the treatment of colon carcinoma. Understanding the mechanism of gossypol-induced cytotoxicity on tumor cells can be helpful for including this drug in clinical use (Wang *et al*., 2000).

Racemic gossypol, composed of both (-)-gossypol and (+)-gossypol, is used in herbal medicines in China. Studies on melanoma, breast cancer and colon cancers have shown that racemic gossypol is well tolerated and is moderately effective in reducing tumor volume (Poznak *et al*., 2001; Blackstaffe *et al*., 1997). Keith and his coworkers has also demonstrated that (-)-gossypol can inhibit tumor growth (Keith *et al*., 2006). Since gossypol confers antibiosis type of resistance to the cotton plant against different insects, it is also used as pesticide (Anonymous, 1982).

Gossypol has been the source of scientific interest for over a century. Recent changes in both cotton fiber and cottonseed products markets have focused renewed interest on potential alternate uses for gossypol contained in the seed.

MATERIALS AND METHODS

Sample collection: Samples of different brands of edible oils and ghee prepared from cottonseed oil were purchased from the local market of Multan city of Pakistan. A total of sixteen samples were collected. Out of which 8 samples were of oils and 8 of ghee. The trade names and manufacturers name will not be mentioned so that their business may not be affected.

Extraction of gossypol: For extraction of gossypol three grams of seed kernels obtained manually were crushed and extracted with diethyl ether (5 x 20 ml) (Nazarova and Glushenkova, 1983). The solvent was evaporated at low temperature till an oily material containing gossypol was obtained. This was stored for further use.

Gossypol was extracted with aqueous acetone (Botsoglou, 1991) with the same method. The residual left after the extraction of free gossypol with aqueous acetone was soaked in 2M HCl solution (75 ml) for 10 min and then refluxed for 30 min. After cooling, the solution was filtered. The residue was washed with absolute ethanol (15 ml). The filtrate was extracted with chloroform (4 x 30 ml). Then chloroform was evaporated from the extract at low temperature till an oily material containing gossypol was obtained.

Detection of gossypol by chemical tests: Specific chemical tests were performed for detection of gossypol in samples of edible oils and ghee. For this purpose 5 gram of each sample of oil and ghee was dissolved in small volume of ethanol in 25 ml conical flask and final volume was made up to the mark by adding more ethanol. Two ml of each sample solution was taken in the test tube separately and equal amount of solid antimony chloride was added in each test tube and mixed thoroughly.

Similarly, tests were performed with stannic chloride and lead acetate. The same tests were also performed with the extract of cottonseed as standard.

Thin layer chromatographic studies: Thin layer chromatography was also employed for qualitative analysis of gossypol in the samples of oils and ghee following Ventalchalam's method (Ventalchalam *et al*., 1980). TLC plates were prepared using silica gel F_{254} as adsorbent. The thickness of the plates was 0.02 mm. 1 g of each sample was dissolved in 5ml of diethyl ether. Then equal volume of the diluted samples and ether extract of cottonseeds (standard) were spotted on the plates with the fine capillary jet. The solvent system composed of benzene, dioxane and acetic acid (91:10:4) was used for the development of chromatoplates. The plates were taken out from the tank after two hours and dried in air thoroughly.

Spectrophotometric measurements for quantitative analysis of gossypol: Absorption spectra of cottonseed extract and samples in chloroform were recorded in 250-350 nm regions in quartz cell using UV-visible spectrometer. In this method reaction of the analyte with chromogenic reagent is not required, since the second derivative transformation and measurement of the conventional analytical band around 300 nm permits direct quantification of gossypol in sample extracts (Botsoglou, 1991). For this purpose 1 g of each sample was dissolved in 100 ml chloroform. The absorbance of each sample was noted directly at 290 nm.

RESULTS

Qualitative analysis of gossypol: For detection of gossypol in the samples of edible oils and ghee as well as in pure extract of cottonseeds, three specific chemical tests with $SbCl_3$, Pb $(CH_3COO)_2$ and $SnCl_3$ were performed. Turbid reddish complex appeared in case of

Table 1: Results of detection of gossypol through biochemical tests, TLC and Spectrophotometric studies

Samples	$SnCl_3$ Test	$SbCl_3$ Test	$Pb(CH_3COO)_2$ Test	TLC analysis of samples	Absorbance at 290 nm
Ghee 1	+	+	+	Light violet	1.212
Ghee 2	+	+	+	Light violet	1.338
Ghee 3	+	+	+	Light violet	0.788
Ghee 4	+	+	+	Lightest violet	0.529
Ghee 5	++	++	++	Dark violet	1.126
Ghee 6	+	+	+	Light violet	0.528
Ghee 7	+	+	+	Light violet	0.732
Ghee 8	+	+	+	Lightest violet	0.686
Oil 1	++	++	++	Dark violet	1.034
Oil 2	+	+	+	Lightest violet	0.791
Oil 3	+	+	+	Light violet	0.894
Oil 4	++	++	++	Dark violet	1.460
Oil 5	++	++	++	Dark violet	1.714
Oil 6	+	+	+	Light violet	1.498
Oil 7	+++	+++	+++	Dark violet	1.980
Oil 8	+	+	+	Light violet	1.466

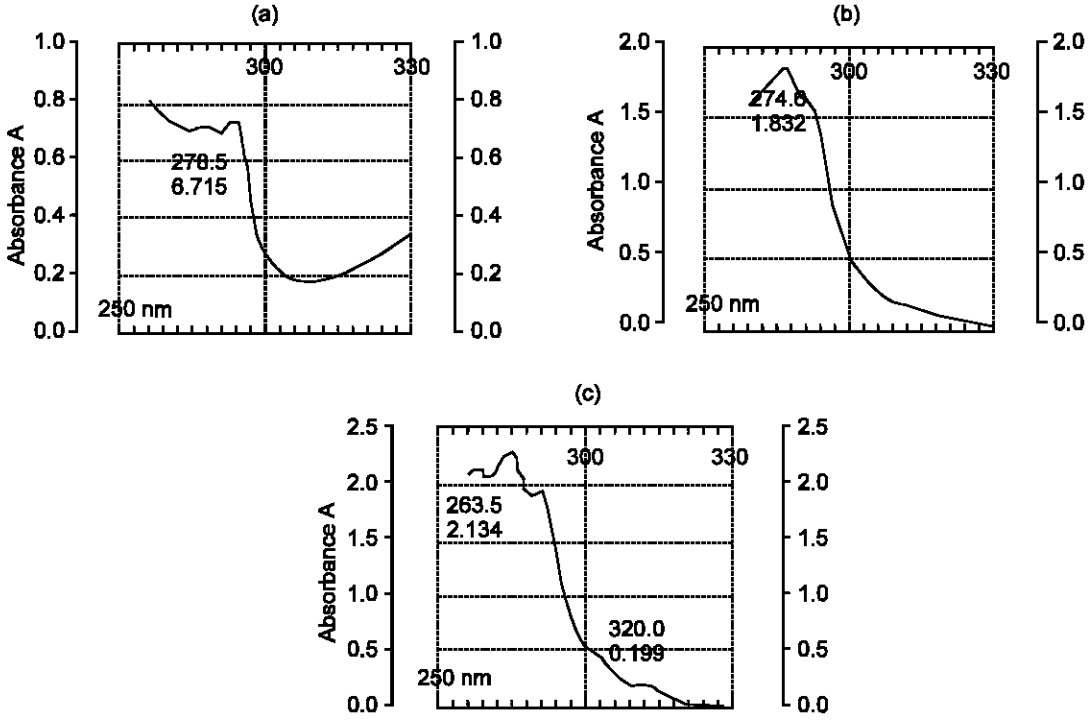

Fig. 2: Absorption spectra of (a) Cottonseed extract (b) Ghee sample 1 (c) Oil sample 8 in chloroform

$SbCl_3$ after 15 min. While reddish precipitate appeared after 10 min in case of test with Stannic chloride. Test with lead acetate gave yellowish precipitate that appeared after 20 min. The intensity of colour increased with the passage of time in case of all tests. Since cottonseed is a richest source of gossypol, therefore its extract was used as standard in these studies. Gossypol was detected in all the samples since these gave positive test with all the reagents with different intensities comparable to that of cottonseed extract. The results are given in Table 1.

For further confirmation of gossypol in the samples TLC was performed. A single light blue spot was detected on chromatoplates under UV-light. The Rf values of the spot in all the samples was approximately 0.97, which was comparable to that of the spot in the standard pure cottonseed extract. It is therefore concluded that gossypol is present in all the brands of oils and ghee having cottonseed oil.

Quantitative analysis of gossypol by spectrophotometry: Because of the phenolic nature of gossypol, spectrophotometric measurements of cottonseed extract and samples were made in UV region (Fig. 2). It was observed that cottonseed extract had spectral maxima at 278.5 and 288 nm having

highest extinction coefficient at 288 nm. All the samples also have two spectral maxima at 273-274 nm and 285 nm having higher extinction coefficient at 273-274 nm, comparable to that of pure gossypol reported in literature, thus permitting the direct quantification of gossypol. The appearance of spectral maxima in this region of UV light is indicative of gossypol in the samples. The spectrophotometric studies were carried directly at 290 nm to avoid the interference of other aromatic compounds that usually show absorbance at about 270 nm. Results are given in Table 1.

DISCUSSION

Gossypol is a phenolic compound that is why spectrophotometric method was applied for its detection. The reason for this is that all phenolic compounds are aromatic and they show intense absorption in the UV region (200-350 nm) of the spectrum. Spectral methods are therefore especially important for identification and quantitative analysis of phenolic compounds (Harborne, 1985).

Overall during these studies it was observed that the amount of gossypol was less in ghee samples than in oils due to conversion of free gossypol to bound gossypol during the process of ghee formation including hydrogenation of oils. The variation in the contents of gossypol in various brands of oils and ghee may be due to different methods of oil extraction and refining.

In food the accepted level of gossypol is 0.045% (Cherry, *et al*., 1981). Different brands of edible oils and ghee are used for cooking and frying of food and also used in sweets, bakery products etc. If gossypol is accumulated in human body, it may cause harmful effects. It is therefore suggested that one should be careful when using the products of cottonseed.

Food and animal feed industries must minimize cotton-derived product levels to prevent toxicity. Scientists at Texas A&M University have genetically modified cotton plants that contain less gossypol in the seed, but the compound remains in the stems and leaves. This protects the plant from pests. This gossypol-free cottonseed can be used as a high-quality protein source for human as well as animals. (USDA/Agricultural Research Service, 2007).

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Proximate and Mineral Compositions of *Dioscorea rotundata* (White Yam) and *Colocasia esculenta* (White Cocoyam)

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Abstract: *Dioscorea rotundata* (white yam) and *Colocasia esculenta* (white cocoyam) were analyzed for their proximate and mineral compositions. The result showed that *Dioscorea rotundata* has a moisture content 54.50%, ash content 1.4%, crude fat content 2.70%, crude protein content 0.087%, crude fibre content 0.70%, carbohydrate content 40.61%, available energy 731.75 kJ; while *Colocasia esculenta* has moisture content 38.50%, ash content 1.60%, crude fat content 1.05%, crude protein content 0.066% crude fibre content 1.0%, carbohydrate content 57.78%, available energy 1022.27 kJ. The mineral content of the analyzed samples showed that *Dioscorea rotundata* and *Colocasia esculenta* were rich in iron of values 81.85 mg/100 g and 59.07 mg/100 g respectively. The copper content of the samples were 10.06 mg/100 g and 6.72 mg/100 g for *Dioscorea rotundata* and *Colocasia esculenta* respectively.

Key words: *Dioscorea rotundata*, *Colocasia esculenta*, proximate analysis, mineral compositions

INTRODUCTION

Dioscorea rotundata (white yam) is grown in West Africa especially Nigeria. It is about 1.6 m in height and weighs about 2-5 kg depending on size. The tuber has a rough skin usually dark to light brown in colour. This rough skin can be peeled with minimal degree of difficulty. The yam become edible only if it is well washed and properly cooked. These steps are necessary in order to reduce the anti-nutritional components of yam before consumption (Dumnnt and Vernier, 1997). Planting of yam tuber starts from march and harvesting is between September and October and late harvesting is between November and December for those not living in coastal region (FAO, 1990). The Igbos of Eastern Nigeria call white yam Jiaga, the Yorubas of Western Nigeria call it Isu ewura and Hausas of Northern Nigeria call it Doya. Yam in not only a preferred high energy food, but a king crop tied up with the socio-cultural life of the people in West Africa especially Nigeria.

Colocasia esculenta (white cocoyam) is a tropical root crop grown in Nigeria. It is planted between the month of March and April. It is harvested between August and September. The Igbos of Eastern Nigeria call it Ede Ocha, while the Yorubas of Western Nigeria call it Isu kobo and the Hausas of Northern Nigeria call it Gwasa or Makani.

White yam and white cocoyam are tropical tubers that store edible material in subterranean roots, corms or tubers. They consist of starch which is the only quantitative important digestible polysaccharides being regarded nutritionally superior to low molecular weight carbohydrate or sugar (Malcolm, 1990). Starch is an important ingredient in food and non-food industries such as paper, plastic, adhesive, textile and pharmaceutical industries. Tubers processing is aimed at obtaining products that are stable in terms of longitivity, nutrition and palatability (Oladebeye *et al*., 2008a). The aim of present study is to compare the proximate and mineral compositions of *Dioscorea rotundata* and *Colocasia esculenta* since some tribes in Nigeria do not eat cocoyam due to cultural and superstitious believe that it is a taboo for somebody to eat cocoyam.

MATERIALS AND METHODS

Sample collection and preparation: Fresh samples of *Dioscorea rotundata* (white yam) and *Colocasia esculenta* (white cocoyam) were obtained from Nkwo Amafor market in Ohaji Local Government Area of Imo State, Nigeria. Both samples were thoroughly washed, peeled, air dried and ground with manual blender. The powdered samples of D*ioscorea rotundata* and *Colocasia esculenta* were finally sieved through 250 µm mesh and stored in plastic container until use.

Proximate analysis: The moisture, ash and crude fibre contents of samples of *Dioscorea rotundata* and *Colocasia esculenta* were determined using Standard Chemical Methods described by Association of Official Analytical Chemistry (AOAC, 1990) by drying 2g each of the sample at 105^{o}C for 24 h for moisture content determination. The ash content was determined by incineration of 2 g of each sample in a muffle furnace at 500^{o}C for 2 h. Soxhlet extraction technique using

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petroleum ether (40-50°C) was used to evaluate the fat contents of the samples (Pearson *et al*., 1981). Kjedahl method was used to determine the crude protein contents of the samples as described by (AOAC, 1990). The contents of carbohydrate of the samples were estimated by difference (% carbohydrate = 100% - sum of percentage of moisture, ash, fat, crude fibre and crude protein contents).

Mineral analysis: Mineral composition of the samples were determined according to methods recommended by Association of Official Analytical Chemists (AOAC, 1990). 1g each of the sample of *Dioscorea rotundata* and *Colocasia esculenta* was digested using 12 cm^3 of mixture of HNO_3, H_2SO_4 and $HClO_4$ (9:2:1 v/v) (Sahrawat *et al*., 2002). Copper, iron, zinc, sodium, potassium, calcium and magnesium were analyzed by Atomic Absorption Spectrophotometer (Pye-Unicam 969, Cambridge, UK). Phosphorus contents of the samples were determined using Flame photometer.

RESULTS AND DISCUSSION

The results of the proximate composition of flour samples of *Dioscorea rotundata* and *Colocasia esculenta* were depicted in Table 1. *Dioscorea rotundata* and *Colocasia esculenta* have moisture content 54.50% and 38.50% respectively. Generally the moisture content of the two samples were high, indicating that the samples are prone to microbial attack in the course of storage. The moisture content of the two samples however indicates that the samples could not be stored favourably for a long period of time because they will deteriorate. It has been reported (Oladebeye *et al*., 2008a) that sweet potato and red cocoyam have moisture content 8.72% and 9.02% respectively. The result of the analysis shows that flours of white yam and white cocoyam have ash content 1.40% and 1.60% respectively. Ash is a measure of total mineral content in the samples. The result indicates that the samples could be a source of mineral elements having nutritional importance.

Table 1 shows the fat content 2.70% and 1.05% in flours of white yam and white cocoyam respectively. That fat supplies most of the energy required by man (Osborne and Voogt, 1978) suggests that *Dioscorea rotundata* is a better source of calories than *Colocasia esculenta*. Fat serve as energy store in the body. It can be broken down in the body to release glycerol and free fatty acids. The glycerol can be converted to glucose by the liver and used as a source of energy. It has been reported (Oladebeye *et al*., 2008b) that flours of rice, millet and wheat have fat content 0.75%, 0.79% and 3.03% respectively. The result indicates that fat content of white yam and white cocoyam were higher than rice and millet except wheat flour. It has been reported that 1 g of fat provide 37 kcal of energy (Gaman and Sherrington, 1990). The crude protein content of *Dioscorea rotundata* and *Colocasia esculenta* were low of values 0.087% and 0.066% respectively. Whereas crude protein content of 1.41% and 1.63% have been reported (Oladabeye *et al*., 2008a) in red cocoyam and sweet potato respectively. The result of the analysis indicates that crude protein content of the two samples were very low. Therefore, *Dioscorea rotundata* and *Colocasia esculenta* are not good sources of protein.

Table 1: Proximate composition of *Dioscorea rotundata* and *Colocasia esculenta* samples (% dry weight)[a]

Parameters	*Dioscorea rotundata* (White yam)	*Colocasia esculenta* (White cocoyam)
Moisture contents	54.50±0.03	38.50±0.04
Ash contents	1.40±0.02	1.60±0.01
Crude fat contents	2.70±0.02	1.05±0.01
Crude protein contents	0.087±0.03	0.066±0.04
Crude fibre contents	0.70±0.01	1.00±0.01
Available carbohydrate	40.61±0.02	57.78± 0.02
Available energy (kJ)[b]	731.75±0.14	1022.27±0.14

[a]Values are mean±standard deviation of triplicate determinations. [b]Calculated Metabolisable energy (kJ/100 g sample):(Protein x 17 + fat x 37 + carbohydrate x 17)

The crude fibre content of flour samples of *Dioscorea rotundata* and *Colocasia esculenta* were 0.70% and 1.00% respectively. The result indicates that white cocoyam has higher fibre content than white yam. Report (Oladebeye *et al*., 2008a) have shown that fibre content of red cocoyam and sweet potato were 0.50% and 0.75% respectively. Fibre has useful role in providing roughage that aids digestin (Eva, 1983). Dietary fibre reduces the risks of cardiovascular diseases. Report have shown that increase in fibre consumption might have contributed to the reduction in the incidence of certain diseases such as diabetes, coronary heart disease, colon cancer and various digestive disorder (Augustin *et al*., 1978). Fibre consumption also soften stools and lowers plasma cholesterol level in the body (Norman and Joseph, 1995). The carbohydrate contents of white yam and white cocoyam were 40.61% and 57.78% respectively. It has been reported that carbohydrate content of red cocoyam and sweet potato were 86.69% and 86.90% respectively. The result of the analysis shows that carbohydrate content of white yam and white cocoyam were low when compared to values reported for red cocoyam and sweet potato. Carbohydrate supplies energy to cells such as brain, muscles and blood. It contribute to fat metabolism and spare proteins as an energy source and act as mild natural laxative for human beings and generally add to the bulk of the diet (Gordon, 2000; Gaman and Sherrington, 1996). The calculated metabolizable energy values show high value of 1022.27 kJ for *Colocasia esculenta* and 731.75 kJ for *Dioscorea rotundata*. The high energy value of *Colocasia esculenta* may be attributed to high carbohydrate content.

Table 2: Mineral composition of *Dioscorea rotundata* and *Colocasia esculenta* samples (mg/100 g)[a]

Minerals	*Dioscorea rotundata* (White yam)	*Colocasia esculenta* (White cocoyam)
Sodium	185.15±0.05	270.83±0.04
Potassium	209.13±0.03	345.32±0.02
Calcium	132.02±0.04	87.14±0.02
Magnesium	45.90±0.02	28.02±0.03
Iron	81.85±0.01	59.07±0.03
Copper	10.06±0.05	6.72±0.04
Zinc	5.46±0.02	1.30±0.03
Phosphorus	54.00±0.04	36.00±0.02
Na/k	0.89	0.78
Ca/P	2.44	2.42
Ca/Mg	2.88	3.11

[a]Values are mean±standard deviation of triplicate determinations

Minerals are important component of diet because of their physiological and metabolic function in the body. The result presented in Table 2 shows that *Dioscorea rotundata* and *Colocasia esculenta* have sodium content 185.15 mg/100 g and 270.83 mg/100 g respectively. Sodium is an important mineral that assist in the regulation of body fluid and in the maintenance of electric potential in the body tissue. The World Health Organization (WHO) recommended intake of sodium per day is 500 mg for adult and 400 mg for children (WHO, 1973). The result indicates that sodium content of *Dioscorea rotundata* and *Colocasia esculenta* were below WHO recommended standard. This study shows that white yam and white cocoyam have potassium content 209.13 mg/100 g and 345.32 mg/100 g respectively. Potassium is important in the regulation of heart beat, neurotransmission and water balance of the body. The WHO recommended intake of potassium per day is 2000 mg for adult and 1600 mg for children. This study revealed that potassium content of white yam and white cocoyam were below WHO standard.

Calcium is an important mineral required for bone formation and neurological function of the body. *Dioscorea rotundata* and *Colocasia esculenta* have calcium content 132.02 mg/100 g and 87.14 mg/100 g respectively. The recommended daily intake of calcium by WHO is 800 mg for both adult and children. This study indicates that white yam and white cocoyam were below WHO standard. *Dioscorea rotundata* and *Colocasia esculenta* have magnesium content 45.90 mg/100 g and 28.02 mg/100 g respectively. Magnesium plays essential role in calcium metabolism in bones and also involve in prevention of circulatory diseases. It helps in regulating blood pressure and insulin releases (Onyiriuka *et al*., 1997; Umar *et al*., 2005). Recommended Dietary Allowance (RDA) for magnesium in adult is 350 mg/day, while children is 170 mg/day. The result revealed that the values obtained from white yam and white cocoyam were far below the recommended values. Therefore white yam and white cocoyam cannot be regarded as a rich source of magnesium.

Table 2 shows iron content 81.85 mg/100 g and 59.07 mg/100g for *Dioscorea rotundata* and *Colocasia esculenta* respectively. The recommended dietary allowance for iron in adult and children is 10 mg/day, while female adult is 15 mg/day. This study indicates that both white yam and white cocoyam were rich in iron and far above the recommended standard. Iron is required for blood formation and it is important for normal functioning of the central nervous system (Adeyeye and Fagbohon, 2005). It also facilitates the oxidation of carbohydrate, protein and fats. The copper content of *Dioscorea rotundata* were higher than that of *Colocasia esculenta* of values 10.06 mg/100 g and 6.72 mg/100 g respectively. The values of the two samples were higher than the recommendation dietary allowance of 3 mg/day for adult and 2 mg/day for children. Copper is required in the body for enzyme production and biological electron transport.

The result of this study shows that *Dioscorea rotundata* and *Colocasia esculenta* have zinc content 5.46 mg/100 g and 1.30 mg/100 g respectively. The WHO recommended standard for zinc in adult and children are 15 mg/day and 10 mg/day respectively. The values of the two samples studied were below WHO standard. Zinc is an essential micronutrient associated with number of enzymes, especially those associated with synthesis of ribonucleic acid (Guil-guerrero *et al*., 1998). Zinc deficiency limits the rate of recovery for protein energy in malnourished children (Hambridge, 1986). The phosphorus content of white yam and white cocoyam were 54.00 mg/100 g and 36.00 mg/100 g respectively. The RDA for phosphorus in adult and children is 800 mg/day. The values of phosphorus in both samples were far below recommended standard.

The ratio of sodium to potassium in the body is of great concern for prevention of high blood pressure. Na/k ratio less than one is recommended. The ratio of Na/k were less than one in *Dioscorea rotundata* and *Colocasia esculenta*, therefore the two samples would not promote high blood pressure. The result of the analysis indicates that Ca/P ratio were similar in *Dioscorea rotundata* and *Colocasia esculenta* of values 2.44 and 2.42 respectively. If the Ca/P ratio is low (low calcium, high phosphorus intake), more than the normal amount of the calcium may be lost in the urine. Food is considered "good" if Ca/P ratio is above one and "poor" if the ratio is less than 0.5, while Ca/P ratio above two helps to increase the absorption of calcium in the small intestine. This study indicates that both white yam and white cocoyam are good source of calcium and phosphorus. The Ca/Mg ratio in *Dioscorea rotundata* and *Colocasia esculenta* were high of values 2.88 and 3.11 respectively, above the recommended value of 1.00 (NRC, 1989).

Conclusion: The result of the analysis shows that *Dioscorea rotundata* and *Colocasia esculenta* were rich in carbohydrate. This study revealed that *Colocasia esculenta* has higher available energy more than *Dioscorea rotundata*. The result revealed that the two samples were rich in iron when compare to RDA for both adult and children. Also *Dioscorea rotundata* and *Colocasia esculenta* were rich in copper when compared to WHO standard. The Ca/P ratio indicates that both *Dioscorea rotundata* and *Colocasia esculenta* are good food sources.

From the result of the analysis it is recommended that people should eat white cocoyam as staple food like white yam, especially those tribes in Nigeria who do not eat cocoyam because of superstitious believe that it is a taboo for somebody to eat cocoyam.

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Nutritional Status of Affluent School Children of Dera Ismail Khan: Is under Nutrition Common?

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Abstract: The present study assesses the frequency of underweight in primary school children from families of high socioeconomic status in Dera Ismail Khan City, Pakistan. It included 322 children, 221 (68.63%) boys and 101 (31.37%) girls. The schools such as The Qurtruba, The City and The Educators were selected as they are the only institutions that cater wards of high socio economic group in Dera Ismail Khan. They were living in healthy environments, have excellent nutrition and easy access to health facilities. Thorough medical examination excluded those suffering from chronic health ailments. Height and weight of each child was taken. BMI was calculated according to the Quatelet's Index and body mass status according to WHO S´ criteria. The BMI-for-age percentile was derived by putting BMI values on gender specific CDC S´ growth charts 2-20 years. Children falling below 5th percentile were declared as under weight and those >95th percentile as obese. Frequency of under weight in primary school children was found 5.59% (in sample): 4.03% for boys and 1.55% for girls. Boys were more than two times under weight than girls. This may be due to absence of gender discrimination; easy access of girls to kitchen and food stored in the house. It also reflects the changing attitude of these families about the status of women; improvement in the level of education, economics and knowledge about nutrition.

Key words: Body mass status, primary school children, affluent families and Dera Ismail Khan

INTRODUCTION

Good nutrition is the corner stone for survival, health and development of current and succeeding generations. Well nourished children perform better in school, grow into healthy adults and in turn give their children a better start in life. Well nourished women; face fewer risks during pregnancy, child birth and their children set off on firmer development paths, both physically and mentally (UNICEF, Report 40, 2006).

Malnutrition refers to deficiencies, excess or imbalances in intake of energy, proteins and/or other nutrients. Malnutrition includes under nutrition and over nutrition. Under nutrition is the result of food intake that is continuously insufficient to meet dietary energy requirements, poor absorption and/or poor biological use of nutrients consumed. This usually results in loss of weight or under weight. Over nutrition refers to a chronic condition where intake of food is in excess of dietary energy requirements resulting in over weight and/or obesity (WHO-Growth standards, 2002). Underweight; a poor anthropometric status (weight for age/BMI-for-age percentile), is mainly a consequence of inadequate diet and frequent infections (Diarrhea and Respiratory), leading to deficiencies in calories; proteins, vitamins and minerals. Underweight remains a pervasive problem in developing countries where poverty is a strong underline determinant, contributing to house hold food insecurity; poor child care, maternal under nutrition, unhealthy environments and poor health care. All ages are at risk but underweight is more prevalent under five. Underweight children are at increased risk of mortality from infectious illnesses such as tuberculosis, diarrhea, pneumonia, measles and malaria. The effects of under weight/under nutrition on the immune system are wide ranging. The infectious illnesses also tend to be more frequent and severe in underweight children. There is an increased risk of death from mild to moderate under nutrition. Chronic under nutrition in the first two or three years of life can also lead to long term developmental defects. Underweight was estimated to cause 3.7 million deaths in 2000, about 1.8 million deaths occurred in Africa and 1.2 million in South East Asia((WHO-under nutrition, 2002). Conflicts and natural disasters in many countries have further exacerbated the situation. Increase in the number of underweight children in Africa also reflects a rapid rate of population growth as well as AIDS (UNICEF malnutrition, 2006).

Weight change is the first indication of over or under nutrition. Weight generally reflects recent changes and is an indicator of short term nutritional status. The stature (length or height) is slower to respond. A

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decrease in weight indicates acute while decrease in height/length indicates chronic under nutrition (MacLean and Graham, 1982).

Disparities exist between children living in a region or a country or a particular geographic area or among certain population sub groups. Children in rural areas are nearly twice as likely to be underweight as children in urban areas, but high underweight magnitude in urban slums in many developing countries still gives cause for concern (UNICEF-Birth Place, 2007). Significant disparities exist between rich and poor children. On average, poor children are twice as likely to be underweight as rich children. The greatest disparities between rich and poor are found in Latin America and Caribbean, where children living in poorest house holds are 3.6 times more likely to be underweight than children from the richest house holds. The lowest disparities are found in East Asia/Pacific fallowed by Central & Eastern Europe (CEE)/Common Wealth of Independent States and Sub-Saharan Africa (UNICEF-.House Hold, 2007). Little difference in underweight magnitude exists between boys and girls in every region except South Asia. In South Asia, 47% of girls are underweight compared to 44% of boys (Sirhari *et al*., 2006). The present study was planned to figure out the magnitude of under weight children (6-11), attending different schools that cater wards of affluent families in Dera Ismail Khan City, Pakistan.

MATERIALS AND METHODS

The present study aimed to assess the magnitude of underweight in primary school children at Dera Ismail Khan. It was carried out in 3 primary sections of schools, having wards of high socio-economic status. The study included 322 school children: 221 (68.63%) boys and 101 (31.37%) girls. Thorough clinical examination of the children excluded those suffering from chronic health diseases. Written permission was obtained from the parents and principals of the institutions. Participation was voluntary.

Weight measurements of children were taken (in kilogram) in stocking feet and light clothing. Stature was measured (in meters) with a wall mounted steel tape possessing a moveable head-board. Children were measured with heels, buttocks and shoulders touching the wall, looking straight ahead and with bare feet. Body Mass Index (BMI) of each child was calculated according to Quatelet's Index. BMI-for-age percentile was used in children and adolescents than BMI. Body mass status was determined according to WHO criteria. Children, falling <5th percentile were considered underweight (WHO, 1995).

RESULTS

This study involved 322 school children (6-11), 221(68.63%) boys and 101 (31.37%) girls. All the children belonged to high socio-economic group. Gender wise distribution of children in different schools is shown in Table 1. School wise distribution of both genders showed maximum participation of The Qurtruba School 131(boys = 81 and girls = 50) followed by The City school 129 (boys = 99 and girl = 30) and The Educators 62 (boys = 41 and girls = 21). Children falling below the 5th percentile in BMI-for-age, were considered underweight according to WHO criteria (WHO, 1995).

Table 2 shows the institutional and gender wise distribution of underweight children. Amongst 18 (5.59%) underweight children, 13 (5.88%) were boys and 5 (4.95%) girls. No significant gender difference was noted for the percentage of underweight children. The maximum number of under weight children (8.06%) was found in The Educators followed by Qurtruba School (6.87%) and The City School (3.10%). These institutions were located in the urban areas with good environmental conditions; have easy access to nutritious diet, health facilities and media. Maximum number of under weight boys (7.40%) were recorded in Qurtruba while 7.32% in Educator and 4.04% in the City school. Among girls, 9.52% girls of Educator were under weight whereas 6% girls in The Qurtruba and none in The City school (UNICEF Nutrition, 2006).

Table 1: Institutional and gender wise distribution of children (n = 322)

		Boys		Girls	
School	Total No. of children	Number	% of boys	Number	% of girls
The qurtuba	131	81	61.83	50	38.17
The city	129	99	76.74	30	23.26
The educators	62	41	66.13	21	33.87
Total	322	221	68.63	101	31.37

Table 2: Institutional and gender wise distribution of underweight school children (n = 18)

	Total		Boys		Girls	
Schools	No.	Underweight (%)	No.	Underweight (%)	No.	Underweight (%)
The qurtruba	9	6.87	6	7.41	3	6.00
The city	4	3.10	4	4.04	0	0.0
The educators	5	8.06	3	7.32	2	9.52
Total	18	5.59	13	5.88	5	5.95

Table 3: Age and gender wise distribution of the sample in schools

	The qurtuba		The city		The educators		Total
Age in years	Boys	Girls	Boys	Girls	Boys	Girls	Boys + Girls
6	07	03	28	0	0	0	68
7	07	04	0	01	18	15	45
8	19	12	13	07	08	02	61
9	09	09	16	07	03	03	47
10	28	13	19	11	06	0	77
11	11	09	23	04	06	01	54
Total	81	50	99	30	41	21	322
% of gender in each school	61.83	38.17	76.74	23.26	66.13	33.87	-

Table 4: Institutional and age wise distribution of underweight school children

	The qurtruba		The city		The educators		Total		
Age (Years)	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	% of Total
06	0	0	0	0	0	0	0	0	0.00
07	2	0	0	0	2	2	4	2	33.33
08	0	0	1	0	0	0	1	0	5.56
09	1	1	0	0	0	0	1	1	11.11
10	2	2	1	0	0	0	3	2	27.78
11	1	0	2	0	1	0	4	0	22.22
Total	6	3	4	0	3	2	13	5	100.00

Table 3 revealed the age and gender wise distributions of children (n = 322). Children were 77 (23.91%) at 10 years, 68 (21.11%) at the age of 6 and 61 (18.94%) at 8 years. Both the ages (6 and 10 years) are the periods of growth spurts, negative energy balance would result in reduction of body weight. Participation of the girls 101 (31.36%) was reasonable.

Table 4 presents the age wise distribution of underweight affluent school children (6-11 years) with a total of 18 (boys: 13 and girls: 5). Boys were more than twice the number of girls, reflecting better nutrition of girls. The number of underweight school children were maximum (33.33%) at the age of 07 years, followed by 27.78% at the age of 11 years and minimum at 06 years. Increase in physical activity, poor dietary habits and growth spurt is common at age 5-7 and 10-13 years in children. Early maturing girls may begin their growth spurt as early as 7-8 years and early maturing boys may begin growing at the age of 9-11 years. If they are not provided with sufficient supply of nutrients, they will not gain weight in short term and length (height) in long run (Gordon and Wald Law, 2003).

DISCUSSION

The present study was undertaken to find out the frequency of underweight primary school children of Dera Ismail Khan, Pakistan from high socioeconomic group. The institutions selected for the study was private and having branches throughout the country. The annual expenditure is unaffordable by the middle class and poor families. It was assumed that under nutrition might exist among the high socioeconomic group. The study involved 322 children, 321 (68.63%) boys' and 101 (31.37%) girls. 18 children (5.1%) were found to be underweight, 13 (4.03%) boys and 5 (1.55%) girls. Gender difference for underweight was statistically significant. It is contrary to what is observed that underweight in girls and gender difference is highest in South Asia. Findings of the present study can be compared with the findings of Nawal-Al-Hamad *et al.* (2006), that observed much lower rates for underweight in school children of Kuwait. Their study included 5047 children (boys: 2522 and girls: 2525), aged 5-10 years, as apart of nutritional survey in Kuwait through Organization of Food and Agriculture (FAO). Underweight in school children was observed boys 3.1% and girls: 1.5%. Gender difference was in agreement with the present study (5% VS 1.55%). Lower rates for underweight in children might be due to better nutritional and socioeconomic conditions in Kuwait. Lower rates in girls also reflect that there is no gender discrimination in Kuwait. Cheryl *et al.* (2006) have also reported the lower rates for the underweight in children (2.7%), as prevalence of under weight in National Health and Nutrition Examination Survey: 2003-2006. The report has been issued by National Center for Health Statistics, USA.

Ismail *et al.* (2005) have observed higher rates for underweight in school children 7.3% (boys: 10.6% and girls: 4.65%) in the National Nutritional Survey based on anthropometry for Egypt (1985-2005). Gender difference was also remarkable. Results of the present study can also be compared with the observation by Sharma *et al.* (2006) that underweight in school children (6-16) in Hyderabad, India was 10.13%. Higher rates for the observed parameter might be due to poor dietary habits, unawareness about nutritional education and intake of junk food. Fong Ming Moy *et al.* (2004) have also

conducted the similar studies in Kuala Lumpur, Malaysia, investigating 1194 children of 5th grade with an average age of 11 years and reported the frequency of underweight children 14.8% (boys 16.1% and girls: 13.3%). Majority of the children had professional fathers. However, 43.7% came from families having more than 4 or 5 siblings, 20.8% had more than 5 and 35.5% had 3 or fewer siblings. Higher rates for the observed under nutrition might be due to different ethnic groups, genetic predisposition, dietary cultures and religious restrictions.

Conclusion and recommendations: Body weight (weight for age) is the earliest, simplest and most important anthropometric measurement to be adopted as an indicator for nutritional status in routine clinical examination of the children. A classification of varying degrees of malnutrition is based on this indicator. This classification is also linked to ultimate health outcome-mortality. Weight-for-age is important clinically and is used to assess the recent malnutrition in communities. Underweight in children reflects the level of socio-economic development as well as that of education and health delivery system. It exposes those sections of society where under nutrition is prevalent and needs to be rectified. It requires educating the concerned society about nutrition; sanitation, environmental conditions and child care.

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Effect of Processing on the Proximate Composition of the Dehulled and Undehulled Mungbean [*Vigna radiata* (L.) Wilczek] Flours

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Abstract: The effects of some processing treatments on the proximate composition of dehulled and undehulled mungbean seeds flours were investigated. The mungbean seeds were subjected to boiling, toasting and sprouting, at different intervals before milling into flour. The flour samples were analyzed for proximate composition using standard methods and the dehulled samples were found to possess better proximate compositions than the undehulled samples, except for crude fiber. Results showed that increase in boiling time (30, 45, 60 and 90 min) did not significantly ($p>0.05$) increase the moisture (10.30-10.65%) and carbohydrate (57.16-65.05%) contents but rather decreased the fat, ash, crude fiber and energy values. The undehulled mungbean boiled for 45 min had the highest crude protein (21.86%), the 30 min boiled sample had the highest fat content (2.20%), while the carbohydrate was highest (66.17%) in the 90min boiled sample. However, the dehulled sample boiled for 30 min had the highest crude protein (26.27%) and fat (2.27%), whereas the dehulled sample boiled for 90 min highest carbohydrate content (65.05%). The 30 min toasted sample had the highest crude protein (19.70%) while the 90 min toasted sample had the highest fat (2.43%) and carbohydrate (69.88%). However, the dehulled sample toasted for 30 min had the highest protein (24.52%) while the 90 min toasted sample had the highest fat (2.07%) and carbohydrate (71.99%). The undehulled sample sprouted for 72h had the highest crude protein (37.17%), while the 24 h sprouted sample had the highest fat (2.32%) and carbohydrate (49.08%). However, for the dehulled sample, increase in sprouting time (24-36 h) increased the moisture (10.43-10.69%) and crude protein (35.90-36.50%) contents but reduced the fat (1.35-1.23%), fiber (2.90-2.83%), carbohydrate (49.02-47.88%) and energy values (351.83-348.59 kcal/g).

Key words: Mungbean seeds, flour, processing, proximate composition

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the lesser known legumes which originated from South East Asia (India) and has been mostly grown in Asian countries like Thailand, Burma, Indonesia and Philippines. It is now widely cultivated in Africa, South America, Australia and the United States (Kay, 1979; Kim *et al*., 2007). It is an important pulse crop, a dicotyledon also commonly referred to as green or golden gram, moong, Ludou (Chinese), green soy and chop bean which belong to the family of Leguminosae or Fabacea and is related to cowpea (Kay, 1979; Charmaine, 1998; Agugo, 2003; Leung, 2007). Opoku *et al*. (2003) reported that about 90% of the world production of mungbean (*Vigna radiata* (L.) Wilczek) is produced in Indo-Burma region while Fery (2002) reported that about 70% of the world production of mungbean is in India. The seed colour is usually dark olive green, bright green skin or yellow and the beans are small, cylindrical or ovoid, globular or oblong in shape, but some cultivars produced brown or speckled black seed (Rubatzky and Yamaguchi, 1997; Anonymous, 2008a). There are 2000 varieties of mungbean, among them yellow, gold and black mungbean (Charmaine, 1998). Structurally, mungbean may consist of 12.1% seed coat, 2.3% embryo and 85.6% cotyledons (Singh *et al*., 1968).

Plate 1: Mungbean seeds

Mungbean [*Vigna radiata* (L) Wilczek] has high nutritional potentials and has been recently introduced

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in Nigeria (Agugo, 2003; Mensah and Olukoya, 2007). The consumption of mung proteins can fulfil the essential amino acids requirements with the exception of the sulphur-containing amino acids (Khalil, 2006). Mungbean is rich in dietary fiber, carbohydrates, energy, vitamins, minerals-thiamin, iron, magnesium, phosphorus, potassium, copper and are a good source of folate, however, riboflavin, niacin are found in trace amount (Khalil, 2006; Charmaine, 1998; Anonymous, 2008a). They are also rich in lysine, 5.24-5.85 g/100 g of protein (Adel *et al*., 1980); 1.9 g/100 g of protein (Calloway *et al*., 1994), but deficient in methionine in contrast with the high methionine value observed for rice bean (Andersen, 2007). The high lysine content of mungbeans makes it a good complementary food for rice-based diets, in which lysine is usually the first limiting amino acid (Chen *et al*., 1987). Mungbean has excellent digestibility and freedom from flatulence has made it suitable for infant feed formulation, recuperating patients and the aged people (Kay, 1979; Agugo and Onimawo, 2008). Mungbean has several food uses- It can be eaten alone or combined with rice to make 'Khichari' or combined with vegetables and greens to make hearty soups (Anonymous, 2008a). They are versatile enough to provide a base for sweet making and the paste is used for a variety of sweets, jellies, snacks like cakes, doughnuts and sweet drinks (Weinberg, 2002). Mungbean also has medicinal uses-anti-poisonous, anti-hypertensive, treatment of various ailments like hepatitis, gastritis, heat rash etc. (Leung, 2007; Huijie *et al*., 2003).

The antinutritional factors limit the food applications of mungbean therefore; dehulling the seeds before milling has been used to overcome this problem (Thompson *et al*., 1976; El-Adawy, 2002; Opoku *et al*., 2003). The impacts of traditional processing methods on the nutrients in mungbean were also reported by Bau *et al*. (1997); Bhatty *et al*. (2000); Mubarak (2005) and Agugo and Onimawo (2008). Furthermore, Chau *et al*. (1997); Apata and Ologhobo (1989); Mubarak (2005); Udom (2007) and Agugo and Onimawo (2008) evaluated the effects of processing on the food toxicants in mungbean, throwing some light on the level of reduction in antinutrients achieved by various processing methods. Chau *et al*. (1997); Agugo and Onimawo (2008) reported that cooking improves the protein quality by destruction or inactivation of the heat-labile antinutritional factors. With the increased interest in the exploitation of this mungbean, this present study is designed to evaluate the effect of processing on the proximate composition of dehulled and undehulled mungbean flour.

MATERIALS AND METHODS

The mungbeans (*Vigna radiata* (L.) Wilczek) seeds were obtained from the Crop Science Department of Michael Okpara University of Agriculture, Umudike, Nigeria. All chemical reagents used for the experiment were of analytical grade and were purchased from Joechem Chemical store, Nsukka and Hoslab, Umuahia, Nigeria.

Seeds pretreatment: The dry cleaned mungbean seeds were divided into two sets; one was left undehulled and the other one was dehulled. The undehulled sample was further sub-divided into four sets; one was kept raw (untreated), the second set was boiled, the third set was toasted while the fourth set was germinated (sprouted) as shown in Fig. 1. The dehulled sample was also sub-divided into three sets; one set was kept raw without any further treatment while the other two sets were subjected to boiling and toasting respectively. The fourth set was sprouted and then carefully dehulled as shown in Fig. 2. All these treatments were given to the mungbeans after soaking for 12 h. The mungbean seeds were crushed to smaller fragments with the corona manual grinder after drying in the oven (65°C) and afterwards milled with a blender, using an 80 mesh sieve to sift the flour. All flour samples were stored in air-tight plastic bags until required for analysis.

Flour processing

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

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

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

Sprouting: The germination was carried out by spreading the undehulled seeds soaked in distilled water (1:3 w/v) for 12 h at room temperature (~25°C), weighing 800 g in between jute cloth and allowed to

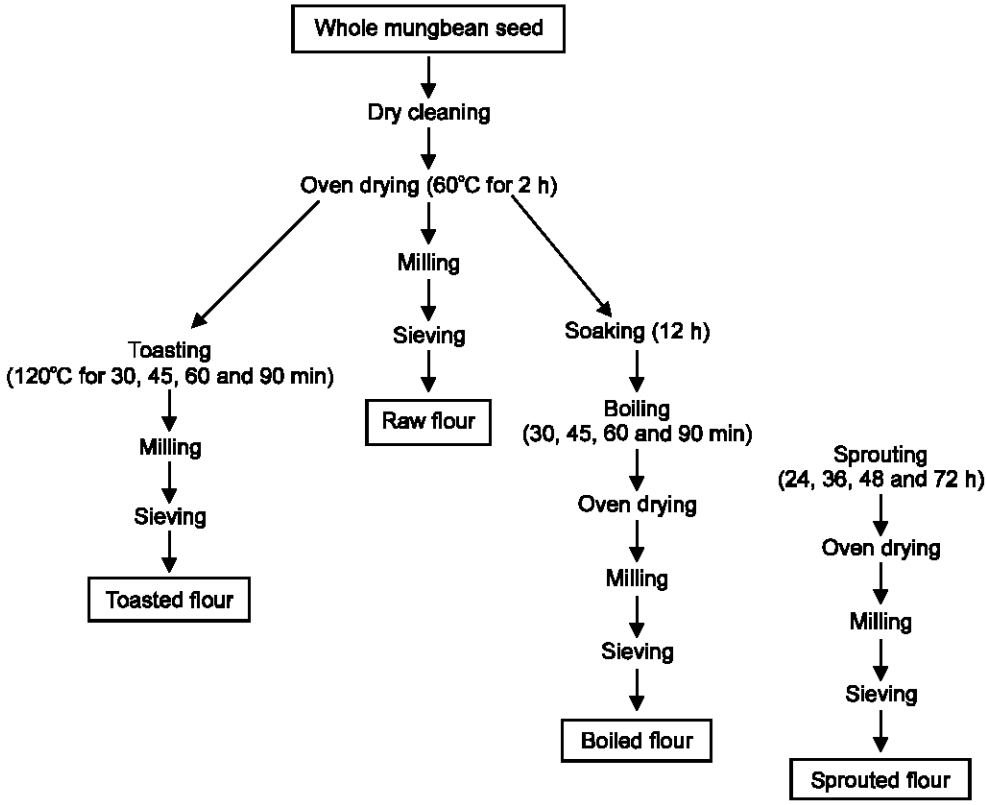

Fig. 1: Flow chart of the undehulled mungbean flour samples

sprout in the dark for 24, 36, 48 and 72 h respectively. The seeds were kept wet throughout germination by spraying them with distilled water every 12 h. The sprouted mungbeans were harvested, rinsed twice in distilled water and oven dried at 65°C for 9 h and cooling followed. The dried seeds were subjected to dry milling and passed through 80 mesh sieve. The flour was cooled and packaged for analysis thereafter in air-tight plastic bags. The dehulled mungbeans were equally germinated under the same conditions for 24 and 36 h, dried, milled and packaged for analysis.

The proximate analysis of the samples for moisture, ash and crude fat determination were done in triplicates using the air oven, dry ashing and soxhlet extraction methods described by AOAC (1990); Nielson (2002); Onwuka (2005). The crude protein (Nx 6.25) was determined by using micro kjeldahl method reported by AOAC (1990); Kirk and Sawyer (1991). The carbohydrate content was determined by difference, a method reported by Onwuka (2005). In this method, carbohydrate content was obtained by calculation having estimated all the other fractions by proximate analysis i.e. % Available carbohydrates = 100 - (% moisture + % ash + % protein + % fiber + % fat).

The energy value was calculated using the Atwater factor method [(9 x fat) + (4 x carbohydrate) + (4 x protein)] as described by Osborne and Voogt (1978); Eneche (1991); Chinma and Igyor (2007) and Nwabueze (2007). The proportion of protein, fat and carbohydrate were multiplied by their physiological fuel values of 4, 9 and 4 kcal, respectively and the sum of the product was taken.

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

RESULTS AND DISCUSSION

Table 1 presents the effect of processing on the proximate composition of undehulled mungbean seeds flour.

Moisture content: The moisture content of the raw undehulled mungbean flour was 10.25% (Table 1) however; other researchers had earlier reported that raw mungbean had 8.25-10% moisture content (Bhatty *et al.*, 2000). Nevertheless, Mubarak (2005) reported lower value (9.75%). The moisture contents ranged from 10.21% for 30 min boiled sample to 10.55% for the 90 min boiled sample. Increase in boiling time of the mung seeds did not result in any significant ($p>0.05$)

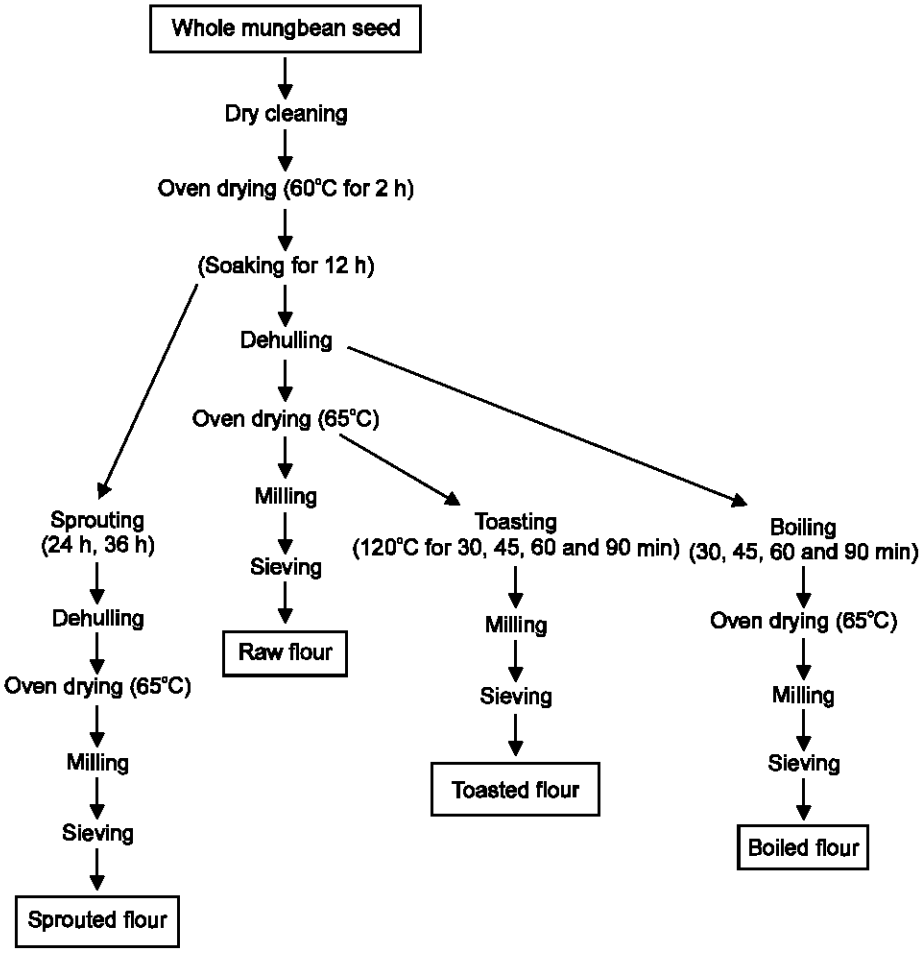

Fig. 2: Flow chart of the dehulled mungbean flour samples

increase in the moisture content of the flours. But generally, boiling was found to slightly increase the flour moisture content. These observations were in agreement with those reported by Bau *et al*. (1997) for soybeans and Mubarak (2005) for mungbeans. Boiling increased the moisture content due to the absorption of water by the seeds during cooking (Mubarak, 2005). There was a slight reduction in the moisture contents of the toasted flour but no significant difference ($p>0.05$) existed among them. The sample toasted for 90 min had the least moisture content of 5.36% which could be as a result of the high temperature and time it was subjected to (120°C and 90 min respectively) and it significantly differed from the moisture content of the raw flour.

The sprouting treatments significantly ($p<0.05$) increased the moisture content of the undehulled flours compared to the raw flour (Table 1), although no marked increase was observed among the samples sprouted for 36, 48 and 72 h (12.33, 12.51 and 12.58% respectively). This increase could be attributed to the hydration of the seeds during soaking, before the germination (Del Rosario and Flores, 1981). Similar report has been made by Mubarak (2005) for germinated mungbean seeds flour.

Crude protein: There were significant ($p<0.05$) differences among the protein contents of the raw and boiled undehulled mungbean flours. The raw flour had 24.08% crude protein which fell between the range 20.97-31.32% reported by Anwar *et al*. (2007).On the contrary, Calloway *et al*. (1994) reported a much lower value of 20.3%. Boiling significantly ($p<0.05$) reduced the protein value of the flour with increase in time. These decreases might be attributed to leaching during boiling. Similar report had been given earlier for boiled soybean and mungbean flour as well as fluted pumpkin seed flour (Kylen and McCready, 1975; Fagbemi, 2007).

Toasting the mungbean seeds reduced the protein from 24.08-19.70% and increase in toasting time significantly reduced ($p<0.05$) the flour protein. The least protein value of 13.13% was observed for the 90 min toasted mung flour (Table 1). This result may be due to the high temperature of toasting, being a dry heat processing

Table 1: Effect of some processing treatments on the proximate composition of the undehulled mungbean (*Vigna radiata* (L) Wilczek) flour

Samples	Moisture	Dry matter	Crude protein	Crude fat	Total Ash	Crude fibre	Carbohydrate	Energy value (kcal/g)
	(%)							
Raw undehulled	10.25^{abc}±0.05	89.75^{bcd}±0.05	24.08^{a}±0.15	1.93^{de}±0.04	3.00^{b}±0.55	5.00^{a}±0.00	55.74^{cde}±0.57	336.65^{i}±0.00
Boiled								
30 min	10.21^{abc}±0.01	89.62^{bcd}±0.29	21.02^{g}±0.40	2.20^{bc}±0.30	1.50^{d}±0.00	3.80^{g}±0.00	61.27^{abc}±0.33	348.96^{b}±0.00
45 min	10.52^{abc}±0.32	89.38^{bcd}±0.18	21.86±0.15	1.33^{f}±0.20	1.85^{d}±0.00	5.45^{c}±0.05	58.99^{bcd}±0.42	335.37^{k}±0.00
60 min	10.32^{abc}±2.87	89.68^{bcd}±2.87	19.71^{h}±0.30	1.20±0.03	1.80^{d}±0.00	4.05^{f}±0.05	62.92^{abc}±2.74	341.12±0.00
90 min	10.55^{abc}±1.66	89.45^{bcd}±1.66	17.34±0.24	0.53^{h}±0.04	1.50^{d}±0.00	4.10^{f}±0.00	66.17^{ab}±1.64	338.81^{h}±0.00
Toasted (120°C)								
30 min	8.28^{bcd}±0.46	91.72^{abc}±0.46	19.70^{h}±0.10	1.20±0.03	4.20^{a}±0.00	3.40^{h}±0.00	63.63^{abc}±1.01	344.12^{d}±0.00
45 min	7.56^{cd}±4.91	92.44^{ab}±4.91	17.51±0.09	1.23±0.03	4.25^{a}±0.00	4.15^{f}±0.50	65.30^{abc}±5.00	342.31^{e}±0.00
60 min	6.38^{d}±0.08	93.62^{a}±0.08	15.35±0.16	2.04^{cd}±0.06	4.40^{a}±0.65	5.30^{cd}±0.00	67.23^{ab}±1.56	348.68^{c}±0.00
90 min	5.36^{d}±1.95	94.64^{a}±1.95	13.13^{i}±0.02	2.43^{a}±0.07	4.00^{a}±0.00	5.20^{d}±0.00	69.88^{a}±2.00	353.64^{a}±0.23
Sprouted								
24 h	11.40^{ab}±1.20	88.60^{cd}±1.20	30.65^{d}±0.11	2.32^{ab}±0.29	1.65^{d}±0.00	4.90^{a}±0.00	49.08^{ef}±1.07	339.80^{g}±0.01
36 h	12.33^{a}±0.28	87.67^{d}±0.28	32.56^{c}±0.46	1.76^{e}±0.49	1.80^{d}±0.00	2.03±0.13	49.52^{def}±0.70	344.16^{d}±0.02
48 h	12.51^{a}±0.35	87.49^{d}±0.35	35.02^{b}±0.05	1.13±0.03	1.70^{d}±0.00	9.20^{b}±0.00	40.45±0.28	312.05^{j}±0.00
72 h	12.58^{a}±0.03	87.42^{d}±0.03	37.17^{a}±0.15	0.80^{g}±0.08	2.35^{c}±0.00	13.13^{b}±0.32	44.97±18.42	335.76^{f}±0.00

Values represent means ± standard deviation of triplicate determinations. Means with the same superscript on the same column are not significantly different (p>0.05)

method, as well as duration of toasting. Similar report was made by Fagbemi (2007) on toasted fluted pumpkin seed flour.

The crude protein of the raw sample (24.08%) significantly differed (p<0.05) from that of the sprouted samples and increase in sprouting hours resulted to increased crude protein agreed with the report made by Bau *et al.* (1997) for sprouted soybeans, El-Adawy (2002) germinated chickpea, Mubarak (2005) for germinated mungbean flour, Fagbemi (2007) for germinated fluted pumpkin seeds. Also, Finney *et al.* (1982) had earlier reported an increase in the protein content of whole germinated mungbean seed flour to be 32.89%.

The increase in the protein content of the sprouted flour samples was mainly due to the use of seed components during the germination process (Mubarak, 2005), breakdown of complex proteins into simpler form and breakdown of nutritionally undesirable constituents (Chavan and Kadam, 1989). The metabolic activities of resting seeds increase as soon as they are hydrated during soaking. The complex biochemical changes that occur during hydration and sprouting lead the protein constituents being broken down by enzymes into simple compounds that are used to make new compounds. The increased hydrolytic activities of the enzymes caused by sprouting resulted in improvements in the contents of the total protein due to the disappearance of starch (Anonymous, 2008b). According to Morgan *et al.* (1992), the absorption of nitrate facilitates the metabolism of the nitrogenous compounds from carbohydrate reserves; thus increasing crude protein levels.

Crude fat: The fat content of the raw sample (1.93%) fell within the range (1.85%-2.83%) reported by Bhatty *et al.* (2000) and Mubarak (2005) and it differed significantly (p<0.05) from the boiled samples. Increase in boiling time of the seeds significantly decreased the fat contents of the flour samples except the samples boiled for 45 min (1.33%) and 60 min (1.20%) which had no significant difference (p>0.05) among them. A slight reduction in fat (1.85-1.82%) of cooked undehulled mungbean seeds has been indicated by Mubarak (2005) and the decrease was attributed to their diffusion into cooking water. Furthermore, Ukwuru (2003) observed a reduction in the fat content of cooked soy flour to be due to the leaching into boiling water.

There was no significant difference (p>0.05) between the fat content of the undehulled mungbean flour toasted for 30 and 45 min respectively with the values 1.20 and 1.23%. It was equally observed that these samples had no significant difference (p>0.05) between the fat content of the flour samples boiled for 45 and 60 min respectively with values 1.33 and 1.2% respectively. It was however, observed that toasting for 60 and 90 min significantly (p<0.05) increased the fat content of the undehulled mung flour to 2.04 and 2.43% respectively. This result was in agreement with the report of Fagbemi (2007) for the increased the fat content of the raw flour from 7.56-9.73%.

Sprouting the mungbean seeds for 24 h was observed to increase the fat content of the flour significantly (p<0.05) from 1.93-2.32%. But the 36, 48 and 72 h sprouted seed flours had their fat contents significantly reduced (p<0.05) with values of 1.76, 1.13 and 0.80% respectively. Mubarak (2005) also reported a significant (p<0.05) decrease in the fat content of the germinated mungbean seed flour from 1.85-1.45%. Fagbemi (2007) also reported a significant (p<0.05) reduction in the fat content of germinated fluted pumpkin seed flour. These decreases could be attributed to the use of fat as an energy source to start germination (Mubarak, 2005).

Total ash: The raw sample had ash content (3.0%) close to the value reported by Mubarak (2005) for

undehulled mungbean seeds flour (3.76%). However, boiling treatments reduced the ash contents although no marked reduction with increase in boiling time was observed.

Increase in toasting time had no significant ($p>0.05$) difference in the ash contents of the undehulled mungbean flours. Toasting for 30, 45, 60 and 90 min increased the ash contents to 4.2, 4.25, 4.4 and 4.0% respectively. Fagbemi (2007) also observed that toasting increased the ash content of fluted pumpkin seeds flour. Sprouting significantly ($p<0.05$) reduced the ash content of the undehulled mung flour, although no significant difference ($p>0.05$) existed among the ash content of the 24, 36 and 48 h sprouted seed flour. The reduction in ash content was in agreement with those reported by El-Beltagy (1996) for germinated mung seeds flour and El-Adawy (2002) for germinated chickpea flour.

Crude fiber: The fiber content of the raw sample (5.0%) was slightly higher than that reported by Mubarak (2005)-4.63% and significantly differed from the crude fiber of the boiled samples with 45 min boiled flour having the highest (5.45%).

Increase in toasting time increased the crude fibre content of the undehulled mung flours significantly except for the samples toasted for 60 and 90 min which had no significant difference ($p>0.05$) among them. Similarly, no significant increase was observed in the toasted fluted pumpkin seed flour (Fagbemi, 2007).

The crude fiber content of the 48 and 72 h sprouted seed flour were the highest, 9.2 and 13.13% respectively. The results corresponded with the findings of Chavan and Kadam (1989); Anonymous (2008b) where they reported that germination increases the crude fiber in seeds due to the disappearance of starch. The crude fiber, a major constituent of cell walls increases both in percentage and real terms, with the synthesis of structural carbohydrates, such as cellulose and hemicellulose (Peer and Leeson, 1985); Cuddeford (1989).

Total carbohydrates: The carbohydrates were significantly ($p<0.05$) increased by boiling treatments probably due to the heat involved which reduced the other components as a result of denaturation and leaching, leading to an increase in carbohydrate contents.

Increase in toasting time, increased the total carbohydrate of the undehulled mung flour although no significant difference ($p>0.05$) was observed among them. Toasting for 30, 45, 60 and 90 min resulted in total carbohydrate contents of 63.63, 65.30, 67.23 and 69.88% respectively.

The total carbohydrates of the sprouted undehulled mungbean seeds flour reduced but no significant difference ($p>0.05$) existed between them. This result was in agreement with the findings of Finney *et al.* (1982); Mubarak (2005) which showed that sprouting resulted to a reduction in the carbohydrate content of mungbean.

Energy value: The energy value of the raw undehulled mungbean flour was observed to be 336.65 kcal/g and it significantly differed ($p<0.05$) from the energy values of the boiled flour samples. Calloway *et al.* (1994) had earlier reported the energy value of 306 kcal/g raw mung flour. Increase in boiling time resulted to significant differences ($p<0.05$) in the energy values of the boiled samples and the values obtained were as a result of the protein and fat contents of the flours since they were calculated using the Atwater factor.

However, increase in toasting time resulted to a significant increase in the energy values of the undehulled mung flours (Table 1). Significant differences ($p<0.05$) in energy values also existed among the sprouted samples.

Dry matter: Significant difference ($p<0.05$) existed between the dry matter contents of the raw sample (89.75%), the toasted and the sprouted samples (Table 1). However, toasting at different intervals did not significantly increase the dry matter and also sprouting at different length of time did not markedly reduce the dry matter of undehulled mung flours. The reduction in the dry matter (total solids) could be relatively attributed to the high moisture content of the sprouted samples. However, a decrease in the seeds had been attributed to the increased contents of total proteins, fat, sugars, ß-group vitamins and certain essential amino acids (Anonymous, 2008b).

Table 2 presents the effect of some processing treatments on the proximate composition of dehulled mungbean seeds flour.

Moisture content: The moisture content of the raw dehulled mungbean seeds flour was 10.11%, however, other reports showed mungbean flour to contain 8.78% (Afzal, 1978); 8.25% moisture content (Bhatty *et al.*, 2000); 9.30% (Augustine and Klein, 1989; Agugo and Onimawo, 2008); 10.10% (Mubarak, 2005). The moisture content of the raw flour was observed to qualify for the standard expected of dry legumes, 10.15% as reported by USDA (1999), since moisture content is a quality factor for preservation, stability of products and convenience in packaging. According to Echendu *et al.* (2009), low moisture content enhances keeping quality of flours. Even though boiling slightly increased the moisture content of the dehulled mungbean flour, increase in boiling time did not significantly increase ($p>0.05$) the moisture content of the flour. Mubarak (2005) also reported that boiling had no significant ($p>0.05$) effect on the moisture content of dehulled

Table 2: Effect of some processing treatments on the proximate composition of the dehulled mungbean (*Vigna radiata* (L) Wilczek) flour

Samples	Moisture	Dry matter	Crude protein	Crude fat	Total Ash	Crude fibre	Carbohydrate	Energy value (kcal/g)
	----------	----------	----------	(%) ----------	----------	----------	----------	
Raw dehulled	10.11^{ab}±0.999	89.98^{ab}±0.98	28.38^{c}±0.21	1.89^{c}±0.02	1.20^{a}±0.00	4.05^{c}±0.25	54.47^{a}±0.66	348.41±0.00
Boiled								
30 min	10.30^{a}±0.06	89.702^{b}±0.06	26.27^{d}±0.03	2.27^{a}±0.01	1.10^{b}±0.00	3.90^{cd}±0.20	57.16^{de}±0.24	354.15^{c}±0.03
45 min	10.91^{a}±0.05	89.09^{b}±0.05	25.36^{e}±0.16	2.24^{a}±0.01	0.20^{b}±0.50	4.005^{c}±0.00	57.29^{de}±0.08	350.16^{c}±0.00
60 min	10.64^{a}±2.08	89.36^{b}±2.08	22.77^{g}±0.04	1.15^{b}±0.02	0.18^{b}±0.30	3.70^{d}±0.00	61.60^{bc}±2.09	347.83±0.00
90 min	10.65^{a}±2.10	89.35^{b}±2.10	18.83^{i}±0.30	0.76^{b}±0.06	0.90^{b}±0.00	3.81^{de}±0.01	65.05^{b}±2.00	342.36^{e}±0.00
Toasted (120°C)								
30 min	7.66^{ab}±2.70	92.34^{ab}±2.70	24.52^{f}±0.09	1.47^{d}±0.04	1.75^{a}±0.20	4.32^{b}±0.01	60.29^{cd}±2.69	352.47^{d}±0.02
45 min	7.26^{ab}±4.61	92.74^{ab}±4.61	21.00^{h}±0.02	1.52^{d}±0.02	2.10^{a}±0.05	4.95^{a}±0.05	63.18^{bc}±4.60	350.41^{g}±0.00
60 min	6.58^{b}±0.08	93.42^{a}±0.08	17.52^{j}±0.12	2.06^{b}±0.08	1.33^{d}±0.08	2.05^{b}±0.05	71.18^{a}±1.31	373.34^{a}±0.00
90 min	6.45^{b}±0.86	93.55^{a}±0.86	15.33^{k}±0.11	2.07^{b}±0.01	1.45^{c}±0.05	3.40^{f}±0.00	71.99^{a}±1.92	367.91^{b}±0.00
Sprouted								
24 h	10.43^{a}±0.08	89.57^{b}±0.08	35.90^{b}±0.09	1.35^{b}±0.02	0.40^{b}±0.05	2.90^{g}±0.00	49.02^{f}±0.15	351.83^{f}±0.02
36 h	10.69^{a}±2.24	89.31^{b}±2.24	36.50^{a}±0.31	1.23^{b}±0.03	0.88^{b}±0.25	2.83^{g}±0.03	47.88^{f}±2.16	348.59^{h}±0.00

Values represent means ± standard deviation of triplicate determinations. Means with the same superscript on the same column are not significantly different (p>0.05)

mungbean flour (10.13%). The slight increase in moisture was due to the water absorbed during dehulling and boiling.

The flour samples toasted at different intervals had comparable moisture content with the raw flour and this could be due to the high temperature of toasting (120°C), which increased the rate of drying. Sprouting treatments did not markedly increase the moisture content of the dehulled mungbean seeds flour and no significant difference (p>0.05) existed among the 24 and 36 h sprouted flours (10.43 and 10.69%) respectively. However, Mubarak (2005) reported that germination resulted to an increase in the moisture content of the mung flour from 10.10-11.10%. The increase in moisture could be attributed to hydration of the seeds during soaking and germination (Del Rosario and Flores, 1981).

Crude protein: As shown in Table 2, the crude protein of the raw sample (28.38%) fell within the range observed by other researchers. Dehulled mungbean seeds flour had protein content of 20-27% (Thompson, 1977); 24.95-28.04% (Adel *et al*., 1980); 22% (Muller, 1988); 23-29% (Augustine and Klein, 1989); 20-25% (Chen, 1990); 24.5% (Calloway *et al*., 1994); 25% (Bhatty *et al*., 2000); 27.6% (Mubarak, 2005); 25.09% (Agugo and Onimawo, 2008). All these reports had shown that dehulled mungbean flour contained protein which was higher than or equal to some of the well-known legumes such as bambara groundnuts (18-20.73%), cowpea (22%), peanuts (24.3-27.0%) (Muller, 1988; Sosulski, 1983; Akinjayeju and Francis, 2007); rapeseed (25%), sunflower flour (28.7%), melon seed (*Colocynthis citrullus*) 28.44%; (Akobundu *et al*., 1982). However, the protein content of the dehulled mungbean seeds flour was lower than that of soybeans (55.56%) (Sosulski *et al*., 1976); 35.1-36% (Muller, 1988); 45.3-46.8% (Ukwuru, 2003); 38-40% (Rastogi and Singh, 1989).

There were significant reduction (p<0.05) among the protein contents of the boiled dehulled mungbean seeds flour with increasing time and the sample boiled for 90 min had the least crude protein (18.83%). A significant reduction (p<0.05) in protein content of dehulled mungbean seeds flour (27.6-26.8%) had been reported by Mubarak (2005) to be caused by boiling the seeds. This decrease has been attributed to leaching during boiling, also due to proteases-resistant complex linkage formation and recombination of amino acid residues (Kylen and McCready, 1975; Fagbemi, 2007).

As shown in Table 2, the crude protein content of the raw sample (28.38%) which fell within the range (20-29%) (Augustine and Klein, 1989; Chen, 1990). Increase in toasting time resulted to significant reduction (p>0.05) in the protein content of the dehulled mungbean seeds flour with the sample toasted for 90 min having the least protein value of 15.33%. The greater reduction in the protein content of the toasted mungbean flours could be as a result of polymerization of amino acid that occur on roasting (dry heat processing) (Kato *et al*., 1985) or due to the formation of meladin at high temperature. The crude protein of the sample was significantly (p<0.05) increased by 24 and 36 h sprouting (35.9 and 36.5%) respectively. Mubarak (2005) similarly reported a significant (p<0.05) increase in crude protein value of germinated mungbean seeds (from 27.6-30.0%). This was in agreement with the report of Anonymous (2008) which attributed the protein increase to protein synthesis during germination or due to inclusion of microbial cells into the flour. Other researchers reported increase in protein for sprouted cowpea (Akinjayeju and Adekanye, 2006) and increase in protein for sprouted bambara nuts (Akinjayeju and Francis, 2007). The increases in crude protein could be due to enzyme hydrolysis of the insoluble protein available (Echendu *et al*., 2009). It has also been reported that increase in protein during germination was due to the release of free amino acids after enzymatic hydrolysis for the synthesis of new protein (Bliss, 1975).

Fat: The fat content of the raw sample (1.89%) significantly differed (p<0.05) from that of the boiled samples. Other researchers presented fat content of

raw dehulled mungbean flour to be 2.83% (Bhatty *et al*., 2000); 0.86-0.96% (Adel *et al*., 1980); 1.2-1.3% (Chen, 1990); 1.0-1.5% (Savage and Deo, 2000); 1.82% (Mubarak, 2005); 1.45% (Agugo and Onimawo 2008). All these variations could be due to difference in variety or probably the varying extent of dehulling (Bhatty *et al*., 2000). The fat content of the dehulled mung flour did not differ much from that found in some other legumes except some legumes which are oil seeds like peanuts, soybeans (Sosulski *et al*., 1976) 11.6%; Ukwuru (2003) 18-19.6%. The low fat levels of legume grains is because its major function is to maintain the integrity of cell wall and other forms of proteins (Chikwendu, 2003).
The boiling treatments markedly reduced the fat content with increasing time although no significant difference ($p>0.05$) existed between the fat contents of the mung seeds flour boiled for 30 and 45 min with values of 2.27 and 2.24% respectively. Similar results were obtained by Mubarak (2005); Agugo and Onimawo (2008).
Toasting for 30 and 45 min reduced the fat content significant ($p<0.05$) to 1.47 and 1.52% respectively though there was no marked differences between them. The fat increased in the 60 and 90 min toasted flours to 2.06 and 2.07% respectively. Agugo and Onimawo (2008) reported a reduction in the fat content of toasted mungbean seeds flour while Fagbemi (2007) reported an increase in the fat content of toasted fluted pumpkin seeds flour.
The fat contents of the 24 and 36 h sprouted samples were reduced (1.35 and 1.23%) significantly ($p<0.05$). Similar observation was made by Mubarak (2005) for germinated mungbean seeds which reduced fat content to 1.45%. Furthermore, Fagbemi (2007) observed a significant ($p<0.05$) reduction in fat content of germinated seeds flour. The decreases could be caused by the use of fat as an energy source to start germination (Mubarak, 2005). Contrarily, Chavan and Kadam (1989); Anonymous (2008b) indicated that sprouting increases the fat content of seeds due to disappearance of starch. Akinjayeju and Francis (2007) attributed fat reduction during soaking to hydrolysis of fat to fatty acids and glycerol by lipolytic enzymes.

Ash: The ash content of the raw sample (1.2%) was lower than those obtained by Adel *et al*. (1980), 3.31-4.05%; Bhatty *et al*. (2000), 4.63%; Agugo and Onimawo (2008), 3.43% for mungbean flour and 6.1% (Sosulski *et al*., 1976); 3.0-4.2% (Ukwuru, 2003) for raw soyflour. Increase in boiling time did not markedly reduce the ash content of the dehulled mung flour.
Significant ($p<0.05$) difference existed among the toasted samples and this result was in agreement with the report of Fagbemi (2007) for the total ash of the toasted fluted pumpkin seeds flour.
The ash was reduced by sprouting treatments which confirms the report of Mubarak (2005) for germinated mungbean seeds flour. Also, Chikwendu (2003) reported an increase in ash content of groundbean germinated for 72 h due to endogenous enzyme hydrolysis of complex organic compounds to release more nutrients leaving antinutrients to leach into the germination medium.

Crude fiber: The crude fiber content of the raw sample (4.05%) which fell within the range 0.57-5.01% reported by Adel *et al*. (1980). However, higher fiber content of mungbean flour was reported by Agugo and Onimawo (2008), 8.95%. The variation observed in the fiber content of raw mungbean was probably due to the varying extent of dehulling (Bhatty *et al*., 2000). According to Maforimbo (2001), the fiber contents of the dehulled soybean seeds were significantly lower than the whole seeds (undehulled seeds), suggesting that fiber is more concentrated on the outer seed coat (testa) than in the endosperm. Agugo and Onimawo (2008) reported that high fiber content makes mungbean a good digestive food. Raw soybeans flour was reported to have crude fiber content of 3.2% (Sosulski *et al*., 1976), 4.3-4.5% (Ukwuru, 2003). Boiling treatments did not significantly ($p>0.05$) reduce the crude fiber of dehulled mungbean flour and this result was in agreement with the report of Agugo and Onimawo (2008) for boiled mungbean flour. There were significant difference ($p<0.05$) between the crude fiber contents of the raw and toasted mungbean flour. Also toasting at different length of times resulted to a significant difference ($p<0.05$) among the crude fiber contents of the mungbean flour. Toasting for 30, 45, 60 and 90 min resulted to crude fiber contents of 4.32, 4.95, 2.05 and 3.4% respectively. According to Agugo and Onimawo (2008), toasting increased the crude fiber content of mungbean flour significantly.
The crude fiber was reduced by sprouting treatments which confirms the report of Mubarak (2005) for germinated mungbean seeds flour. However, on the contrary, Peer and Leeson (1985); Cuddeford (1989) stated that sprouting increased the crude fiber contents of seeds. The increase in fiber due to germination might be that the microfloura enzymes hydrolyzed complex carbohydrate to release fiber which subsequently decreased carbohydrate.

Carbohydrate: The carbohydrate content of the raw dehulled mungbean was found to be 54.47%. However, much higher total carbohydrate content have been observed by Mubarak (2005) to be 62.9%; Agugo and Onimawo (2008), 61.47%; Adel *et al*. (1980), 64.15-66.32%. Lower total carbohydrates have been reported by Muller (1988) to be 35%. The variations could be attributed to the processing methods (Agugo and Onimawo, 2008).
Boiling treatments were found to significantly ($p<0.05$) increase the total carbohydrate content of the dehulled

mungbean seeds flour especially with increase in boiling time. Boiling for 30, 45, 60 and 90 min increased the total carbohydrates to 57.16, 57.29, 61.60 and 65.05% respectively. These results were in agreement with the findings of Mubarak (2005); Agugo and Onimawo (2008) for boiled mungbean seeds flour.

Increase in toasting time resulted to significant ($p<0.05$) increase in the total carbohydrates of the dehulled mungbean flour. Toasting for 30, 45, 60 and 90 min gave the total carbohydrate contents of 60.29, 63.18, 71.18 and 71.99% respectively. Similar result was obtained by Agugo and Onimawo (2008) for toasted mungbean flour which showed that toasting significantly increased the total carbohydrate content.

Sprouting treatments were found to significantly ($p<0.05$) reduce the total carbohydrate of the dehulled mungbean seeds flour although, no significant difference existed among the 24 and 36 h sprouted samples(49.02 and 47.88%) respectively. This result corresponded with the report of Mubarak (2005) for germinated samples which showed a significant reduction in the total carbohydrate (from 62.9-61.7%). This could be due to increased starch digestibilities promoted by improved hydrolytic activities of the enzymes during sprouting. Akinjayeju and Francis (2007) reported the carbohydrate by alpha-amylases to simple and more soluble sugars needed by the growing seeds. The decrease in carbohydrate due to germination might also be due to the use of carbohydrate for metabolism by the young seedling (Obizoba and Atu, 1993).

Conclusion: It was noted from this study that, as expected, the dehulled samples had better proximate composition than the undehulled samples because dehulling generally, although not with marked incremental values, improved the proximate compositions of the mungbean flour except the crude fiber values which were much higher in the undehulled mungbean flour. Apparently, boiling treatments increased the moisture and carbohydrate contents but reduced the protein, fat, ash, fiber and energy values. Toasting treatments reduced moisture and protein values but increased carbohydrates and fat contents. However, sprouting treatments increased moisture, crude protein but reduced fat, ash, fiber, energy values and carbohydrates.

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Chemical Characteristics of Drinking Water of Peshawar

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Abstract: Ninety drinking water samples of fifteen each from three urban and three rural areas of Peshawar were collected and analyzed for their heavy metals, minerals, SO_4, NO_3 and Cl. In the urban area were Hayatabad, City and Sadder while from rural area Shikhmohamadi, Palosi and Jagra. Among the heavy metals and minerals Pb, Cr, Fe, Zn, Mn, Ca, Cu and Mg were determined by atomic absorption spectrophotometer, Na and K by flame photometer, Cl by titration method, SO_4 by spectrophotometer and NO_3 by nitrogen analyzer. The mean value of Pb, Cr, Fe, Zn, Cu and Mn among different locations were, 0.15±0.11, 0.21±0.05, 0.68±0.15, 0.09±0.14, 0.11±0.03 and 0.01±0.01 mg/L respectively. The mean value of Na, Mg, K and Ca were 26.61±3.70, 23.47±4.24, 3.11±1.77 and 40.96±16.94 mg/L respectively. For SO_4, NO_3 and Cl mean value 12±6.54, 5±1.26 mg/L and 14±4.90 mg/L respectively. Minerals content result obtained indicated that their level in different locations were in safe level as recommended by WHO and for heavy metals they are above the safe limit as recommended by WHO. Hence considered unfit for drinking purpose and it was concluded that the water from Peshawar region should be checked and monitored regularly in order to omit all possible sources of contamination or to reduce it.

Key words: Drinking water, heavy metals, minerals, contamination

INTRODUCTION

Water is clear, colorless, odorless and tasteless liquid, essential for most plant and animal life and the most widely used of all solvents. Freezing point 0°C (32°F); boiling point 100°C (212°F); specific gravity (4°C) 1.0000; weight per gallon (15°C) 8.338 pounds (3.782 kilograms).

Water is second to oxygen as being essential for life. People can survive days, weeks, or even longer without food, but only about four days without water. The average adult consumes and excretes about 2.5-3 quarts of water a day. Some of this water is supplied through foods but most is consumed through beverages. It is generally recommended that adults consume 6-8 cups (48-64 ounces) of liquids daily.

Water makes up about 60% of the human body Most of the living tissue of a human being is made up of water; it constitutes about 92% of blood plasma, about 80% of muscle tissue, about 60% of red blood cells and over half of most other tissues. It is also an important component of the tissues of most other living things (Turgut *et al*., 2005).

It covers some 70% of the earth's surface, with only 3% being from fresh water sources. With the world population growing and the increasing pollution of our natural resources, we are facing a water crisis. The World Health Organization has estimated that over 1 billion people lack access to safe drinking water and about 4000 children die every day from water borne disease (Virkutyte and Sillanpa, 2006).

As world population grows, drinking water is becoming increasingly scarce. Even in countries with sufficient supplies, this resource is often contaminated, especially in the world's threshold and developing nations. There are 1.2 billion people who do not have enough drinking water and 2.4 billion who are not connected to a sewage system.

Polluted water has direct effect on human health while sewerage and industrial effluents, have indirect effect through consumption of foodstuffs being irrigated with such type of discharge. According to World Health Organization more than 80% human diseases are water born. In developing countries 80% of the population has not easy excess to pure drinking water and more than 5 million human being die with and early death annually as a result of illness linked to unsafe drinking water unclean domestic environments and improper excreta disposed. Water pollutants mainly consist of heavy metals, microorganism, fertilizer and thousand of toxic organic compounds (WHO, 1999). Heavy metals consist of Cd, Cr, Cu, Pb, Ni, Fe, Mn, Hg, Zn, Al, Se as well as metals of group III and IV, which have toxic effect on human physiology. Along with the heavy metals the next most important pollutants are microorganisms. Surface and ground water contains bacteria, protozoa and other synthetic microorganisms. These affect water quality by assimilating NO_3, CO_2 and also solubilize some heavy metals in water. These also produce toxicants in ground water. They cause many epidemic diseases in human (Khan *et al*., 2000).

More than 2.6 billion people-forty percent of the world's population-lack basic sanitation facilities and over one billion people still use unsafe drinking water sources. As a result, thousands of children die every day from

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diarrhoea and other water-, sanitation- and hygiene-related diseases and many more suffer and are weakened by illness.

The World Health Organization (WHO, 1972-73) estimates that 500 million diarrhea cases reportedly take place each year in children less than five years in Asia, Africa and Latin America. The extent of enteric diseases in different areas depends upon the extent to which water is exposed to contamination. The incidence of typhoid fever, bacillary dysentery, infectious hepatitis and other enteric infections are common and are transmitted through contaminated water. Cholera is still a wide spread water borne disease in some developing countries. There are numerous other diseases that are transmitted through polluted water. It has been shown that cancer may be caused by the accumulation of certain materials carried out by water to human organs (DAWN, 1989).

The excess of cadmium accumulated in the kidney causes hypertension as is evident from study conducted on animals. The deficiency of chromium in drinking water favour atherosclerotic diseases in human. The compounds of chlorobenzenes and chlorophyll's may affect taste and odor of water.

Pakistan's current population of 141 million is expected to grow to about 221 million by the year 2025. This increase in population will have direct impact on the water sector for meeting the domestic, industrial and agricultural needs. Pakistan has now essentially exhausted its available water resources and is on the verge of becoming a water deficit country. The per capita water availability has dropped from 5,600-1,000 m^3. The quality of groundwater and surface-water is low and is further deteriorating because of unchecked disposal of untreated municipal and industrial waste water and excessive use of fertilizers and insecticides. Water quality monitoring and information management is lacking, even though it's crucial to any water quality improvement program (Shakirullah *et al*., 2005).

It is estimated that by 2025 world water consumption will rise by 40%. This is why investments are urgently needed in the entire water infrastructure around the world, especially in mega cities. An additional main user of water is industry, whose plants can only be kept running with the aid of a wide range of process waters (Sidhu and Warner, 2003).

The purpose of present study to observed the physicochemical and bacteriological characteristics of Peshawar.

The objective of the study was:

- Determine mineral (Na, K, Ca, Mg, Mn, Fe, Zn, Pb, Cr, Cl, SO_4, NO_3) contents in drinking water.

MATERIALS AND METHODS

The water samples were tested for chemical analysis. Drinking water samples from different areas of Peshawar were tested for heavy metals, minerals, SO_4, NO_3 and Cl, in order to evaluate its quality. Urban and Rural areas of Peshawar were selected randomly. Urban area constitutes Hayyatabad, Sadder and City and while rural constitutes Sheikh Mohammadi, Jagra and Palosi.

From each location 15 water samples were collected in one liter capacity plastic bottles for chemical analysis. Before sampling the bottles were washed with detergents, followed by tape water and finally several time with distilled water. The water at the samples site was allowed to flow for some time then the bottles were rinsed two to three times by this water and one liter was taken as sample from drinking water. The samples were properly tagged indicating code. These samples were air tightened and stored in refrigerator.

Chemical analysis

Heavy metals and minerals: 100 ml water sample was taken and were analyzed for heavy metals. Among the heavy metals and minerals Pb, Cu, Zn, Fe, Cr, Mg, Mn and Ca were determined by atomic absorption spectrophotometer (model parkin Elmer 2380) while Na and K were determined by flam photometer.

Determination of SO_4: 10 ml of samples was added with 1 ml of 6 N HCl followed by 5 ml 70% sorbitol mixed and added with 1 g $BaCl_2$ and again mixed. Reading was noted at 470 nm wavelength on spectrophotometer. Standard were prepared with the same procedure and SO_4 were determined by Turbedemetric method (AOAC 2003 method no 973.57).

Determination of NO_3: 20 ml of water sample was distilled with MgO+DA which was collected in 5 ml Boric acid mixed indicator and it was then titrated against 0.005 M HCl and NO_3 were determined by Kjeldahal method (AOAC 2003 method no 973.48).

Determination of Cl: 10 ml sample was taken and two to three drops of phenolphthalein indicator and titrated against 0.05 N $AgNO_3$ till the disappear of pink color and Cl were determined by Mercuric Nitrate method (AOAC 2003 method no 973.51).

Statistical analysis: Physical, chemical and becterlogoical data were checked by using Epi-info statistical software. Descriptive statistical analysis was carried out in order to check the mean, standard deviation and range in collected data. Difference between urban and rural was checked by F-test (ANOVA).

RESULTS AND DISCUSSION

Water sample collected from Peshawar valley were analyzed for their chemical characteristics. The heavy metals, minerals, SO_4, NO_3 and Cl were determined. Among the heavy metals Pb, Cu, Zn, Fe, Cr, Mn in minerals Na, K, Mg and Ca were analyzed.

Table 1: Minerals content of water sample

Area	Na mg/L	K mg/L	Mg mg/L
	Mean±SD		
Total urban (n = 45)	24.89±3.77	2.71±0.59	20.67±3.81
Hayatabad (n = 15)	26.87±4.63	2.73±0.46	16.46±0.58
Sadder (n = 15)	23.53±2.85	2.80±0.77	21.21±3.27
City (n = 15)	24.27±2.94	2.80±0.77	24.33±0.96
Total rural (n = 45)	28.33±2.72	3.51±2.38	26.27±2.39
Sheikh Mohammadi (n = 15)	26.13±1.13	5.87±2.90	26.34±1.78
Jagra (n = 15)	29.60±1.18	2.20±0.41	27.28±1.48
Palosi (n = 15)	29.27±3.58	2.47±0.52	25.20±3.19
Grand total (n = 90)	26.61±3.70	3.11±1.77	23.47±4.24

Table 1 represent the mineral content of water sample. Among the mineral content Na, K and Mg were analyzed for their mineral chemical characteristics and then compared with WHO standards

Sodium in drinking water: Sodium was found in water sample of Peshawar valley in the range of 17.00-33.00 mg/L with Total mean Na value 26.61 mg/L which is lower than the WHO maximum acceptable concentration i.e. 250 mg/L, mean value of Na for Hayatabad, Sadder, City, Sheikh Mohammdi, Jagra and Palosi were 26.87, 23.53, 24.27, 26.13, 29.60 and 29.27 mg/L recorded respectively.

Na in drinking water is not of a health concern for most people. But may be an issue for someone with heart disease, hypertension, kidney disease, circulatory illness or on sodium controlled diet. Studies have shown that reducing salt intake will lower blood pressure in people with hypertension but it can be conclusively inferred that increased sodium will cause hypertension (Radojevic, 1999).

It is unlikely that sodium alone is carcinogenic. However, a high-salt diet may enhance the carcinogenic potency of chemicals such as *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine in drinking water by causing irritation of the gastroduodenal tract, thus increasing the exposure of epithelial cells to the carcinogen and resulting in an increased incidence of gastric tumours (Takahashi, 1983). Although it is generally agreed that sodium is essential to human life, there is no agreement on the minimum daily requirement. However, it has been estimated that a total daily intake of 120-400 mg will meet the daily needs of growing infants and young children and 500 mg those of adults (NRC Washington, 1989).

In general, sodium salts are not acutely toxic because of the efficiency with which mature kidneys excrete sodium. However, acute effects and death have been reported following accidental overdoses of sodium chloride. Acute effects may include nausea, vomiting, convulsions, muscular twitching and rigidity and cerebral and pulmonary oedema. Excessive salt intake seriously aggravates chronic congestive heart failure, and ill effects due to high levels of sodium in drinking-water have been documented. (Department of National Health and Welfare, 1992).

The effects on infants are different from those in adults because of the immaturity of infant kidneys. Infants with severe gastrointestinal infections can suffer from fluid loss, leading to dehydration and raised sodium levels in the plasma (hypernatraemia), permanent neurological damage is common under such conditions. Addition of cows' milk or tap water containing high levels of sodium to solid food may exacerbate the effects (Sax *et al*., 1985) Whereas reducing the sodium intake can reduce the blood pressure of some individuals with hypertension, this is not effective in all cases. In addition, some data for both humans and animals suggest that the action of sodium may be at least partly modified by the level of the accompanying anion as well as that of other cations. Although several studies suggest that high levels of sodium in drinking-water are associated with increased blood pressure in children.

Although there is an association between hypertension and certain diseases, such as coronary heart disease, genetic differences in susceptibility, possibly protective minerals (potassium and calcium) and methodological weaknesses in experiments make it difficult to quantify the relationship and sodium in drinking-water generally makes only a small contribution to total dietary sodium. No firm conclusions can therefore be drawn at present as to the importance of sodium in drinking-water and its possible association with disease.

Potassium: Potassium ranks seventh among the elements in order of abundance, yet its concentration in most drinking water reached to the range of 2.00-9.00 mg/L with total mean K value 3.11 mg/L of Peshawar valley which is in recommended level of WHO i.e. 12.00 mg/L. mean value of K for Hayatabad, sadder, City, sheikh Mohammdi, Jagra and Palosi were 2.60, 2.73, 2.80, 5.80, 2.20 and 2.47 mg/L.

Excessive loss of extra cellular fluid may result in potassium deficiency. The loss may be due to vomiting, diarrhea, excessive diuresis or prolonged malnutrition. The chief features of deficiency are muscular weakness and mental apathy. In hypokalemia cardiac failure can result from depletion of ionized potassium in heart muscle (Vogel *et al*., 1999).

In hyperkalemia the serum level is elevated, resulting from kidney failure to clear ionized potassium. The

symptoms are mental confusion, numbness of extremities, poor respiration and weakening of heart action. The strongest epidemiological argument in favor of a water magnesium effect was provided by Dr. Paul Hunter based upon analytical studies in Taiwan (by Dr. Chun-Yuh Yang, who also spoke at the meeting) and Sweden (Rubenowitz) that reported a reduction of CVD mortality risk with increasing potassium levels in drinking water, but there were no strong associations with water hardness or calcium levels. The potassium benefits seemed to level off at about 10 mg/L in the five analytical studies that were reviewed. There are other larger CVD risk factors, however. There was a consistent finding of reduced rates of CVD associated with consumption of drinking water with increasing levels of magnesium. Even a small percentage benefit, if real, would have a very significant impact on death rates and public health.

Magnesium: Mg is one of the earth's most common element and from highly soluble salt. high concentration of Mg is undesirable in potable water as it causes scale formation and cathartic and diuretic effect. Especially when associated with Sulphate. Many earlier research worker have studied Mg in the drinking water and also reported its toxicity towards living things. The level of Mg of water sample analyzed in the range of 15.34-29.93 mg/L with grand mean value of 23.47 mg/L, which is in the safe limit recommended by WHO i.e. 36.45 mg/L. Mg mean value for Hayatabad, sadder, City, sheikh Mohammdi, Jagra and Palosi were 16.46 m^{-1}, 21.21m^{-1}, 24.33 m^{-1}, 26.34 m^{-1}, 27.28 mg/L and 25.20 mg/L respectively.

The nutritional essentiality and benefits from sufficient dietary intakes of magnesium are well established but quantitatively imprecise. Most of the epidemiology studies conducted since the mid-1950s support the hypothesis that extra magnesium in drinking water can contribute to reduced Cardiovascular Disease (CVD) and other health benefits in populations. This is the so-called 'hard water cardiovascular disease benefits hypothesis' (National Institute of Occupational Safety and Health, 1991).

Magnesium is an essential co-factor for more than 350 enzyme systems and it is involved in energy metabolism, nucleic acid synthesis, cellular balance, cardiovascular health and hormonal functions. Low magnesium intake has been associated with osteoporosis, increased calcium balance, insulin resistance, metabolic syndrome, increased oxidant stress and increased risk of cardiovascular disease (Tuthill and Calabrese, 1991).

The adult human body contains about 24 grams of magnesium, about half in bone and the remainder in soft tissue; only about 0.3% is in serum. There is no simple, rapid and accurate test to assess a person's magnesium status (Armstrong, 2002).

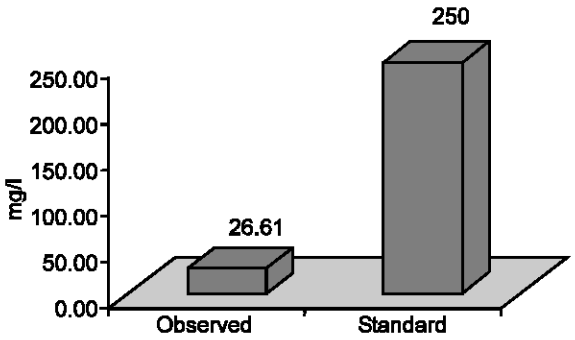

Fig. 1: Na concentration (observed vs standard)

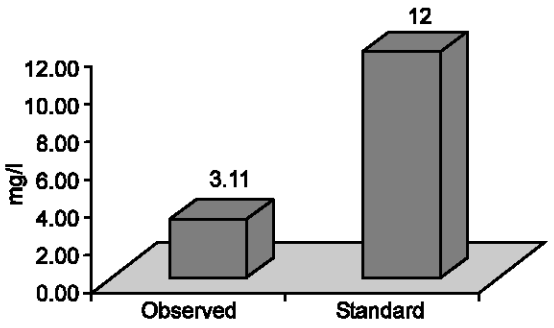

Fig. 2: K concentration (observed vs standard)

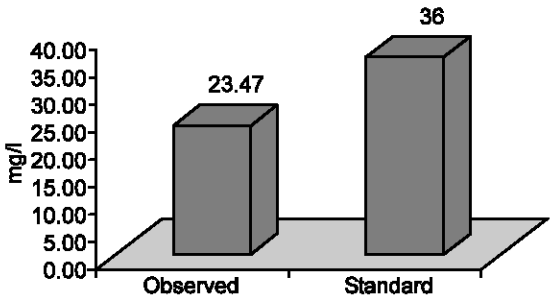

Fig. 3: Mg concentration (observed vs standard)

Calcium: Ca is present in all natural water as its level depends upon the type of rocks through which the water passes. It is usually present in the form of Carbonates, Bicarbonates, Sulphate, Chlorides and Nitrate. Ca contribute the hardness of water but also essential for human diet.

The concentration of Ca found in the water sample of Peshawar valley ranged from 12.30-87.57mg/L with grand mean of 40.96 mg/L which is in the safe limit recommended by WHO i.e. is 100 mg/L. Mean value for Hayatabad, sadder, City, sheikh Mohammdi, Jagra and Palosi were 24.74 m^{-1}, 34.36 m^{-1}, 38.05 m^{-1}, 52.04 m^{-1}, 50.35 mg/L and 46.19 mg/L respectively.

Calcium plays important roles in bone structure, muscle contraction, nerve impulses transmission, blood

Table 2: Micronutrient of water sample

Area	Ca mg/L	Zn mg/L	Cu mg/L
	Mean±SD		
Total urban (n = 45)	32.38±11.03	0.08±0.13	0.09±0.03
Hayatabad (n = 15)	24.74±6.73	0.10±0.14	0.07±0.01
Sadder (n = 15)	34.36±12.51	0.04±0.06	0.10±0.03
City (n = 15)	38.05±8.89	0.11±0.17	0.11±0.01
Total rural (n = 45)	49.53±17.57	0.10±0.14	0.14±0.01
Sheikh Mohammadi (n = 15)	52.04±18.89	0.04±0.13	0.13±0.01
Jagra (n = 15)	50.35±15.99	0.12±0.14	0.13±0.01
Palosi (n = 15)	46.19±18.38	0.14±0.15	0.14±0.01
Grand total (n = 90)	40.96±16.94	0.09±0.14	0.11±0.03

Table 2 represent the mineral content of water sample. Among the mineral content Ca, Zn and Cu were analyzed for their mineral chemical characteristics and then compared with WHO standards

clotting and cell signaling; 99% of calcium is in bone and teeth and the remainder is in soft tissue. Low intake is associated with osteoporosis, rickets and hypertension. Consumption in drinking water also reduces the risk of kidney stones, probably by complexing with oxalates in the diet that compose some types of kidney stones (Kurtz and Morris, 1993). U.S. EPA noted that the mineral content of drinking waters varies widely throughout the world as well as within countries. Some examples ranged from about 2-89 mg/L for calcium.

Above the recommended limit of WHO it may create the problem like deposition in water system and excessive scales formation.

Zinc: Zn content concentration in water sample collected from Peshawar valley varies. Zn value ranges from 0.00-0.57 mg/L. Mean value for Hayatabad, sadder, City, sheikh Mohammdi, Jagra and Palosi were 0.10, 0.04, 0.11, 0.04, 0.12 and 0.14 mg/L respectively which is below the recommended level of WHO i.e. 15 mg/L.

Zinc is an essential micronutrient of plant and animals. It plays a significant role in the enzymatic system of human body e.g. the enzyme like aldolase, alkaline phosphates, etc depend totally on zinc. It is also essential for the normal functioning of the cells including protein synthesis, carbohydrate metabolism, cell growth and cell division (ATA and TF, 1996).

The zinc deficiency results into retardation of growth, anorexia, lesions of the skin and impaired development and function of reproductive organs. On the other hand when zinc concentration is exceeded then it causes fever, depression, malaisc, cough, vomiting, salvation and headache. However its toxicity is less than that of other heavy metals like Cd, As and Sb.

Copper: Cu content contamination was not widely effected by area. Its mean content among different locations ranges from 0.01-0.15 mg/L. Mean value for Hayatabad, sadder, City, Sheikh Mohammadi, Jagra and Palosi were 0.07, 0.10, 0.11, 0.13, 0.13 and 0.14 mg/L respectively with the grand mean value 0.11 mg/L is in toxic range, as it was higher than the maximum toxic

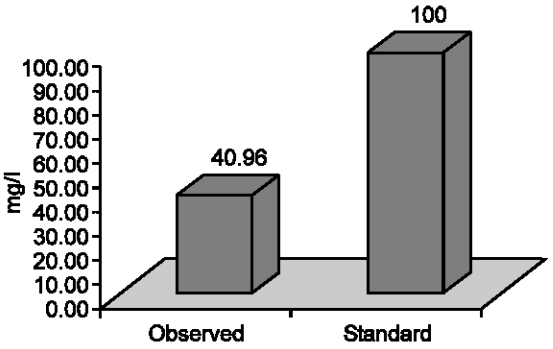

Fig. 4: Ca concentration (observed vs standard)

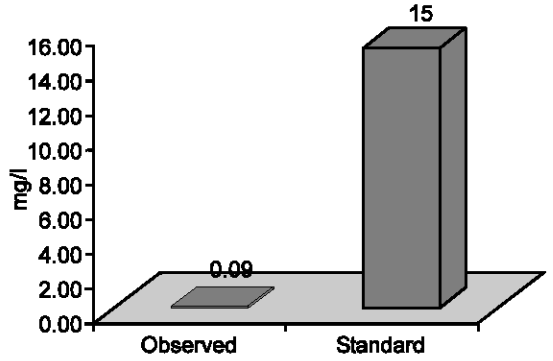

Fig. 5: Zn concentration (observed vs standard)

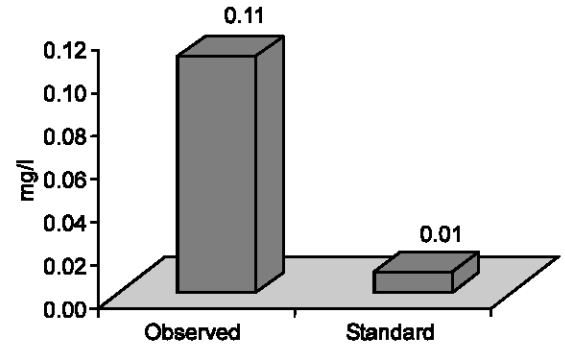

Fig. 6: Cu concentration (observed vs standard)

level (0.01 mg/L) with recommended by WHO (1999) this amount agrees with the result of Afia Zia *et al.* (2006) who reported its range from 0.011-0.0199 mg/L Cu content.

Copper concentrations in drinking-water often increase during distribution, especially in systems with an acid pH or high-carbonate waters with an alkaline pH (US EPA, 1995).

Cu rarely occurs in natural water. Most copper contamination in drinking water happens in the water delivery system, as a result of corrosion of the copper pipes or fittings. Copper level above the permissible limit can cause a bitter metallic taste in eater and result in blue green stains on plumbing fixtures. Stomach intestinal distress such as nausea, vomiting, diarrhea, stomach cramp and liver and kidney damage are the health problem associated with Cu contaminated drinking water (Zacarias, 2001).

Cu is also essential micronutrient and is required by the body in very small amount. People with Wilson's disease are more sensitive to copper deficiency, infants and children up to ten years old have a greater sensitivity to copper (Yarze, 1999).

Lead: Table 3 shows Pb concentration, which varies from location to location. This variation in Pb concentration is due to outdated piping, Pb value ranged from 0.00-0.41 mg/L with mean value of 0.15 mg/L which is above the recommended level of WHO i.e. 0.05 mg/L (WHO 1999) Mean value for Hayatabad, sadder, City, Sheikh Mohammadi, Jagra and Palosi were 0.18 m^{-1}, 0.18 m^{-1}, 0.15 m^{-1}, 0.11 m^{-1}, 0.14 mg/L and 0.15 mg/L respectively this value agree with the result of (Afia Zia *et al.*, 2006) who reported its value from 0.162-0.421 mg/L. The mean concentrations of Pb in the entire water sample were high than the recommended value because of corrosion of Pb based solder pipe joints, galvanized pipes and fittings (Ihsanullah *et al.*, 1999).

Lead is metal found in natural deposits, can't be seen, smelled, or tasted. It is found in food, paint, dust, soil, smoke and even in drinking water. In water main cause of Pb contamination is old piping and industrial discharge. Children and pregnant women are very susceptible to health risk from lead in drinking water (EPA, 2002).

Most lead contamination of drinking water occurs when soft acid water corrodes lead or galvanized pipe or corrodes solder used in pipe fittings. Lead is used in insecticide and in high-octane gasoline. But its main source of contamination is vehicle discharge and industrial effluent, which contaminate normal water bodies resulting serious ground water contamination through leaching.

Lead broadly effect human organs and systems. The most sensitive is Central Nervous System (CNS) particularly in children. Lead also damage kidney and immune system. The exposure of unborn children due to mother is also dangerous which results harmful effects include pre-mature birth, smaller babies and decreased mental ability in the infants, learning difficulties and reduce growth in young children. Lead can cause stroke, kidney disease, and cancer form a lifetime exposure at level above the MCL (Yang *et al.*, 1999).

Iron: Iron is one of the most troublesome element in water supplies. Rainwater as it infiltrates the soil and underlying geologic formation dissolve iron, causing it to seep into aquifers that serves as sources of groundwater for wells.

Table 4 show the Fe concentration in samples of drinking water of Peshawar valley. Fe concentration ranged from 0.42-0.99 mg/L with grand mean of 0.68 mg/L Fe concentration Mean value for Hayatabad, sadder, City, Sheikh Mohammadi, Jagra and Palosi were 0.50, 0.53, 0.62, 0.74, 0.79 mg/L and 0.88 mg/L respectively which is below the recommended level of WHO i.e. 0.3 mg/L.

Iron limit for drinking water are based on aesthetic parameters rather than on toxicity. Iron is mainly present in water in two forms either soluble ferrous ion or the insoluble ferric ion. Water containing ferrous is clear and colorless, when exposed to air in the pressure tank or atmosphere, the water turns cloudy and a reddish substance begins to form. This sediment is the oxidized or ferric form of iron that dissolved in water. Iron is not hazardous to health, but it is considered a secondary or aesthetics contaminant (EPA, 2002).

Iron is essential for good health, iron helps to transport oxygen in the blood, dissolve ferrous iron gives disagreeable taste. When the iron combines with tea, coffee and other beverages it produce an inky, black, appearance and a harsh unacceptable taste.

Chromium: Cr concentrations in drinking water sample were found in the range of 0.12-0.35 mg/L with grand mean 0.21 gmL^{-1}. Mean value for Hayatabad, sadder, City, Sheikh Mohammadi, Jagra and Palosi were 0.21, 0.21, 0.24, 0.20, 0.20 and 0.19 mg/L respectively which is above the WHO recommended limit i.e. 0.050 mg/L.

The higher concentration was found in City due to congested old piping, low mean concentration was found in Palosi and it was mainly that the source of drinking water are surface wells of home water supply.

The tap water contamination may be due to corrosion of Cr discharge from steel and pulp mills, erosion of natural deposits. Cr is a metal found in nature with other metal deposits. They are mainly used in metals alloys like stainless steel, protective coating on metal, magnetic tapes and pigments for paint, cement, paper, rubber, composite floor covering and materials. It's soluble from are used in wood protective (Winter, 2003).

Table 3: Heavy metals of water sample

Area	Pb mg/L	Fe mg/L	Cr mg/L	Mn mg/L
	Mean±SD			
Total urban (n = 45)	0.17±0.10	0.55±0.07	0.22±0.05	0.01±0.01
Hayatabad (n = 15)	0.18±0.12	0.50±0.06	0.21±0.05	0.01±0.01
Sadder (n = 15)	0.18±0.09	0.53±0.04	0.21±0.05	0.01±0.01
City (n = 15)	0.15±0.10	0.62±0.05	0.24±0.04	0.00±0.00
Total rural (n = 45)	0.14±0.11	2.76±13.15	0.20±0.04	0.01±0.01
Sheikh Mohammadi (n = 15)	0.11±0.10	0.74±0.09	0.20±0.04	0.01±0.02
Jagra (n = 15)	0.14±0.12	0.79±0.11	0.20±0.04	0.01±0.01
Palosi (n = 15)	0.15±0.10	0.88±0.07	0.19±0.05	0.01±0.02
Grand total (n = 90)	0.15±0.11	0.68±0.15	0.21±0.05	0.01±0.01

Table 3 represent the heavy metals analyzed in the drinking water of Peshawar valley. Among the heavy metals Pb, Fe, Cr and Mn were analyzed

Table 4: Chemical content of water sample

Area	NO_3 mg/L	SO_4 mg/L	Cl mg/L
	Mean±SD		
Total urban (n = 45)	4.25±1.10	9.50±3.48	14.96±4.58
Hayatabad (n = 15)	4±1.31	8±1.84	17±4.55
Sadder (n = 15)	4±0.97	9±2.03	13±4.11
City (n = 15)	4±1.07	12±4.65	15±4.34
Total rural (n = 45)	5.46±1.11	14.93±7.70	12.56±4.96
Sheikh Mohammadi (n = 15)	5±0.87	16±6.09	12±1.60
Jagra (n = 15)	6±1.26	17±4.89	13±6.81
Palosi (n = 15)	5±1.09	12±10.47	13±5.14
Grand total (n = 90)	5±1.26	12±6.54	14±4.90

Table 4 indicates the chemical content of drinking water sample collected from Peshawar valley. Among the chemical content NO_3, SO_4 and Cl were analyzed

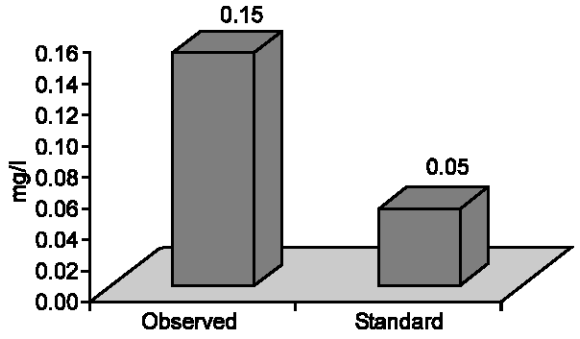

Fig. 7: Pb concentration(observed vs standard)

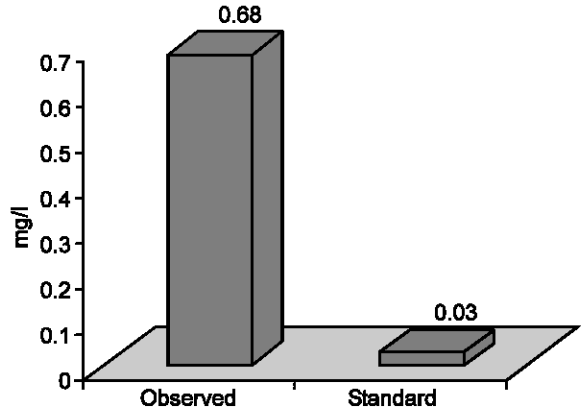

Fig. 8: Fe concentration(observed vs standard)

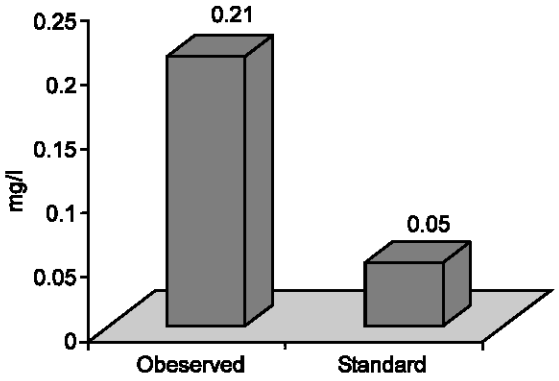

Fig. 9: Cr concentration(observed vs standard)

Ingestion of Cr in large amount can cause stomach upset and ulcer, convulsions, kidney and liver damage and even death (WHO, 1999).

The Cr harmfulness to the fetus or its ability to reproduction is unknown but experiment done on mice showed that large amount of Cr ingested had reproductive problems and offspring with birth defects. A life time exposure to Cr at level above the MCL can cause damage to liver, kidney, circulatory and nerve tissues and skin irritation.

Ingestion of 1-5 g of "chromate" (not further specified) results in severe acute effects such as gastrointestinal disorders, hemorrhagic diathesis and convulsions.

Death may occur following cardiovascular shock in some occupational studies, increased incidences of genotoxic effects such as chromosomal aberrations and sister chromatid exchanges have been found in workers exposed to chromium (VI) compounds and mortality due to lung cancer (Janus and Krajnc, 2000).

Manganese: The concentration of Mn found in the water sample of Peshawar valley ranged from 0.00-0.05 mg/L with grand mean of 0.01 mg/L which is in the safe limit recommended by WHO i.e 0.05 mg/L. Mean value for Hayatabad, sadder, City, Sheikh Mohammadi, Jagra and Palosi were 0.01, 0.01, 0.00, 0.01, 0.01 mg/L and 0.01 mg/L respectively.

The deficiency of Mn could occur in humans. The symptoms were weight loss, transient dermatitis, occasional nausea and vomiting, change in hair and beard color and slow growth of hair and beard. Studied of Mn deficiency in animals revealed the effects on reproductive capacity, pancreatic function and other aspects of carbohydrate metabolism. There is 10-20 µg of Mn in the adult human body while serum concentration reported to range from 1-200 µg/L (Henkin, 1976).

Mn toxicity has been seen in minors as a result of absorption of Mn through the respiratory tract after prolonged exposure to dust. The excess accumulates in the liver and central nervous system. Symptoms resemble those found in Parkinson's and Wilson's disease (Yarze, 1999).

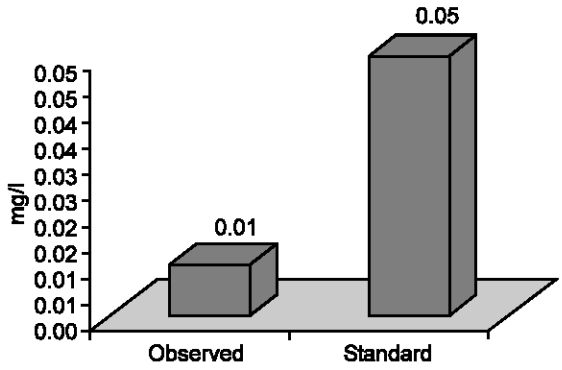

Fig. 10: Mn concentration(observed vs standard)

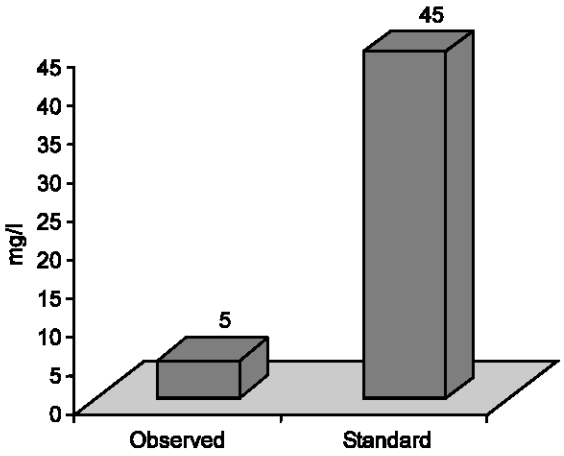

Fig. 11: Nitrate concentration (observed vs standard)

Nitrate: Nitrogen is the nutrient applied in the largest quantities for lawn and garden care and crop production. In addition to fertilizer, nitrogen passes naturally to the soil from decaying plant and animals' residue. In the soil, bacteria convert various forms of nitrogen to nitrates. This is desirable as greater extent of the nitrogen used by plant is absorbed in the nitrate form. However nitrate is highly leeachable and readily moves with water through the soil profile. If there is excessive rainfall or over irrigation, nitrate will be leached below the plants root zone and eventually reach ground water. Nitrate nitrogen in ground water may result from point sources such as sewage disposal system and livestock facilities as wells as from non point sources such as fertilized cropland, parks, golf courses, lawns and gardens. Proper site selection for the location for domestic water well and proper construction can reduce potential nitrate contamination of drinking water sources (Weinberg, 2006).

The concentration of drinking water sample collected from Peshawar valley in the range of 2-8 mg/L with mean Nitrate value of 5 mg/L. Mean value of nitrate for Hayatabad, sadder, City, Sheikh Mohammadi, Jagra and Palosi were 4, 4, 4, 5, 6 and 5 mg/L respectively which is in the permissible limit recommended by WHO i.e 45 mg/L. All the water sample are below the recommended limit of WHO which is safe for health.

When the level of Nitrate exceeds the permissible limit then the primary health hazard from drinking water with Nitrate Nitrogen may occur when Nitrate is transformed to Nitrite in the digestive system. The Nitrite oxidizes iron in the hemoglobin of the red blood cells to form methemoglobin and causes methemoglobineria or blue baby syndrome (a situation in which blood lack the ability to carry sufficient oxygen to he individual body cells causing the veins and skin to appear blue). Most children over one year of age have the ability to rapidly convert methemoglobin back to oxy hemoglobin, hence the total amount of methemoglobin with in red blood cells remains low in spite of relatively high level of Nitrate/Nitrite uptake. However in infants under six months of age the enzyme systems for reducing methemoglobin to oxyhemoglobin are incompletely developed and methemoglobineria can occur. This also may happen in older individuals who have genetically impaired enzyme system for metabolizing methemoglobin (Yang *et al*., 1999).

Sulphate: Sulphate is a combination of sulfur and oxygen and is a part of naturally occurring minerals in some soil and rock formation that contain groundwater. The mineral dissolved over time and is released into ground water. Sulfur reducing bacteria , which use sulfur as energy source are the primary producer of large quantities of hydrogen sulfide. These bacteria chemically change natural sulfates in water to hydrogen sulfide. Sulfur reducing bacteria live in oxygen deficient environments such as deep wells, plumbing system, and water softener and water heaters. These bacteria usually flourish on the hot water side of water distribution system. Hydrogen sulfide gas also occurs naturally in some groundwater, deposits of organic matter such as decaying plant material. It is found in deep or shallow wells and also can enter surface water through springs, although it quickly escapes to the atmosphere. Hydrogen sulfide often is present in those wells which are drilled in shale or sandstone or near coal or peat deposits or oil fields (Virkutyte and Sillanpa, 2006).

Table 4 indicted the Sulphate level of drinking water sample of Peshawar valley which is in the range of 2-36 mg/L with grand mean of 12 mg/L. Mean value of sulfate for Hayatabad, sadder, City, sheikh Mohammdi, Jagra and Palosi were 8, 9, 12, 16, 17 and 12 mg/L respectively. The permissible limit for sulfate by WHO is 250 mg/L .so all the water sample in the permissible range of WHO and hence safe for drinking purpose.

Chloride: Chloride is a major constituent of most waters. It is normally present in low concentrations in surface waters, while groundwater will contain varying amounts of chloride depending on the surrounding geology.

Chloride is widely distributed in the environment, generally as sodium chloride (NaCl), potassium chloride (KCl) and calcium chloride ($CaCl_2$). The weathering and leaching of sedimentary rocks and soils and the dissolution of salt deposits release chlorides into water.

Chloride in drinking water is generally not harmful to human beings until high concentrations are reached, although it may be harmful to some people suffering from heart or kidney disease. Restrictions on chloride concentrations in drinking water are generally based on palatability requirements rather than on health. The Guidelines for Canadian Drinking Water Quality 1989 has set the aesthetic objective for chloride in drinking water at 250 mg/L.

Chloride in drinking water may impart a salty taste at concentrations as low as 100 mg/L, however, the limit of 250 mg/L is considered the taste threshold for the average individual (Kurtz and Morris, 1993).

The concentration of chloride in drinking water sample collected from Peshawar valley in the range of 0.00-26 mg/L with grand mean of chloride value of 14 mg/L.

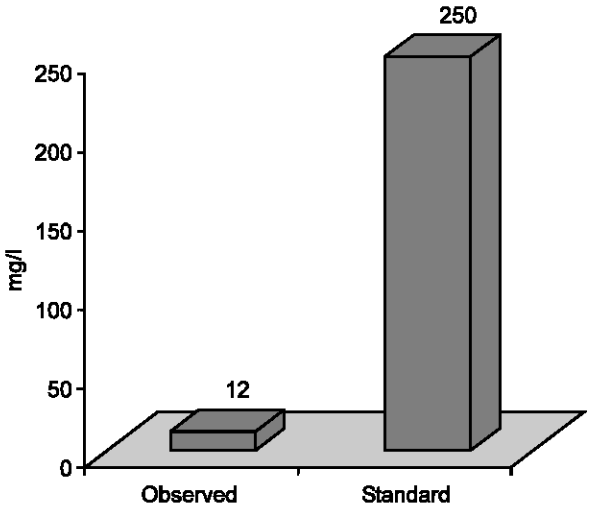

Fig. 12: Sulphate concentration (observed vs standard)

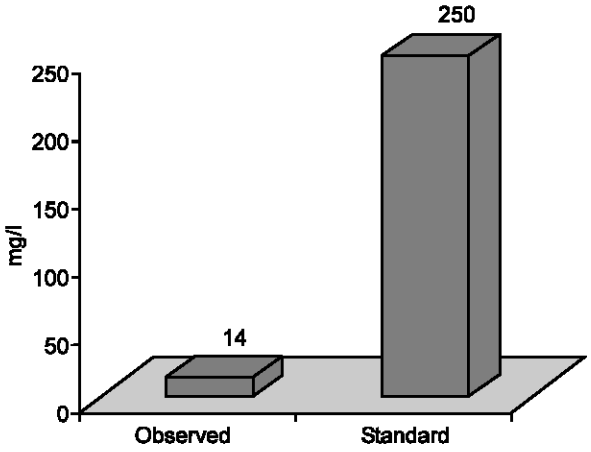

Fig. 13: Chloride concentration (observed vs standard)

Mean value of chloride for Hayatabad, sadder, City, sheikh Mohammdi, Jagra and Palosi were 17, 13 15, 12, 13 and 13 mg/L respectively. The permissible limit for chloride in drinking water is 250 mg/L. Hence all the water simples are in safe level as recommended by WHO.

Besides imparting a salty taste, chloride can affect the taste of coffee brewed in water containing a high concentration of chloride. Chlorides also appear to exert a significant effect on the rate of corrosion of steel and aluminum and can therefore affect some metals used in water handling systems.

A high level of chloride may cause gastrointestinal problems, irritation, diarrhea and dehydration (WHO, 1999).

Conclusion: The main source of contamination is the untreated sewerage water. In Peshawar City these

sewage effluents are discharged directly into non-bricked and uncommented drains, sewage carrier streams and Naray Khwar running across the palosi. From there due to the seepage of polluted water, it percolates into the underground fresh water body and contaminates it. Moreover, in irrigated farming lands the chemicals, fertilizers and pesticides are used in the fields and most of these chemicals residues reached the ground water table. Smoke and wastes of vehicles are deposited on the soil surface and in rainy season it results in considerable rise in the contamination of shallow well's water. The other possible sources of heavy metals pollution, for all categories include leaching from time old water supply pipes which are rusted, Pb/Cd based solder pipes, joints and use of sub-standard chemicals for water treatments.

In Hayyatabad and Palosi the main source of heavy metal contamination may be the near by flowing Palosi drain, as it is uncemented and carries the industrial effluent discharged in to it directly from Hayyatabad industrial estate. In Peshawar the sewerage and industrial effluents are also disposed into canals or rivers, which are used for irrigation in the vicinity of Peshawar and results in the contamination of irrigation as well as drinking water. In City and Saddar the main source of water pollution is the vehicle discharged and the polluted environment and their accumulation in old piping system and solder pipes and joints. For Sheikh Mohammadi and Jagra the main source of water contamination is the leaching of chemical and synthetic fertilizer in water table as there are more agriculture land for cultivation and the improper irrigation system.

The drinking water form all the study areas is contaminated with heavy metals, so it is essential that the supply of water for human consumption should be free from unpleasant or harmful impurity and for this reason it should be subjected to various treatments to render it safe for the use of man. The study indicates from the background values that it should be monitored regularly in order to evaluate the toxic, logical significance of commonly used water for drinking.

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Physical and Bacteriological Characteristics of Drinking Water of Peshawar

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Abstract: Ninety drinking water samples of fifteen each from three urban and three rural areas of Peshawar were collected and analyzed for their pH, Electrical Conductivity (EC), turbidity, coliform and *E. coli* were determined for bacteriological assessment and its quality. In the urban area were Hayatabad, City and Sadder while from rural area Shikhmohamadi, Palosi and Jagra. Coliform and *E. coli* were by Most Probable Number (MPN) and Ejakman method respectively. pH, EC and turbidity mean value were 7.44±0.16, 0.64±0.08 μscm^{-1}, 0.88±0.77 FTU respectively. Total Coliform ranged from 0.00-150.00 with mean value of 37.79, while *E. coli* was present (+ve) in 26% sample and absent (-ve) in 74% drinking water sample. All the physical parameters were in the safe limit recommended by WHO. Hence considered unfit for drinking purpose and it was concluded that the water from Peshawar region should be checked and monitored regularly in order to omit all possible sources of contamination or to reduce it.

Key words: Drinking water, pH, electrical conductivity, turbidity, coliform

INTRODUCTION

Water is clear, colorless, odorless and tasteless liquid, essential for most plant and animal life and the most widely used of all solvents. Freezing point 0°C (32°F); boiling point 100°C (212°F); specific gravity (4°C) 1.0000; weight per gallon (15°C) 8.338 pounds (3.782 kilograms).

Water is second to oxygen as being essential for life. People can survive days, weeks, or even longer without food, but only about four days without water. The average adult consumes and excretes about 2.5-3 quarts of water a day. Some of this water is supplied through foods but most is consumed through beverages. It is generally recommended that adults consume 6-8 cups (48-64 ounces) of liquids daily.

Water makes up about 60% of the human body Most of the living tissue of a human being is made up of water; it constitutes about 92% of blood plasma, about 80% of muscle tissue, about 60% of red blood cells and over half of most other tissues. It is also an important component of the tissues of most other living things (Turgut *et al*., 2005).

It covers some 70% of the earth's surface, with only 3% being from fresh water sources. With the world population growing and the increasing pollution of our natural resources, we are facing a water crisis. The World Health Organization has estimated that over 1 billion people lack access to safe drinking water and about 4000 children die every day from water borne disease (Virkutyte and Sillanpa, 2006).

As world population grows, drinking water is becoming increasingly scarce. Even in countries with sufficient supplies, this resource is often contaminated, especially in the world's threshold and developing nations. There are 1.2 billion people who do not have enough drinking water and 2.4 billion who are not connected to a sewage system.

Polluted water has direct effect on human health while sewerage and industrial effluents, have indirect effect through consumption of foodstuffs being irrigated with such type of discharge. According to World Health Organization more than 80% human diseases are water born. In developing countries 80% of the population has not easy excess to pure drinking water and more than 5 million human being die with and early death annually as a result of illness linked to unsafe drinking water unclean domestic environments and improper excreta disposed. Water pollutants mainly consist of heavy metals, microorganism, fertilizer and thousand of toxic organic compounds (WHO, 1999). Heavy metals consist of Cd, Cr, Cu, Pb, Ni, Fe, Mn, Hg, Zn, Al, Se as well as metals of group III and IV, which have toxic effect on human physiology. Along with the heavy metals the next most important pollutants are microorganisms. Surface and ground water contains bacteria, protozoa and other synthetic microorganisms. These affect water quality by assimilating NO_3, CO_2 and also solubilize some heavy metals in water. These also produce toxicants in ground water. They cause many epidemic diseases in human (Khan *et al*., 2000).

More than 2.6 billion people-forty percent of the world's population-lack basic sanitation facilities and over one billion people still use unsafe drinking water sources. As a result, thousands of children die every day from diarrhoea and other water-, sanitation- and hygiene-related diseases and many more suffer and are weakened by illness.

The World Health Organization estimates that 500 million diarrhea cases reportedly take place each year in children less than five years in Asia, Africa and Latin

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America. The extent of enteric diseases in different areas depends upon the extent to which water is exposed to contamination. The incidence of typhoid fever, bacillary dysentery, infectious hepatitis and other enteric infections are common and are transmitted through contaminated water. Cholera is still a wide spread water borne disease in some developing countries. There are numerous other diseases that are transmitted through polluted water. It has been shown that cancer may be caused by the accumulation of certain materials carried out by water to human organs (Afia *et al*., 2006).

The excess of cadmium accumulated in the kidney causes hypertension as is evident from study conducted on animals. The deficiency of chromium in drinking water favour atherosclerotic diseases in human. The compounds of chlorobenzenes and chlorophyll's may affect taste and odor of water.

Pakistan's current population of 141 million is expected to grow to about 221 million by the year 2025. This increase in population will have direct impact on the water sector for meeting the domestic, industrial and agricultural needs. Pakistan has now essentially exhausted its available water resources and is on the verge of becoming a water deficit country. The per capita water availability has dropped from 5,600-1,000 m^3. The quality of groundwater and surface-water is low and is further deteriorating because of unchecked disposal of untreated municipal and industrial waste water and excessive use of fertilizers and insecticides. Water quality monitoring and information management is lacking, even though it's crucial to any water quality improvement program (Shakirullah *et al*., 2005).

It is estimated that by 2025 world water consumption will rise by 40%. This is why investments are urgently needed in the entire water infrastructure around the world, especially in mega cities. An additional main user of water is industry, whose plants can only be kept running with the aid of a wide range of process waters (Sidhu and Warner, 2003).

The purpose of present study to observed the physical and bacteriological characteristics of Peshawar.

The objective of the study was:

- Examine the physiochemical characteristics such as pH, conductivity and turbidity of drinking water
- Assess bacteriological quality of drinking water by Coliform and *E. coli* count

MATERIALS AND METHODS

The water samples were tested for physical and bacteriological analysis. Drinking water samples from different areas of Peshawar were tested for pH, EC, turbidity and pathogens in order to evaluate its quality. Urban and Rural areas of Peshawar were selected randomly. Urban area constitutes Hayyatabad, Sadder and City and while rural constitutes Sheikh Mohammadi, Jagra and Palosi.

From each location 15 water samples were collected in one liter capacity plastic bottles for physical analysis and in 100 ml sterilized glass bottles for bacteriological analysis. Before sampling the bottles were washed with detergents, followed by tape water and finally several time with distilled water. The water at the samples site was allowed to flow for some time then the bottles were rinsed two to three times by this water and one liter was taken as sample from drinking water. The samples were properly tagged indicating code. These samples were air tightened and stored in refrigerator.

Physical characteristics

pH: I standardized instrument with standard with pH near that of specimen and then with two other to check the accuracy of electrodes then analyze water sample as soon as possible and let the bottles to be closed till analysis, then 10 ml water sample was taken in clean beaker and pH was measured by pH meter using fisher automatic tetrameter model 36-pH meter (AOAC, 2003 method no 973.41).

Turbidity: Turbidity was measured by using turbidity meter Wag-WT-300 by taking 1 ml water sample in cuvate. The result was displayed by turbidity meter and noted.

EC: 10 ml water sample was taken from water sample collected in a tube and EC of all drinking water sample were determined by using EC meter LF-91.

Microorganisms: Total coliform was counted (most probable number method) while just the existence of *E. coli* was tested (Tandon *et al*., 2005).

Statistical analysis: Physical, chemical and becterlogoical data were checked by using Epi-info statistical software. Descriptive statistical analysis was carried out in order to check the mean, standard deviation and range in collected data. Difference between urban and rural was checked by F-test (ANOVA).

Bacterial test: For total coliform count MacConkey broth was used as culture media. A duplicate water samples along with samples for chemical analysis collected in clean sterilized glass bottles for the becterlogoical analysis and tested at Food Analysis Lab City Hospital Kohat Road Peshawar, where the test for total coliform and *E. coli* were also conducted.

Water analysis (Coliform/100 ml): Media used for microbial colony development was MacConkey Broth and actual method was MPN (most probable number), for this purpose water samples were collected from water sources in sterilized glass bottles in 100 ml capacity.

Media preparation: Prepared two types of Media i.e. single strength and double strength media. Double Strength Broth was prepared by weighting 8 gm media and dissolved it in 100 ml distilled water. 10 ml Broth was distributed in 5 test tubes sealed with cotton plugs. Before adding the Durham tube was placed in every test tube in inverted position. Single Strength Broth was prepared by weighting 4 gm of broth in 100 ml distilled water. Add 5 ml broth to each 10 test tubes and Durham tubes then sealed. All test tubes along with other required material are sterilized in autoclave at 121°C for 15 min. After cooling, 10 ml water sample was added to each 5 test tubes having double strand Broth (10 ml). Then 1 ml testing water sample was added to each 5-test tubes having 5 ml single strand Broth (5 ml). Put 0.1 ml testing water sample into remaining test tubes of single strand. Then all these sample were put for Incubation in incubator at 30-37°C for 18-24 h and observed growth, of acid and gas. After completion of incubation period Count the number of positive tubes in each set of 5 test tubes (Appendix 1) and consulted the appropriate number in the table (Tandon *et al*., 2005).

Confirmatory test (Ejakman test): Prepared sterilized *E. coli* media for *E. coli* in same way as for coliform and inoculate the positive tube in the same manner as for coliform i.e. from 10 ml coliform positive to 5 test tubes, from 5 ml coliform positive to 5 test tubes and from 0.1 ml coliform positive to 5 test tubes having EC media. Incubate it at 44°C for 24 h. If acid and gas were found, means that contamination is feacal one and *E. coli* is also present.

RESULTS AND DISCUSSION

Water sample collected from Peshawar valley were analyzed for their physical and bacteriological characteristics. Among the physical characteristics pH, EC and turbidity were measured. For bacteriological characteristics total coliform count and only existence of *E. coli* was performed. The results obtained were summarized in the Tables 1 and 2.

Table 1 indicates the physical properties of water sample collected from six different locations of Peshawar. In all locations the main source of drinking water is tube wells. Among the physical parameters pH, EC and turbidity were analyzed and compared with WHO standards.

pH: pH is the measure of the activity of the hydrogen ion (H+) and is reported as the reciprocal of the logarithm of the hydrogen ion activity. Therefore, a water with a pH of 7 has 10^{-7} moles per liter of hydrogen ions; whereas, a pH of 6 is 10^{-6} moles per liter. The pH scale ranges from 0-14. In general, a water with a pH < 7 is considered acidic and with a pH > 7 is considered basic. The normal range for pH in surface water systems is 6.5-8.5 and for groundwater systems 6-8.5. Alkalinity is a measure of the capacity of the water to resists a change in pH that would tend to make the water more acidic. The measurement of alkalinity and pH is needed to determine the corrosivity of the water.

pH was found in water sample of Peshawar valley in the range of 7.04-7.85 with Total mean pH value 7.41. Which is in the safe limit as recommended by the WHO, which is from 6.5-8.5. Mean value of pH for Hayatabad, Sadder, City, SheikhMohammadi, Jagra and Palosi were 7.55, 7.43, 7.41, 7.46, 7.40 and 7.37 respectively.

In general, water with a low pH (< 6.5) could be acidic, soft and corrosive. Therefore, the water could leach metal ions such as iron, manganese, copper, lead and zinc from the aquifer, plumbing fixtures and piping. Therefore, a water with a low pH could contain elevated levels of toxic metals, cause premature damage to metal piping and have associated aesthetic problems such as a metallic or sour taste, staining of laundry and the characteristic “blue-green” staining of sinks and drains. The primary way to treat the problem of low pH water is with the use of a neutralizer. The neutralizer feeds a solution into the water to prevent the water from reacting with the house plumbing or contributing to electrolytic corrosion; a typical neutralizing chemical is soda ash. Neutralizing with soda ash increases the sodium content of the water (Shakirullah *et al*., 2003).

Water with a pH > 8.5 could indicate that the water is hard. Hard water does not pose a health risk, but can cause aesthetic problems. These problems include: Formation of a "scale" or precipitate on piping and fixtures causing water pressures and interior diameter of piping to decrease Causes an alkali taste to the water and can make coffee taste bitter.

Table 1: Physical properties of water sample

Area	pH Mean±SD	EC µscm^{-1} Mean±SD	Turbidity (FTU) Mean±SD
Total urban (n = 45)	7.46±0.18	0.65±0.10	0.53±0.54
Hayatabad (n = 15)	7.55±0.23	0.56±0.06	0.29±0.38
Sadder (n = 15)	7.43±0.16	0.70±0.07	1.01±0.56
City (n = 15)	7.41±0.12	0.70±0.09	0.29±0.31
Total rural (n = 45)	7.41±0.13	0.64±0.06	1.23±0.80
SheikhMohammadi (n = 15)	7.46±0.08	0.69±0.07	0.50±0.57
Jagra (n = 15)	7.40±0.12	0.62±0.03	1.37±0.58
Palosi (n = 15)	7.37±0.17	0.60±0.03	1.82±0.61
Grand total (n = 90)	7.44±0.16	0.64±0.08	0.88±0.77

Formation of a scale or deposit on dishes, utensils and laundry basins; difficulty in getting soaps and detergents to foam and formation of insoluble precipitates on clothing, etc. and decreases efficiency of electric water heaters.

Exposure to extreme pH values results in irritation to the eyes, skin and mucous membranes. Eye irritation and exacerbation of skin disorders have been associated with pH values greater than 11. In addition, solutions of pH 10-12.5 have been reported to cause hair fibres to swell. In sensitive individuals, gastrointestinal irritation may also occur. Exposure to low pH values can also result in similar effects. Below pH 4, redness and irritation of the eyes have been reported, the severity of which increases with decreasing pH. Below pH 2.5 damage to the epithelium is irreversible and extensive. In addition, because pH can affect the degree of corrosion of metals as well as disinfection efficiency, it may have an indirect effect on health (WHO, 1999).

Electrical conductivity: Conductivity of a substance is defined as the ability or power to conduct or transmit heat, electricity, or sound. Pure water is not a good conductor of electricity. Ordinary distilled water in equilibrium with carbon dioxide of the air has a conductivity of about 0.10 μscm^{-1}. Because the electrical current is transported by the ions in solution, the conductivity increases as the concentration of ions increases. Thus conductivity increases as water dissolved ionic species.

EC was found in water sample of Peshawar valley in the range of 0.47-0.82 μscm^{-1} with Total mean EC value 0.64 μscm^{-1}. The recommended limit of WHO for EC ranges from 0.5-1.5 μscm^{-1}. Mean value of EC for Hayatabad, Sadder, City, SheikhMohammadi, Jagra and Palosi were 0.56, 0.70, 0.70, 0.69, 0.62 and 0.60 μscm^{-1} respectively. All the water sample were in the safe limit as recommended by WHO.

Turbidity: Turbidity is a measure of the degree to which the water looses it's transparency due to the presence of suspended particulates. The more total suspended solids in the water, the murkier it seems and the higher the turbidity.

Turbidity is considered as a good measure of the quality of water. There are various parameters influencing the cloudiness of water. Some of these are Phytoplankton, Sediments from erosion, resuspended sediments from the bottom (frequently stir up by bottom feeders like carp), Waste discharge, Algal growth and Urban runoff (EPA, 2002).

Turbidity was found in water sample of Peshawar valley in the range of 0.00-2.90 FTU with Total mean turbidity value 0.88 FTU. Mean value of turbidity for Hayatabad, Sadder, City, SheikhMohammadi, Jagra and Palosi were 0.29, 1.01, 0.29, 0.50, 0.37 and 1.31 FTU respectively. The suspended particles absorb heat from the sunlight, making turbid waters become warmer and so reducing the concentration of oxygen in the water (oxygen dissolves better in colder water). Some organisms also can't survive in warmer water (Rosborg *et al*., 2003).

As a consequence of the particles settling to the bottom, shallow lakes fill in faster and insect larvae are covered and suffocated, gill structures get clogged or damaged. The main impact is merely aesthetic: nobody likes the look of dirty water. But also, it is essential to eliminate the turbidity of water in order to effectively disinfect it for drinking purposes. This adds some extra cost to the treatment of surface water supplies. The suspended particles also help the attachment of heavy metals and many other toxic organic compounds and pesticides (EPA, 2002).

Pathogenic microorganism: Table 2 show pathogenic bacteria which directly effect the human and causes acute and chronic diseases.

In drinking water sample of Peshawar valley total coliform count ranged from 0-150 coliform per 100 ml, with grand mean of 38 coliform per 100 ml. while *E. coli* were present in 26% of the samples. Mean value of Coliform count for Hayatabad, Sadder, City, Sheikh Mohammdi, Jagra and Palosi were 7, 45, 64, 13, 64 and 35 coliform per 100 ml.

The WHO recommended level for Coliform count should be less than 10 per 100 ml while presence of *E. coli* must be negative in the all cases (WHO, 1999).

The presence of bacteria and pathogenic (disease causing) organism are a concern when considering the safety of drinking water. Pathogenic organisms can cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera and other illnesses.

Human and animal wastes are a primary source of bacteria in water. These sources of bacterial contamination include runoff from feedlots, pastures, dog runs and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks and sewage treatment facilities. Bacteria from these sources can enter in wells that are either open at the land surface, or don't have water tight casings or caps, or don't have seal in the annular space (the space between the wall of the drilled well and the outside of he well casing). Insects, rodents and animals entering the well are other sources of contamination. Old wells were dug by hand and lined (cased) with rocks or bricks. These wells usually have large openings and casings that often are not well sealed. This makes it easy for insects, rodents, or animals to enter the well. Another way through which bacteria can enter the water supply is through inundation or infiltration by flood waters or by surface runoff. Flood water commonly contains high level of bacteria. Small depressions filled with flood water provide and excellent breeding ground for bacteria (Ley and Samant, 2003).

Table 2: Bacteriological characteristics of drinking water

Area	Location	Coloform/100 ml Mean±SD	*E. coli* +Ve	*E. coli* -Ve
Urban (n = 45)				
	Hayatabad (n = 15)	7±5.66	0	15
	Sadder (n = 15)	45±40.51	4	11
	City (n = 15)	64±39.50	8	7
Total urban (n = 45)		38.38±40.13	12	33
Rural (n = 45)				
	SheikhMohammadi (n = 15)	13±7.09	0	15
	Jagra (n = 15)	64±30.30	9	6
	Palosi (n = 15)	35±19.13	3	12
Total rural (n = 45)		37.20±29.60	12	33
Grand total (n = 90)		37.79±35.07	24	66

Fecal Coliform is a portion of the Coliform bacteria group originating in the intestinal tract of warm-blooded animals that pass into the environment as feces. Fecal Coliform often is used as an indicator of the bacteriological safety of a domestic water supply.

Coliform bacteria may not cause disease, but can be indicator of pathogenic organisms that causes diseases. The latter could cause intestinal infection, dysentery, hepatitis, typhoid fever, cholera and other illnesses. However these illnesses are not limited to disease causing organism in drinking water. Other factors not associated with drinking water may be the cause. Intestinal infections and dysentery are generally considered minor health problems. They can, however prove fatal to infants, the elderly, and those who are ill. Today typhoid, hepatitis and cholera are rarely encountered in the developed countries.

Conclusion: The main source of contamination is the untreated sewerage water. In Peshawar City these sewage effluents are discharged directly into non-bricked and uncommented drains, sewage carrier streams and Naray Khwar running across the palosi. From there due to the seepage of polluted water, it percolates into the underground fresh water body and contaminates it. Moreover, in irrigated farming lands the chemicals, fertilizers and pesticides are used in the fields and most of these chemicals residues reached the ground water table. Smoke and wastes of vehicles are deposited on the soil surface and in rainy season it results in considerable rise in the contamination of shallow well's water. The other possible sources of heavy metals pollution, for all categories include leaching from time old water supply pipes which are rusted, Pb/Cd based solder pipes, joints and use of sub-standard chemicals for water treatments.

In Hayyatabad and Palosi the main source of heavy metal contamination may be the near by flowing Palosi drain, as it is uncemented and carries the industrial effluent discharged in to it directly from Hayyatabad industrial estate. In Peshawar the sewerage and industrial effluents are also disposed into canals or rivers, which are used for irrigation in the vicinity of Peshawar and results in the contamination of irrigation as well as drinking water. In City and Saddar the main source of water pollution is the vehicle discharged and the polluted environment and their accumulation in old piping system and solder pipes and joints. For SheikhMohammadi and Jagra the main source of water contamination is the leaching of chemical and synthetic fertilizer in water table as there are more agriculture land for cultivation and the improper irrigation system.

The drinking water form all the study areas is contaminated with heavy metals, so it is essential that the supply of water for human consumption should be free from unpleasant or harmful impurity and for this reason it should be subjected to various treatments to render it safe for the use of man. The study indicates from the background values that it should be monitored regularly in order to evaluate the toxic, logical significance of commonly used water for drinking.

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Nutritional Evaluation of Millet-beniseed Composite Based *Kunun-zaki*

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Abstract: *Kunun-zaki* was prepared using millet as the base and supplemented with beniseed at varied level (0-50%). The effect of added beniseed on the physio-chemical (moisture, ash, protein, fat and carbohydrate), sensory (colour, taste, odour/aroma, mouth feel and general acceptability) and microbial qualities were evaluated. The added beniseed decreased the moisture, ash and carbohydrate composition from 86.23-85.03, 0.33-0.22 and 10.21-7.23%, respectively with increasing concentration (0-50%), while fat and protein increased from 1.03-2.03% and 2.21-5.44% respectively with increasing concentration (0-50%). The added beniseed paste had a high positive correlation coefficient (r = 0.75-0.95) with the increase in the protein, fat and energy content. There were significant differences in the assessed sensory qualities (colour, odour, taste, texture) at 20% and above added beniseed paste which corresponds with 3.32%, 1.26% and 64.06 kcal of protein, fats and energy content respectively. At this acceptable level the protein content of the products has been improved by 50% if digested and absorbed.

Key words: Nutritional, evaluation, millet-beniseed composite, *Kunun-zaki*

INTRODUCTION

Kunun-Zaki is a traditional beverage made from grains such as millet (*Pennisetum spp*), Sorghum bicolor, maize (*Zea mays*) etc. *Kunun-zaki* is the most popularly consumed non-alcoholic drink in Nigeria, taken for its thirst quenching property and used for entertainment at homes and during unique ceremonies like Salah and Christmas. Major types of *kunun zaki* include: *Kunun zaki, kunun gyada, kunun akamu, kunun tsamiya* and *kunun baule* (Gaffa, 2000).

Research has shown that cereal could be used in composite form in the production of *Kunun zaki* but millet and sorghum grains are the most commonly used basic raw material. The preferred ratio of mixing is 1:2 (w/w) sorghum/millet. The traditional production process involves steeping the grains in local house hold utensils such as buckets, drums, calabashes or earthen ware vessels (Adeyemi and Umar, 1994). The steeping duration depends on the cereal used but may vary between 12-72 h for millet/sorghum and maize respectively (Gaffa, 2000). Grinding of the steeped grains mixed with species (ginger, clove, red and/or black pepper) is done with local milling machine and part of the slurry (3/4 volume) is gelatinized with boiling hot water (Onuorah *et al*., 1987a). The remaining part of the slurry (1/4 volume) ungelainized containing liquefied agents (sweet potato tuber paste, malted rice, extract of *Cadaba farinosa* stem) is mixed with the gelatinized portion when the temperature is about 60-70°C. The mixture is altogether left overnight at room temperature for chance fermentation and filtered using local sieve the next morning. The filtrate, *Kunun-zaki* is consumed as a beverage with or without addition of sugar as a sweetener. The whole process lasts about 24 h. The nutrient content and microbiological quality of this product had been reported (Gaffa *et al*., 2002b; Onuorah *et al*., 1987b). The consumption rate of the beverage has also been studied (Gaffa *et al*., 2002a). The gross chemical composition of *Kunun-zaki* is 87.85-89% moisture content, 9.84-12% carbohydrate, 1.56-3% protein, 0.10-0.30% fat and 0.61-0.75% ash (Adeyemi and Umar, 1994; Badifu *et al*., 1999) indicating that the drink is low in protein.

Owing to the high demand for this product and the high consumption rate, it is thought that the present traditional production process is outdated, inefficient, time consuming and with product quality varying between batches. In this present study, attempts have been made to improve on the traditional production process with the hope of maintaining nutrients and improving microbiological quality of the final product. The nutrient and sensory qualities of *Kunun-zaki* from new process has been analyzed and compared with the traditional process.

Problems associated with *Kunun-zaki* production include non-uniformity in the production method, poor sanitary conditions and short shelf-life of the product. The low protein content of *Kunun-zaki* and its general acceptability should be a thing of concern.

This work is aimed at producing a high qualitative *Kunun-zaki* in terms of nutrients using locally available and less used raw materials as beniseed. The objective of the research which includes: Production of beniseed-millet composite *Kunun-zaki* of varied, substitution using beniseed grain (5-50%), evaluating the nutrient content by determining the chemical composition,

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evaluating the sensory quality (taste, colour, odour/aroma, texture and general acceptability) and microbial analysis of the products.

MATERIALS AND METHODS

The millet *(Pinnesetum nigritarum)*, beniseed *(Seamum indicum)* red pepper *(Capsicum anuum)*, sweet potatoes *(Ipomoea batatas)*, ginger (*Zingiber officinale)*, and sucrose were purchased at Muda Lawal market in Bauchi state.

The millet rains were steeped in water (at 30°C for 12 h), washed, wet milled with added spices (clove, ginger, red pepper) and divided into unequal parts (1:4). The larger parts (3/4) was gelatinized (by addition of hot water, 1:3 of paste to hot water), cooled to 40°C and added to the ungelatinized portion, mixed thoroughly, supplemented with benised (5, 10, 15, 20, 25, 50%), fermented (left for 12 h), filtered (cloth sieve), sweetened(addition of 4% sucrose) and packed (Fig. 1, Table 1) ready for analysis.

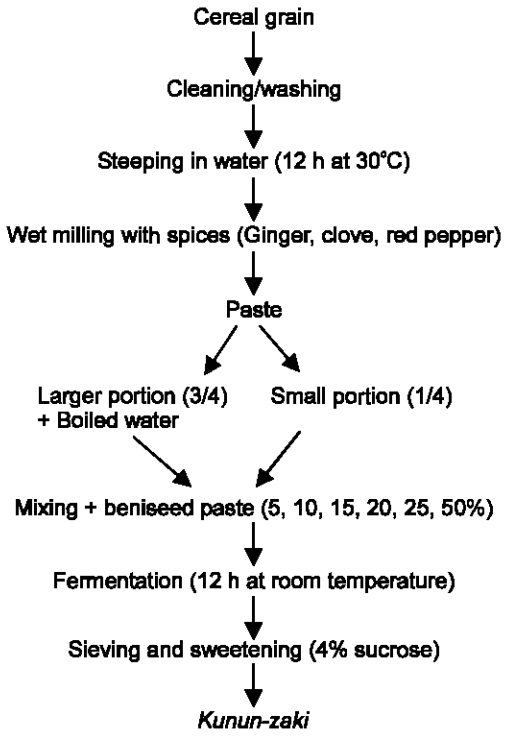

Fig. 1: Innovation in the traditional *Kunun-zaki* production process

Table 1: Recipe for improved *Kunun zaki* production

	Samples in grams (g)					
Materials	A	B	C	D	E	F
Millet	100.0	90.0	85.0	80.0	75.0	50.0
Beniseed	0.0	10.0	15.0	20.0	25.0	50.0
Clove	0.5	0.5	0.5	0.5	0.5	0.5
Ginger	4.0	4.0	4.0	4.0	4.0	4.0
Chilli pepper	0.5	0.5	0.5	0.5	0.5	0.5
Sweet potato	15.0	15.0	15.0	15.0	15.0	15.0
Sugar	10.0	10.0	10.0	10.0	10.0	10.0

The chemical composition (moisture, protein, fats, ash and carbohydrate), microbial and sensory quality of the products were analyzed (AOAC, 1990; Jidean and Jideani, 2006; Lamon, 1977).

Sensory evaluation was carried out on each coded products. Assessed qualities include: colour, taste, odour and aroma, after mouth feel and general acceptability. Twenty (20) untrained panelists were selected at random from Department of Food Science and Technology, Federal Polytechnic, Bauchi. Evaluation was based on the above named quality parameters and were assessed accordingly. A nine (9) point Hedonic scale was used (1 and 9 for extremely like and extremely dislike, respectively). Coded samples of the same sizes and at the same temperature (30°C) were served in transparent glass cup to judge in each panel cupboard under fluorescence light. Data collected were subjected to analysis of variance at $p \leq 0.05$ (Lamon, 1977).

RESULTS AND DISCUSSION

The effects of added beniseed paste to millet based *Kunun zaki* are summarized in Table 2, 3 and 4.

Effect of added beniseed paste on chemical composition of *Kunun zaki*: The moisture content of the *Kunun-zaki* generally decrease from 86.23-85.08% with increase in percentage added beniseed paste (0-50%). Thus 100% millets based *Kunun-zaki* had the highest moisture content (86.23%) while the least (85.05%) value was obtained for sample containing 15% beniseed. The generally high moisture content agrees with the finding of Enegbede (1999) and Sopade and Kassum (1992) that *Kunun-zaki* generally contain 85.8% moisture. The moisture content of any food could be an index of its water activity. Frazier and Westhoff (1976), reported that bacteria requires more moisture than yeast and yeast than mould. This implies that spoilage organisms likely to survive in this could be major causes of the short shelf-life of *Kunun-zaki*. The ash content decreased from 0.33-0.22% with increasing beniseed concentration (Table 2). The *Kunun-zaki* sample with 0% beniseed content the highest ash content of 0.33% while that of 50% had the least with 0.22%. The amount of ash content compared with the work done by Sopade and Kassum (1992), that *Kunun-zaki* contain 1.5% of ash content. This might had probably resulted from the fact that beniseed is rich in mineral.

The crude fat of the *Kunun-zaki* increased from 1.03-2.03% with the addition of beniseed (0-50%) as stated in the Table 2. The *Kunun-zaki* sample with 50% beniseed had the highest fat content (2.03%) while that of 0% beniseed had the least fat content (1.03%). This result agrees with the findings of Sopade and Kassum (1992), that *Kunun-zaki* has a fat content of 1%. The increased concentration of beniseed from 0-50% could

Table 2: Effect of added beniseed on the chemical properties of *Kunun zaki*

Material		Physicochemical properties (%)					
Beniseed	Millet	Moisture content	Ash	Fat	Protein	Carbohydrate	Energy (kcal)
0	100	86.23	0.33	1.03	2.21	10.21	58.95
5	95	85.98	0.26	1.07	2.64	10.05	60.39
15	85	85.65	0.24	1.25	2.72	9.94	61.89
20	80	85.32	0.24	1.26	3.32	9.86	64.06
25	75	85.21	0.22	1.29	4.08	8.80	63.13
50	50	85.08	0.22	2.03	5.44	7.23	68.95

Table 3: Sensory quality of millet-beniseed based *Kunun zaki*

Material (%)		Sensory quality				
Beniseed	Millet	Color	Taste	Odour	After mouth feel	G. Acceptability
0	100	7.6^{a}	7.9^{a}	7.75^{a}	8.0^{a}	8.0^{a}
5	95	6.9^{a}	6.95^{ab}	6.95^{ab}	6.8^{ab}	7.0^{ab}
15	85	7.2^{a}	6.9^{ab}	6.65^{ab}	6.25^{bc}	7.0^{ab}
20	80	7.05^{a}	6.10^{b}	6.15^{bc}	5.9^{cd}	5.85^{b}
25	75	6.55^{a}	5.25^{c}	5.25^{cd}	5.35^{d}	4.90^{d}
50	50	5.65^{b}	3.85^{d}	4.50^{d}	3.95^{e}	4.05^{c}

Average mean score equal to or greater than the corresponding L.S.D along the same column are significant difference, $p \leq 0.05$.
LSD = Least Significant Difference

Table 4: Microbial load of the products

Beniseed (%)	Millet	pH	Total microbial count	Bacterial count	Characteristics	Microorganisms
0	100	3.66	1.2×10^{5}	1.2×10^{3}	Bluish green flucose malted mycelia, conidiosphore bearing phialides (flask shaped) that produce spore	*Aspergillus*
5	95	3.37	2.0×10^{5}	1.4×10^{3}	Green/black mycelia, spore on flask shaped sterigmata	*P. digitatum*
15	85	3.46	2.0×10^{5}	1.5×10^{3}	Small dryshining mucoid colourless opaque, G^{+},LF colonies on MC	*Streptococcus pyogenes*
20	80	3.33	2.0×10^{5}	1.5×10^{3}	Round white colonies in chain	*Staphilococcus*
25	75	3.32	2.0×10^{5}	1.6×10^{3}	Short rods in single and two branch colonies with space in middle	*Lactobacillus*
50	50	3.39	2.1×10^{5}	1.6×10^{3}	Small dry shining mucoid colourless opaque G^{+}, LF colonies on MC	*Streptococcus pyogenes*

have resulted in the high fat content of the *Kunun-zaki* due to the high fat content of the added beniseed. This agrees with the reported work of Oresanya and Koleoso (1990), that beniseed is very high in fat content (57.15-63.40%).

The crude protein content of *Kunun-zaki* produced increased from 2.21-5.44% with addition of beniseed (Table 2). The *Kunun-zaki* sample with 50% beniseed level had the highest protein content (5.44%) while that of 0% beniseed level had the least protein (2.21%). The general high protein content agree with the findings of Douglas and Glenn (1982), that beniseed is very rich in protein, therefore could boost the protein content of *Kunun-zaki*.

The carbohydrate content in *Kunun-zaki* is found to be higher at 0% beniseed (10.21%) while the sample with 50% had the least carbohydrate content (7.23%) with addition of beniseed (Table 2). The result agrees with the findings of Sopade and Kassum (1992), that *Kunun-zaki* contain 12.2% of carbohydrate. It indicated that, the nitrified material (beniseed) which contains relatively lower carbohydrate could have affected the carbohydrate content in the *kunun-zaki* by reducing its contents and increases the protein of the *Kunun-zaki*.

Effect of added beniseed paste on the sensory quality of *Kunun zaki*: The average mean scores of colour decreased from 7.60-5.60 with increasing percentage of added beniseed (0-50%) as shown in Table 3. Statistical analysis show no significant difference between 0-25% but there was significant difference in colour at above 25% beniseed, p = 0.05. The colour changes with increasing concentration of beniseed added could be due to the golden yellow colour of added beniseed which defer from normal colour of locally produced *Kunun-zaki* (Oresanya and Koleoso, 1990).

As the concentration of the beniseed increased from 0-50%, the average mean score for taste decreased from 7.9-3.85. There were no significant difference in the average means scores of taste on addition of beniseed at less than 15%, p = 0.05. The effect of beniseed on *Kunun-zaki* of above 15% could be as a result of

the inherent bitter taste of beniseed. This result agrees with the findings of Douglas and Glenn (1982), that beniseed have a slight bitter taste.

The result of the average mean score of the odour/aroma of the *Kunun-zaki* decreased from 7.75-4.5 with increasing level (0-50%) of added beniseed. There was no significant difference at less than 15% added beniseed, p = 0.05. This result agrees with the findings of Douglas and Glenn (1982), that beniseed (sesame) have an inherent off flavor.

The average mean score of texture decreased from 8.0-3.95 with the concentration of beniseed (0-50%) as shown in Table 3. This could be as a result of the texture of the beniseed which is not as fine as that of *Kunun-zaki*. This result agrees with the finding of (Sweiss, 1983), that beniseed is rich in fibre (6.3-8.6%).

The average mean score of general acceptability decreased from 8.0 to 4.05 with increase in concentration of beniseed (0-50%) as shown in Table 3. The sample with 0% beniseed (100% millet) had the highest acceptability (8.0%), while sample with 50% beniseed proportion had the least acceptability (4.05%).

Effect of added beniseed paste on the Microbial quality of *Kunun zaki*: The effect of the added beniseed on the *Kunun zaki* is summarized in Table 4. The total microbial count and bacterial count increased from 1.2 x 10^5 to 2.1 x 10^5 and 1.2 x 10^3 to 1.6 x 10^3 cfu with the percentage added beniseed paste.

Microbial isolates obtained from the *Kunun-zaki include; Streptococcus* species, P*ennicillium digitatum, Lactobacillus leichmanni*, *Lactobacillus fermentum* and *Aspergillus*. The presence of *Lactobacillus leichmanni and* Lactobacillus fermentum could be due to the fermentation process as confirmed by early researcher (Akoma *et al*., 2006) while the presence of *Aspergillus spp*. suggest contamination for leguminous grains. However, the level of total microbial count is still lower than the maximum acceptable count of 1.0 x 10^7 cfu the beverage (Kolawole *et al*., 2007). The generally high moisture content of *Kunun-zaki* could encourage microbial spoilage if not properly treated. The adoption of pasteurization and appropriate packaging is hereby suggested.

Conclusion and recommendation: The research work has shown that addition of Beniseed paste did increase the protein and fat content of *Kunun-zaki*. However, the acceptability was mostly preferred at 20% and below which corresponds with an increase in the protein content (50.22%) and fat content (22.3%). This can be said to be a great improvement in the nutrient content if it can be digested and assimilated to the body. The relative high fat content though could improve the nutrient, but can pose problem as it coulds encourages rancidity in the product. The use of defatted beniseed is therefore encouraged.

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