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Factors affecting whipping ability of fresh and stale egg

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ABSTRACT

The foaming property of egg white and yolk has been evaluated at different speeds of beater and beating time with their optimal values. The amount of air incorporation decreased with the passage of time in almost all the cases. Similarly, speed No. 2 (approximately 1080 rpm) of egg beater gave maximum air incorporation in 2 minutes. Stale egg has better air incorporation ability than the fresh egg while the yolk has negligible ability to incorporate air as compared to the egg white. The results indicated that the foaming ability or amount of air incorporation could be improved by choosing the proper beating time and speed.

Keywords: Foaming ability, foam stability, egg white, egg yolk

INTRODUCTION

Foams are agglomerations of gas bubbles that are separated from each other by thin liquid films. They are gas in liquid dispersions with lyophilic groups face towards the liquid phase while lyophobic groups are oriented toward the gas phase.

Foams need to be stable in food system for persistence in quality. The foam stability depends on chemical nature of foam forming components and nature of surrounding molecules present in food. The protein-foam stability is related to triangular properties of a protein i.e. adsorption at gas-liquid surface, denaturation and coagulation (Cumper 1953).

Proteins dispersed in egg white are the surface active agents that are responsible for foam formation. The proteins not only lower the surface tension of egg. White but many of them are easily denatured at the surface. They coagulate at the gas – liquid interface and form a network that gives some rigidity and stability to the foam.

Nakamura (1963) found that proteins that foam well are easily surface –denatured. The possible roles of the various proteins in egg white for foam formation were suggested by MacDonnell *et al* (1955) after fractionating the total proteins and using each fraction to make an angel food cake.

Globulins appeared to be good in foam formation, producing small gas bubbles and a large volume (Allen 2006). Ovomucin was not a good foamer by itself but stabilized the foam produced by globulins because it was rapidly insolubilized at the bubble surface. All other proteins in the egg white get heat denatured bulk to form a supporting matrix for the baked cake.

Egg white foams may be beaten to various volumes. A very soft under-beaten foam has relatively large gas bubbles and is somewhat unstable because surface denaturation of the proteins has not been sufficiently extensive; the film around the bubbles of air is not rigid enough to support the foam on standing. Considerable drainage of liquid from the films occurs and some of the bubbles coalesce (Martin 1980).

On the other hand, in foam that has been beaten until it appears dry, dull and curdled, the proteins in the surface films are over coagulated and the film loses its elasticity. The air bubbles in an over beaten film tend to coalesce as the films are ruptured. Liquid drains from the foam on standing (Martin 1980). Thus under beating and over beating both produce unstable foams.

Fine wires and thin blades produce foams with fine air cells. With electric beaters operated at high speed (Plancken 2007), overbeating may readily occur. A good foam volume was reported by Bailey in 1935, to be more easily attained with a power-operated beater than with a hand beater when whipping thick viscous whites. Electric beaters are so forceful that they give a shearing action rather than a folding over motion that is necessary to produce foam. Electric blenders do not produce good foams for the same reason.

Greater foam volume has been reported for egg whites that are beaten at room temperature than for those beaten at refrigerator temperature (Henry and Barbour 1933; John; Hansen 1931). The surface tension as well as the viscosity of egg whites is

lowered by increasing the temperature. This may at least partially explain the difference due to the temperature of beating.

The conditions of storage for eggs affect their foam quality (Meehan *et al* 1962). Sugar (Vassilios 2007; Hanning 1945), time and method of adding sugar (Cathy 2005), acids or acid salts (Edwin and Rojas 2006; Flawia 2007; Nakamura 1961; Sechler 1959), such as cream of tartar, citric acid and acetic acid (Paul and Palmer 1972), salt (Hanning 1945 and Sechler *et al* 1959) and fat (Bailey 1935) also affects the foaming ability of egg white. The objective of the present study was to determine the affecting extent of beating time and speed of electrical egg beaters for the air incorporation in eggs (Barmore 1934). Also to determine the difference of air holding capacity between fresh and stale eggs.

MATERIALS AND METHODS

Samples Fresh eggs were purchased from the local market. The egg samples were stored in accordance with the experimental conditions.

Analysis of air holding capacity of fresh egg white by changing beating time and speed: Each egg was broken such that it's white was easily separated from yolk in to the beakers having radius of 3.45cm and weighed it (RH 60-70%, 30°C)

Height of liquid egg white in a beaker was determined by measuring scale. Beating of eggs were done by the electric beaters having whipping blades, keeping the speed of egg beater at speed no. 2 (~1080 rpm), constant through out the analysis. Change in weight and volume were measured after 0.5, 01, 1.5 and 2 minutes interval. The amount of air incorporated into the foam determined by calculation of specific gravity i.e. weight per unit volume or specific volume i.e. volume per unit weight (Paul and Palmer 1972 and Martin 1980) and subtracting the initial value from final one.

Took five egg white in beakers individually and beat each for constant time i.e. 2 minutes at speed no.1~820 rpm, speed no. 2~1080 rpm, speed no. 3~1340 rpm, speed no. 4~1600 rpm and speed no. 5~1860 rpm individually. The amount of air incorporated into the foam was determined as described above.

Analysis of air holding capacity of fresh egg yolk by changing beating time and speed: Beating of yolk was done by the electric beater having whisking blades, keeping the speed of egg beater at speed no. 2 ~1080 rpm, constant through out the analysis. Change in weight and volume were measured after 01, 02, 03 and 04 minutes interval. The amount of air incorporated into the foam was determined as described above.

Took five egg yolks in individual beakers and beat each egg yolk for constant time i.e. 2 minutes at speed no.1~820 rpm, speed no. 2~1080 rpm, speed no. 3~1340 rpm, speed no. 4~1600 rpm and speed no. 5~1860 rpm. The amount of air incorporated into the foam was determined as described earlier.

Staling of eggs: Eggs are purchased from the local market and kept for stalling for four weeks (RH 60-70%, 30°C) exposed to air. Staling is analyzed by the floating of eggs in water bath.

Analysis of air holding capacity of stale egg white and yolk by changing beating time: Each stale egg was broken very carefully such that egg white and yolk was easily separated in a beaker. Height of liquid stale egg white in a beaker was determined by measuring scale. Beating of eggs were done by the electric beaters having whisking blades, keeping the egg beater at speed no. 2~1080 rpm, constant through out the analysis. Change in weight and volume were measured after 0.5, 01, 1.5 and 2 minutes interval. Same procedure was followed for stale egg yolk.

RESULTS AND DISCUSSION

By increasing the beating time (Pernell 2002) at constant speed, air incorporation increases (Rysova *et al* 1989) (Fig 1)

When egg white is beaten and air is incorporated, one part of the protein molecule is attracted to the water through hydrogen bonds while other parts of the molecule will align themselves, so that they are in contact with the pocket of air, since they have no attraction for the water. This action causes the molecule which are normally compact, to unfold. Once unfolded (denatured), the protein molecule bound with each other forming a network, which holds the water in place while shielding it from the pocket of air. Denatured proteins interact readily through a variety of physical and chemical bonds to produce aggregated protein films that enhance the entrapment of air bubbles in beaten egg. Aggregated protein particles play an important role in the stability of egg white foam by holding water in the lamellae and providing structural rigidity and elasticity. Aggregated avouching is an especially important contributor to foam stability (Nakamura 1966).

After certain time foam started decreasing as over beating (Cunningham 1976) caused the collapsing of air cells (Fig 1). The individual bubbles pressed against each other in foam are separated by very thin films that are approximately parallel to the plane of the bubble surface. Certain regions of the films around the gas bubbles however are thickened and have a more concave shape. After foam is formed, certain factors operate to decrease its stability. Because of the more concave shapes of the thicker regions of liquid around the gas bubbles a capillary pressure is produced between these areas and the thinner portions of the film. This tends to drain the liquid from the thin films into the thicker portions. In addition, since the curvature of the thickened ribs often varies, capillary transfer of liquid may take place from some thicker parts (with smaller curvature) to others. Hydrostatic pressure also operates in foam to cause the liquid to drain towards the bottom of the foam. As a result of these two factors, foam gradually lose some of the liquid contained in them, a process called synergetic or weeping and the film become thinner. The breakdown or collapse of foam is due to the eventual rupture of the films surrounding the gas bubbles.

Different speeds of egg beater can effect the foaming of egg white (Henry 1933). As we know that egg beaters are so forceful that they gave a shearing action rather than a folding over motion that is necessary to produce foam. Speed No. 2 incorporated the maximum air in egg white at constant time i.e. 2 minutes (Fig 2).

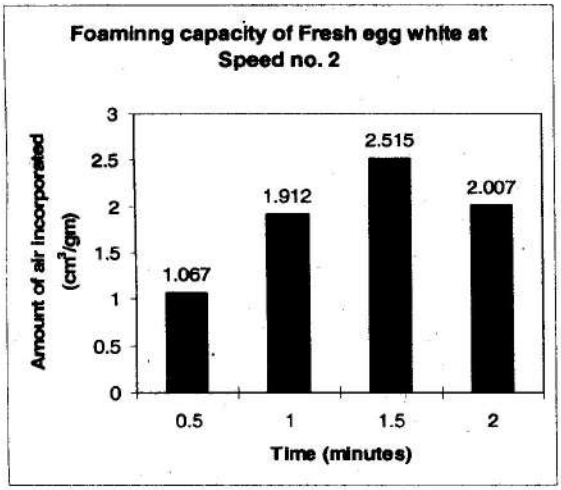

Fig 1. Effect of beating time on air holding capacity of fresh egg white

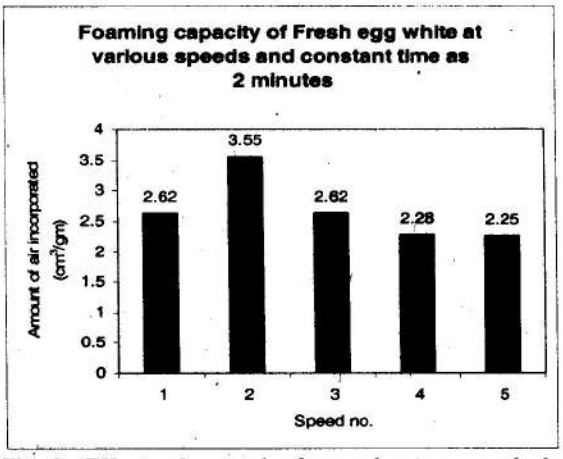

Fig 2. Effect of speed of egg beater on air holding capacity of fresh egg white

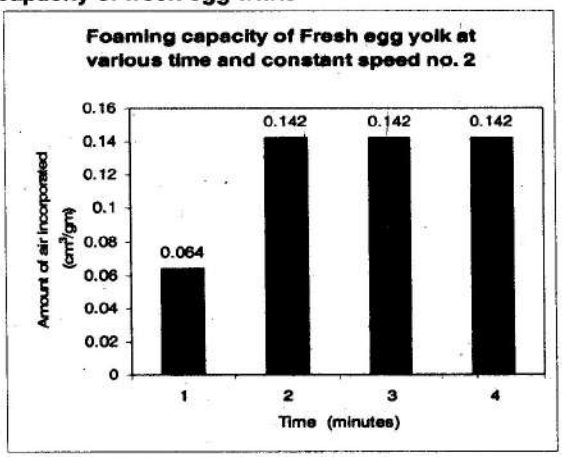

Fig 3. Effect of beating time on air holding capacity of fresh egg yolk

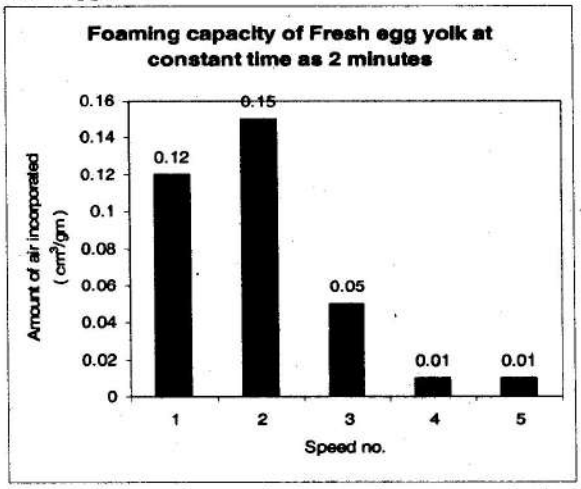

Fig 4. Effect of speed of egg beater on air holding capacity of fresh egg yolk

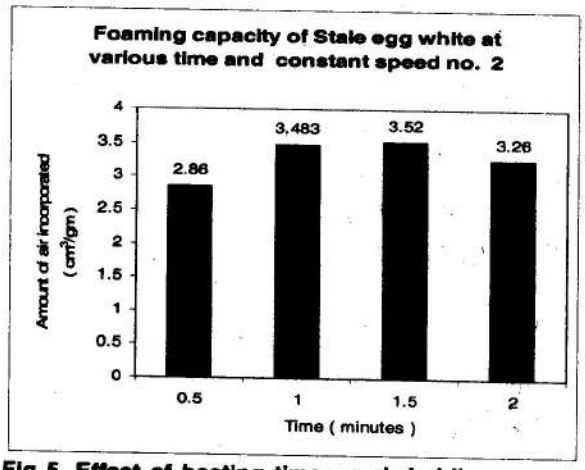

Fig 5. Effect of beating time on air holding capacity of stale egg white

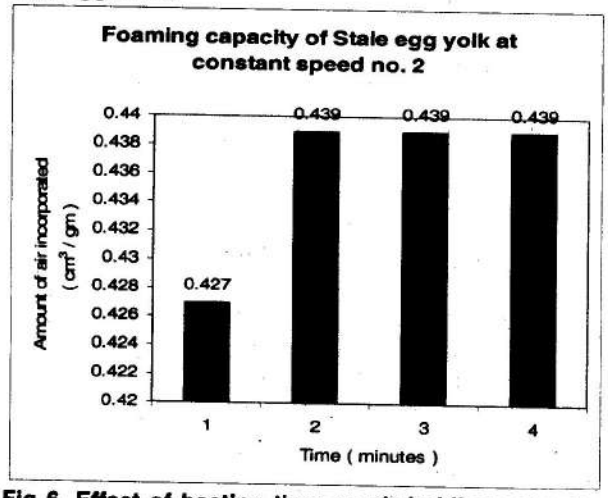

Fig 6. Effect of beating time on air holding capacity of stale egg yolk

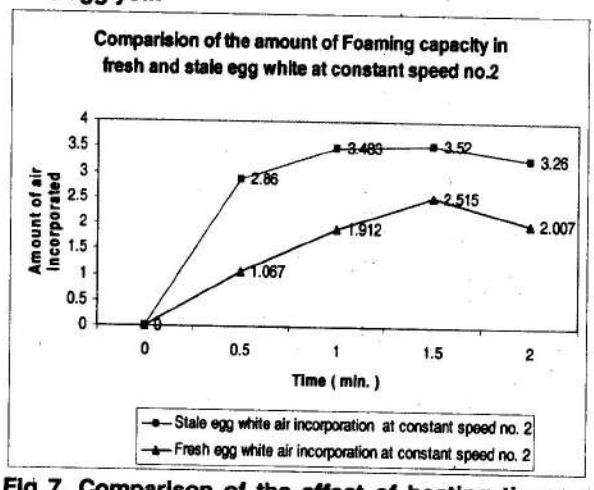

Fig 7. Comparison of the effect of beating time on air holding capacity of fresh and stale egg white

Fresh egg yolk achieved the maximum whipping within 2 minutes at speed number 2. As egg yolk has very less foaming ability (Fig 3). Due to the high contents of cholesterol and emulsifying agent lecithin (Joplin, 1954) egg yolk has the negligible whipping ability (Hung *et al* 2003).

Egg beater speed No. 2 gave the maximum egg yolk foaming at constant beating time 2 minutes in comparison with speed No. 1, 3, 4 and 5 (Fig. 4). Stale egg white attained maximum foaming (Sauter *et al.*, 1972) within 1.5 minutes of beating at constant speed number 2 and become decreased on over beating (Fig 5).

Stale egg yolk also has less ability to incorporate air. A maximum foaming is occurred within 2 minutes after which no more change occurred (Fig. 6). The pH of yolk is 5.9-6.1 in fresh eggs which increased to about 6.8 after storage (Paul and Palmer 1972). On storage the viteline membrane weakens and stretches. The network of fibers on the surface of the membrane, that are apparently part of the chalzeferous layer, tend to dissipate as the pH of the albumen rises (Fromm 1967).

Stale egg white has maximum foaming ability as compared to fresh egg white (Silversides *et al* 2004, John *et al* 1931) (Fig.7). During staling, thick white is gradually converted in to thin white. The rise in pH (7.6 to 9.7) of albumen causes a break down in the gel structure of the thick white. Under alkaline condition, complexing of ovomucin with another egg white protein lysozyme decreases, dissociation of a complex between ovomucin and lysozyme, a breaking of disulfide bonds, loss of carbohydrates from ovomucin molecule occurs which enhance the whipping ability of stale egg. Stale egg white foam is more stable than foam of fresh egg white. The drainage of liquid from the egg white occurred more early with compare to the foam of stale egg white (Fig 7)

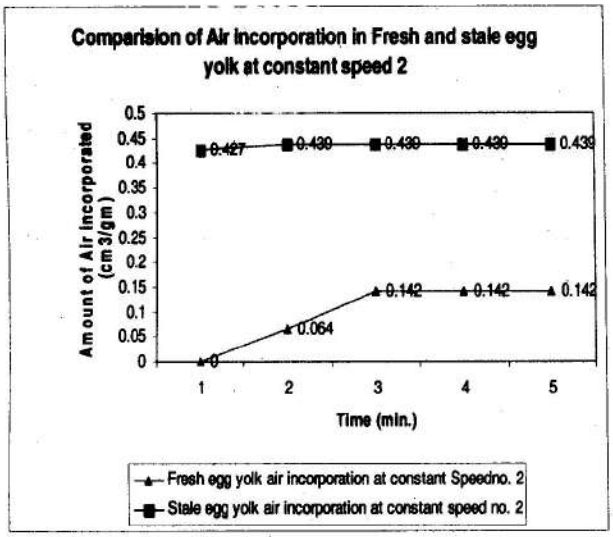

Fig.8. Comparison of the effect of beating time on air holding capacity of fresh and stale egg yolk

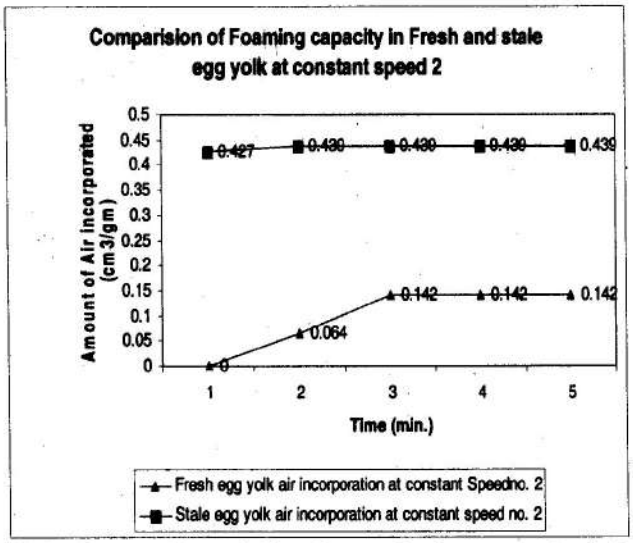

Fig.9. Comparison of the change in the amount of air incorporation

Similarly stale egg yolk incorporate more air with respect to fresh egg yolk (Fig. 8). On staling water contents of egg white moves towards yolk, increases the water contents of yolk. Stale egg yolk foam is stabilized more early than foam of fresh egg white (Fig. 8).

The maximum air incorporation occurred within starting 2 minutes and then little incorporation of air. After certain interval of time the volume of foam is reduced (Fig. 9).

CONCLUSION

It has been concluded that whipping ability of egg is not only dependent on chemical factors but also on physical means for example the speed of egg beater and the beating time. The sequence of speed number's efficiency is 2 > 1 > 3 > 4 > 5. The efficiency sequence of beating time for fresh egg white is 1.5 > 2 > 1 > 0.5 minutes at constant speed no. 2. The efficiency sequence of beating time for stale egg white is 1.5 > 1 > 2 > 0.5 minutes at constant speed no. 2. While the difference of whipping ability of egg yolk fresh and stale both is very negligible.

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Nutritional evaluation of red palm oil in comparison with other oils

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ABSTRACT

Nutritional evaluation of red palm oil (RPO), sunflower oil (SFO) and vegetable ghee (VG) was conducted for 6 weeks, through rat-bioassays. Experimental diets were prepared on the basis of 30% and 40% fat energy levels. The nutritional parameters, included in the study were weight gain, blood lipid profiles i.e., total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) triglycerides (TG), phospholipids (PL) and fecal excretion for total cholesterol and bile acid. Results revealed a lower plasma lipid profile in rats fed on RPO diet than those fed on other experimental diets. The HDL-cholesterol concentration was found pronouncedly higher ($p<0.05$) in RPO fed animals. The fecal excretion of cholesterol was not significantly ($p<0.05$) different in RPO and SFO fed groups, but was lower in VG fed group. Faecal bile acid excretion was slightly higher in RPO (12.81 µmol/g) than SFO (12.09 µmol/g) fed groups and lowest concentration (6.38 µmol/g) was observed in rats consumed VG diet.

Keywords: Red palm oil, rat bioassay, lipid profile, fecal bile acids

INTRODUCTION

The role of dietary fats and oils in human nutrition is one of the most important areas of concern and investigation in the field of nutritional science. Dietary fat and the nutrients associated with it play a critical role in the health and functioning of the human body. The information regarding nutritional implications of dietary fats and oils can profoundly influence the consumption of various other foods and ultimately health and physiological conditions of humans (FAO 1993).The consumption of saturated fat in the diet elevate the cholesterol concentration and could produce hypercholesterolemia both in animals and humans (Renaud *et al* 1974).

Fats and oils are components of normal diets and consumed by the vast majority of the population on a regular basis. An authoritative panel of the FAO/WHO rightly recommended that fat should provide a maximum of 32 calorie percent but should preferably not below than 20 calorie percent. A further recommendation of this committee was to obtain equal amounts of energy in the ratio 1:1:1 from the saturates, monounsaturates and polyunsaturates (FAO/WHO 1977). The evidence from animal and of human studies is consistent in suggesting that habitual intake of diet rich in saturated fat and low in polyunsaturated ones is a primary causative factor in hyper-cholesterolemia and atherosclerosis, which lead to heart attacks, strokes and sudden death (Hayes 1997; O'Holohan 1992).

In Pakistan, edible oil is mainly obtained from cottonseed, canola/rape seed, and sunflower, while soybean and palm oil is imported. Red palm oil (RPO) has recently been introduced by Malaysian Palm Oil BOARD (MPOB) which is naturally balanced edible oil regarding fatty acid composition. It retains about 70% and 90% of the original levels of carotene and vitamin E, respectively (Choo *et al* 1993). The role of β-carotene in enhancing immunity and anti-toxicant defences and of vitamin E in exhibiting hypocholesteremic effect, have also been demonstrated (Vannucchi*et al.* 1977; Tan *et al.* 1991).In view of increased use of different oils & fats and occurrence of heart related problems, it was therefore considered necessary to conduct research on nutritional evaluation of commonly consumed edible oils in comparison with red palm oil.

MATERIAL AND METHODS

Sample of RPO was obtained from MPOB Malaysia, through their regional office at Karachi, while SFO and VG was procured from local market. Experimental diets (Table 1) were prepared on the basis of 30% (normal) and 40% (high) fat energy levels for each oil or fat. The diets were almost isocaloric, isonitrogenous and isofibrous.

Male albino rats were obtained from National Institute of Health (NIH), Islamabad. Rats were divided into 6 groups depending upon the treatments. Each treatment was replicated twice with 5 rats in each case. Animals were housed in steel cages and maintained at almost uniform temperature conditions. Feed and water was provided *ad-libitum*. Animals

were weighed weekly and slaughtered at the end of experiment for collection of blood. The feces from each group were collected weekly, oven dried, ground and stored in polyethylene pouches for subsequent analysis of total cholesterol and bile acids. The blood was mixed with anticoagulant, centrifuged to obtain plasma, and analysed for lipid profile i.e. total Cholesterol (TC), Low Density Lipoprotein cholesterol (LDL-G), high density lipoprotein cholesterol (HDL-C) triglycerides (TG) and phospholipids (PL), using analytical kits of Boehringer Mannheim, (Germany). For fecal lipids analysis 10% (w/v) homogenate in isopropanol was made, kept at 4 °C for 48 hours and then centrifuged. The aliquots of the supernatant was analysed for lipid parameters. The saturation of each oil/fat was determined by Gas chromatograph (Perkin –Elmer Model 3920) presented in Table 2.

Table 1. Components of experimental diets with normal and high fat energy

Ingredients	Red Palm Oil	Sunflower Oil	Vegetable Ghee
Wheat	22.50	22.50	22.50
Wheat bran	27.00	27.00	27.00
Fish meal	15.00	15.00	15.00
Dried milk	20.00	20.00	20.00
Oil/fat (30%)	12.50	12.50	12.50
(40%)	17.20	17.20	17.20
Salt	1.50	1.50	1.50
Molasses	1.50	1.50	1.50
Vitamin/mineral mixture	1.50	1.50	1.50
Protein (%)	20.75	20.75	20.75
Fiber (%)	3.70	3.70	3.70
Total Fat (%)	13.11	13.11	13.11
Calories /100g	400	400	400

Table 2. Saturation of experimental oils and /fat

Dietary oil/fat	Saturates	Monounsaturates	Polyunsatuartes
	14:0+ 16:0 +18:0	18:1	18:2 +18:3
RPO	35.8	40.21	11.39
SFO	9.78	23.25	66.44
VG	43.5	35.35	19.88
Mean	**29.56**	**32.94**	**32.57**
CV	**60.54**	**26.52**	**90.99**

Values are averages of 3 independent determinations

RESULT AND DISCUSSION

Data regarding the effects of feeding RPO, SFO and VG diets on body weight are shown in Fig-1. Results clearly indicated that at higher dietary fat energy level the RPO and SFO fed animals fed animals showed similar growth pattern. Representative body weight (Fig-1) showed that these oils were more digestible than hydrogenated fat therefore they permitted adequate gain in the body weight. Our findings are well supported by the earlier findings of Ng *et al* (1987) who reported that palm oil and soybean oil fed animals showed comparable growth

The present results are also in agreement with the results of Taufiq *et al* (1997) while comparing the digestibility of corn oil, palm oil, soybean oil and vegetable ghee observed a comparable weight gain in corn oil and palm oil fed rats and minimum in VG fed animals. Data regarding the effect of dietary fat sources and fat energy levels on total serum

cholesterol of rats are given in Table 3. It was noted that dietary fat source and energy levels significantly ($p<0.05$) influenced TC concentration in the blood picture of experimental animals. Highest value (160.68 mg/dl) was noted in group fed on VG, followed by SFO (156.24 mg/dL), while the lowest cholesterol level (140.86 mg/dL) was found in group fed on RPO diet.

Similar results were reported by Sundram and Basiron (1996), Chandrasekharan (1997) and Serbinova *et al* (1992). They stated that the cholesterol lowering effect of palm oil is primarily due to profound concentration of β-carotene and vitamin E and were less due to the fatty acid composition. The tocotrienols but not the tocopherols have been reported to suppress cholesterol production in liver, thereby lowering blood cholesterol and athrogenic LDL - cholesterol in animal and human subjects (Qureshi *et al* 1986). The serum HDL- C I levels was found maximum in rats fed on RPO diet (62.2 mg/dl) followed by SFO fed animals (48.02 mg/dl), while minimum values (40.56mg/dl) was noted in rats consuming diet having 30% fat energy from VG. The data clearly showed that consumption of RPO significantly ($p<0.05$) increased the HDL-C as compared to SFO and VG test diets at both (30 and40%)dietary fat energy levels. The ratio of TC to HDL-C (TC/HDL) was minimum (2.26) in blood of rats fed on RPO diet, while VG and SFO fed groups showed almost similar values i.e. 3.84 and 3.34 respectively. Composition of fatty acid in RPO may have contributed to the significant ($p<0.05$) increase in the HDL and reduction in TC by HDL-C ratio. The RPO does not need hydrogenation for the majority of food uses, while in case of VG hydrogenation of polyunsaturated fatty acids results in changes in the structure and properties of fat. The trans fatty acids formed, raise the levels of the harmful LDL-C in the blood stream, and decrease the beneficial HDL (Mensink and Katan 1990).

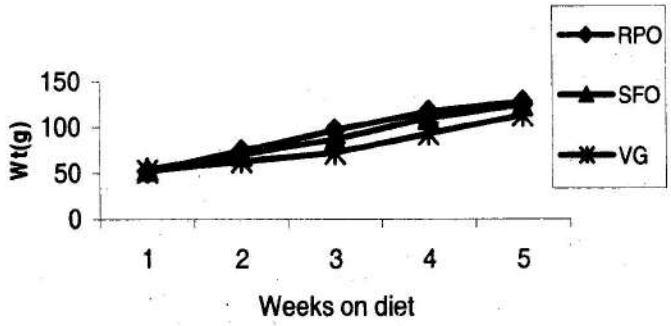

Fig. 1. Body weight gain on different experimental diets

The effect of dietary fat saturation on plasma LDL-C concentrations of rats fed on RPO, SFO and VG (30 and 40%) diets showed that lowest concentration (28.80 mg/dl) was observed in rats fed on RPO, followed by SFO (45.33mg/dl) and VG (61.45 mg/dl). The ratio of LDL/HDL indicated that the RPO diet caused a significant reduction in the ratio of LDL/HDL cholesterol. The hypochlesterolemic effect of RPO may be exerted through the action of tocotrienoles, which are found abundantly in RPO as compared to other test fats. Similar results were observed by Nestle *et al* (1992) and Zock *et al* (1992) they found an increase in LDL-C and decrease in HDL-C levels upon consumption of trans fatty acid diets.

The dietary fats greatly affected the TG concentration, the data revealed that the dietary fat saturation significantly ($p<0.05$) effected the triglycerides concentration. Rats fed on VG diet showed maximum plasma TG concentration (302.6 mg/dl) followed by SFO while RPO fed rats showed minimum values.

Dietary fat sources and the fatty acid composition of the test oil significantly ($p<0.05$) affect the phospholipids (PL) concentration. It is evident from table that the PL values were markedly lower in rats fed on RPO diet than those fed on SFO and VG diets. Hostmark *et al* (1989) studied the effect of feeding of various diets (coconut oil, sunflower oil, and fish body oil, cod liver oil or low fat/high sucrose diet) on plasma lipids and observed lower concentration of phospholipids in groups fed on marine oil and cod liver oil as compared to PUFA group of plant origin

The fecal excretion for total cholesterol and bile acids was determined at the end of the experiment and illustrated in Table-4. Results revealed that the fecal excretion of cholesterol was not significantly ($p<0.05$) different in RPO and SFO groups, but was lower in

the VG group. Fecal bile acid excretion was slightly higher in RPO (12.81 µmol/g) than SFO (12.09 µmol/g) fed group of animals and lower concentration (6.38 µmol/g) was observed in VG fed rats. This may be due to the fact that RPO was easily digested and well absorbed as compared to other test oils.

Subsequent studies (Mattson *et al* 1952) showed that pancreatic lipase specifically hydrolyze the fatty acids esterified in the 1-and 3 position of a triglycerides and that a high content of palmitic acid at the 2nd position of a fat favours its absorption (Tomarecili *et al* 1968 and Filer *et al* 1969). Generally, only a small percentage of the total Palmitic acid of vegetable fats is present in the 2nd position of the triglyceride molecule. Similar results were observed by Hostmark *et al (*1988) who studied the effect of various high fat diets or a low fat/high sucrose diet on plasma lipids, lipoproteins, and fecal excretion of neutral sterols and bile acids in the coconut fed group.

The results obtained on rats revealed that feeding RPO containing diets leads to consistently lower serum and liver TC, LDL-C,TG,PL but higher HDL-C level than feeding isoproteinous, isofibrous and equienergetic amounts of SFO and VG containing diets and that the RPO may be more hypolipemic than PUFA containing oil of plant origin. A possible explanation for this apparently paradoxical action which attributes to the high content of tocotrienols present in Red palm oil (780-1080ug/g). Animal studies (Qureshi *et al* 1986) have also demonstrated that tocotrienols inhibit enzymes of the cholesterol biosynthetic pathway, including 3-Hydrooxy-3-Methylglutaryl coenzyme A (HMGCoA) reductase and cholesterol 7α-hydroxylase.

Table 3: Effect of dietary oils/fat on lipid profiles of rats.

Characteristics	Dietary Oils/Fats					
Plasma Lipids(mg/dl)	RPO Energy (%)		SFO Energy (%)		VG Energy (%)	
	30	40	30	40	30	40
TC	140.86d	156.44c	156.24c	168.54b	160.68c	176.63a
LDL -C	24.45e	33.15d	43.55c	47.11c	57.07b	66.01a
HDL -C	62.21b	68.92a	48.02c	49.03c	40.56d	47.25c
TG	255.40e	258.80e	273.76d	283.47c	297.16b	308.12a
PL	229.51e	229.51e	235.05d	246.39c	253.07b	262.06a

abcd=in each oil values sharing common letters were not significantly different ($p<0.05$)

Table 4: Fecal Excretion of neutral sterols and bile acids

Parameters	Dietary Oils/fats		
	RPO	SFO	VG
TC (mg/g)	3.487a	3.025b	2.347c
Bile acid (µmol/g)	12.09b	12.81a	6.380c

LSD Fat Energy Level (5%) =0.7160
LSD Fat Source (5%) = 1.013

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Submerged production of alkaline protease by newly isolated Bacillus sp.

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ABSTRACT

Bacillus magaterium was used for alkaline protease production by submerged fermentation in shaking flask using wheat bran as a substrate. The optimum cultural conditions for protease were found at 24h of fermentation period at 45°C with 2% inoculum size. Addition of soybean meal as a carbon source and casein and NH_4HCO_3 as organic and inorganic source respectively enhance enzyme productivity. Crude enzyme was obtained after centrifugation of the fermented broth at 10,000 rpm for 10 minutes at 4°C.

Keywords: Cultural conditions, Protease production, Bacillus sp.

INTRODUCTION

Proteases represent the class of enzymes that occupy a pivotal position with respect to their physiological roles as well as their commercial applications. More than 75% of industrial enzymes are hydrolases. Protein-degrading enzymes constitute about 40% of all enzymes sales (Leisola *et al* 2001). They perform both degradative and synthetic functions. A number of eukaryotic and prokaryotic organisms are reported to produce proteolytic enzymes (Sakka *et al* 1986).The biosynthesis of proteolytic enzymes by microorganisms is not only of scientific but also of great practical importance. *Bacillus subtilis* produces both neutral and alkaline protease (Dhandapani and Vijayaragavan 1994).

Proteases produced from *Bacillus subtilis* have wider specificity than that of trypsin and chymotrypsin of animal origin. They are present in a large variety of commercially available enzymes differing in biological source, activity, purity, physical form and characteristics such as pH and temperature optima (Cheetham 1995). Microbial alkaline proteases dominate the worldwide enzyme market, accounting for two-thirds share of the detergent industries (Gupta *et al* 2002). Alkaline proteases useful for detergent applications were mostly active in the pH range 8-12 and at temperatures between 50 and 70°C (Perez *et al* 1999).

MATERIAL AND METHODS

Microorganism: The strain *Bacillus megaterium* isolated from soil sample containing decaying organic matter was used for alkaline protease production by submerged fermentation process (SFP).

Inoculum preparation: Inoculum was prepared by taking a loopful culture of 24 h old from slant to the 50 mL nutrient broth (Oxoid) in 250 mL Erlenmeyer flask. The pH of the medium was maintained at 10 with 1N HCl/NaOH before sterilization at 121°C for 15 min. After sterilization the inoculated medium was incubated at 40 °C for 24 h with the continuous agitation of 140 rpm for the propagation of bacteria up to 10^{8-10} cells/mL.

Fermentation medium: The medium used for the production of alkaline protease contained (%): 1.0 g Wheat bran, 0.5 g yeast extract, 0.5 K_2HPO_4, 0.1g NaCl, 0.05 g $MgSO_4$ and 0.05 g $CaCl_2$. The pH of the fermentation medium was also maintained at 10 with 1N HCl/NaOH before sterilization at 121°C for 15 min. The inoculum size of 1% (v/v) was added into the growth medium and incubated at 40°C for 24 h with the continuous agitation of 140rpm in a water bath shaker (Eyela Japan). After 24 h, the broth was centrifuged at 10,000 rpm for 15 min at 4°C to get clear enzyme solution for subsequent studies.

OPTIMIZATION OF PROCESS PARAMETERS

Effect of incubation period: To investigate the optimum period for maximum enzyme yield, the fermentation experiments were carried up to 72h in triplicates. The samples were collected at regular intervals of 12h.

Effect of incubation temperature Fermentation was carried out at different incubation temperature ranges from 30°C to 50 °C to find the suitable incubation temperature for optimum production of alkaline protease by *B. megatarium*.

Effect of initial pH: Effect of initial pH on production was studied by changing the growth medium pH from 5-11 with 1N HCl/NaOH before sterilization at 121°C for 15 min.

Effect of inoculum size: Various sizes of inoculum ranging from 1% to 6 % (v/v) were evaluated for optimum production of alkaline protease by *B. megaterium.*

OPTIMIZATION OF CARBON AND NITROGEN SOURCES

Effect of Carbon sources: Different carbon sources such as wheat bran, soybean meal, rice husk, corn steep liquor and cotton seed meal was used to examine the enhanced production of protease.

Effect of Nitrogen sources: Among nitrogen sources, different organic (yeast extract, peptone, casein, skim milk, tryptone) and inorganic ($NaNO_3$, NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$, NH_4HCO_3) were tested for the maximum enzyme production.

ANALYTICAL PROCEDURE

Protease assay; The protease activity was measured by using Kunitz (1947) method. The 100 µl of enzyme solution was incubated with 200 µl of 1% (w/v) casein Hammerstein grade at 60°C for 15 min. After incubation the reaction was stopped by the addition of 300 µl of 10% (w/v) TCA. The whole mixture was centrifuged at 10,000rpm for 15 min at 4°C. The absorbance of the filtrate was measured against blank at 280 nm. One unit enzyme activity was defined as the amount of enzyme that releases 1 µg of tyrosine per mL per min under the above assay conditions.

Protein estimation: Protein contents of the inoculated broth were estimated by the method of Lowery (1951) by using BSA as a standard. Specific enzyme activity was expressed as units/mg of protein.

RESULTS AND DISCUSSION

OPTIMIZATION OF PROCESS PARAMETERS

Effect of incubation period: The suitable incubation period played an important role in optimum production of any biotechnological product during fermentation. Therefore, fermentation experiments were carried out throughout up to 72h and the samples were examined after regular intervals of 12h. Maximum enzyme production (156.0 µ/mL) was observed after 24 hr of incubation (Fig. 1). Uyar and Baysalb (2004) worked on alkaline protease production from *Bacillus sp.* Under solid state fermentation and reported the optimum fermentation period of 24h. Dutta and Banarjee (2006) also observed optimum production of protease after 24h of incubation period.

Effect of incubation temperature: Fermentation was carried out at different incubation temperature ranges from 30 °C to 50 °C . It was observed that maximum protease production (185.0 µ/mL) was found at 40°C (Fig. 2). Shaheen *et al* (2008) observed maximum protease production at temperature of 50°C. However, maximum enzyme production at 60°C by various microbes has also been reported by some investigators (Sookkeo *et al* 2000; Kobayashi 1995; Olajuyigbe and Ajele 2005). The variations in incubation temperature might be existed due to difference in types of microbial species.

Effect of initial medium pH : The growth and other metabolic activities of the microorganisms for extracelluar products depend upon the environmental pH, therefore protease production at various pH values ranging from 5 to 11 were studied (Fig. 3). The results revealed highest protease production (243.5 µ/mL) at pH 10 after 24 h of incubation. The optimum protease production at pH 8 has been reported in earlier investigations by some workers (Al-shehri *et al* (2004). Olajuyigbe and Ajele (2005), Muderrizade *et al* (2001) and Kumar (2002) found the highest alkaline protease yield at pH 11.5 by *Bacillus sp.* All these findings indicate that suitable pH of growth medium is necessary for the maximum production of alkaline protease.

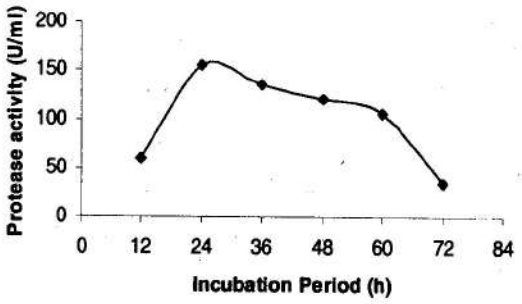

Fig 1 Effect of incubation period on alkaline protease production by *B. megaterium* in submerged fermentation (SF)

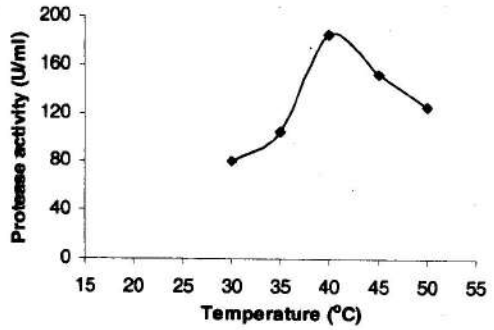

Fig 2 Effect of incubation temperature on Protease production by *B. megaterium* in Submerged fermentation (SF)

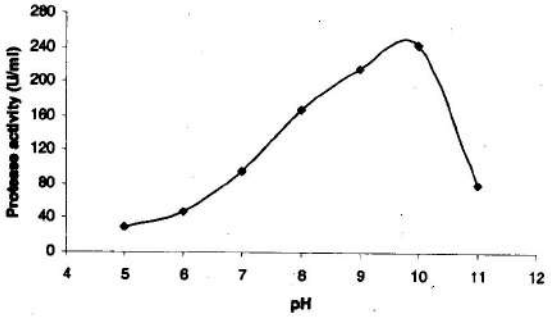

Fig 3. Effect of initial medium pH on Protease production by *B. megaterium* in submerged fermentation (SF)

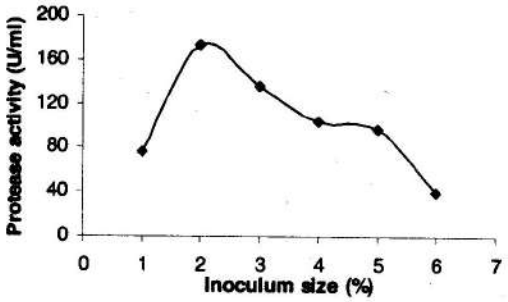

Fig 4. Effect of inoculum size on Protease production by *B. megaterium* in submerged fermentation (SF)

Effect of inoculum size: Alkaline protease production was studied by inoculating the culture media with various size of inoculum, and the maximum activity (174.0 µ/mL) was found at 2% of inoculum size (Fig.4). However, a further increase in inoculum size decreased enzyme production by *B. megaterium*. Elibol and Moreira (2005) observed the inoculum size of 2.5% was optimum for alkaline protease production using *Teredinobacter turnirae* under solid state fermentation. Dutta and banarjee (2006) reported the inoculum size of 1.5% best for protease production by using strains of Pseudomonas sp. Elibol *et al* (1995) reported the size and age of inoculum may affect the microbial process.

Effect of Carbon sources: Different carbon sources such as wheat bran, soybean meal, rice husk, corn steep liquor and cotton seed meal was used to evaluate the suitable carbon source for protease by *B. megaterium*(Fig.4). Maximum yield (236.0 µ/mL) was found when soybean meal was used as a sole carbon source. Al-Shehri *et al* (2004) worked on protease production by using *B.licheniformis* and reported maximum protease activity using soybean meal as carbon source. Nadeem *et al* (2008) quoted glucose was best source of carbon for alkaline protease production. Saurabh *et al* (2007) used wheat bran as best source of carbon for protease production.

Effect of Nitrogen sources: Among nitrogen sources, different organic (yeast extract, peptone, casein, skim milk, tryptone) and inorganic ($NaNO_3$, NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$, NH_4HCO_3) was tested for the maximum enzyme production. Highest protease activities of 197.3 µ/mL and 219.0 µ/mL with casein and NH_4HCO_3 as organic and inorganic nitrogen source respectively. Al-shehri *et al* (2004) reported the maximum protease production using peptone as organic nitrogen source and $NaNO_3$ as inorganic source. Nadeem *et al* (2008) reported the yeast extract as organic source of nitrogen was best for alkaline protease production.

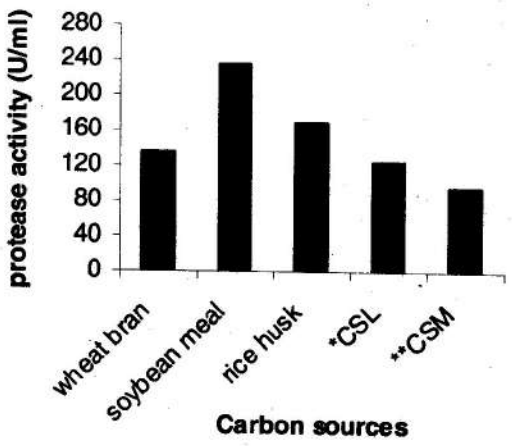

Fig 5. Effect of inoculum size on Protease production by *B. megaterium* in submerged fermentation (SF)
*Corn Steep Liquor, **Cotton Seed Meal

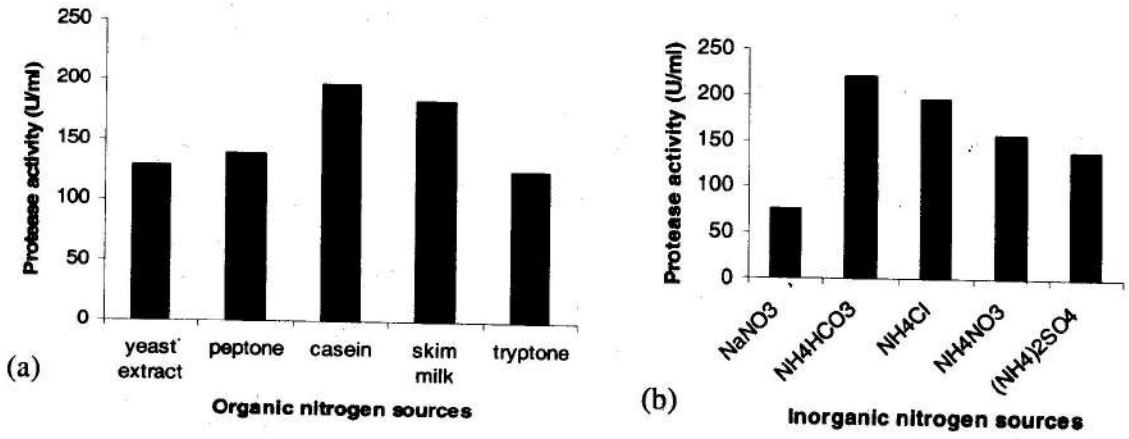

Fig 5. (a) Effect of organic nitrogen sources on protease production by *B. megaterium* in submerged fermentation (SF)

(b) Effect of inorganic nitrogen sources on protease production by *B. megaterium* in submerged fermentation (SF)

CONCLUSIONS

The results of the study indicate that the suitable culture conditions play an important role in yield improvement of alkaline protease production by *B. megaterium* during submerged fermentation. Maximum alkaline protease activity (259 µ/mL) was obtained after complete optimization of culture conditions at shake flasks level. The results of the present study will be used as baseline for further scaling up of protease production in lab scale fermentor.

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Microbial quality of food snacks and drinking water in Islamabad schools and colleges

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ABSTRACT

This study has socio-economic benefit monitoring the health standards of population especially the young generation. The main objective of this study was to determine the microbiological quality of various food items available in different schools and colleges of Islamabad. For this purpose ten different schools and colleges were selected for sampling water and food items. These samples were analyzed for total plate count (TPC), Total coliform, Fecal coliform, *E.coli*. yeast & mould. The data revealed that out of 30 water samples 12 samples were found within permissible limits and for food out of 10 only 3 samples were found to be rang while remaining were highly contaminated and unfit for human consumption.

Keywords: Snack food, microbial quality, Islamabad

INTRODUCTION

Food is the most fundamental need of human being. It is only nutritive when it is pure, fresh and free from hazardous matter such as pathogenic bacteria and sub-standard food colors. Infected or contaminated food endangers health and impairs quality of life. According to WHO more than 80% human diseases are due to contaminated food & water. Pakistan is struggling hard to provide its citizens with basic amenities but clean drinking water is not available to great number of people mainly because of rising level of pollution in the environment, poor un-keeping of water supply lines and faulty drainage system. In Pakistan more than 140 million people today lack access to adequate supply of safe water for household use. Contaminated food and polluted water causes diseases like dysentery, hepatitis, cholera, typhoid, fever and bacteriosis (IDRC 1988, Awan 1983).

Drinking polluted water causes 80% of the childhood diseases leading to death of children. There is a dire need to educate public for use of clean potable water so that the water borne diseases especially in case of children are avoided. Water polluted with faces is often contaminated with a group of bacteria belonging to family enterobacteriaceae, so called because of their normal habitat is the intestinal tract of man and other animals. From medical point of view this family is further divided into two groups (a) organisms occurring exclusively as intestinal pathogens e.g. *Salmonella* and *Shigella* (b) organisms occurring primarily as intestinal commensals, e.g. coliform and Proteus. *E. coli* is a typical organism of the intestinal tract and is present in large numbers in the faces of man and other animals. Water is a basic constituent of almost all the food items, so infected or contaminated water endangers health, impairs quality of life and is proved to be a serious hazard in Pakistan (Khan 1986). Several researchers have reported different pollutants in drinking water sampled from different regions of Pakistan. Ahmed and Saleem (1983) analyzed drinking water of NWFP for chemical pollutants. Bacteriological quality of drinking water from rural areas of Pakistan was determined by Wadud *et al* (1992). Occurrence of pathogenic microorganisms has been studied in water supplies of different areas of Peshawar and it was reported that 50% water samples were highly contaminated and unfit for human consumption (Khan *et al* 2000). Water quality in different areas of Punjab including selected parts of Rawalpindi and Islamabad has been studied for nitrate contamination and nitrate level were found to exceed the Canadian standard for drinking water (Ashraf *et al* 1986). Pakistan Council of Research in Water Resources carried out a survey to determine quality of drinking water in Rawalpindi and Islamabad. Minerals, heavy metals and microbiological contamination were analyzed and it was reported that water was fit for consumption with respect to physiochemical and aesthetic water qualities. However, 75% samples in Islamabad were found unsafe for human consumption due to bacterial contamination, based on total coliform quantification (PCRWR 2001).

According to FAO (1992) recent involvement of *E. coli* in several cases of food poisoning suggests that this organism, rather than fecal coliform group, should be used as an indicator of sanitary quality.

Microbiological standards for drinking water in most developed countries rely on the detection of total coliform and *E. coli* as markers for enteric organism (Karatz *et al* 1999). The main objective of the reported study was to determine the microbiological quality of various food items available in tuck shops of different schools and colleges of Islamabad.

MATERIALS AND METHODS

Samples were collected from seven different schools and three colleges. Water samples were collected from taps, general water cooler and canteen water cooler, while food sample was obtained from school/college canteens. Three water and one food snack samples (Samosa) were collected from each school and college. Total plate count, coliform, *E.*coli, yeast and moulds were determined by the method described in FAO (1992). According to this method food (Samosa) was homogenized with butter field's phosphate buffer (pH 7.2). Serial dilutions of the food were prepared. 1 mL volumes were transferred to petri dishes with plate count agar and mixed with medium in triplicate. After incubation colonies formed on the surface and in the medium were counted. The total count was calculated from the mean count of triplicate of Petri dishes, taking the dilution in to consideration. Similarly yeast growth was checked on plate count agar + chloramphenicol (added as antibacterial) Petri plate incubated at 25°C and mould on potato dextrose agar. Coliform and *E. coli* in food items were determined by Most Probable Number (MPN) method (FAO 1992). Water analysis was carried out by multiple tube method. In this method double strength and single strength MacConkey broth were prepared. Measured volumes of water to be tested were added to tubes containing differential medium. After incubation, each tube which received one or more viable organism in the inoculums showed growth and the most probable number of organisms in the original sample was estimated from the tubes giving positive reaction. Most probable number (MPN) coliforms per 100 mL of water sample was calculated from the relevant MPN Table.

RESULTS AND DISCUSSION

As per WHO standards following are the maximum permissible limits in cooked food Aerobic plate count (APC)10,000/g, coliform <10/g, *E. coli* absent; yeast and mould absent and in drinking water total coliform ,*E. coli* should be absent and APC <100/ml (WHO, 1994). A school wise comparison of WHO standards and results of this study are given in Table 1(water analysis) and Table 2 (food analysis).

In tap water sample collected from G-6/3 college, TPC, coliform, and fecal coliforms were higher than the permissible limits while in general water and canteen water cooler samples TPC, coliform , fecal coliform , *E. coli* were within permissible limit except coliform in canteen water cooler sample. In food sample collected from canteen, all the parameters fell within permissible limit. TPC of Tap water collected from Federal Govt. Girls Secondary School No. 12 had higher bacterial count while other counts were within range. In general water and canteen water cooler TPC, Coliform, fecal coilform and *E. coli* were found to be higher than the permissible limit. Although food sample contained low bacterial count, yeast and mould were also absent but *E. coli* was detected and coliform level was also high in this sample. So this sample was unfit for human consumption. Results of bacteriological analysis of tap and general water cooler samples collected from F.G. Higher Secondary School No. 15 were unsatisfactory due to high level of total plate count, food sample was also contaminated with *E. coli* and highly polluted with coliform bacteria. All the samples collected from F.G. Junior Model School for Girls were fit for consumption because their total plate count, coliform & *E. coli* were within the permissible range. In food sample, *E. coli* was absent, total plate count and coliform was within range. From F.G. Boys Model School G-6/4 general water and canteen water cooler samples were highly contaminated (TPC, total coliform were much higher and *E. coli* was also present). Although *E. coli* was absent in food sample it was highly contaminated with yeast and TPC.

Total plate count was low in all water samples received from F.G. Model Boys School G-7/3, but coliform and *E. coli* were present in these samples. Food samples were also contained with *E. coli* and coliform. Tap water and canteen water cooler samples from F.G. Model School G-9/4 were highly contaminated with TPC, total coliform, *E. coli* and fecal coliform while general water cooler and food samples were fit for consumption. All water samples of F-8/3, G-10/4 and G-11/1 Colleges for boys were found to be within permissible limit. TPC, total coliform, *E. coli,* fecal coliform and yeast were higher in food samples of F.G Model school F-8/3. Whereas, food sample of G-10/4 was contaminated with coliform, *E. coli,* fecal coliform and yeast. Food sample collected from G-11/1 was highly polluted with coliform, *E. coli* and fecal coliform, while yeast and mould were absent.

Table 1. Data of drinking water of schools and colleges

Location	Parameters	F.G College for Boys, G-6/3, Islamabad	F.G. Girls Sec. School No. 12	F.G Higher Secondary School for Boys, No. 15	F.G Junior Model Public School for Girls	F.G Model School G-6/4, Islamabad	F.G. Model School G-7/3, Islamabad	F.G. Model School G-9/4, Islamabad	F.G. Model School F-8/3, Islamabad	Islamabad Model College for Boys G-10/4, Islamabad	Islamabad Model College for Boys G-11/1, Islamabad
Tap water	TPC	76500	560	333	Nil	Nil	20	250	25	23	50
	Coliform	23	Nil	Nil	Nil	Nil	35	1800	Nil	Nil	Nil
	Fecal Coliform	23	Nil	Nil	Nil	Nil	25	1800	Nil	Nil	Nil
	E.coli	Nil	Nil	Nil	Nil	Nil	25	35	Nil	Nil	Nil
General water cooler	TPC	75	747	245	30	1370000	50	Nil	45	30	10
	Coliform	Nil	15	Nil	Nil	1800	13	Nil	Nil	Nil	Nil
	Fecal Coliform	Nil	3.6	Nil	Nil	1800	6	Nil	Nil	Nil	Nil
	E.coli	Nil	3.6	Nil	Nil	1800	6	Nil	Nil	Nil	Nil
Canteen water cooler	TPC	85	675	18	20	123000	90	1800	85	90	95
	Coliform	23	20	Nil	Nil	1800	13	1800	Nil	Nil	Nil
	Fecal Coliform	9.1	9	Nil	Nil	1800	13	225	Nil	Nil	Nil
	E.coli	Nil	23	Nil	Nil	1800	10	25	Nil	Nil	Nil

WHO permissible limits for water
TPC =Total Plate Count (cfu/100mL) <100/mL, Coliform= MPN/100mL absent, *E. coli* = MPN/100mL absent

Table 2. Data of food items of schools and colleges

Location	Parameters	F.G College for Boys, G-6/3, Islamabad	F.G. Girls Sec. School No. 12	F.G Higher Secondary School for Boys, No. 15	F.G Junior Model Public School for Girls	F.G Model School G-6/4, Islamabad	F.G. Model School G-7/3, Islamabad	F.G. Model School G-9/4, Islamabad	F.G. Model School F-8/3, Islamabad	Islamabad Model College for Boys G-10/4, Islamabad	Islamabad Model College for Boys G-11/1, Islamabad
Canteen	TPC	8800	9150	8300	2000	1400000	3000	2000	13500	12000	195000
	Coliform	Nil	15	460	9	7.2	40	9.2	210	240	75
	Fecal Coliform	Nil	15	93	9	Nil	20	9.2	210	240	75
	E. coli	Nil	9	93	Nil	Nil	20	Nil	Nil	Nil	21
	Yeast	Nil	Nil	2	Nil	15	3	Nil	10	15	Nil
	Mould	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

WHO permissible limits for cooked food
TPC= Total Plate Count (cfu/g) 10,000/g, Coliform= MPN/g <10/g, *E.coli* = MPN/g absent
Mould & Yeast = cfu/g absent

Overall TPC was found higher in water samples from five out of ten schools and colleges. *E. coli* contamination was detected in four out of ten and fecal coliform were present in five out of ten schools and colleges mostly in cooler water samples. Higher contamination in cooler water was probably due to unhygienic method for dispensing water e.g. it was observed while taking samples that a plastic glass hanged with the cooler was used repeatedly to take out water from the container instead of dispensing water through the cooler tap. It is also possible that children handle coolers with unwashed hands. In food sample overall TPC was high in three out of ten, coliform and fecal coliform in six out of ten and *E. coli* in four out of ten school and college samples.

Data differed widely depending upon the location from where these samples were taken. These results are based on a limited number of random samples which do not indicate the true situation. Therefore, it is required that a comprehensive survey is conducted according to standard statistical procedures to get a clear picture of the existing microbial contamination in food and water that is being consumed by the children. Nevertheless these initial investigations indicate the need for continuous monitoring of the status of pollution of water and food items sold in school and college canteens or tuck shops. Children are our future, we must be careful for their health because strong people build a strong nation. The present study can be used as a base line by health management authorities and also may enable the concerned authorities to pay attention to this important issue of common man's concern. In this regard, an effective programme to control the biological quality of drinking water and food items is highly recommended. Remedial steps are needed for the improvement in the quantity of food item

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Phosphate fertilizers as natural environmental pollutants in the staple food and cancer risk in Pakistan

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ABSTRACT

Wheat is a staple food in Pakistan. Production of wheat is increased with extensive use of phosphate based fertilizers in agricultural fields. Activity mass concentration of primordial radionuclides due to use of phosphate fertilizers in soil enhances the external gamma dose and due to the consumption of wheat food grown on these soils also increases the internal dose. Different types of soil, including saline and normal soil in the districts of Lahore and Faisalabad in Punjab were selected for this study. The technique of gamma ray spectroscopy was used for the determination of s levels of radioactivity ^{226}Ra, ^{232}Th, ^{40}K, and the nuclear fallout ^{137}Cs in soil, wheat and wheat made products. Radioactivity in flour, chapatti, bread, nan and rusk was determined. Maximum activity was found in the chapatti sample of highly fertilized soil. Calculations were made for the determination of external absorbed dose in air from soil and internal absorbed dose in human body due to the consumption of wheat and wheat products. The average value of wheat consumption of 140 kg was used in the estimation of ingestion dose. The value of the ingestion dose due to highly fertilized soil was 206.1 μSv y^{-1}, while the total ingestion dose due to unfertilized farm's food was 146.3 μSv y^{-1}. Risk assessment to man due to ingestion of wheat was also calculated. The cancer risk assessment due to ingestion of wheat food grown on highly fertilized soils comes as additional sufferings of 14 persons in cancer per million.

Keywords: ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs, Gamma Ray Spectroscopy, Wheat Consumption Ingestion Dose and Risk Assessment.

INTRODUCTION

In order to study the Impact of natural radioactivity on the food we eat, we have to study the impact of natural radioactive pollution due to phosphate fertilizers on soils and their uptake by plants. Different studies surveys in many countries of the world have done to assess the background radiation status of the soil of the particular area (UNSCEAR 1988, 2000). Man obtains his food mainly from soil. Phosphate fertilizers have relatively high concentrations of natural radionuclides, particularly isotope of radium (^{226}Ra), which is a daughter product of uranium (^{238}U). The natural source of phosphorus is phosphate rock, found in sedimentary rock formations. Wheat is the most important food crop of the world. The largest cropped area is devoted to wheat crop and the quantity produced is more than any other crops in the world. In Pakistan, wheat is most important single crop. Wheat is a staple food of Pakistani diet, which provides about 72% of the total calories and protein in the daily average diet (Khan *et al*, 1984). About 80% of the total wheat is consumed in the form of unleavened flat bread locally known as "Chapatti" while the rest 20% goes for other bakery products like bread, rusk, nan, etc. (Shafi,1994). The intake of food produced in the cultivated and uncultivated soils is the largest contributor to radiation doses received by human beings.

The possible effects of ionizing radiation due to naturally occurring radionuclides have been a cause of growing concern. Initiation and development of radiation injury starts, from sub-cellular apparatus such as molecules of proteins, carbohydrates, fats, inorganic salts, membrane system, etc. The result to the sub-cellular structure and constituents provokes a chain of developments finally resulting in and manifestation of morphological and functional changes in the cell. The damage develops into tissue damage leading to the possible malfunctioning of the organ/organ systems and ultimately organism as a whole (UNSCEAR 1988, 2000). On temporal scale the physical absorption of energy on molecular level takes 10^{-6} seconds, cellular changes in the cell are affected in 10^{-5} seconds, cellular damage and physiological changes takes place in second to hours and days and long term, the delayed effect may take years to appear (UNSCEAR 2000).

The main objective of the study was to determine the uptake of the natural and man made radioisotope from

the soil to wheat produce. The presence of radionuclides in soil is a source of radioactivity intake of human beings by direct and indirect ways. The diet is a source of intake of radium radioactivity and human exposure.

EXPERIMENTAL

Site selection: The area under investigation consisted of four sites including two saline, one normal with and one without fertilizers treatments. These sites were selected in such a way that most of them were already been receiving fertilizer for the last many years in different amounts. Fertilizers were applied by means of spreader machines, so that each soil profile receive uniform amount of fertilizer. the sampling was done at the two bio saline research stations of nuclear institute for agriculture and biology (niab) and normal fertilized soil getting 500 kg ha^{-1} y^{-1} of phosphate fertilizers and where regular cultivation practices have been going on for last thirty-five years, named as site 1. The location of the area is 31°24/ n and 73° 05/ e (Faisalabad, web). The area is a part of natural unit known as the indus plains, which represent a vast geosynclines lying between the himalayan foothills and center core of the indian subcontinent.

First Bio Saline station was established by NIAB in 1990 near the city of Lahore, in the province of Punjab, where regular cultivation practices by adding 400 kg ha^{-1} y^{-1} have been going on for the last 22 years; was regarded as site 2. The district lies at 31° 15' and 31° 45' latitude and 74° 01' and 74° 39' longitude (Lahore, web). The district is bounded by Sheikhupura, on the east by India and on the south by Kasur. The Lahore district, is the second largest district of the country, and is regarded as the cultural nucleus of Punjab. The soil of study area consists of ten hectares, along the famous Bari Rakh Branch (BRB) canal also called Rakh Branch. The name of this village (where study site-2 lies) is Rakh Dera Chal, which is situated at 30 km from the historical city of Lahore. The other area Pakka Anna, village (study sites 2 and 3) under study consisted of 10 hectares of saline soil located at a distance of 34 km in the south west of famous city of Faisalabad, in the Punjab province of Pakistan. This area is called the Bio Saline Research station number 2, which has been established by Nuclear Institute for Agriculture and Biology in 1992, about 10 years after the establishment of first station, the land was acquired from government of the Punjab in 1992. The variation of soil salinity is very high. The position of the area is 31° 24' latitude and 73° 05' longitudes at an elevation of 190 m from sea level. It was not cultivated earlier but laying barren since decades. The shallow ground water is brackish, having high salt concentration. It is thus unfit for irrigation. Pakka Anna station contained two types of soils. The saline soil cultivated with 237 kg ha^{-1} y^{-1} of phosphate fertilizers is regarded as site 3. The soil cultivated for one year without fertilizer was named as barren or virgin soil and regarded as site 4.

EXPERIMENTAL METHODS

Sample collection and treatment: Soil sampling was carried out in the months of May–June in 2003. Sampling from the soil patches was done using the standard sampling methods (IAEA 1989). The area was divided into 25 locations. The sampling was done from 0–25 cm with an increment of 5 cm. The chosen sampling sites were plain land, from where the vegetation was removed. Total number of soil samples was 125. Wheat grown in the respective soils was also taken. Wheat samples were divided into wheat flour, chapatti, bread, nan and rusk. In this way total number of wheat samples was 100. The samples were mixed to prepare one representative soil sample as per standard sampling methodology (IAEA 1989). The samples were properly marked, cataloged and brought to Health Physics Laboratory at NIAB, Faisalabad, Pakistan.

The collected samples were dried on plastic sheets at room temperature for several days (Knoll, 1988). To remove moisture, samples were heated in an electric oven at 110 °C up to 48 hours depending on the depth of soil until the sample attained constant weight. After drying the samples were crushed, ground and pulverized to a predetermined particle size by the analytical requirements and then passed through a sieve of 2 mm mesh size. The homogenized soil samples were packed in plastic containers having same geometry as that for the reference materials as dictated by the calibration requirement (Debertin *et al* 1988). These containers were sealed hermetically so that ^{222}Rn produced from ^{226}Ra decay would not result in gas leakage. After ensuring secular equilibrium among the progenies of ^{238}U and ^{232}Th series (two months), these sealed samples were ready for analysis.

Radiometric analysis: Radiometric analysis of these samples was performed using PC based, high resolution gamma spectrometry system comprising of High Purity Germanium (HPGe) coaxial detector (relative efficiency: 30%, active volume: 180 cub.cm. with beryllium-end window and FWHM: 2.0 keV at 1332 keV for ^{60}Co) (9-10). The detector was shielded by 8 cm thick lead having inner lining of 0.5 cm thick

copper plate covered with 1 mm aluminum to absorb the X-rays from lead and copper. The inner size of shielding cavity was 30 × 30 × 30 cm (Knoll, 1988). The detector was given high voltage through preamplifier which was then connected to amplifier to computer based Multi channel analyzer through ADC (analogue to digital converter).

The system was calibrated using IAEA soil-6. The counting was performed for 65000 seconds both for reference materials and the soil samples. The spectra were analyzed by commercially available software GENIE-2000 obtained from Canberra, USA. The detection efficiency of the system 'η' was calculated for each peak corresponding to the energies given in (Debertin *et al*, 1988) using the relation:

$$\eta = \frac{C}{A \times y \times t} \quad (1)$$

where C = C_t – C_b = net peak counts, here C_t is the peak area (with reference material) and C_b is background counts for the respective peak; y = percent abundance (% yield); t = collection time (sec). The detection efficiency 'η' was plotted as a function of γ ray energy (E) on the log–log graph paper (Knoll, 1988). A polynomial of degree 2 was fitted on the experimental data which is given as follows:

$$\log\eta = 9.002\text{-}1.923(\log E) + 6.448\times10^{-2}(\log E)^2 \quad (2)$$

The reliability of counting efficiency was confirmed using reference material soil-375. The results were within an error of 3–5 %. The lowest limit of detection (LOD) for ^{40}K, ^{137}Cs, ^{232}Th and ^{226}Ra were determined for all tested radioisotopes. Spectrum for every sample was collected for 65000 seconds. The analysis was done with the help of the computer software Gene–2000 and activity mass concentrations for ^{40}K, ^{137}Cs, ^{226}Ra and ^{232}Th was determined using the following relation:

$$A_s = \frac{C}{\eta \times y \times t \times m} \quad (3)$$

Where m is mass of the sample (kg); and the other factors have the same meanings as are in eqn (1).

After the spectrum collection, count rates for each detected photo peak and activity per unit mass (specific activity) for the detected nuclides were calculated. For the radionuclide considered, if there are more than one peaks in the energy range of analysis (100–2000 keV), then the peak activities are averaged and the result is the weighted average nuclide activity. The total uncertainty of the radioactivity measurements, which is also applicable to the calculated gamma dose and effective dose rates, was typically in the range 3–10% (Shirly 1986).

Ingestion dose: Annual effective dose to man from consumption of wheat grain was calculated (UNSCEAR, 2000) using following equation:

$$D = A_s \times I \times C_F \quad (4)$$

Where, D is annual dose (Sv y^{-1}), A_s is specific activity (Bq kg^{-1}), I is annual intake (kg), and C_F is dose conversion factor (Sv Bq^{-1}). In Pakistan, wheat grain is consumed in the form of "Chapatti", bread, nan, rusk, biscuits and other bakery items. Grain product is the major diet of the people of Pakistan. The consumption of grain product is relatively more in village population than those who live in cities; therefore, the annual consumption of the product varies from 100 kg to 150 kg. Average annual consumption of grain product reported by United Nations Scientific Committee on Effects Atomic Radiations (UNSCEAR 2000) is 140 kg, therefore, 140 kg per year has been considered for the estimation of radiation dose to the adult population in Pakistan.

RESULTS AND DISCUSSION

Rocks are radioactive due to naturally occurring radioactive material (NORM) in the earth's crust. The radioactivity of rocks ultimately shifts to soil. The levels to terrestrial background radiation are related to the type of rock from which the soils originate (INSCEAR 1988). Only the radionuclides present at the time of creation of earth with considerable half-lives and their decay products can be found even today on the earth, e.g. ^{238}U and ^{232}Th series and ^{40}K, (Shirely 1988). The manmade radioactivity as a result of nuclear explosions has also contaminated the earth's crust. Natural and manmade radioactivity was investigated in the soil and the wheat products of the area under study by mean of gamma ray spectrometer. To assess the implications of the extended use of phosphate fertilizers, on human health, we performed radioactivity measurements in soil, wheat and wheat products from pastures treated with and without phosphates fertilizers. The natural radionuclides of interest are the uranium and thorium series and ^{40}K whereas the manmade radionuclide ^{137}Cs, which was determined in the cultivated, fertilized soil of the Nuclear Institute for Agriculture and Biology (NIAB) in the city of Faisalabad, BSRS-1 in Lahore and BSRS-II in district of Faisalabad in the Punjab province of Pakistan. The measured activity concentration of the radionuclides of concern is given in Table- 1. Activity levels were found to follow log

normal distribution. The natural environmental higher levels of radiation are associated with igneous rocks, such as granite and lower level with sedimentary rocks. The highest concentration of ^{40}K was found in the sedimentary muddy material. In subcontinent soils the contents of potassium are more. This may be due to presence of radioactivity in fertilizers (Tufail *et al* 2006a).

Table-1: Activity concentration of different radionuclides in soils treated with different amounts of fertilizers.

Sample ID	Depth (cm)	Activity concentration (Bq kg^{-1})			
		^{40}K	^{232}Th	^{226}Ra	^{137}Cs
Normal Soil of Faisalabad (S-1)					
N1	0—5	642.6	58.0	31.4	3.1
N2	5—10	614.4	60.2	29.1	2.5
N3	10—15	629.8	61.6	38.6	2.7
N4	15—20	660.5	60.3	32.6	2.4
N5	20—25	670.7	55.8	29.1	2.1
Mean		643.6	57.18	34.4	2.1
Saline Fertilized Soil Lahore (S-2)					
L1	0—5	586.3	48.5	34.7	< LLD
L2	5—10	594.9	59.7	29.2	< LLD
L3	10—15	575.8	51.6	35.5	< LLD
L4	15—20	602.6	52.6	26.2	< LLD
L5	20—25	590.3	54.5	26.5	< LLD
Mean		597.5	55.1	30.60	< LLD
Saline Soil of Faisalabad (S-3)					
F1	0—5	583.7	50.6	20.6	5.03
F2	5—10	600.6	55.8	32.4	4.55
F3	10—15	599.5	61.9	30.3	5.15
F4	15—20	545.8	45.6	32.6	4.05
F5	20—25	563.2	57.41	27.3	3.98
Mean		563.9	49.3	26.4	5.03
Saline Barren Soil of Faisalabad (S-4)					
V1	0—5	547.8	43.3	16.2	3.57
V2	5—10	499.2	42.5	20.0	3.63
V3	10—15	542.7	51.3	20.5	< LLD
V4	15—20	596.5	43.7	21.3	< LLD
V5	20—25	604.2	50.6	20.6	< LLD
Mean		550.0	42.6	20.9	1.25

The average concentration of ^{40}K in the NIAB, Lahore fertilized, Faisalabad fertilized saline, and undisturbed saline soil of Faisalabad determined were, 643.60, 597.50, 563.90 and 550.0 Bq kg^{-1} respectively. Besides potassium, the other naturally occurring radionuclides measured, were ^{226}Ra and ^{232}Th. Radium-226 (a member of ^{238}U series having half life of 1620 y) is considered as the highly radiotoxic natural radionuclide. The average value of the measured activity of ^{226}Ra in the fertile and virgin soil of NIAB, Lahore, and Pakka Anna were 34.40, 30.60, 26.40 and 20.90 Bq kg^{-1}, respectively. The average measured specific activity of ^{232}Th ($T_{1/2} = 1.4x10^{10}$ y) for the above mentioned soils were 57.18, 55.10, 49.30 and 42.60 Bq kg^{-1} respectively. The data shows that the average activity value of ^{232}Th is about two times higher than that of ^{226}Ra this may be due to longer half life of ^{232}Th than ^{226}Ra. The activity concentration of ^{40}K in soil is in order of magnitude higher than that of ^{226}Ra and ^{232}Th for all soils. It has been known since early in this century that phosphate rocks contain substantial concentration of uranium, thorium, radium, and their decay products (Tufail *et al*, 2006a). Since phosphate rock is an important raw material used for the manufacturing of different types of phosphatic fertilizers, therefore, when this rock is processed into fertilizers, most of the uranium and some of the radium accompanies the fertilizers (Tufail *et al* 2006b) It has also been estimated that phosphatic fertilizers applied to the agriculture fields in recommended amounts could raise radioactivity level in soils (Nasim-Akhtar *et al*, 2005). The average activity of ^{137}Cs in all the above soils samples was found to be 2.56, BDL, 4.50 and 1.25 Bq kg^{-1} respectively. The reasons of existence of ^{137}Cs in these soils are that these soils may have the nuclear fallout in the past. The land was fertilized and

cultivated regularly; the excess amount may be leached out by irrigation water.

The average values of activity of ^{40}K for the wheat grown in all the above mentioned soils obtained were 120.84, 117.50, 102.52 and 96.20 Bq kg^{-1}, respectively. The values of ^{40}K in chapatti from the wheat in the same soils were 107.75, 97.51, 87.53 and 52.53 Bq kg^{-1}, respectively. Activity comparison of the bread samples from the wheat of the same soils was 71.50, 67.50, 59.00 and 40.50 Bq kg^{-1} respectively. The values measured in nan were 65.02, 55.02, 50.01and 40.00 Bq kg^{-1} respectively. The levels of activity in rusk samples were 57.29, 54.68, 45.01 and 33.06 Bq kg^{-1}. The values of all other determined radionuclides have been shown in Table-2. It is clear from the data that the radioactivity taken up by the wheat grains ranges from 10-13 % of the total activity present in the soil. While the value of the manmade radioisotope ^{137}Cs varies from 20-35% of the total activity measured in soil.

Ingestion dose was calculated and values for four selected sites are shown in the Table-3. The soil getting 500 kg/ha/yr for 35 years of continuous use of the phosphate fertilizers was regarded as the highly fertilized area. The ingestion dose due to consumption of wheat grown on this area calculated as 206.1 μSv y^{-1}. It is clear from the data the contribution of ^{40}K to ingestion dose was greater then 50 %. The remaining 41- 49 % dose was shared equally by ^{232}Th and ^{226}Ra respectively, in all selected sites. The least value of ingestion dose due to wheat consumption grown on unfertilized soils came as 146.3 μSv y^{-1}. The difference between the ingestion doses due to fertilizers use is 60 μSv y^{-1}. Doses of ^{262}Ra and ^{232}Th are greater than the range specified by UNSCEAR (2000). By using the values of ingestion doses and external gamma doses cancer risk assessment was done. By using the total population of the Lahore, Faisalabad and Pakka Anna as six million, 5 million and 10000 persons respectively who consume the wheat grown there. The calculations were made by using mathematical formulations as given in (Akhtar, 2006). Applying the conversion factors given in UNSCEAR report, by using eqn. 4, the ingestion dose due to each radionuclide has been computed and is presented in Table 4.

Table 2: Activity mass concentration in different wheat food stuff

Sampling Site	Radionuclide	Activity mass concentration (Bq kg^{-1})				
		Soil	Wheat Products			
			Chapatti	Bread	Nan	Rusk
Site 1	^{40}K	643.60	107.75	71.50	65.02	57.29
	^{232}Th	57.18	1.25	1.20	1.01	0.91
	^{226}Ra	34.45	0.97	0.86	0.71	0.58
Site 2	^{40}K	597.50	97.51	67.50	55.02	54.68
	^{232}Th	55.18	1.23	1.05	0.95	0.75
	^{226}Ra	30.60	0.89	0.78	0.67	0.50
Site 3	^{40}K	563.96	87.53	59.00	50.01	45.01
	^{232}Th	49.38	1.00	0.98	0.85	0.74
	^{226}Ra	26.40	0.94	0.73	0.57	0.50
Site 4	^{40}K	550.08	52.53	40.50	40.00	33.06
	^{232}Th	42.68	0.45	0.45	0.37	0.25
	^{226}Ra	20.96	0.36	0.36	0.25	0.20

Table-3: Ingestion doses due to consumption of wheat food grown on fertilizer soils.

Location	Committed dose (μSv y^{-1})			Total
	^{40}K	^{226}Ra	^{232}Th	
Site 1	108.8	49.0	48.3	206.1 ± 2.34
Site 2	105.8	39.2	40.3	185.2 ± 2.30
Site 3	92.3	39.2	46.4	177.9 ± 1.99
Site 4	86.6	25.9	33.8	146.3 ± 1.50
UNSCEAR,2000 Reference value	170	8.0	0.36	

Table 4: Excess cancer risks in the study areas (life time cancer risk factor = 0.07) (11)

Sites	External Dose (Sv)	Internal Dose (Sv)	Total (Sv)	Risk Coefficient	Excess cancer risk in one million.
S-1	0.6745×10^{-3}	206×10^{-6}	88×10^{-5}	6.16×10^{-5}	62 ± 2.5
S-2	0.630×10^{-3}	185×10^{-6}	81×10^{-5}	5.67×10^{-5}	57 ± 2.3
S-3	0.573×10^{-3}	177×10^{-6}	75×10^{-5}	5.25×10^{-5}	5 ± 0.15
S-4	0.508×10^{-3}	146×10^{-6}	65×10^{-5}	4.55×10^{-5}	4 ± 0.10

From the Table it is clear that the risk coefficient has a direct link with the number of sieverts. The dose for the highly fertilized farms of Faisalabad is greater then the other study sites. The public of Faisalabad is at a more risk then public around Lahore. The public of Pakka Anna is at least risk than the population around Lahore and Faisalabad. If the wheat food consumed is from the unfertilized fields, then risk coefficient is minimum. The data shows that excess risk per year is maximum at the consumption of wheat food stuff grown on the highly fertilized soils. The excess risk is estimated as death of 14 persons per year in the population of one million. The excess cancer risk for Dera Rakh Chall (District Lahore) population has 13 persons per year in 1 million (Akhtar 2006). The Pakka Anna population has less fear of excess cancer, 12 in I million, by using wheat food from less fertilized area. The least amount of risk (10 in 1 million) was posed by the population consuming food from unfertilized fields. The net excess cancer risk form the consumption of food in fertilized fields comes out as 4 persons in one million.

CONCLUSION

The activity concentration determined on the highly fertilized area was maximum for all radionuclides determined. Activity determination on other sides also confirms the enhancement of radiation levels due to the application of phosphate fertilizers. The net rise in the activity of highly fertilized soil (site-1) for ^{40}K, ^{232}Th, and ^{226}Ra came as 93.00, 15.50 and 13.40 $Bqkg^{-1}$. The food stuff of wheat grown on fertilized fields follows the patterns for all sides as:

Grain < Chapatti < Bread < Nan < Rusk

The ingestion dose committed dose by the use wheat food only due to fertilizer came as 60.8 $\mu Sv^{-1} y^{-1}$. On the basis of ingestion dose risk assessment was made. The excess risk is estimated as death of 14 persons per year in the population of one million due to the use of the wheat grown in the highly fertilized area. The data shows that the use of fertilizers is also a reason of great number of deaths of public due to cancer now days.

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Comparative evaluation of sorbatox and bentonite for detoxification of aflatoxin contaminated layer feed

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ABSTRACT

Aflatoxins are considered as unavoidable contaminants of food and animals feed, since their production cannot be prevented successfully using current agricultural practices. It causes severe loses to poultry industry in recent times. The use of aflatoxin adsorbents as feed supplements is one of the most promising approaches to reduce aflatoxicosis in poultry. To avoid adverse effects of aflatoxin sorbatox and bentonite, mycotoxin binders were used and their comparative evaluation for detoxification of aflatoxin contaminated layer feed was studied. In this study total 108 white Leghorn layers of 43 weeks old were used and randomly divided in to six groups (ration) A, B, C, D, E and F. Each group was subdivided into three replicates and each replicate had 6 birds. The ration A served as –ve control having no aflatoxin, B served as +ve control having 50 ppb aflatoxin, the ration C and D had 50 ppb aflatoxin + 0.1%, 0.2% sorbatox while E and F had 50 ppb aflatoxin + 2.5, 5.0% bentonite. Their egg was collected at 14, 21 and 28 days of post vaccination. During experiment their egg production, feed consumption, egg weight, egg shell weight, egg shell thickness, and mortality were recorded. It was found that aflatoxin level of 50 ppb had no significant effect on the overall performance of white leghorn. There was also no significant difference between the controls and sorbatox at the rate of 0.2% of the feed and bentonite at the rate of 5.0% ($P>0.01$).

Keywords: Aflatoxin, mycotoxin binder, sorbatox, bentonite, poultry feed

INTRODUCTION

The importance of feed safety as an integral component of food security has been recognized during the last decade. Natural toxins produced by molds or fungus have threatened the quality and safety of food. The fungi produced two types of metabolites during their metabolism, primary and secondary metabolites. Primary metabolites for their own life while secondary metabolites are non-essentials for their life and are toxic in nature called mycotoxin. There are 350 kinds of mycotoxin, of them one is classified as aflatoxin (Chin and Tan 2006).

A poultry feed mainly consists of cereal grains and agro industrial byproducts. Among the cereal grains, corn grain is main source of energy and highly susceptible to fungi growth under appropriate temperature and humidity. The higher level of mycotoxin in cottonseed meal, corn gluten meal, corn grain and peanut meal affects the quality of the finished feed (Sun *et al* 2006). In this way mycotoxin contaminates the feed and affects the animal health and ultimately the human health (Bintvihok *et al* 2003). *Aspergillums flavus* produced four structurally related aflatoxin namely B1, B2, G1 and G2. B1 is considered to be one of the most hepatoxin and hepatocarcinogenic compound for poultry (Martins 2008). Aflatoxin produced a disease in chicks called aflatoxicosis, which is characterized by increased stress, decreased growth rates, decreased egg production and increased susceptibility of other diseases. The chronic symptoms of aflatoxicosis are enlarged hemorrhagic liver, paleness of kidney, anemia, accumulation of fat in the organs, anorexia, reduced feed efficiency, decreased resistance against diseases and increased mortality (Solmaz *et al* 2006; Ortatatli and Oguz 2001)

A number of efforts had been done to reduce aflatoxin from feed and food. It can be reduced by physical and chemical methods. Aleksandra *et al* 2005) conducted studies for adsorption of mycotoxin by organozeolites and observed encouraging results. Ali *et al* (2000) also reported that decontamination of aflatoxin could be done with ammonia treatment for broiler chicks.

The chemical detoxification of aflatoxin in poultry diet has been found more effective than the others (Huwig *et al* 2001). These chemicals are the feed additives and thoroughly mixed in feed. Upon mixing these chemicals immediately fix themselves with the

aflatoxin molecules because of opposite charge. These chemicals are actually agents (clay minerals) having high ability to adsorb toxins in the intestine and expel them out from the animal body through feces. (Noemi and Jozsef 2006). One specific compound with the name Na-Ca-Alumino Silicates has been demonstrated to significantly reduce the availability of aflatoxin from feed of both layer and broilers (Phillips *et al* 2002). High adsorbent capability of smectite minerals has found wide-range applications not only in industry, but also as excellent adsorptive materials of heavy metals and bacteria (Hassen *et al* 2003; Katsumata *et al* 2003). Bentonite in animal diet acts as gut protectants (enterosorbents), which rapidly and preferentially binds aflatoxin from the digestive tract and thus reduce their absorption (Piyarat and Johanna 2004).

The study was conducted to study the comparative evaluation of most effective mycotoxin binder and the effects of aflatoxin, using contaminated corn layer diet.

MATERIALS AND METHODS

The feed and mycotoxin binder sorbatox and bentonite were purchased from local market. Aflatoxin contaminated corn and other ingredients were mixed carefully according to experimental ration composition with the cooperation of local feed mill as shown in Table 1.

Proximate Composition: The chemical composition of corn was estimated by using AOAC methods (2005). Percentages of moisture, crude protein, ether extract, crude fiber and ash were determined by standard methods in the laboratory before and after contamination.

Table 1. Composition of Layer Ration.

Ingredients	Percentage (%)
Corn	60.00
Soyabean Meal	5.50
Canola Meal	9.90
Fish Meal	4.00
Corn Gluten Meal 60%	4.17
Molasses	4.00
Chips (CaCO3)	8.73
Oil	1.16
Di-Calcium Phosphate (DCP)	1.18
Premix	1.00
L-Lysine	0.27
DL-Methione	0.09

Preparation of aflatoxin contaminated corn: For the production of aflatoxin the corn grains were ground and spread over the jute cloth and moisture was increased by sprinkling of water. The temperature was maintained as in the early days of October and increased moisture provided by humid conditions suitable for the growth of fungus on corn. The corn was covered by the green colored fungi within 12 days of incubation. The corn was dried to remove the excessive moisture and mixed homogenously. The samples of corn were collected for analysis of aflatoxin level by using direct competitive ELISA (Brinton and Miller 1987). The contaminated corn was mixed in the mixer with other ingredients to prepare the experimental rations B, C, D, E and F of layer birds according to experimental formulation and their overall aflatoxin level was found to be 50.7, 52.8, 51.2, 49.9 and 42.4 ppb, respectively. An average aflatoxin level of these groups was taken 50ppb. The ration A in which aflatoxin contaminated corn was not added served as negative control.

In this experiment 108 white Leghorn layers of 43 weeks old were used. The layers were randomly divided in to six groups; each group was divided in three replicates. Thus:

Total number. of layers	= 108
Groups	= 6
Replicates in each group	= 3
Number of birds in each replicates	= 6

Initially, the layers were kept for 14 days for adaptation, then the layers were fed on experimental ration from 46th week to 54th week of age. The proper managemental practices like vaccination, ventilation and lighting schedule were practiced through out the experimental period. All the birds were vaccinated against Newcastle disease using Lasota strain of Newcastle disease virus through drinking water. Egg samples were collected after 14 days, 21 days and 28 days respectively.

Experimental Rations: In this experiment six groups of white Leghorn layer birds were maintained and 6 experimental rations A, B, C, D, E and F were prepared by mixing all the ingredients in required amount. The aflatoxin contaminated corn was added in all the rations except group A which was devoid of mycotoxin binder. The group B had aflatoxin contaminated corn and also lacked mycotoxin binder, it served as positive control for comparison with other groups C, D, E and F. The experimental rations C, D, E and F consisted of 60 % aflatoxin contaminated corn with mycotoxin binder sorbatox (0.1, 0.2% of the feed) and bentonite (2.5, 5.0% of the feed) respectively.

All the rations had same composition, isocaloric and isonitrogenous but the mycotoxin binders were added according to their doses in the premixes of layer rations C, D, E and F as mentioned above.

Experimental Parameters: During the experimental period eggs were randomly collected to study the Feed consumption; Egg production; Feed conversion ratio (FCR); Egg weight; Egg shell weight; Egg shell thickness; Mortality and Cost per dozen eggs (economics).

Statistical Analysis: The data were subjected to statistical analysis using one way analysis of variance (Steel *et al* 1996).

RESULTS AND DISCUSSION

Results regarding proximate composition before and after contamination with aflatoxin are shown in Table 2.

Table 2. Proximate Composition of Layer Ration.

Description	Estimation (%)	
	Before Contamination	After Contamination
Moisture	12 ± 0.08	12.30 ± 0.08
Protein (N*6.25)	16.5 ± 0.5	16.30± 0.5
Ether Extract	3.5 ± 0.3	3.3 ± 0.3
Crude Fiber	3.3 ± 0.2	3.0 ± 0.2
Ash	5.0 ± 0.6	5.2 ± 0.6
M.E K Cal/ Kg	2690	2690
Calcium	3.20	3.20
Available Phosphorous	0.40	0.40
Linolenic Acid	1.30	1.30
Lysine	0.85	0.85
Methionine	0.35	0.35

Data are represented as ± standard deviation

These results indicate that there was some decrease in percentage protein, ash, ether extract, crude fiber and some increase in percentage of moisture but there were no effects on the calcium, phosphorus, linolenic acid, lysine and methionine contents. So there is no significant effect of aflatoxin up to the 50ppb level. These results coincide with the results of Ali *et al* (2000).

Group A: (Negative Control): The birds were given feed without aflatoxin contaminated corn and mycotoxin binder, the average, feed consumption was 660g/hen/week, egg production 92.10%/hen/week, feed conversion ratio 1.45/hen/week, egg weight 60.70g/hen/week, egg shell weight 6.80g/hen/week and egg shell thickness was 0.42mm/hen/week.

Group B: (Positive Control): The birds were given feed having aflatoxin level at 50ppb and without mycotoxin binder, the average feed consumption was 638g/hen/week, egg production was 80.00%/hen/week, feed conversion ratio was 1.56/hen/week, egg weight was 59.40g/hen/week, egg shell weight was 5.92g/hen/week and egg shell thickness was 0.35mm/hen/week.

Group C: The birds were given feed having aflatoxin level at 50ppb and mycotoxin binder sorbatox 0.1% of the feed, the average, feed consumption was 643g/hen/week, egg production was 85.20%/hen/week, feed conversion ratio was 1.53/hen/week, egg weight was 59.60g/hen/week, the egg shell weight was 6.30g/hen/week egg shell thickness was 0.37mm/hen/week.

Group D: The birds were given feed having aflatoxin level at 50ppb and mycotoxin binder sorbatox 0.2% of the feed, the average, feed consumption, egg production, feed conversion ratio, egg weight, egg shell weight and egg shell thickness were 653g/hen/week, 88.30%/hen/week,1.50/hen/week, 60.00g/hen/week, 6.50g/hen/week and 0.40mm/hen/week respectively.

Group E: The birds were given feed having aflatoxin level at 50ppb and mycotoxin binder bentonite with 2.5% of the feed, the average, feed consumption, egg production, feed conversion ratio, egg weight, egg shell weight and egg shell thickness were 651g/hen/week, 86.40%/hen/week, 1.54/hen/week, 59.50g/hen/week, 6.40g/hen/week and 0.39mm/hen/week respectively.

Group F: The birds were given feed having aflatoxin level at 50ppb and mycotoxin binder bentonite 5.0% of the feed, the average, feed consumption, egg production, feed conversion ratio, egg weight, egg shell weight and egg shell thickness were 656g/hen/week, 89.50%/hen/week, 1.52/hen/week, 60.25g/hen/week, 6.60g/hen/week and 0.41mm/hen/week respectively (Table 3).

Mortality: There was only 1% mortality observed throughout the experiment.

Economics (Cost per Dozen Eggs): The economics of different experimental groups was also calculated. Price of ration of A, B, C, D, E and F were 9.40, 9.43, 9.60, 9.78, 9.53 and 9.55 Rupees/dozen respectively. Apparently the ration A was the cheapest. The cost for rations A, B, C, D, E and F was 14.60, 16.0, 16.45, 15.40, 15.10 and 15.0 rupees/dozen eggs respectively.

Table 3. Effects of mycotoxin binders on feed intake, egg Production and feed conversion ratio, egg weight, egg shell weight and egg shell thickness.

Groups	Feed intake g/hen/week	Egg production (%/hen/week)	FCR	Egg Wt (g)	Shell Wt (g)	Egg shell Thickness (mm)
A	660	92.00	1.45	60.70	6.80	0.42
B	638	80.00	1.56	59.40	5.92	0.35
C	643	85.00	1.53	59.60	6.30	0.37
D	653	88.00	1.50	60.00	6.50	0.40
E	651	86.00	1.54	59.50	6.40	0.39
F	656	89.00	1.52	60.25	6.60	0.41

Group A: Feed without aflatoxin and mycotoxin binder (-ve control)
Group B: Feed with 50ppb aflatoxin and without mycotoxin binder (+ve control)
Group C: Feed with 50ppb aflatoxin + sorbatox 0.1% of the feed
Group D: Feed with 50ppb aflatoxin + sorbatox 0.2% of the feed
Group E: Feed with 50ppb aflatoxin + bentonite 1.0% of the feed
Group F: Feed with 50ppb aflatoxin + bentonite 2.0% of the feed

DISCUSSION

The maximum egg production, feed consumption and minimum FCR was observed in group A (the group served as negative control, which had no aflatoxin and mycotoxin binder), the minimum egg production and feed consumption was in group B. the group A, D, E and F had a little difference in feed consumption, egg production and FCR. Statistically there was no significant difference ($P > 0.01$) among the groups for feed consumption, egg production and FCR. The results of study are in agreement with Muthiah *et al* (1998) that decrease in feed consumption, and egg production was observed when aflatoxin B1 was added in the feed. Ghosh *et al* (1990) reported that when feed was contaminated with aflatoxin, poor feed consumption and reduced egg production was observed. Eraslani *et al* (2004) also reported that aflatoxin in feed significantly reduce the egg production. The reasons of non significant difference for this parameter might be due to the aflatoxin level 50ppb not enough to produce the significant effect.

From this experiment it was found that the highest egg weight was observed in group A and lowest in group B. The group A, D, and F had almost same value of egg weight and group B, C and E had apparently minor difference. The similar results were observed by Fatima *et al* (2000) and Rizzi *et al* (2003).

The results of egg shell weight in this experiment showed that highest value was observed in group A and F. The lowest value observed in group B. The value of group C, D, and E were close to the group A. In group F in which 5% of the feed bentonite used appeared to be the most effective mycotoxin binder against the aflatoxin. The results were agreement with the Belive *et al* (1998) who observed that no significant difference was observed in the egg shell weight of layer, when feed was contaminated with 18ppm mycotoxin. These results are also similar as Trchova *et al* (2004).

The highest egg shell thickness was observed in group A and F, while the lowest in group B. The value of group D and F of egg shell thickness were almost comparable to group A in which no aflatoxin was added. The egg shell thickness of group C was almost similar to the group E. There was no significant difference in egg shell thickness among the groups. The results were in agreement with Kubeana *et al* (1987) who observed that no significant difference was observed in egg shell thickness of white leghorn when feed was contaminated at 18ppm mycotoxin level.

There was only 1% mortality observed through out the experiment as layers were carefully managed and properly ventilated. The reason might be that the layers could have produced some resistance against the adverse effects of aflatoxin. This resistance is related with their age, proper level of protein and metabolized energy. High level of aflatoxin could have caused the mortality as observed by Mani (1992) who reported high mortality when chicks were given feed contaminated with higher level of aflatoxin 0.75-2.00 ppm. The ration A was found to be the cheapest as 24.00 Rs per dozen. The ration C was found to be more expensive.

CONCLUSIONS

From the results of this experiment, it is concluded that, aflatoxin level of 50ppb has no significant effect on the overall performance of the white Leghorn hen ($P > 0.01$) and it is evident that the sorbatox at the rate of 0.2% of the feed and bentonite at the rate of 5% produced almost the similar results when compared with the control group (negative control).

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Status of iodine in different age groups of school children in some selected areas of District Gilgit

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ABSTRACT

The objective of this study was to evaluate the urinary iodine concentration in some selected areas of District Gilgit to estimate the prevalence of endemic goiter in the. The sample population was a total 40 referred to Food Laboratory for urinary iodine measurement. Urinary iodine levels were determined using spectrophotometeric techniques with wavelength of 405nm. The results reveled that out of forty samples from selected areas (Jalalabad Oshikhandass, Danyore, Sultanabad) of District Gilgit, no deficiency of iodine in 18 samples, mild in 18 and moderate in 4 while the median urinary iodine level aged 5-6 (7.42 µg/dL), 7-8 (10.21 µg/dL), 9-10 (10.02 µg/dL), 11-12 (9.7 µg/dL) and 13 -14 (7.06 µg/dL) respectively. When these results were compared with the standard guideline developed by ICCIDD /WHO/ UNICEF, it was observed that the District Gilgit surveyed were in mild stage of IDD severity and in this stage the need for correction is important

Keywords: Urinary iodine, Iodine deficiency, School children, District Gilgit.

INTRODUCTION

Iodine deficiency disorders (IDDs) are one of the worldwide public health problems of today. Their effect is hidden and profound affecting quality of life (Tiwari 1995). Globally 2.2 billion people live in areas with iodine deficiency and risk its complications.

Iodine deficiency is recognized as the most important preventable cause of mental defect in the world today. Communities living in an iodine-deficient environment face a major block to their human and social development. Correction of iodine deficiency is indicated as a major contribution to development (Hetzel 1993). In the Eastern Mediterranean Region (EMR) of WHO at least 10 of the 22 Member States (Afghanistan, Egypt, Islamic Republic of Iran, Iraq, Libyan Arab Jamahiriya, Lebanon, Pakistan, Syrian Arab Republic, Sudan and Tunisia) have high to alarming rates of prevalence of goitre in certain areas (Alexandria 1990) .

Iodine is an essential element required by the thyroid gland for the synthesis of thyroid hormones. The thyroid hormones play a crucial role in the regulation of the growth, development and metabolism of nearly all tissues of the body. The normal adult intake of iodine should be at least 100 mg day±1, more in pregnant and lactating women. Below this level, insufficient amounts of thyroid hormones may be produced resulting in recognized manifestation of iodine deficiency for which the term `iodine deficiency disorders' (IDDs) has been coined. The IDDs include abortions, stillbirths, low birth weight, deaf-mutism, short stature, mental retardation and goiter and its complications.

The aim of this study was to assess the iodine status of District Gilgit schoolchildren aged 5-14 years. As subjects, school-aged children are readily accessible, enthusiastic and honest participants, and the indicators used to assess iodine deficiency in this group are simple to obtain and well defined.

MATERIALS AND METHODS

For assessing the status of iodine in school children 40 children were included in the study. A total of 8 clusters were randomly selected from District Gilgit (Oshikhandass, Jalalabad, Danyore, and Sultanabad) (two clusters from each village) randomly selected five children having different ages 5-6, 7-8, 9-10, 11-12,13-14 years respectively, in primary, middle or high school in the area were considered as cluster. In this way 10 schools were considered (40 children) were selected for clinical examination. Further 10% sub samples from these were selected for urine examination.

Selection of Clusters (Schools): The cluster i.e. Primary, MiddLe or high schools were selected from each of the four villages, Danyore, Sultanabad, Oshikhandass, and Jalalabad. Since there was no existing sample frame, and therefore initially a listing of all primary, middLe and high schools were developed. The primary, middLe and high schools were included in order to select children between

ages of 5-14 years. Schools were selected by random strata.

Selection of children: Children with in each school were selected using (systemic selection) methodology. The total number of eligible children aged (5-14 years) was divided by 5 (the sample size for the cluster) to calculate the sampling interval "r". The first child was chosen by selecting a random interval "I" and the value of interval "r". The next child was selected by adding the first random number to the sampling interval. The process was repeated until all the 5 children were selected.

Collection of urine sample: After the selection of five children in each cluster, the urine sample was collected. A random number between 1 and 5 was selected for each cluster for the selection of urine sample among 5 selected children. Each urine sample was collected in a small plastic bottle containing few drops of toluene as a preservative. The bottle was then coded. All urine samples were brought to the laboratory for determination of iodine. Urinary iodine concentration were measured, using strong choleric acid for urine digestion at 120°C heating oven, iodine was determine by its catalytic reduction of ceric ammonium sulfate in the presence of arsenious acid known as sandell-kolthoff digestion reaction as followed equation,

$$2Ce^{4+} + 2I \longrightarrow 2Ce^{3+} + I_2$$

$$I_2 + As^{3+} \longrightarrow As^{5+} + 2I^-$$

The iodine concentration was determined using spectrophotometer technique with wavelength of 405nm (Dunn *et al* 1993; Sandell and Kolhoff 1937).

RESULTS AND DISCUSSION

The levels of median urinary iodine in forty urine sample of school going children ranged aged 5-6 (7.42 µg/dL), 7-8 (10.21 µg/dL), 9-10 (10.02 µg/dL), 11-12 (9.7 µg/dL) and 13 -14 (7.06 µg/dL) (Table 2). The overall prevalence of mild, moderate and severe iodine deficiency among schoolchildren (5-14 years) examined in selected areas of District Gilgit. The result s reveled that out of forty samples of selected areas (Jalalabad Oshikhandas, Danyore, Sultanabad) of District Gilgit no deficiency of iodine (18 samples), mild (18 samples), moderate (4 samples) and severe none (Table 3). On area-wise comparing the results of these urinary iodine level with the WHO/ UNICEF/ICCIDD (1993) epidemiological criteria for assessing the severity of IDD on median urinary iodine level, it was observed that in Jalalabad there was 1 moderate and 6 mild cases of IDD among 10 urine samples analyzed. In Oshikhandas there were 1 moderate and 5 mild case, in Danyore 2 moderate and 3 mild cases of IDD while in Sultanabad 4 mild cases of IDD were observed (Table 3).

Iodine deficiency disorders are still a major public health problem in many countries of the world in spite of the fact that the technology available for their prevention makes the problem the most amenable of the nutritional deficiencies to quick and effective control. The prevention and control of IDD, because of its dramatic impact on the quality of life, productivity and educibility of millions, would make a major contribution to the development of countries whose people are at risk of developing IDD. In addition, it would contribute significantly to the attainment of the World Health Organization's goal of health for all by the year 2000 (Hetzal 1987).

Implementing practical and effective surveillance is essential to control micronutrient deficiencies successfully. One of the main purposes of IDD surveillance is to determine the prevalence of such disorders and to identify high-risk populations and risk factors. This is important in order to develop and monitor programmes aimed at eliminating IDD.

Dunn and Vander Haar (1991) International Council for Control of Iodine Deficiency Disorders (ICCIDD)/WHO / UNICEF has developed a guideline for showing IDD severity and its need for correction Table -1. This guideline shows different stages of severity, mild stage moderate stage and severe stage of I.D.D.

The assessment of iodine status urinary iodine level used in this study. Which were found to be mild. WHO has define IDD as public health problem in a population , if the goiter prevalence rate in school children is greater than 5% and a major national plan has to be launched to combat the situation (Gurney M and Vander Haar 1993) .

These results indicate that while no severe IDD problem among the child population residing in District Gilgit, mild severity of IDD was detected by goiter based on median urinary iodine level was widespread. The Government of Pakistan is committed to eradicate IDD through introduction and continued use of iodized salt. In this regard a crash programmed of iodized oil injection / capsule has been under operation since 1987. Similarly a salt iodization programme has also been in operation to cover a target population of about 20 million populations residing in areas of endemicity. The iodized salt in these areas is being sold at par with salt of common

salt and additional cost of production is born by Government. This programmed has resulted in reduction of goiter in the Northern Areas by about 60%. Beside this 46% of the target population has been covered (Hussain 1995).

Table:-1 Proposed epidemiological criteria for assessing the severity of IDD based on median urinary iodine level (WHO & ICCIDD, 1997).

Median urinary iodine µg/ dL	IDD Stage
< 2.0	Severe
2.0 – 4.9	Moderate
5.0-9.9	Mild
10 ->10	No deficiency

Table:-2 Urinary Iodine level of different age group of school going children of District Gilgit

Parameters Age	Id_1	Id_2	Id_3	Id_4	Id_5	Id_6	Id_7	Id_8	Max	X	Min
5-6	4.3	12.7	3.3	12.1	11.1	2.4	11.1	2.4	12.7	7.42	4.2
7-8	6. 6	6.4	10.5	11.2	6.8	14.9	6.8	14.9	14.9	10.21	6.4
9-10	13.7	7.8	6.9	10.8	13.2	7.3	13.2	7.3	13.7	10.02	6.9
11-12	9.2	12.1	6.4	6.3	13.4	8.6	13.4	8.6	13.4	9.7	6.3
13-14	6.2	7.1	6.5	6.9	10.7	4.2	10.7	4.2	10.7	7.06	4.2

Id_1 F.G Boys H/S Jalalabad, Id_2 = F.G Girls H/S Jalalabad, Id_3 = F.G Boys H/S Oshikhandas, Id_4 = F.G Girls H/S Oshikhandas, Id_5 = F.G Boys/H school Danyore, Id_6 = F.G Girls/H school Danyore, Id_7 = English Grammar School Sultanabad, Id_8 = F.G Boys School Sultanabad

Table:-3 Severity of IDD based on the urinary Iodine level

Severity	Jalalabad	Oshikhandas	Danyore	Sultan Abad	Total
No deficiency	3	4	5	6	18
Mild	6	5	3	4	18
Moderate	1	1	2	0	4
Severe	0	0	0	0	0
Total	10	10	10	10	40

RESULTS AND DISCUSSION

The levels of median urinary iodine in forty urine sample of school going children ranged aged 5-6 (7.42 µg/dL), 7-8 (10.21 µg/dL), 9-10 (10.02 µg/dL), 11-12 (9.7 µg/dL) and 13 -14 (7.06 µg/dL) (Table 2). The overall prevalence of mild, moderate and severe iodine deficiency among schoolchildren (5-14 years) examined in selected areas of District Gilgit. The result reveled that out of forty samples of selected areas (Jalalabad Oshikhandas, Danyore, Sultanabad) of District Gilgit No deficiency of iodine (18 samples) , mild (18 samples), moderate (4 samples) and severe none (Table 3). On areas wise Comparing the results of these urinary iodine level with the WHO/ UNICEF/ICCIDD (1993) epidemiological criteria for assessing the severity of IDD on median urinary iodine level, it was observed that in Jalalabad there was 1 moderate and 6 mild cases of IDD among 10 urine samples analyzed. In Oshikhandas there were 1 moderate and 5 mild case, in Danyore 2 moderate and 3 mild cases of IDD while in Sultanabad 4 mild cases of IDD were observed (Table 3).

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Determination of selected metals in drinking water of water purification plants in Gilgit city

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ABSTRACT

In order to ascertain water quality for human consumption, trace and bulk metals pH and electric conductivity were evaluated in the water purification plant of Gilgit city. The samples were determined by atomic absorption spectrophotometer. Result indicated that low variation existed among the pH and electric conductivity (pH 7.42-7.92, electric conductivity 0.12–0.19). The mean level of trace metals (mg/L) ranges thus 0.025 (Cd), 0.007 (Cr) 0.006 (Pb), 0.011 (Ni), 0.017 (Cu), 0.308 (Mn), 0.215 (Fe), 0.112 (Zn) and mean level of bulk metal was 2.73 (Na), 5.126 (K), 39.52 (Ca), and 10.75 (Mg). Comparisons of these water samples obtained from water purification plants with WHO limits showed that the mean level of all metals were below the maximum permissible levels for drinking water.

Keywords: Trace and bulk metals, water purification plant, WHO limits, Gilgit

INTRODUCTION

The human body is primarily composed of water which contributes approximately 90% of blood plasma, 80% of muscles tissues, 60% of red blood cells and more than 50% of most other tissues. Water is extremely important nutrient for body. Its function varies from regulating body temperature to transporting waste products. Today's health conscious society, now more than ever, focuses on importance of water. (Shalina and Eisenbnerg 2004)

Water covers about 73% of the earth's surface. It is the major constituent of the lithosphere and atmosphere and it is an essential requirement of all living organisms. The largest water requirement is for municipal use but standard of purity required for this purpose is one of the prime factors in deciding the growth of towns and cities as well as industries (WHO 1984).

Interest in water analysis is due to the enormous importance of water to all categories of living things. It is necessary for the healthy development of man, animals and plants. Developing countries are witnessing changes in ground water, which constitute another source of portable water. The preference for ground water to surface water must be due to the purification of the latter prior to distribution (Adeyeye 2004).

Drinking water plays an important role in the bodily intake of true element by human. Even through some trace elements are essential to man, at elevated levels essential as well as non essential element can cause morphological abnormalities: reduce growth increase mortality and mutagenic effects (Nkono 1998, Adeyeye 2000).

The toxicity of metals is dependant on their solubility and this in turn depends on pH and on the presence of different types of anions and other cations. Water pollution has been a subject of active investigation for a long time. Interest in this has grown because of the perceived hazardous effects of trace element.

The aim of this study was to assess trace and bulk metals determination of drinking water of water purification plants in Gilgit city

MATERIAL AND METHODS

Sample collection: Water samples were collected form six different location of water purification plant in Gilgit. The water samples were collected in two liter polythene bottles. Samples for analysis were acidified prior to analysis with a few drops of HNO_3 to keep the metals in solution while pH and electric conductivity were determined through pH meter and conductivity meter.

Sample analysis: For metals analysis, the water samples where acidified with nitric acid. A 100 cm^3 aliquot of the sample was digested with HNO_3 in a beaker at 120C until a clear solution was obtained (Abulude 2005). All samples were use to determine the trace and bulk metals using genosys 10uv (Thermospectrum) model atomic absorption spectrophometer. Mean, maximum and minimum values were calculated.

RESULTS AND DISCUSSIONS

In order to ascertain water quality for human consumption, pH, electric conductivity, trace and bulk metals were evaluated in the water purification plant of Gilgit city. Result indelicate that low variation existed among the pH and electric conductivity (Range pH 7.42-7.92, electric conductivity 0.12–0.19). Maximum pH vale was recorded in Kashorate samples and minimum in Jutial while the electric conductivity, maximum in Konadas and minimum in DHQ samples. (Fig: 1 and 2).

The mean level of trace metals and bulk metals (mg/l) ranges thus 0.009 (Cd), 0.011 (Cr) 0.008 (Pb), 0.017 (Ni), 0.025 (Cu), 0.418 (Mn), 0.215 (Fe), 0.358 (Zn) and while bulk metals ranges 4.09 (Na), 5.126 (K), 140.51 (Ca) and 30.80 (Mg). Comparisons of these water samples obtained from water purification plants with WHO limits showed that the mean level of all metals were below the maximum permissible levels for drinking water.

The minor mineral components i.e. copper, zinc, manganese, lead, nickel, cadmium and chromium were analyzed and compared with drinking water standards. Cadmium was present in the range of 0.0015-0.009mg/L. The concentration of lead was in the range of 0.005- 0.008 mg/L. the total weekly intake of cadmium and lead through food, water and air established by WHO (WHO 1996) are 0.3500 and 0.525 mg/L. The levels of these minerals are well below the WHO drinking water standards.

Chromium was present in the range of 0.004-0.011mg/l , lead was found in the range of 0.005-0.008 mg/L and nickel was found in the range of 0.007-0.017 mg/l which is also well below the WHO and PCI drinking water standards. Heavy metals poisoning particularly lead and cadmium have been reported to give rise to quite a number of chemical syndromes. For example cadmium accumulation is associated with hypertension, osteomalacia and itai-itai disease. Lead poisonining have been found to be associated with permanent brain damage, behavior disorder and impairing hearing. (Asaolu 2002). But in our study these metals are well below the WHO standards.

Level of Zinc was in the range of 0.025-0.35mg/L with maximum amount from the sample collected from Jutial. Zinc imparts an undesirable astringent taste. Tests indicates that 5% of population could distinguish between zinc free water and water containing zinc at the level of 4mg/L. water containing zinc at the in the range of 3-5 mg/L also tend to appear opalescent and develop a greasy film when boiled. Vomiting occurs after the consumption of more than 500mg of zinc sulfate (WHO 1996).

Fig.1 pH in water sample of purification plants of water sample

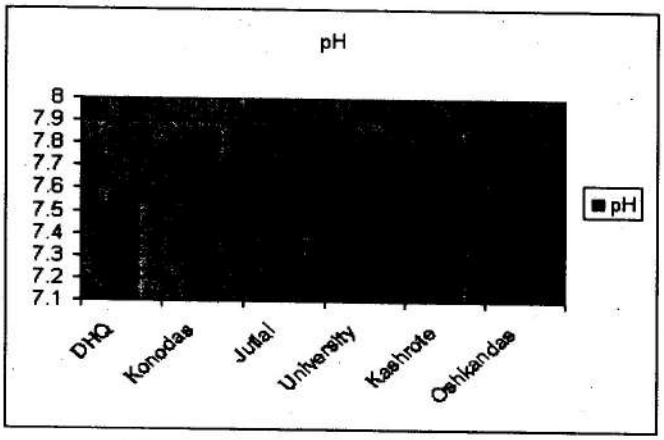
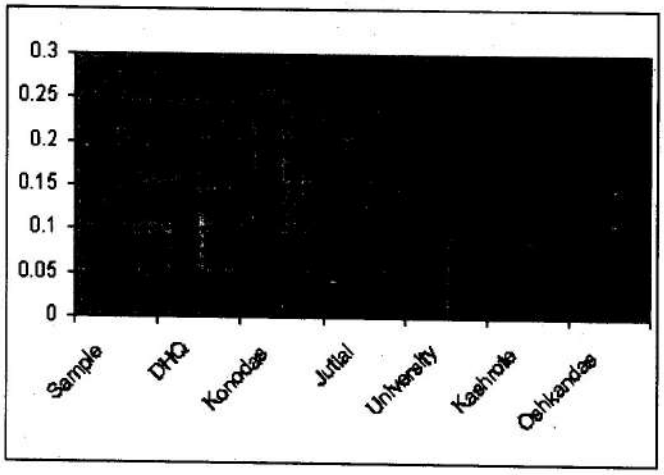


Fig. 2 Electric conductivity in water sample of purification plant



Concentration of bulk metal: Concentration levels (ppm or -mg/L ± SD) of bulk metals (sodium, magnesium, calcium and potassium) of water samples, collected from various water purification plants are given in Table 3.

Concentration of sodium was in the range of 1.525-4.095mg/L with maximum concentration detected from the sample of Kashrote filtration plant. American Heart Association implied that a limit of 20 mg sodium per liter might be adopted, in order to afford protection to those individuals with heart and kidney ailment, who require a low sodium diet. (Edward 1983.)

Sodium compounds naturally end up in water. Not only seas, but also rivers and lakes contain significant amounts of sodium. Concentrations however are much lower, depending on geological conditions.

Potassium was present in the range of 2.467-5.126mg/L. Highest amount was present in the sample collected from jutial, that may be because of rock type present on the side of that area.

Both calcium and magnesium share left / right-sided cell receptors and are essential to human health. Calcium (Ca) and magnesium (Mg) have become the "Gold Standard" when discussing supplements, mineral ratios, paired cell receptors, or many nutrition-related health issues. In general calcium is now the most promoted nutrient by proponents of conventional, nutritional, and alternative medicine - yet at the same time, the assumed need is based purely on the speculation that the body's calcium intake is well below its requirements. Over 300 enzymes that influence the metabolism of carbohydrate, amino acids, nucleic acids and protein, and ion transport, require Mg (Wracker 1980).

In our study calcium was in the range of 3.45-140.27mg/L with maximum concentration in the sample of Konodas. Magnesium was in the range of 0.4-30.80 mg/L.

Table 1 Water quality standards

Parameter	DHQ	Konodas	Jutial	University	Kashrote	Oshkandas	Min	X	Max
Cadmium	0.002	0.0025	0.0015	0.0018	0.0017	0.009	0.0015	0.003	0.009
Chromium	0.01	0.004	0.009	0.007	0.004	0.011	0.004	0.007	0.011
Lead	0.008	0.007	0.007	0.005	0.007	0.005	0.005	0.006	0.008
Nickel	0.009	0.007	0.01	0.015	0.017	0.012	0.007	0.011	0.017
Copper	0.025	0.019	0.01	0.025	0.008	0.015	0.008	0.017	0.025
Manganese	0.418	0.173	0.314	0.414	0.189	0.341	0.173	0.308	0.418
Iron	0.187	0.041	0.0179	0.174	0.088	0.215	0.018	0.120	0.215
Zinc	0.044	0.048	0.146	0.025	0.056	0.358	0.025	0.112	0.358

Table 2. Trace metals content (mg $^{L-1}$) of water samples

Parameters	DHQ	Konodas	Jutial	University	Kishrote	Oshkandas	Min	X	Max
Sodium	1.525	3.99	2.628	1.656	4.095	2.513	1.5	2.7345	4.095
Potassium	2.467	3.973	5.126	2.745	3.958	3.439	2.5	3.618	5.126
Calcium	8.524	140.272	3.453	8.339	72.952	3.565	3.5	39.518	140.27
Magnesium	0.359	30.807	0.745	0.373	26.494	5.732	0.4	10.752	30.807

Table 3. Data of bulk metals in samples of water purification plants of Gilgit city

Water Quality Parameter	WHO Drinking Water Standards (mg/L)	PCI Drinking Water Standards (mg/L)
Cadmium	0.003	0.001
Chromium	0.050	0.05
Lead	0.010	0.05
Nickel	0.020	0.05
Copper	2.00	1.00
Manganese	0.50	0.10
Iron	0.30	0.30

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