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Comparative investigation, formulation and composition of guava (*Psidium guajava* L) slab/leather

Mohammad Nisar Khan Alizai

Pakistan Council of Scientific and Industrial Research Laboratories Complex, Peshawar

ABSTRACT

Guava slabs were made from fresh guava pulp using hot air dehydration. No colour, preservative or essence were added to the product. Comparative investigation of fresh guava pulp and their products were conducted for moisture, dry matter, ash, sugar, soluble pectin and ascorbic acid. Mineral contents (calcium, magnesium, sodium, potassium and phosphorous) were determined. Shelf life of the products was monitored for more than twelve months. No change in colour, flavour or taste was observed during storage at room temperature. Sensory evaluation of the products revealed that guava slab sample-I was excellent scoring sensory (27 points) followed by slab sample-II got (25.5 points). Whereas senosory characteristics fell after 9 months of storage at 25°C .

Key Words: Guava slab, nutrient, minerals.

INTRODUCTION

Guava (*Psidium guajava L.*) is tropical fruit with appealing taste and aroma, abundantly produced in Pakistan. Most of the produce has yellow colour though off white, greenish yellow, reddish white and greenish white are also available in Northern Pakistan. Physical characteristic vary depending upon commodity and cultivars variety. Fruit is with or without seed and contains high amount of ascorbic acid. (Mann and Termazi 1967; Khan *et al* 1991).

There seems to be little utilization of the fruits in commercial product, although one report (Khedkar *et al* 1982) suggests a fraction of the fruit produced is employed in jelly production. Dehydration of fruit has been reported earlier with the objective of its utilization in pectin and jelly (Mapson 1971). Most fruit is wasted due to browning, or during transportation as a result of improper packaging or over loading in the carriers. Shelf life of the fresh fruit is limited, approximately 10-15days. Fruit changes colour on ripening, bruising, cutting or mishandling during transportation. Control of browning in raw guava and fresh juice/concentrates by using polyphenol oxidase inhibitors and their chelating agents have been reported (Alizai and Fazalur Rehman 1993, Alizai and Ahmad 1997). Keeping in view to control the post harvest losses and for the benefit of the growers it is need of hour to make new guava products which have enlarge shelf life and can be handy to carry and easy to consume. For that reason products such as guava slabs have been formulated without using food additives and preservatives. Sensory evaluation and nutritional analysis were conducted to see the efficacy of the products as nutritious and palatable food for human consumption. This product will have attraction in local market and abroad due to natural nutritional contents as compared to synthetic products available in the markets.

MATERIALS AND METHODS

Fresh guava fruit was purchased from the local market and transported to the Laboratory under hygienic conditions. Fruit was thoroughly washed under tap water. Rusted, damaged, bruised and brown portions were discarded during sorting. Good quality, ripe wholesome fruits were cut in to pieces using stainless steel knives and kept in water to prevent browning. Cut pieces were passed through pulper using (3/32 mesh) sieve to remove the seeds and peels. Few pieces of fresh guava (approximately 500 g) from whole lot were selected prior to passing through pulper. These were sealed in polythene and stored in freezer at -20° C for analysis.

Guava slurry obtained from the pulper was of 13°Brix . 1-2 % sugar was added while stirring to sweeten of the product. Apart from (1-2%) cane sugar, (0.1-0.15%) liquid glucose and (0.01%) honey were added with stabilizer and flavour enhancers. Percentage of the honey depended

upon the consistency along with polyphenol oxidase inhibitors to prevent browning and to give shine to the product. Addition of sugar varies and depended upon the variety of the guava because few varieties are more sweet than the others. The mixture was thoroughly blended to a uniform consistency. This was evenly spreaded in stainless steel trays lined with polythene sheet. Each tray contained 1.5 to 2.0 Kg material. Trays were placed in cabinet shelf dryer previously heated to 90-100°C. Temperature was, reduced to 70°C and dryer was run overnight to get the desired level of moisture (approximately 6%). After thirty hours the product was removed from the trays and placed in aerated iron trays to keep further two hours in hot air dehydrator so that both sides of the slab may be dried properly. The product was evenly cut one inch square that weighed approx. 4.0g each, wrapped in slab paper and then packed in polythene bags, sealed, stored at room temperature. Same way guava slabs were made approximately 1.5 x 3.0 inches each and packed in labelled transparent polythene bags and sealed. Product was stored at room temperature and after every fifteen days analysed for colour, odour and texture. Sensory evaluations were also carried out.

Besides the fresh fruits the products such as slabs were analysed separately for its proximate composition (a) freshly made product (b) six months old (c) nine months (d) twelve months and above. The pH was determined using digital Corning 102 pH meter. Moisture, soluble pectin and tannins were estimated by following (Ruck 1975) method. Total soluble solids were measured by using Abbe's refractometer at 20°C. Total acidity was determined by titration against 0.1 N sodium hydroxide solution and results were expressed as percent citric acid. Ascorbic acid and sugars were determined by the method of (AOAC 2000). Ash was made from the seedless fruit at 525°C in furnace. Calcium, magnesium, phosphorous, potassium and sodium were determined from ash. Calcium was determined in solution of ash by precipitating as calcium oxalate, determining titrimeterically while filtrates and washing obtained from the above were used for the magnesium determination. Mg was precipitated, dried, ignited and weighed as magnesium pyrophosphate (AOAC 2000). Phosphorus was determined by using Shimadzu double beam UV 200S spectrophotometer. Jenway PEP 7 Model digital flame photometer was used to estimate sodium and potassium. All the chemicals used were of analytical grade.

RESULTS AND DISCUSSION

Composition of dehydrated guava pulp without addition of sugar alongwith guava slabs stored at room temperature for the period of three, six, nine, twelve and more are given in Table-1. These results are compatible for dehydrated guava beverage base reported by (Khan *et al* 1991 and Singh *et al* 1983). Pectin is somewhat low, obviously due to ripening of the fruit utilized in formulation of the guava slab, while tannin increased on storage. Nonenzymatic browning is due to Maillard reaction which can be restricted by quick processing of the fruits

Table 1: Composition of fresh and dehydrated guava fruit product

S.No.	Sample	Moisture (%)	Ash (%)	Soluble Pectin (%)	Tannins (%)
1.	Freshly dehydrated	4.0	1.15	1.7	0.06
2.	Three months old	4.11	1.16	1.85	0.062
3.	Six months old	4.5	1.16	1.62	0.065
4.	Nine months old	5.62	1.18	1.32	0.075
5.	Twelve months	6.9	0.99	1.26	0.078

at low temperature whereas enzymatic browning can be controlled with polyphenol oxidse inhibitors and their chelating agent or by keeping fruit dipped in water during cutting, slicing, etc. to avoid oxidation during the process (Alizai 1993, 1997).

Enzymatic browning can be controlled in some fruits/vegetable products by blanching to inactivate polyphenol oxidation (Mc Cord and Kilara 1983; Hall1989; Ma silva 1992). Blanching cannot be used in guava because it adversely affects on flavour and texture, consequently other approaches such as exclusion of oxygen and application of browning inhibitors can be useful (Alizai 2000, 2001).

Comparison of nutritional constituent of freshly dehydrated guava fruit products stored for two, three, six, nine, twelve and above at room temperature is given in Table-2. Although analysis were conducted without addition of sugar but found little higher than reported by (Siddiqui and Farooqui 1959; Khan *et al* 1991).

Ascorbic acid content of fresh fruit is known to be variable due to the conditions of the fruit as ascorbic acid (vitamin C) decreases on ripening but analysis of the dehydrated pulp shown in Table-2 is compatible to the finding reported by (Jabbar *et al* 1988, Pollard and Timberlake 1971 and Khan *et al* 1991). Comparatively ascorbic acid (vitamin C) is considered to be effective as its role of polyphenol oxidase inhibitor to control the browning of fruits and vegetables (Saper 1989) but guava is found high in ascorbic acid content and highly prone to browning due to ascorbic acid which completely oxidized to dehydro ascorbic acid by reacting to make quinones which can accumulate and undergo browning (Sapers 1993). Special measures such as packing under anaerobic and storage under controlled atmospheric conditions could improve the situation. Studies were carried out to control the browning with polyphenol oxidase inhibitors in these laboratories (Alizai 2000). Further more sugar which it self is a preservative can be added to product to enhance the shelf life. Slabs prepared are found golden pale yellow or slightly golden yellow with brownish streaks on the side of the dehydrated sheet of guava pulp.

Mineral constituents were determined from formulated guava slabs (Table-3). Data presented for dehydrated guava product is in agreement with those already published for Pakistani species of the guava though wide variation in mineral constituents are reported abroad (Huxsoll et al 1989). Hardlicka (1959) has reported calcium content as 43 mg/100g, (Ma and Garner 1992) found 10 mg/100 g in Australian guava fruit. Similar variations are found in potassium and sodium contents

Table 2: Nutritional constituent of dehydrated guava fruit slab analysed during different period of months stored at room temperature

	Sugars				
Months	**Reducing (%)**	**Non reducing (%)**	**Total (%)**	**Citric acid (%)**	**Ascorbic acid mg/100 g**
Fresh	12.4	84.4	96.8	1.45	215.6
02	12.1	83.4	95.5	1.41	211.8
03	12.9	81.5	94.4	1.40	210.6
06	13.35	80.0	93.35	1.38	210.8
09	13.45	80.9	94.35	1.28	208.8
12	13.25	80.0	93.25	1.20	208.0

Our data shows potassium lower but sodium is higher as reported for Indian and Australian fruit (Shewfelt RL 1986). These variations can be justified if we look into ascorbic acid contents reported in literature which are tenfold higher in guava produced in different areas of the world which may be due to the specie concerned and climatic effect along with soil composition of that particular area. Apart from analytical investigation of fresh guava slabs were formulated and their organoleptic evaluation were conducted at different period of time (Table-4) to see the efficacy of the product and to see whether or not it is perfect food for human consumption.

Table 3: Mineral composition of guava slab

	Calcium mg%	Potassium mg%	Phosphorous mg %	Magnesium mg %	Sodium mg %
Guava fresh Sample I	0.029	0.464	0.014	0.036	0.011
Guava (one month old) Sample II	0.0285	0.465	0.016	0.038	0.0095
Guava (two months old) Sample III	0.0288	0.465	0.015	0.038	0.0102
Guava (three months old) Sample IV	0.0287	0.464	0.015	0.037	0.010

Table 4: Sensory evaluation of guava slab

S.No.	Guava*	Taste, 10	Colour, 10	Texture, 10	Total score, 30
1.	Fresh				
	Sample I	9.0	9.0	9.0	27
	Sample II	8.5	9.0	8.0	25.5
2.	Three months				
	Sample I	8.5	9.0	9.0	26.5
	Sample II	8.5	8.5	8.5	25.5
3.	Six months				
	Sample I	8.5	8.5	8.5	25.5
	Sample II	8.5	8.5	8.5	25.5
4.	Nine months				
	Sample I	7.0	7.5	7.0	21.5
	Sample II	7.5	7.0	7.0	21.5
5.	Twelve months				
	Sample I	6.5	7.0	6.5	20.0
	Sample II	6.5	6.5	6.5	19.5

*Sensory evaluation of each sample was average of fifteen judgements

CONCLUSION

Fresh guava fruit has limited shelf life. Fruit is prone to browning and it deteriorate too quickly. Fruit is usually wasted during transportation or in the farms. To minimize the financial losses to growers and to utilize maximum guava produce, product such as guava slabs were formulated which have long shelf life and will be easily available to ordinary customers. Keeping in view as a natural food product with intact vitamins and minerals the product will compete with synthetic products available in the market.

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Studies on the fatty acid composition of Kinnow peel and seed oil

Sherin Iqbal, Azra Yasmin, Ghulam Mohuddin Paracha

Food Technology Center, PCSIR Laboratories Complex. Peshawar.

ABSTRACT

The oil was obtained from peels and seeds of Citrus Sinensis variety Kinnow (a hybrid) by soxhlet apparatus to investigate the physico-chemical characteristics. Physical characteristics i.e., odor, color, specific gravity, refractive index and acid value were determined according to standard procedures. Gas Chromatographic analysis revealed that peel oil was composed of 61.7% saturated fatty acids and 2.7% unsaturated fatty acids while seed oil content was composed of 76.8% saturated fatty acids and 6.0% unsaturated fatty acids. The major components of peel oil were comprised of capric acid (53.6%), caproic acid (3.7%), lauric acid (1.9%), palmitic acid (1.2%), myristic acid (0.6%), stearic acid (0.2%), oleic acid (0.7%) and linoleic acid (1.9%).

Keywords: Citrus, saturated fatty acids, unsaturated fatty acids, gas liquid chromatography

INTRODUCTION

A variety of citrus fruits such as oranges, kinnows and lemons are available in Pakistan. The fruits are generally processed for fruit juices and squashes and their peels, which are by-product/ waste of the citrus processing industry, are sources for various essential citrus oils. Kefford and Chandler (1970). The peel oils of these fruits are widely used as flavours alone or in combination with other oils in beverages, ice cream, baked foods, pharmaceutical emulsions, confectionery and other food products. These oils are popular for their pleasant and refreshing odors.

Citrus fruits are processed for the extraction of juice and preparation of jam. In industries, million of tonnes citrus peels are wasted during processing. Citrus peels are used for oil extraction throughout the world. (Braddock and Kerston 1976; Lawrence 1990, 1999). In Pakistan most of the peels are wasted and peel oils are extracted to a limited scale and has to be imported to meet the requirements of the country. Citrus oils are utilized in perfumery cosmetics Gunther (1949) pharmaceuticals, soft drinks, beverages, ice cream, confectionery and other food products.

The other by-product of the citrus processing industry is its seeds that are on average amount of nearly 2% of the whole fruit. The citrus seeds yield on average 33% fixed oil, which is rich in essential unsaturated fatty acids. Kefford and Chandler (1970).

As a result of the processing of fruit for squashes, juices and jam, about 40-60% of the weight is left in the form of pulp, rag, seeds and peels as waste materials which are thrown away outside the factory. Due to the fermentable nature, it ferments quickly, creates pollution and disposal problems. These waste materials contain sufficient quantity of nutrients such as proteins, sugars and minerals. Therefore these waste materials can be utilized for various useful products. The seed oils can be used for edible purposes as well as for soap making. These citrus oils are presently being imported annually. Present studies deal with the physico-chemical investigation of Kinnow peel and seed oil.

MATERIALS AND METHODS

Fresh fruits of kinnow were purchased from local market. The fruit was sorted out, washed and dried with cloth. The fruit was cut into halves and juice was extracted through reamer. Seeds and peels were separated, washed and dried at room temperature. Juice was processed for product production. Oil was extracted from seeds and peels. Kinnow seeds and peels were separately crushed coarsely and packed in thimble of soxhlet apparatus. The seeds and peels were extracted with hexane for 4-5 hrs. and solvent was removed under vacuum. Yellow color oil from seeds and dark yellow color oil

from peels have been obtained. Percentage yield of the oil was determined.

Physico-chemical characteristics i.e., specific gravity, refractive index and acid value were determined according to standard methods (Gunther 1948; 1949; AOAC 2000) whereas chemical composition was studied by Gas Liquid Chromatography Uchida *et al* (1984)

Preparation of Methyl Esters

Fresh transmethylating reagent was prepared. 2 mL of transmethylating reagent, 20% methanolic sulphuric acid was added to each tube containing 200 mg oil samples. The tubes were tightly screwed and heated in oven at 80°C for two hours. After cooling 2 ml of distilled water was added to each tubes and the methyl esters were extracted thrice with diethyl ether. The combined extract was concentrated to 1 mL by flashing with nitrogen.

Gas Chromatographic Analysis

Gas chromatographic analysis was conducted on gas chromatography (Perkin Elmer Model 3920) equipped with flame ionization detector (FID) and a non-polar glass column (6 ft x 2 mm) packed with 20% DEGS on chromsorb w80-100 mesh. The temperature of detector and injector were maintained at 250°C and 200°C, respectively. The column oven temperature was maintained at 200°C. The carrier gas used nitrogen with a flow rate of 25 ml/min. Peak area and concentration were calculated by intersmat. Peak area and concentration were identified by comparing the resolution time with authentic standards run under the same parameters as well as by co-injection technique.

RESULTS AND DISCUSSION

The oil was obtained by soxhlet apparatus. The yield of oil from peel and seed was 0.68% and 33.00% respectively. The physico-chemical characteristics and chemical composition of oil was determined. (Tables 1 & 2). The physical properties i.e., specific gravity, refractive index and acid value were determined according to standard methods.

The observed value of refractive index was 1.4734 for peel oil while it was 1.4688 for seed oil. The refractive index of reported sweet orange is 1.48 at 20°C. The resolution and identification of the components of the oil was obtained by GC using co-injection technique and authentic standard samples.

The fatty acid concentrations of kinnow seed and peel oils as methyl esters were also determined. Both saturated and unsaturated fatty acids were determined. The saturated fatty acids included C_6 Capric acid, C_8 Caproic acid, C_{12} Lauric acid, C_{14} Myristic acid, C_{16} Palmitic acid and C_{18} Stearic acid. The unsaturated fatty acids as methyl esters were consists of $C_{18:1}$ Oleic acid and $C_{18:2}$ Linoleic acid. The concentration of saturated fatty acids was determined in percentage. C_6 Capric acid in seed oil was 18.7% whereas in peel oil was 53.9%. C_8 Caproic acid in seed oil was 12.9% and in peel oil was 2.7%. C_{12} Lauric acid in seed oil was 3.8% and in peel oil was 1.9%. C_{14} Myristic acid in seed oil was 6.5% and in peel oil was 0.6%. C_{16} Palmitic acid in seed oil was 26.1% and in peel oil was 1.2%. C_{18} Stearic acid

Table 1: Physico-chemical Characteristics of Kinnow Seed and Peel Oil

S.No	Parameters	Peel Oil	Seed Oil
1.	Yield	0.68%	33.0%
2.	Color	Dark Yellow	Yellow
3.	R.I at 40°C	1.4734	1.4688
4.	Acid Value	3.34%	4.50%
5.	Specific gravity	0.847	0.917

was 8.6% in seed oil whereas it was 0.2% in peel oil.

The concentration of unsaturated fatty acids was also determined in percentage. $C_{18:1}$ oleic acid in seed oil was 3.6% and in peel oil was 0.7%. $C_{18:2}$ linoleic acid in seed oil was 2.3% whereas in peel oil was 1.9%

Table 2: Fatty Acid Concentration of Kinnow Seed and Peel Oil as Methyl Esters

S.No	Fatty acid as methyl esters	Kinnow seed Concentration	Kinnow peel Concentration
A	**Saturated Fatty Acids**	**%age**	**%age**
	C_6 Capric Acid	18.7268	53.9625
	C_8 Caproic Acid	12.92	3.7325
	C_{12} Lauric Acid	3.843	1.9125
	C_{14} Myristic Acid	6.54	0.6582
	C_{16} Palmitic Acid	26.1155	1.2589
	C_{18} Stearic Acid	8.6892	0.2235
	Total	**76.8345**	**61.7481**
B	**Unsaturated Fatty Acids**		
	$C_{18:1}$ Oleic Acid	3.6474	0.7729
	$C_{18:2}$ Linoleic Acid	2.3599	1.9903
	Total	**6.0073**	**2.7632**

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Nutritive value of some bakery products from selected bakeries in Faisalabad

Muhammad Shoaib Akhtar[1], Nadia Zulifqar[2], Nazir Ahmad[3], Nighat Bhatty[2] and Amanat Ali[4]

[1]Department of Physiology and Pharmacology, [2]Department of Home Economics and [3]Department of Animal Reproduction, University of Agriculture, Faisalabad 38040, Pakistan. [4]Department of Food Science and Nutrition, College of Agriculture and Marine Sciences, Sultan Qaboos University, Muscat, Oman.

ABSTRACT

In the baking industry, cakes and biscuits occupy primary position, both for production and utilization, as compared to other bakery products. For healthy eating and planning and for community nutrition, it is important to know the nutritional facts about these products. The nutrient composition and nutritional quality of some bakery products available in Faisalabad, Pakistan was studied. The random samples of three types of cakes and three types of biscuits were purchased directly from these four bakeries and analyzed in our nutrition laboratory. The proximate analysis results showed that moisture content was obviously higher in the cakes than biscuits. The differences in moisture contents of three types of cakes were highly significant ($P<0.01$). The ash, fiber and protein contents were lower in cakes and biscuits, while their carbohydrates and fats contents were highest. A careful look into the ingredients of the cakes and biscuits revealed that they possess flour and oil in very high percentages. Due to higher carbohydrates and fats contents, these products had higher calorific value. In conclusion, since cakes and biscuits have relatively low protein, ash and fiber contents and possess very high carbohydrates and fat contents. Therefore, these bakery products are not at all suitable for frequent consumption by the diabetics and the patients suffering from cardiovascular disease problems.

Key words: Nutritive value, cakes, biscuits, Faisalabad.

INTRODUCTION

Baking industry has its importance among food industry all over the world. In this industry, cakes and biscuits occupy primary position, both for production and utilization, as compared to other bakery products. These are rich in energy and also have substantial amounts of other nutrients. They are used as snacks between meals, as supplementary foods for toddlers and school-going children, and as refreshment item in various ceremonial occasions and other social gatherings. The chemical composition of the bakery products varies with the amount of the ingredients in the products. A previous study (Hussain 1985) has shown 67.2% moisture, 4.1% protein, 3.2% fat, 24.3% nitrogen free extract and 214 Kcal per 100g of kheer, a common sweat dish. White bread contains 39.0% moisture, 7.8% protein, 12.2% fat and 51.9% nitrogen free extract (Goplan *et al* 1981). Cakes and biscuits are generally low in protein, ash and fiber but are very high in carbohydrates and fats. Because of their higher carbohydrate and fat contents, they have higher caloric values. More intakes of such products may cause an energy imbalance and may result in over weight and other nutritional problems (Khattak *et al* 2003). The nutritional information for many bakery products available in the market was lacking while for healthy eating and planning for community nutrition, it is important to know the nutritional facts about these products. The present study reports the nutrient composition and nutritional quality of some bakery products available in Faisalabad.

MATERIALS AND METHODS

Sample Collection: Four bakeries located in Faisalabad were selected for the present study. These bakeries had already been recommended by the Pakistan Export Promotion Bureau to include B-complex vitamins. Random samples of three types of cakes (pineapple, chocolate and

plain) and the three types of biscuits, (sweet, salty and coconut) were collected from each bakery. To obtain information about ingredients and composition, recipes of each type of cakes and biscuits were collected from the cooks of the bakeries. The weights of ten pieces of each selected bakery product from selected shops were determined by a digital balance.

Chemical Analysis: The moisture content of flour and products were estimated by drying the sample in an air forced draft oven at 105 ± 5°C till the weight of the sample became constant (AOAC 2000; Method, 4-15A). The moisture content was calculated according to the following formula.

$$\text{Moisture}(\%) = \frac{\text{Wt. of original sample} - \text{Wt. of dried sample} \times 100}{\text{Wt. of original sample}}$$

Ash estimation was done by direct incineration of the sample taken in a tarred crucible. The crucible was heated on the oxidizing flame till it gave no fumes. The contents were ignited as by the AOAC (2000) method and % ash content was calculated according to the following formula:

$$\text{Ash}(\%) = \frac{\text{Wt. of ash}}{\text{Wt. of sample}} \times 100$$

The crude fiber was determined by taking fat free sample and digesting first with 1.25% H_2So_4, followed by washing with distilled water and then digesting with 1.25% NaOH solution. The residue was washed, dried, weighed and ignited in a muffle furnace at 550°C till white residue was left. Fiber percentage was calculated according to the procedure described in AOAC (2000) method 32-10. The percentage of nitrogen in the sample was determined by using Kjeldhal's method, as described previously (AOAC 2000). The samples were first digested with concentrated H_2So_4 in the presence of digestion tablet of 5 gm for 5-6 hours or till the digested material attained light greenish or transparent color. This material was diluted and distillation was carried out by taking 10 ml of diluted material and 10 ml of 40% NaOH solution in the distillation apparatus. The ammonia thus liberated was collected in 2% boric acid solution containing few drops of methyl red as an indicator. Finally, the distillate was titrated against 0.1N H_2So_4 solution till golden brown end point. The crude protein percentage was calculated by multiplying nitrogen with a factor 5.7. The crude fat content was determined by taking 2g flour sample and extracting in petroleum ether as a solvent in a Soxhlet's apparatus (AOAC 2000). Carbohydrates contents were determined by phenol method where 0.2g of dry sample was taken with 100 ml 0.25N HCl in a conical flask and boiled on a hot plate for an hour. Then it was cooled and pH was maintained at 7.0 by adding NaOH drop by drop; it was filtered and the 100 ml volume was made. Then 0.1 ml was taken as a sample and three solutions prepared as given in Table 1. These three solutions were run on spectrophotometer and absorbance of standard and samples was recorded. Calorific values of the products were calculated by multiplying their carbohydrate, fat and protein contents by factors 4, 9 and 4 respectively, as described earlier (Khattak *et al* 2003).

Statistical Analysis:

Standard errors were calculated and data were analyzed by Analysis of Variance technique (Steel and Torrie 1980). To determine the difference between groups, Duncan Multiple Range test (Duncan 1955) was applied.

RESULTS

The average weight of cakes ranged from 294.0 to 344.4g. Plane cakes had the minimum weight, while chocolate cakes had the maximum weight. The major ingredients of cakes were flour (43%), sugar (19%), fat (17%), eggs (7%), miscellaneous ingredients (8%) and milk (3%).

Salty and sweet biscuits were small in size but size of coconut biscuits was large. The average ingredients of biscuits were: flour (50%), sugar (20%), fats (20%), eggs (2%) and other minor ingredients (8%).

Moisture Content: The moisture contents were highest in pine apple cakes and lowest in plain cakes, the difference was significant ($P<0.05$, Table 1). In biscuits, the moisture contents were

significantly higher (P<0.05) in sweet and salty than in coconut biscuits. The difference between former two types was non significant (Table 2). The moisture contents of cakes (Table 3) and biscuits (Table 4) were also significantly different among four bakeries.

Ash Content: For cakes, ash contents were highest in pine apple cakes, followed by chocolate and plain cakes (Table 1), the difference was significant. For biscuits, ash was highest in coconut type and lowest in sweet type (Table 2), the salty biscuits being intermediate (P<0.05). Ash contents of both cakes and biscuits differed significantly among four bakeries.

Fiber Content: Plain cakes showed the highest fiber contents, followed by pine apple cakes and chocolate cakes (P<0.05, Table 1). Similarly, salty and coconut biscuits had higher fiber contents than sweet biscuits (P<0.05), the difference in fiber content between former two types of biscuits was, however, non significant (Table 2). The fiber contents of both cakes and biscuits differed significantly among bakeries.

Protein Content: Protein content was highest in chocolate cakes and lowest in pine apple cakes, the plain cake was intermediate (P<0.05, Table 1). Similarly, for biscuits, salty biscuits revealed the highest protein level, followed by sweet biscuits and coconut biscuits (P<0.05). All four bakeries differed significantly for protein contents of their cakes (Table 3) and biscuits (Table 4).

Fat Content : Pineapple cakes had the highest fat, while the lowest fat was observed in plain cakes (Table 1); chocolate cakes were intermediate with respect to their fat contents (P<0.05). However, the difference in fat contents among three types of biscuits was non-significant Table 1). Four bakeries differed significantly for fat contents of their cakes (Table 2) but not biscuits (Table 3).

Carbohydrate Content : The carbohydrates contents did not differ among three types of cakes (Table 1). However, for biscuits coconut type had significantly higher carbohydrates that sweet or salty biscuits; the difference between latter two types was non-significant. Effect of bakeries on carbohydrate contents of cakes (Table 3) and biscuits (Table 3) was also significant (P<0.05).

DISCUSSION

Plain cakes had the minimum weight, while chocolate cakes had the maximum weight. The major ingredients of cakes were flour (43%), sugar (19%), fat (17%), eggs (7%), other ingredients (8%) and milk (3%). The major ingredients of cakes and biscuits were flour, sugar, fat and milk. These major ingredients are added for the nutritional and calorific values of cakes and biscuits. The minor ingredients used in cakes and biscuits preparation were fruit, eggs and chocolate. These are basically used for baking quality, taste and name. However, these may also add some nutritional value to the product. The composition of cakes and biscuits in terms of major and minor ingredients can be changed according to the choice of bakers and market demand. New types of cakes can be developed with changed ingredients. Ashraf (1992) reported that dough, which is made by flour, sugar, fat and milk along with minor ingredients, is used in cake preparation. The single most important measurement made on bakery products is moisture content. This controls the shelf life of most products. For soft products, higher moisture is desired; for biscuits and cakes, low initial moisture gives the longest shelf life. In this study, the moisture contents of cakes were higher than biscuits. Pasha *et al* (2002) observed that during the whole storage, there were significant changes in moisture contents and non-significant changes in fat, ash, protein, fiber and NFE contents in bakery products. The protein contents in cakes and biscuits were also very low. The protein contents of different types of cakes and biscuits showed highly significant differences. Tsen *et al* (1973) reported that fortification of wheat flour with soya flour or soya protein isolates resulted in an increase of 60-100% of protein contents of cookies. However, spread factor was reduced considerably and this could be overcome by the use of sodium stearayl-2-lactate. Commercial

cakes and biscuits had low protein contents, probably due to the use of less number of eggs and unimproved quality of wheat protein. The cakes and biscuits of selected bakeries were low in ash, fiber and protein but high in fat and carbohydrate contents. In the ingredients of cakes and biscuits, flour and oil were in high percentage so fat content of cakes and biscuits were significantly high. Plain cakes had lowest fat contents but pine apple and chocolate cakes had highest fat contents. Parcersia *et al* (1999) revealed that saturated fatty acids occurred in the largest proportions in all commercial bakery products (52.8%), followed by mono saturated (23.5%) and poly unsaturated fatty acids (17.2%). A small percentage of trans fatty acids, which were found in all samples, showed a mean value of 5.7%. The carbohydrate contents of cakes and biscuits differed significantly between bakeries but not between types of cakes and biscuits. Higher carbohydrates contents in cakes and biscuits might be due to high quantity compounds of flour and sugar in the ingredients of these products. Differences in the calorific value of cakes and biscuits were highly significant. In a previous study (Khattak *et al* 2003) the chemical composition and caloric values of samples of bakery products were, moisture (4.17-4.3%), ash (0.70-1.92%), fiber (1.55-3.08%), fat (8.02-48.36%), carbohydrate (12.45-56.01%) and protein (3-21%). These data demonstrate that bakery products are low in protein and high in fat. The results of the present study indicate that bakery products available in the market of Faisalabad are low in nutrients particularly proteins. Therefore, nutrient fortification should be done as practiced in some other countries (Weinberg 1976). The inclusion of legumes in the recipes of bakery products can improve protein contents and other nutrients of the products. Cakes and biscuits are low in protein, ash and fiber, but high in carbohydrates and fat contents. Because of higher carbohydrates and fat contents, these products also have high caloric values. In conclusion, bakery products are good supplementary food for children, particularly those who are suffering from energy malnutrition. However, adults should eat these products with care as they may cause over weight and obesity problems due to their high carbohydrates and fat contents.

Table 1: Biochemical constituents of three types of cakes

Product	Plain	Pineapple	Chocolate	Means
Moisture	32.95 ± 4.56c	50.38 ± 4.58a	40.63 ± 2.27b	41.32 ± 2.10
Ash	0.47 ± .04c	0.70 ± 0.01a	0.51 ± 0.02b	0.56 ± 0.02
Fiber	0.35 ± 0.03a	0.24 ± 0.01b	0.11 ± 0.005c	0.25 ± 0.01
Protein	11.182 ± 0.19b	7.89 ± 0.69c	12.01 ± 0.36a	10.36 ± 0.39
Fat	23.22 ± 1.24c	26.82 ± 1.24a	25.69 ± 1.2b	25.24 ± 0.736
Carbohydrate	53.29 ± 4.05a	53.25 ± 4.48a	53.02 ± 4.35a	53.19 ± 2.41
Total caloric value program	440.448	442.691	469.62	449.09

Mean values with different letters within a row differ significantly from one an other (P<0.05)

Table 2: Biochemical constituents of three types of biscuits

Product	Sweet	Salty	Coconut	Means
Moisture	3.6 ± 0.11a	3.47 ± 0.073a	3.20 ± 0.09b	3.42 ± 0.059
Ash	0.39 ± 0.01c	0.47 ± 0.01b	0.49 ± 0.01a	0.45 ± 0.01
Fiber	0.09 ± 0.003b	0.11 ± 0.004a	0.115 ± 0.003a	0.105 ± 0.003
Protein	11.19 ± 1.18b	12.63 ± 1.34a	9.93 ± 0.72c	11.25 ± 0.65
Fat	23.53 ± 0.41a	24.01 ± 0.44a	25.31 ± 0.49a	24.28 ± 0.28
Carbohydrate	53.11 ± 1.59b	53.65 ± 1.69b	60.18 ± 2.27a	55.65 ± 1.18
Total caloric value per gram	411.35	428.36	435.07	424.8

Mean values with different letters within a row differ significantly from one an other (P<0.05)

Table 3: Biochemical constituents of cakes of different bakeries

Bakeries	B_1	B_2	B_3	B_4	Means
Moisture	35.36 ± 3.82c	46.8 ± 3.13a	39.24 ± 5.21b	35.36 ± 4.07c	41.32 ± 2.10
Ash	0.46 ± 0.047d	0.66 ± 0.03a	0.51 ± 0.054c	0.60 ± 0.03b	0.56 ± 0.02
Fiber	0.21 ± 0.021d	0.25 ± 0.01b	0.33 ± 0.53a	0.23 ± 0.01c	0.25 ± 0.001
Protein	10.75 ± 0.14b	11.64 ± 0.40a	9.089 ± 0.91d	9.975 ± 1.07c	10.36 ± 0.39
Fat	24.03 ± 0.81b	13.62 ± 1.01a	53.80 ± 0.81c	33.93 ± 0.54c	53.19 ± 2.410
Carbohydrate	51.41 ± 0.81b	31.93 ± 0.69a	22.20 ± 0.61b	22.82 ± 0.71c	25.24 ± 0.74
Total caloric value per gram	434.5	574.59	425.8	352.8	449.03

Mean values with different letters within a row differ significantly from one an other (P<0.05)

Table 4: Biochemical constituent's values of biscuits of different bakeries

Bakeries	B_1	B_2	B_3	B_4	Means
Moisture	3.54 ± 0.12a	3.60 ± 0.08a	3.16 ± 0.11b	3.40 ± 0.12b	3.42 ± 0.06
Ash	0.44 ± 0.02b	0.44 ± 0.02b	0.48 ± 0.01a	0.44 ± 0.03b	0.45 ± 0.01
Fiber	0.12 ± 0.005a	0.10 ± 0.004b	0.10 ± 0.008b	0.10 ± 0.004b	0.105 ± 0.003
Protein	11.289 ± 1.75c	7.42 ± 0.88d	12.01 ± 0.37b	14.29 ± 0.67a	11.25 ± 0.65
Fat	24.473±0.671a	33.98 ± 0.462a	23.70 ± 0.55a	24.997±0.561a	24.28 ± 0.28
Carbohydrate	60.01 ± 2.59a	57.27 ± 1.32a	58.42 ± 1.22a	46.88 ± 1.15b	55.65 ± 1.18
Total caloric value per gram	475.28	439.76	391.39	451.43	422.79

Mean values with different letters within a row differ significantly from one an other (P<0.05)

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Effect of interchanging starch and protein (gluten) fractions of two wheat varieties on the rheological and bread making quality

Faqir Muhammad Anjum, Asif Sultan, Muhammad Issa Khan, Imran Pasha, Shahzad Hussain, Ali Asghar and Moazzam Rafiq Khan

National Institute of Food Science & Technology, University of Agriculture Faisalabad, Pakistan 38040.

ABSTRACT

The wheat gluten proteins play a key role in determining the unique properties of wheat flour dough. The gluten comprises of gliadin and glutenin proteins. Two wheat varieties that are AS 2002 and SH 2002 were included in the study. Total protein contents of AS 2002 and SH 2002 were found to be 13.24 and 11.44% respectively. Wet and dry gluten of two varieties were 36.80 & 12.18% for AS 2002 and 32.17 & 10.73 % for SH 2002 respectively. Three fractions namely starch, glutenin and gliadin of one variety were replaced with identical fraction of the other variety. The rheological studies revealed significant differences in their characteristics such as water absorption, arrival time, dough development time, departure time, dough stability, tolerance index and softening of dough. Peak height and mixing time also exhibit significant differences. Different parameters of bread quality were found significantly varied. T_2 (SH2002) followed by T_1 (AS2002) and T_3 ($S_1g_1G_1$) obtained highest scores respectively on the nine points Hedonic scale. The flour combination having only starch and gliadin showed the poor results regarding the quality of bread. It is also found that flour combination without gliadin or glutenin does not give better loaf volume, crust color, grain and texture of bread. Replacement of AS2002 starch with the starch of SH 2002 gives better results with reference to the bread making quality. Wheat variety SH 2002 was found better for bread making.

Key words: Starch, glutenin, gliadin, interchanging, rheology & bread quality.

INTRODUCTION

Wheat is the most favoured of all plants. The unique properties of its endosperm protein, called gluten, make wheat flour singularly adaptable for bread and a variety of other baked products and give the wheat its pre-eminent position as the most basic food of the world. Study of the wheat grain proteins is as old as protein chemistry itself, gluten and gliadin were in the original brief list of compounds. By that time gluten was recognized as a simply prepared, relatively pure protein because of the much earlier demonstration that gluten could be prepared by washing the starch and water soluble components from dough by kneading it under running water (Bailey, 1941). Further study gave the name "gliadin" to the protein of gluten that was soluble in alcohol and called the alcohol-insoluble fraction "glutenin". Osborne (1907) built on these contributions and devised a method based on differential solubility to distinguish albumin, globulin, gliadin and glutenin from one and other. Wheat has a unique position as a staple diet of millions of peoples all over world and is consumed in various forms of bread. The quality of wheat products is largely determined by the quality and quantity of protein in the flour. The quantity of protein is generally influenced by environmental factors, whereas the quality of protein is more of the heritable character Pyler (1988). For this reason, it has drawn the attention of scientists to look for the deficiency and non-availability of some of the essential amino acids in wheat proteins. The biologists (Plant Breeders) resort to selective breeding by employing genetic engineering techniques in order to improve protein quality of wheat. A number of wheat cultivars have been developed after 1965 in Punjab province for commercial exploitation. These cultivars have enhanced the grain production to a significant extent but little or no emphasis has been paid to improve the wheat quality. There is no information available about the Pakistani wheats on different quality parameters related to their genetic make-up. However, some information is available about

with bread quality. The proteins of gluten are the basis of the unique ability of wheat flour to be baked into leavened bread, for the rubber like cohesive properties of gluten whch are essential for retaining the bubbles of carbon dioxide generated during fermentation or chemical leavening. Thus gluten content and quality are prime factors in determining the bread making quality of wheat flour. However, total protein content is more often used to assess quality of the wheat. The protein content of a sample of a whole wheat grain is generally about 1% higher than that of the flour milled from the grain, because the protein content is slightly higher in the grain than in the endosperm as a whole. Recent reviews by Bushuk (1971), Pomeranz (1980) and Macritchie (1987) emphasized the extensive amount of work being conducted on the properties of the gluten proteins by solubility fractionation techniques. Another model emphasizing the role of interpolypeptide disulfide bonds in glutenin was proposed by Graveland *et al.* (1985). Since it is an established fact that assessment of baking behavior of wheat flour depends upon the quantity and the presence of relative proportions of various protein fractions.

Beside the gluten proteins, the starch isolated from different wheat cultivars also had an active role in determining dough rheological characteristics. The gluten proteins constitute the predominant fraction controlling the viscoelastic properties of wheat flour dough. Petrofsky and Hoseney (1995) stated that on dry weight basis wheat flour contain starch 80%, protein 14%, lipids 4-5% and almost 2% pentosans. This research project was design to determine the effect of interchanging starch and protein fractions of two newly evolved varieties on rheological and functional properties of bread.

MATERIALS AND METHODS

Procurement of wheat varieties

Two wheat varieties AS2002 and SH2002 were collected from Ayub Agriculture Research Institute (AARI), Faisalabad.

Milling of wheat

Ten Kg wheat grains of each wheat cultivars were tempered to 15.5% moisture level. The amount of water required to temper the wheat grains was computed according to the expression given in (AACC 2000).

$$\text{Vol. of water to be added} = \frac{100 - \text{Original moisture}}{100 - \text{Desired moisture}} - 1 \times \text{Wt. of wheat sample}$$

After tempering, the wheat grain samples were milled by using Quadrumat Senior Lab mill to get break flour, reduction flour, shorts and bran milling fractions.

Wet and dry gluten

Wet and Dry gluten of reduction and break flour was carried out according to the method as described in AACC (2000).

Sedimentation test

Sedimentation test for each flour sample was carried out according to the method as described in AACC (2000).

Proximate analysis of Composite wheat flour

Composite wheat flour was analyzed for crude fat, crude fiber, crude protein, ash, moisture content and NFE according to the methods described in AACC (2000).

Isolation of starch

Starch of wheat flour was isolated by following the method as described by Oda and Schofield (1997).

Protein estimation (Composition and fractionation)

Major fractions of protein i.e. albumin, globulin, gliadin and glutenin were isolated on the basis of their solubility in different solvents according to the modified method as described by Anjum (1976).

Preparation of flour

Flours were prepared by interchanging wheat flour gluten gliadin and starch of two varieties as given in Table 1

Table 1: Treatments combination used in study

Treatments	Combination
T_1 (Starch AS2002 + Gliadin AS2002 + Gluten AS2002)	S1g1G1
T_2 (Starch SH2002 + Gliadin SH2002 + Gluten SH2002)	S2g2G2
T_3 (Starch AS2002 + Gliadin SH2002 + Gluten SH2002)	S1g2G2
T_4 (Starch AS2002 + Gliadin AS2002 + Gluten SH2002)	S1g1G2
T_5 (Starch AS2002 + Gliadin SH2002 + Gluten AS2002)	S1g2G1
T_6 (Starch SH2002 + Gliadin AS2002 + Gluten AS2002)	S2g1G1
T_7 (Starch SH2002 + Gliadin SH2002 + Gluten AS2002)	S2g2G1
T_8 (Starch SH2002 + Gliadin AS2002 + Gluten SH2002)	S2g1G2
T_9 (Starch AS2002 + Gliadin AS2002)	S1g1
T_{10} (Starch SH2002 + Gliadin SH2002)	S2g2
T_{11} (Starch AS2002 + Gluten AS2002)	S1G1
T_{12} (Starch SH2002 + Gluten SH2002)	S2G2

Physical dough properties

For Farinographic studies Barbender Farinograph was used to prepare farinogram of different treatment in order to access water absorption, dough development time, dough departure time, dough stability according to methods as described in AACC 2000.

Mixographic studies were carried out in order to access the peak height and dough development time by their respective methods as described in AACC 2000.

Preparation of breads

The breads for different treatments were prepared by straight dough method as given in AACC (2000) method No. 10-10B.

The recipe followed is given on flour weight basis as under:

Flour	100 g
Sugar	3 g
Salt	1 g
Yeast	1 g
Shortening	5 g
Water	According to water absorption

Sensory evaluation of the bread

To assess the quality and acceptability, the breads were presented to the panel of judges and the sensory evaluation were carried out for

taste, aroma, texture, flavor, grain size, crust color, crumb color, volume and overall acceptability characteristics according to the methods as described by Land and Shepherd (1988).

Statistical analysis

The data obtained from each parameter were subjected to statistical analysis to determine the level of significance between quality parameters of different straight grade flours by using completely randomized design and means were compared according to the appropriate methods described in Steel *et al* (1997).

RESULTS AND DISCUSSION

Both wheat varieties i.e. AS-2002 and SH-2002 were subjected to proximate analysis prior to the substitution of their starch and protein (gluten) fractions in order their effect on the bread functional properties. Proximate composition result of SH-2002 and AS-2002 are depicted in Table 2 indicates that AS-2002 (13.10%) contain higher moisture contents than the SH-2002 (12.99%). Both these varieties also varied much in their protein contents. Protein content is more in AS-2002 (13.24) as compared to SH-2002 that contains 11.44 %. Similar trend was observed in case of ash contents for these two varieties as AS-2002 contains higher amount (0.63%) compared to SH-2002 in which the value for ash content are 0.54%. The difference in chemical composition in both these varieties is attributed to genetic make up, soil type, agronomic practices and environmental condition of the crop growing area. Ahmad (1994) examined six Pakistani wheat varieties and was of view that moisture, protein and ash contents varied from variety to variety. Whole wheat flour of some wheat varieties were analyzed by Mashhood (1993) who also gives results for these parameters in range that indicates that these varies from variety to variety. Soil types, Agronomic practices and environmental condition also contribute their part to the variation in chemical composition.

Protein and starch of both varieties were fractionated on the basis of solubility. These fractions of the varieties were combined in different combination in order to asses their effect on the gluten forming ability of the flour. Gluten is the hydrated product of two protein fractions of wheat i.e. glutenin and gliadin which developed after mechanical force is applied to the hydrated flour. Wheat protein quality is determined by its gluten forming ability. Gliadin fraction of the gluten has significant effect in the bread making quality of wheat flour. Higher amount of wet and dry gluten was found in AS-2002 with respect to the SH-2002, which is result of higher amount of protein in AS-2002 as shown in Table 2. The amount of wet and dry gluten in AS-2002 is 36.80 and 12.18 % respectively while these values for SH-2002 are 32.17 and 10.73 % respectively. Similar to protein content variation in gluten is outcome of variation in genetic make up, soil type, agronomic practices and environmental condition growth locations. Wheat varieties grown in the same location but with different genetic make up show variation in gluten contents (Huebner 1970 and Kim 1979). Higher gluten contents of AS-2002 suggest that it may be used as source of gluten protein source in the bakery product that requires a strong gluten network in the dough. Toufeili *et al* (1999) suggested that fortification of an average quality flour with the gliadin and glutenin from the poor and good quality flours at the levels of 1% and 2% (protein to flour mass) induced marked differences in the mechanical properties of the bread. Interchanging the gliadin and glutenin fractions between the reconstituted flours showed that the glutenin fraction is largely responsible for the differences in the bread making performance.

Table 2: Chemical composition of two varieties

Variety	Moisture (%)	Ash (%)	Protein (%)	Wet Gluten (%)	Dry Gluten (%)
AS-2002	13.10	0.63	13.24	36.80	12.18
SH-2002	12.99	0.54	11.44	32.16	10.73

Table 3: Farinographic characteristics of flour combinations

Treatment	W.A (%)	AT (min)	DDT (min)	DT (min)	DS (min)	TI (BU)	SD (BU)
T_1	57.4 a	3.5 ab	11.0 a	21.5 a	18.0 a	50 ef	100 cd
T_2	56.4 ab	4.0 a	10.0 ab	22.0 a	18.0 a	30 fg	30 e
T_3	53.8 bcd	1.5 cd	8.0 bc	16.0 b	15.0 b	50 ef	110 cd
T_4	53.0 ad	1.5 cd	3.5 e	6.0 fg	5.0 ef	160 ab	200 ab
T_5	54.2 abcd	2.0 c	4.5 de	11.0 cde	9.0 cd	76 d	150 bc
T_6	56.6 abc	2.0 c	7.5 bc	11.0 cde	9.0 cd	100 c	140 bcd
T_7	56.6 ab	3.0 b	8.0 bc	14.5 bc	11.5 c	60 de	160 bc
T_8	56.0 abc	2.0 c	6.5 cd	10.0 def	8.0 d	80 cd	150 bc
T_9	55.3 abcd	1.5 cd	3.5 e	13.0 bcd	11.5 c	20 g	80 de
T_{10}	52.2 d	1.5 cd	3.0 e	4.5 g	3.0 f	180 a	240 a
T_{11}	55.6 abc	1.5 cd	3.0 e	8.0 efg	6.5 de	60 de	160 bc
T_{12}	55.8 abc	1.0 d	2.5 e	5.0 g	4.0 ef	140 b	260 a

Table 4: Mixographic characteristics of flour combinations

Treatment	Peak height (BU)	Mixing Time (min)
T_1	30 bc	3.45 ab
T_2	29 bcd	3.30 ab
T_3	25 cd	3.15 abc
T_4	28 bcd	2.45 bcd
T_5	35 ab	2.15 cde
T_6	28 bcd	3.15 abc
T_7	40 a	4.00 a
T_8	35 ab	3.45 ab
T_9	28 bcd	1.45 de
T_{10}	22 d	1.30 e
T_{11}	25 cd	1.45 de
T_{12}	27 cd	1.50 de

Physical dough properties were determined by subjecting both varieties to farinographic and mixographic studies with different combination of starch, gliadin and glutenin fraction. Results regarding farinographic parameters i.e. water absorption, arrival timer, dough development time, departure time, dough stability, mixing tolerance index and softening of dough are depicted in Table 3. It is evident from the results that T1 ($S_1g_1G_1$) showed higher water absorption capacity as compared to other treatments of the study. It is reported that water absorption capacity of dough increases with increase in protein contents and improvement in gluten quality (Matz, 1972) but other factors like damaged starch also affect the water absorption capacity. Stronger wheat variety flour has the ability to absorb and retain larger quantities of water as compare to soft wheat flours (Kent, 1983). Lowest water absorption value was found for T_{10} (S_1g_1). Higher values for arrival time, dough development time, dough departure time and dough stability were observed for T2 ($S_2g_2G_2$) followed by T1 ($S_1g_1G_1$) that indicate their good quality protein and regarded as strong wheat. Higher mixing tolerance index values 180 and 160 B.U. were found in case of T_{10} (S_1g_1) and T_4 ($S_1g_1G_2$) respectively. Higher MT values indicate low quality protein and subsequently weaker wheat while flour with low MT is regarded as stronger wheat. T1 ($S_1g_1G_1$), T2 ($S_2g_2G_2$), T3 ($S_1g_2G_2$) and T9 (S_1g_1) exhibit lower values for the MT. Similarly lower values i.e. 30 and 80 B. U. for softening of dough were observed in case of T2 ($S_2g_2G_2$) and T9 (S_1g_1) respectively. Lower values of dough softening index also an indicator of stronger wheat varieties. Wheat flour with different ratio of three fractions that is starch, gliadin and glutenin were also subjected to mixographic studies to know about the peak height and mixing time. All the three parameters of mixograph varied significantly for the different treatment with varied amount of glutenin, gliadin and starch fraction. Dough resistance and mixing peak time is strongly correlated to the HMW glutenin composition whereas dough extensibility is correlated to protein contents. Loaf volume is positively affected by dough extensibility whereas protein quality has no significant effect. Maximum peak height was observed for T_7 (40.0) while lower value was found for T_{10} (22.0) as predicted in Table 4. Higher values for peak height indicate stronger wheat flour with a good quality of gluten protein. Mixing time of different type of flours also varied significantly with maximum values for T_7 (4.0) while mixing time of 1.30 minutes was observed for T_{10}. Mixing time is also determinant of wheat flour protein and overall quality of wheat. Mixing tolerance as measured by resistance breakdown and bandwidth breakdown decrease as a result of addition of gliadin and glutenin fractions. Peak dough resistance values increased with addition of individual groups of gliadin and gluten to the base flour. Gliadins produce least positives effects on peak dough resistance. Addition of gliadin and its subgroups substantially improved loaf volumes of pan bread (Khatkar *and* Schofield 1997). Higher values are indicative of stronger wheat while lower values are found for weaker wheat. Finney (1965) suggest that wheat for good quality bread should have higher water absorption, medium to medium long mating requirements, satisfactory mixing tolerance and dough handling properties. Mixing time for Pakistani wheat varieties varied between 1.10 to 3.0 minutes while peak height remains in the range of 41.1 to 51.5% (Anjum 2000).

Sensory evaluation of product by panel of judges for certain parameters was carried out to assess the acceptability of product by the consumers. Breads prepared from flours of different treatment were presented to panel of judges for the evaluation of their external and internal characteristics. Results regarding the color of crust, symmetry of form, evenness of bake, characters of crust, break and shreds are predicted in Table 5(a). High score values for external characteristics of bread are obtained by T_2, T_3 and T_1 respectively. External characteristics of bread are responsible for physical appearance and eye appeal of the product to the consumers. Internal parameters of bread are grain size, crumb color, aroma, taste, mastication and texture that are indicative

of bread eating quality. Data explicated in Table 5(b) show that T_2 gain maximum score for all the internal parameters of bread followed by T_3 and T_1. Gluten protein of wheat is largely responsible for the unique ability of wheat flour to form viscoelastic dough yielding baked products with a porous texture. Protein contents and qualitative differences in protein composition significantly affect the bread characteristics (Tronsmo 2002).

Table 5: Sensory evaluation of bread

External characteristics

Treatment	Color of crust	Symmetry of form	Evenness of bake	Character of crust	Break and shreds
T_1	6.90 a	2.10 a	2.10 a	2.00 a	2.20 a
T_2	7.10 a	2.40 a	2.40 a	2.00 a	2.30 a
T_3	7.00 a	2.20 a	2.10 a	2.20 a	2.00 a
T_4	6.70 a	2.00 a	2.00 a	2.00 a	2.00 a
T_5	6.80 a	1.80 a	1.70 a	1.90 a	1.60 a
T_6	6.50 a	1.80 a	1.70 a	1.90 a	1.70 a
T_7	6.60 a	1.90 a	1.80 a	1.80 a	1.80 a
T_8	6.40 a	1.90 a	1.80 a	1.80 a	1.90 a
T_9	3.50 b	0.60 b	0.50 b	0.40 b	0.50 b
T_{10}	3.40 b	0.50 b	0.60 b	0.30 b	0.70 b
T_{11}	3.20 b	0.60 b	0.50 b	0.30 b	0.60 b
T_{12}	3.50 b	0.50 b	0.60 b	0.40 b	0.60 b

Internal characteristics

Treatments	Grain	Crumb color	Aroma	Taste	Mastication	Texture
T_1	7.50 a	7.60 a	7.40 ab	11.00 a	7.30 a	12.00 ab
T_2	8.00 a	8.10 a	8.00 a	13.00 a	7.90 a	13.00 a
T_3	7.86 a	7.90 a	7.70 a	12.00 a	7.80 a	12.00 ab
T_4	6.90 a	7.00 ab	6.80 abc	10.50 a	6.90 ab	11.00 ab
T_5	6.80 ab	6.90 ab	6.60 abc	10.20 a	6.70 abc	11.20 ab
T_6	6.70 ab	6.80 abc	6.80 abc	10.30 a	6.90 ab	10.90 ab
T_7	6.90 ab	7.00 ab	6.80 abc	10.40 a	6.80 abc	10.40 b
T_8	6.80 ab	6.90 ab	6.70 abc	10.20 a	6.70 abc	10.20 b
T_9	4.00 c	4.50 d	4.20 c	5.00 b	4.10 c	3.90 c
T_{10}	4.33 c	4.60 cd	4.30 c	5.50 b	4.20 bc	3.80 c
T_{11}	5.00 bc	4.80 bcd	4.50 c	6.00 b	4.50 bc	3.70 c
T_{12}	4.00 c	4.90 bcd	4.60 bc	7.00 b	4.40 bc	3.60 c

It is conclude from the results that flour combination which has only starch and gliadin or starch and glutenin in their formulation have poor bread baking quality. It indicates that both protein fractions i.e. gliadin and glutenin are required for the proper gluten development. Bread prepared from these flours does not have better loaf volume, crust color, grain and texture. Flours with these three fractions i.e. starch, gliadin and glutenin does not show significant variation in their bread making quality from the parent flours. Wheat variety SH 2002 was found better with regard to bread baking quality. It was further found that if only starch of AS 2002 is replaced with the starch of SH 2002 it gives better result with reference to baking quality of bread.

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Quality evaluation of different ice cream brands available in local market

Nuzhat Huma, Mian Anjum Murtaza, Omer Mukhtar Tarar, Ghulam Mueen ud Din and Shahid Mahmood

National Institute of Food Science and Technology, University of Agriculture, Faisalabad.

ABSTRACT

In Pakistan, due to poor enforcement of food regulations and lack of implementation of standards related to hygienic quality of ice cream, the consumers are deprived of getting quality ice cream. This study was under taken to assess the nutritional and microbial quality of ice creams sold in the local markets. Ten ice cream samples (five commercial brands, three vendor produced and two home made) were collected from the local market and analyzed for microbial contamination and physico-chemical characteristics. All samples showed microbial contamination with Total Plate Count in the range 107 – 109 cfu/ml, total coliforms, fecal coliforms and *Eschericia coli* 43 – 110 MPN. Out of all ten samples two commercial brands were found less contaminated. Although none of the ice cream samples, met the international nutritional standards, commercial brands were found better than vendor produced ice creams. The nutritional composition of commercial brands mentioned on the packing matched the analysis report. The results of this study suggest that there is a dire need for the enforcement of rules and regulations in the country regarding hygienic quality of food produces.

Key words: Ice cream, total plate count, total coliform, faecal coliform

INTRODUCTION

Ice cream and related aerated frozen desserts are complex colloidal systems comprised of, in their frozen state: ice crystals; air bubbles; partially coalesced fat globules and aggregates; all in discrete phase surrounded by an unfrozen continuous matrix of sugars, proteins, salts, polysaccharides and water (Goff, 2002). According to the Pakistan Pure Food Rules, 1965, ice cream must contain not less than 10% by weight fat, 36% total solids and 0.5% stabilizer (Awan 2007).

It was around 1920 that the value of ice cream as an essential food was generally recognized and the product has become immensely popular since then. Its production has increased rapidly in most countries (Bhandari, 2001). The ice cream market is changing with increasing share of commercial branded ice cream in the market and with a switch of emphasis from cheaper commodity ice cream towards value added products. Ice cream manufacturers are learning how to maximize benefits offered by value added ingredients (Anon 1987). Although richness in nutritive constituents of ice cream has been realized by all, the microbial contamination problem associated with the production, handling and storage of this food is very complex. Many food poisoning cases have been associated with the consumption of contaminated ice cream sold in local markets (Korel *et al* 2002). Ice cream may get contaminated with the microbial and biological agents at various stages during production, transportation, storage and handling during selling. These microbial agents are traced back to ingredients, post pasteurization and environmental factors such as air, faults in storage tanks, cracks in the packaging materials etc (Bigalke and Chappel 1984).

In developed countries, ice cream production is quality controlled to prevent potential threats to public health. Pakistan is still backward in this respect. Due to poor enforcement of food safety regulations related to hygienic quality of ice cream, the consumers of Pakistan are deprived of getting quality ice cream. It is essential to determine that majority of population is consuming ice cream produced by commercial vendors and at home. Consumers should be aware of the nutritional quality and sanitary conditions of the ice cream they are consuming. The objective of this study was to determine and compare the nutritional and microbiological quality of commercial home-made and vendor's ice cream with commercial brands of ice cream available in market.

MATERIALS AND METHODS

Sampling

Ten commercially available ice cream samples were collected in and were coded as per table 1. These samples were analyzed for microbial contamination and physico-chemical characteristics.

Table 1: Description and coding of ice cream samples used in the study

Sample description	Sample No.
Commercial Brand 1	S_1
Commercial Brand 2	S_2
Commercial Brand 3	S_3
Commercial Brand 4	S_4
Commercial Brand 5	S_5
Vendor 1	S_6
Vendor 2	S_7
Vendor 3	S_8
Home made 1	S_9
Home made 2	S_{10}

Microbial analysis

Ice cream sample was homogenized and 1ml of each sample was taken separately to prepare 10-fold serial dilutions by transferring 1 ml of blend and successive dilutions to 9 ml of sterile Butterfield's phosphate buffer in Universal bottles (using separate sterile pipettes). After each dilution contents were mixed for 10 sec over vortex-mixer. These dilutions were then plated /added to different media for the determination of total plate count of ice cream samples. Total Coilforms, Fecal Coliforms and *Escherichia coli* were also determined by using their specific medium.

Physico-chemical analysis

Fat in ice cream was determined with Gerber method. Crude protein, Total ash, pH and moisture contents were determined by the appropriate methods given in AOAC (2000).

Statistical analysis

The data obtained in the research was analyzed through analysis of variance as described by Steel *et al* (1997).

RESULTS AND DISCUSSION

Microbiological Analysis

Since, microbial quality of ice cream constitutes a potential hazard to public health. Evaluation of commercially available ice cream brands, vendor ice cream and home-made ice cream may assist in the assessment and improvement of hygienic precautions adopted during production, packaging, handling and storage. The results of TPC, total coliform, faecal coliform and E. coli are mentioned in Table 2.

Total Plate Count (TPC)

The results obtained using serial dilution agar plate technique revealed that the minimum number of microorganisms was present in commercial brand sample (S_5) and a home made ice cream (S_9) which was 1.87×10^7 and 2.24×10^7 cfu/mL respectively. These numbers exceeded the standard limits i.e.1,00,000 cfu/ml (Hankin and Hanna 1984). All other samples also showed more plate counts than the standard (Table 2). So, none of the samples were within the acceptable limit of public health safety.

Total Coilform

The highest average total coliform was more than 1100 in most of the samples on MPN (Most Probable Number) base. Only commercial brand samples S_5 and S_3 had less coliform count, i.e. 43 and 75 respectively (Table 2). Coliform count is probably of more value as an indicator of poor hygienic condition, poor method of processing, packaging and storage conditions. The total coliform count for ice cream according to international standard should not be more than 10/ml (James and Jay 1978). So this study demonstrated that none of the samples met the recommended international standards.

Faecal Coliforms

The same results were found for faecal coliform which were more than 1100 for MPN of total coliforms in all ice cream samples (commercial, vendor and home made ice cream), therefore all of these were above the standard limits.

E. coli

The data based on MPN in this research indicated that the E. coli was present in all the ice cream samples except S_3 and S_5 at the level of >1100 cfu/mL (Table 2). The S_3 and S_5 contained the *E. coli* at the level of 81 and 50 cfu/mL respectively, whereas according to international standards the presence of *E. coli* in ice cream samples is unacceptable. Therefore, none of the samples met the standards.

Physico-Chemical Analysis

Difference in fat, pH, moisture, ash, protein, TS and SNF were observed among different ice cream samples. Fat directly affects all the physical and chemical properties of ice cream. In this research, the results showed that the minimum percentage of fat was found in vendor ice cream sample (S_6) (Table 3). Commercial brand ice cream samples (S_1, S_2 and S_5) almost met the Pakistani standards for fat. The protein content in ice cream should be 3.5% minimum (Sebastian *et al* 1975). Protein content as determined by Kjeldahl method showed less than standard in S_4 and S_6 followed by S_8 as 1.0, 1.0 and 1.5% respectively. More protein contents were recorded in home made ice creams due to the presence of almond and coconut. None of the samples showed the comparable ash contents as described in standards. The lowest was 0.1% in S_5 and S_1 while the highest was in S_2 (1.1%). The highest moisture was found in S_6 (77.74 %), followed by S_7, S_8 and S_1 which contained 76.98, 7.3.14 and 72.84% moisture, respectively. The standard for moisture is 60.8 % to 65.55 % in ice cream as reported by Wong *et al* (1988). So only, S_5 and S_9 met the standards. The highest total solid content among commercial and home-made ice cream was observed in S_5, S_9 and S_{10} as 35.70, 34.88 and 33.98% respectively. Marshall *et al* (2003) have mentioned that the ice cream should contain 39.3 to 42.0% total solids. So, only S_5 was in agreement to the standards, none of the others met the standard limits. The lowest SNF was in S_7, i.e. 16.02%. All the samples exceeded the maximum limit as described in standards. The highest pH value was recorded in S_5 and S_9, i.e. 6.80. The lowest pH value was observed in S_6 as 5.10.

Table 2: Total Plate Count, Total Colifroms, Faecal Coliforms and *E. coli* in ice cream samples

S.No.	Sample	Mean TPC/mL	Total Coliforms /mL	Faecal Coliforms /mL	*E.coli* /mL
1	S_1	2.06×10^9	>1100	>1100	>1100
2	S_2	5.1×10^9	>1100	>1100	>1100
3	S_3	2.35×10^8	75	62	81
4	S_4	2.2×10^8	>1100	>1100	>1100
5	S_5	2.24×10^7	43	37	50
6	S_6	2.75×10^8	>1100	>1100	>1100
7	S_7	2.32×10^9	>1100	>1100	>1100
8	S_8	2.4×10^{10}	>1100	>1100	>1100
9	S_9	1.87×10^7	>1100	>1100	>1100
10	S_{10}	2.39×10^9	>1100	>1100	>1100

Table 3: Physico-chemical analysis of ice cream samples

Sr. No.	Sample	Fat (%)	Protein (%)	Ash (%)	Moisture (%)	TS (%)	SNF (%)	pH
1	S_1	8.01^{c}	3.62^{d}	0.11^{d}	$72.84b^{c}$	27.16^{e}	19.66^{f}	5.80^{c}
2	S_2	9.50^{b}	2.05^{g}	1.12^{a}	67.86^{cd}	32.14^{c}	22.64e	6.29^{b}
3	S_3	4.00^{g}	3.05^{e}	0.42^{c}	69.72^{c}	30.28^{d}	$26.28b^{c}$	6.23^{b}
4	S_4	5.05^{f}	1.07^{i}	0.50^{b}	67.88^{cd}	32.12^{c}	27.12^{b}	5.30^{d}
5	S_5	10.02^{a}	4.46^{c}	0.10^{d}	64.30^{d}	35.70^{a}	25.70^{c}	6.80^{a}
6	S_6	1.04^{i}	1.08^{i}	0.15^{d}	77.74^{a}	22.26^{f}	22.26^{e}	5.10^{e}
7	S_7	7.01^{d}	2.57^{f}	0.14^{d}	76.98^{ab}	23.02^{f}	16.02^{g}	5.24^{d}
8	S_8	4.05^{g}	1.50^{h}	0.55^{b}	73.14^{b}	27.86^{e}	23.86^{d}	5.35^{d}
9	S_9	2.05^{h}	5.35^{b}	1.05^{a}	65.12^{cd}	34.88^{ab}	32.88^{a}	6.80^{a}
10	S_{10}	5.50^{e}	6.08^{a}	1.02^{a}	$66.02c^{d}$	33.98^{b}	27.48^{b}	6.02^{bc}

Mean values bearing different letters in each column for every factor differ significantly (LSD, $P< 0.05$).

CONCLUSIONS

It could be concluded that the ice creams available in the markets are below the par from national standards and not fit for consumption. The microbial contamination in ice cream, especially for coliforms may be due to unhygienic conditions of production, packaging, transportation and storage. Total plate count in commercial ice cream is less than the vendor and home made ice creams. Home made ice creams were found more nutritious than the commercial and vendor ice creams as later were found very poor in fat, protein, ash, TS and SNF contents.

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Effect of organic feed materials on the quality of chicken meat

S. Ali, S. Usman, M. A. Hussain and W. H. Shah

Food & Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore

ABSTRACT

Day old broiler chicken were procured from commercial hatchery and divided into two groups. These birds were reared on feed having 20% protein, 3.5% fat, 5% ash and energy 2800 Kcal/Kg. Experimental feed was based on cereal grains and vegetable protein with only one source of animal protein i.e. fish meal. No additives, drugs and recycled meat sources were used. In the parallel group chicks were given feed from a commercial source containing cereals, vegetable and animal proteins, feed additives and drugs. These two feeds were given for a period of 6 weeks. During the experimental period temperature of the experimental room was made comfortable to the chicks by using air cooler. Weight gain, feed intake, feed efficiency and dressing percentage was noted. After completion of the experiment, chicks were picked up randomly and slaughtered for dressing percentage. At the end sensory qualities were determined. Results showed that chicken on experimental feed gained less weight. Sensory evaluation indicated that meat of experimental birds was comparable with domestic in respect of aroma, taste of curry, gravy and flavour; while tenderness, texture, juiciness and chewability were comparable with commercial chicken. However, overall acceptability of experimental meat was better than domestic and commercial birds.

Key words: Chicken meat, sensory evaluation, proximate composition

INTRODUCTION

During the last three decades poultry industry has developed very rapidly in Pakistan and has come up in a big way to bridge up the gap of animal protein. In the past only domestic birds were available for meat in small quantity (Heinz 1999). There is a common belief that organic chickens are healthier and have better taste (Ristic 2004). The consumer demands that a food product should be tasty, tender, appealing to the eye, wholesome, safe for consumption, nutritious and at an affordable price. The values and the limits (i.e. how tasty, how tender, etc.) depend on the area/country/region, ethnic group, age, sex, income or standard of living, attitude (tradition, buying behavior, food and eating habits, prejudice, etc.) and psychology. A quality which is acceptable in one society may not be acceptable in another (Fields *et al* 1968). In Nigeria, there is a prejudice against the modern type of broiler because it is considered poor in palatability and much too tender when compared with spent hens of the indigenous chicken strains (Okubanjo and Babalola 1981). Pakistanis prefer the local, dual-purpose desi-bird, but there has been a change in attitude in that the market is now ready to accept the modern broiler, i.e. the specially grown, meat type bird.

The feed materials contain antinutritional materials for example aflatoxins, rancid fat, glucosinolates, gossypol, chlorogenic acid and other antimetabolites (Pathak 1997). These should be detoxified before feeding to the poultry, as these adversely affect the health of birds and quality of chicken meat, which ultimately affects the consumers. That is why farmer is obliged to use medicines, which help in decreasing the chances of disease due to feed containing these types of toxic materials.

Antibiotics have been widely used in rations for boilers in the last decades, both as coccidiostat and growth promoters (Vicente 2004). There is an urgent need for sensitive, reliable multi methods and internationally accepted tolerance for veterinary drugs and coccidiostat. These tolerances should protect both the consumer from toxicological relevant or unnecessary residues in poultry products. There is need to check the quality of meat for residue drugs and contaminants like aflatoxins, pesticides, heavy metals, etc. Therefore, this study was planned to determine the effect of feed on the quality of chicken meat.

MATERIALS AND METHODS

Three hundred broiler chickens of one day age were procured from commercial hatchery and randomly divided into different groups. Before the start of experiment room was white washed and disinfected. Temperature of the experimental room was maintained at 37°C.

Isocaloric and isonitrogenous feeds were prepared. Experimental feed was based on cereal grains, cereal byproducts, vegetable protein and the only animal protein source used was fish meal (Table 1).

Commercial feed contained cereal grains, cereal byproducts, vegetable protein, fish, meat, poultry meals, drugs and feed additives. Both feeds were isocaloric and iso nitrogenous. Feed and water was made available round the clock (Table 1).

Desi (domestic) birds are reared on kitchen wastes and fulfill their dietary needs from outside the house, i.e. from insects, green leafy vegetables and waste thrown away. It takes more time to reach upto the mark of table purpose (age of slaughter). Reasons behind slow growth are:

i) Shortage of feed supply
ii) Genetic potential
iii) Exercise (struggle to search food & feed)

During experimental period following parameters were recorded:

1. Average initial weight/chick (g)
2. Average final weight/chick (g)
3. Average total weight gained/chick (g)
4. Feed efficiency (F.E.)
5. Mortality (%)
6. Dressing percentage (g)
7. Weight of internal organs (g)
8. Organoleptic quality

Proximate composition of feeds and different parts of the chicken meat was carried out by following standard methods of AOAC (2005).

Moisture content of samples were estimated by placing a ground sample (2-5 g) at 100 ± 5°C in an oven for 24 hours, until a constant weight was attained, whereas ash content was determined after ignition at 550 ± 5°C in a muffle furnace for a period of 6 hours. Crude protein content of samples was estimated by following the kjeldhal method. Fat contents were determined after extracting with hexane as described in AOAC (2005).

Sensory quality was determined according to the standard methods by using hedonic scale (Larmond 1977).

Table 1: Composition of experimental and commercial feeds

Ingredients (%)	A-Experimental	B-Commercial
Maize ground	30	40
Wheat ground	24	-
Rice polishing	5	10
Fish meal	4	4
Soybean meal	14	15
Cotton seed meal	3	3
Maize gluten meal 30%	5	-
Maize gluten meal 60%	2	2
Canola meal	7	4
Molasses (cane)	3.5	2
Ground limestone	1	1
Dicalcium phosphate	1	0.7
Vitamin-mineral premix	0.5	0.3
Rice broken	-	10
Rape seed meal	-	3
Poultry protein	-	1
Blood meal	-	1
L-lysine	0.11	0.11
DL-methionine	0.175	0.175
Salt	0.11	0.11
Albac	-	500 g/ton
Endox	-	125 g/ton
Farazolidon	-	50 g/ton
Clincox	-	200 g/ton

RESULTS AND DISCUSSION

The composition of experimental and commercial feed is given in Table 1. The difference between two feeds is that experimental group contains no additives and drugs.

Average weight gained by the chicks fed on experimental and commercial rations was 1500 and 1650 g respectively after six weeks (Table 2).

Results indicated that chicks receiving test feed, containing cereals plus vegetable protein and no drugs and additives gained less weight as compared to chicks consuming commercial feed. The reason of lesser weight may be due to the lack of additives like zinc bacitracin drug which helps in improving the weight gain as has beeh reported earlier (Fernandez *et at* 1973;Johnson and Arscatt 1974; Tesarova *et at* 1974).

Average total feed consumed by the chicks given different feeds indicated that group A (experimental feed) consumed less feed than group B (commercial feed). The increase in commercial feed intake may be due to incorporation of feed additives.

Average total feed efficiency of group A and B was 2.06 and 2.56 respectively (Table 2). Group A showed lesser feed efficiency value as compared with group B taking commercial feed containing animal protein, feed additives and drugs. The improvement in the feed efficiency value of group B can be due to incorporation of feed additives. Similar results were observed in the case of dressing percentage, heart, liver and gizzard weights.

Chemical analysis and sensory evaluation of chicks meats from three sources i.e. chicks fed on experimental and commercial feeds and domestically reared chicks were carried out. The results are reposted in (Tables 3 and 4).

Chemical analysis of different parts of the chicken indicated that moisture contents of breast were lesser in the case of domestic bird

Table 2: Data on average weight gain, feed efficiency, mortality, dressing percentage and weight of internal organs of birds.

Ingredients	A-Experimental	B-Commercial
Number of chicks	150	150
Days of experiment	42	42
Average initial weight/chicks (g)	47	47
Average final weight/chicks (g)	1500	1650
Average total wt gained/chicks (g)	1453	1603
Feed efficiency	2.06	2.56
Mortality%	10	10.50
Dressing percentage	58	60
Heart weight (% body weight)	0.53	0.54
Liver weight (% body weight)	2.66	2.72
Gizzard weight (% body weight)	2.24	2.33

as compared with commercial and experimental birds. Moisture contents of experimental group were comparable with commercial while broiler of commercial, experimental and domestic groups showed more moisture in leg tissues as compared to breast tissues. Mineral contents of tissues of different parts of chicken were also investigated. It was observed that domestic birds had more minerals than experimental while commercial groups had lesser mineral content as compared with experimental. Fat and protein contents of breast and leg tissues of domestic chickens were also higher as compared to breast and leg tissues of commercial and experimental groups while the results of experimental group were comparable with commercial group. Ristic (2004) also observed similar results (Table 2).

Sensory evaluation indicated that color of commercial group was better than experimental

and in case of domestic group color acceptability was the lowest. This can be due to the aging of tissue. In case of domestic group the aroma was better and comparable with experimental birds. Similar results were noted in the case of flavor and taste of meat. Taste of experimental bird meat was comparable with domestic and better than commercial. Texture of meat was very good in case of commercial group and comparable with experimental birds. Similar findings were noted in case of tenderness and juiciness. Chewability was better in case of commercial and experimental groups. Overall acceptability was best in case of experimental group (Table 4).

CONCLUSION

Due to the low quality of commercially available ingredients feed manufacturer are forced to incorporate drugs, which neutralize the ailment factors of the harmful agents present in feed. Presently there is need to strictly follow the withdrawal period of drugs before slaughtering to avoid transfer of antibiotics to the consumers.

From the above results it can be concluded that commercial feeds adversely affected the quality of meat, especially acceptability to the consumers, due to the presence of toxic elements, drugs and other additives as compared with the experimental feeds.

Table 3: Proximate analysis of chicken meat

	Commercial		Experimental		Domestic	
	Breast	Leg	Breast	Leg	Breast	Leg
Moisture	72.25	74.55	72.27	73.77	68.80	72.21
Fat	1.84	2.05	2.10	2.11	4.17	3.95
Protein	24.50	22.33	24.11	23.00	25.48	22.79
Ash	1.41	1.07	1.52	1.12	1.55	1.05

Table 4: Sensory evaluation

Attributes	Commercial	Experimental	Domestic
Color	8.3	8.1	7.2
Aroma	7.4	8.2	8.5
Flavor	7.3	8.3	8.6
Taste of meat	7.5	8.0	8.5
Taste of gravy	7.5	8.4	8.5
Texture of meat fiber	8.6	8.3	7.5
Tenderness	8.7	8.5	7.1
Juiciness	8.4	8.2	6.9
Chew ability	7.8	7.6	6.7
Overall acceptability	7.9	8.1	7.7

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Bacteriological analysis of drinking water of hand pumps in different schools of District Peshawar (Pakistan)

Hamida Abid, Mohammad Nisar Alizai, Javed Ali and Musaddiq Ibrahim

Pakistan Council of Scientific and Industrial Research Laboratories Complex, Peshawar

ABSTRACT

The study was carried out to assess the quality of hand pump drinking water. Twenty-four water samples were collected from different Schools of Peshawar City (Pakistan). These samples were analyzed bacteriologically for total plate count, Coli MPN, Fecal Coli MPN, E coli, Salmonella and *Staphylococcus aureus* using standard methods. The data was compared with the WHO Standards and only 8 % samples were found fit for drinking purposes. 92 % samples were unfit for drinking purpose due to the presence of high bacterial load and pathogenic bacteria. The usage of this water may adversely affect the overall health of the children studying in these schools due to sub standard hand pump water.

Key words: Hand pump, drinking water, bacteria

INTRODUCTION

Water is a precious gift of Allah which has no alternative. Life cannot exist without water. Water is an essential part of human body and other living species/organisms. Apart from its importance as an essential constituent of living organism, it can be a carrier of various harmful organisms. It has been recorded that since the beginning of life water has been a potential carrier of different diseases (Prescott *et al* 2005).

Globally 70 percent of the earth surface is covered with water and yet there is an acute water shortage for drinking and irrigation. This is because three quarters of the water is polluted with salt and one quarter of the water is present in rivers, lakes and in frozen ice caps. Consequently, more than one third of the world population, i.e. 2.4 billion people have no access to clean water (UNIC 2003).

There are three types of water pollution viz; physical, chemical and biological which develops from microorganism. Sewage is the major source of drinking water pollution, which enters the distribution system through leakage, causing serious diseases like cholera, typhoid fever, shigellosis and Gastro intestinal Tract diseases (Pleczar and Kries 1986; Pommervill 2004). Diarrhea diseases are transmitted through contaminated drinking water and cause the death over three million people annually; (Islam *et al* 2005). In Punjab diarrhea ranks second amongst 15 priority infectious diseases in children under 5 years of age clearly indicating the fecal contamination of drinking water supply. It has been recognized that in the great majority of rural water supplies in developing countries fecal contamination is wide spread (WHO 1997). The source of fresh water in Pakistan are glaciers, rivers and lakes but due to the shortage of rains and snowfall, Pakistan also suffer the water shortage. To overcome this situation, the people use under ground water. But before using the underground water it is necessary to check whether the quality of water is good for drinking purpose or not(Khan and Ahmad 2001). According to WHO survey, 60% diseases in the Asian Countries are water borne (Ahmad *et al* 2001). History reveals that pollution of drinking water caused water born diseases, which are capable to wipe out entire population of the cities (Irshad *et al* 2001).

The present study is an attempt to assess the drinking water quality of hand pump at different Schools of District Peshawar (Pakistan). The investigation would be a useful tool for creating awareness amongst the inhabitants specially the young children and their parents, the planners and the decision makers for further improvements in drinking water supply schemes of these schools.

MATERIALS AND METHODS

Twenty-four samples were collected in sterilized Duran Bottles (500 mL) each from different Hand Pumps of the selected Schools of District Peshawar (Pakistan). All the samples were kept in an ice cold box at 4°C at the time of collection and were transferred to the microbiology section of Food Technology Center, PCSIR Laboratories Complex Peshawar. The same approach was adopted on each visit and all samples were collected in duplicate. The samples were analyzed within Two hours after collection for Total Plate Count, Coli MPN, fecal coli MPN, E. coli, *Staphylococcus aureus* and *Salmonella,* using the procedures specified in standard method of (APHA 1998).

Total plate count was determined by the pour plate method using standard methods Agar as a culture medium and incubated at 35°C for 48 hours, using the Memmert incubator. The colonies were counted by Quebec Colony Counter.

The coliform bacteria (Coli MPN) were determined by most probable number (MPN) technique of serially diluted samples 1:10, 1:100 and 1:1000 with 0.1 M strile phoshate buffer. Saline Lactose Broth and Brilliant Green Broth were used as a culture media.

The fecal coliform bacteria were determined by inoculation, the positive tubes from lactose Broth to EC Broth and Incubated at 44.5°C for 24 hours. E. coli were determined by streaking a loop from EC Broth to EMB Agar Plates and incubated at 35°C for 24 to 48 hrs. The colonies were confirmed by E. coli 0157 Pro-Lab Diagnostics kits followed by biochemical tests.

Staphylococcus aureus were determined by using Blood Agar for the isolation of colonies and the isolated colonies were confirmed by commercially available Pro-Lab Diagnostics kits and followed by biochemical tests.

Salmonella were determined by using lactose broth as pre-enrichment medium (25 mL sample + 225 mL lactose broth), incubate at 35°C for 24 hrs. Added 1 mL test portion Preenrichment to 10 mL selenite cystine broths and tetrathionate broth as a selective enrichment medium and incubated at 35°C for 24 hrs. From these two type of media after incubation streaks were made on Bismuth Sulfite Agar, Hektoen enteric agar and xylose lysine desoxycholate agar and incubated at 35°C for 24-48 hrs. The colonies found on these culture media were inoculated for screening on triple sugar iron agar and lysine iron agar and incubated at 35°C for 24 hrs. (TSI Agar) or 48 hrs (LI Agar). Red Slant and yellow butt on TSI or purple butt with or without H_2S_2 production were proceeded for biochemical test and confirmed by Salmonella antisera Pro-Lab kit.

RESULTS AND DISCUSSIONS

Twenty four samples of drinking water were collected from hand pumps of different schools of Peshawar District and analyzed for total plate count, total coli MPN, total fecal coli MPN, *E. coli, Staphylococcus aureus* and salmonella, the results are presented in Table 1. It was observed that 92% of the sample were unfit on the basis of total plate count which ranged from $3.3x\ 10^0$ to $1.2x10^4$ cfu/mL.

These results are in agreement with the investigations and reported data of Ahmad *et al* (1964) who had mentioned high total bacteria count, fecal coci in drinking water of Karachi. Later on Wadud *et al* (1992) investigation showed that most of samples have high level of total bacterial count as well as total fecal coliform. It was found that 42 % samples were unfit on the basis of coliform bacteria. The total Coli MPN of these samples ranged from 15 - >1100 MPN/mL. Similar studies were carried out by Hasni and Qureshi (2004) for drinking quality of water of costal village of Karachi. Cheema *et al* (2003) studied the drinking quality of Muzaffarabad city (AJK) and concluded that 60% samples were not fit for drinking purposes because of coliform bacteria or higher standard plate count. Similar studies were conducted by Khan *et al* (2000). Studies were conducted on the water quality of different sources i.e. tubewells, hand pumps, wells and surface water from Dristrict Bannu. It was concluded that overall quantity of water of Bannu District was

Table 1: Bacteriological analysis of hand pump of different school in District Peshawar

Lab Code	TPC	Coli MPN/mL	Fecal Coli MPN/mL	E. coli	S. aureus	Salmonella
1	9	<3	<3	-ve	+ve	-ve
2	6.5	<3	<3	-ve	-ve	-ve
3	4.5	15	<3	-ve	-ve	-ve
4	3.3×10^{0}	<3	<3	-ve	-ve	-ve
5	2×10^{2}	64	43	-ve	-ve	-ve
6	3.7×10^{2}	<3	<3	-ve	-ve	-ve
7	4.5×10^{2}	<3	<3	-ve	-ve	-ve
8	4.5×10^{2}	<3	<3	-ve	+ve	-ve
9	9.8×10^{2}	<3	<3	-ve	-ve	-ve
10	6.4×10^{2}	>1100	64	-ve	-ve	-ve
11	8.7×10^{2}	<3	<3	-ve	+ve	-ve
12	2.3×10^{2}	<3	<3	-ve	+ve	-ve
13	2.5×10^{3}	<3	<3	-ve	-ve	-ve
14	1×10^{3}	64	43	+ve	+ve	-ve
15	9.7×10^{2}	43	15	+ve	+ve	-ve
16	1.3×10^{3}	<3	<3	-ve	-ve	-ve
17	6×10^{3}	>1100	>1100	+ve	+ve	-ve
18	8×10^{3}	<3	<3	-ve	+ve	-ve
19	1.2×10^{3}	<3	<3	-ve	+ve	-ve
20	4×10^{2}	>1100	>1100	+ve	-ve	-ve
21	1.1×10^{3}	<3	<3	-ve	+ve	-ve
22	1.2×10^{4}	1100	240	+ve	-ve	-ve
23	2×10^{2}	75	23	-ve	-ve	-ve
24	5.3×10^{2}	40	<3	-ve	-ve	-ve

TPC=Total Plate Count

unsuitable for drinking purpose (Khan *et al* 1999). In these investigations among 42% Coli contaminated samples 33% samples were further found to be contaminated with fecal coliform bacteria which ranges from 15 - 1100 MPN/mL. The results were comparable with the previous findings of (Mashiatullah *et al* 1993). The rest of samples have coli MPN and fecal coli MPN < 3 /mL which is the permissible limit of WHO drinking water standard. In these studies 33% fecal contaminated water sample 6 % were further contaminated with E. coli and rest of 72 % were free from *E. coli.*

Fecal coliform and fecal streptococci were considered to originate in the digestive tract of humans and warm blooded animals. The presence of these bacteria in well water, indicated that there is a source of fecal matter entering in the water supply.

The main source of rural well water contamination by bacteria of fecal origin are animal manure, human waste disposal system such as septic units and flood water (ICRC 2005). In Pakistan when the drinking water

source such as hand pumps are drilled, there is no consideration about the distance between the source of water and the potential contamination source. Many hand pumps are drilled close to the toilets in these schools. Water quality of hand pumps and open wells in Punjab, Pakistan was studied by Ali and Ahmad (1994) who investigated the occurrence of fecal coliforms and *E. coli* in these water sources. Similarly the results regarding microbiological analysis are comparable with the findings reported by Aurangzeb and Ali (1991). According to their results 99 % of water samples collected from hand pumps and wells were unfit for human consumption whereas investigation carried out in study it was concluded that 92% samples collected from the hand pumps of different schools of District Peshawar were unfit for drinking purpose.

Table 2: Recommendations for minimum separation distance between wells and potential contaminant source (OMAFRA 1991)

Item	Recommendation for Minimum Distance (m)
Septic Tank	15
Weeping Bed	30
Manure	30
Feed Lot	30
Crop	30
Unused well that has not been properly plugged	30
Lagoon and Land fills	300

OMAFRA for Ontario Ministry of Agriculture, Food and Rural Affair, University of Guelph Canada.

CONCLUSION AND RECOMMENDATIONS

It is obvious that drinking water supplied through hand pumps are largely contaminated and pose serious health risks to the consumers especially the young children of these schools.

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Natural occurrence of mycotoxins in wheat in Pakistan

Salma Khatoon and Nafeesa Qudsia Hanif

Romer Labs. Rawalpindi

ABSTRACT

Wheat samples (n=15) were randomly collected from major wheat stores of government and private sector. These were analyzed for Aflatoxin B1, B2, G1, G2, Zearalenone (ZON), Deoxynivalenol (DON), 3ac-Deoxynivalenol (3ac-DON), 15ac-Deoxynivalenol (15ac-DON), Nivalenol (NIV), Fusarenon-X (FUS-X), T-2 toxin, HT-2 toxin, Diacetoxyscirpenol (DAS), Neosolaniol(Neos) and Ochratoxin A (OTA) by thin layer chromatography at Romer labs Pakistan. The contamination frequency of AfB1 was 33% ranging from 3-5µg/Kg. DON and ZON each were found in 20% samples with a range of 1580-2800 µg/Kg and 250-2200 µg/Kg, respectively. Only one sample was positive for Fus-X (500µg/Kg). All samples were negative for AfB2, G1, G2, 3ac-DON, 15ac-DON NIV, DAS, T-2, HT-2, Neos and OTA. This is the first report in Pakistan about the natural occurrence of a range of *Aspergillus* and *Fusarium* mycotoxins in wheat. The study suggests for the need of monitoring programme to control mycotoxin level in grains as co-occurrence of different mycotoxins has deleterious effects on humans and animal health.

Key Words: Wheat, mycotoxins, TLC, Pakistan

INTRODUCTION

Wheat (*Triticum aestivum*) is the major staple diet of Pakistani population. It ranks first as a cereal crop in terms of its production and consumption. During the year 2005-2006, 21.6 million tons of wheat was produced which is far higher than other major cereal crops including rice and maize (GOP 2006). More than 90% of population obtains about 50 % of the daily calories intake from wheat. Annual per capita consumption of wheat is 124 Kg.

Despite its central position in food chain, fungal invasion/mycotoxin contamination is a major constraint to the wheat crop all over the world. It is colonized by phytopathogenic moulds mainly belonging to the *Fusarium*, *Penicillium*, *Alternaria* and *Aspergillus* genera, which are involved in mycotoxin production. The major fungal species and their mycotoxins are shown in Table 1.

The invading fungi and their toxic secondary metabolites (mycotoxins) results in low production/ yield of crop. It is reported that 10-30% of the harvested grains is lost due to mould infection (Chelkowski 1991).

The FAO has estimated that about 25 % of the grains are affected annually with mycotoxins (CAST 2003). These not only deteriorate the quality of wheat grains by lowering their nutritive value but also pose health risks to humans.

Table 1: Major Mycotoxin producing Fungi

Fungi	Toxin
Aspergillus flavus *Aspergillus paraciticus*	Aflatoxin B_1, B_2, G_1& G_2
Aspergillus ochraceous *Penicillium verrocosum*	Ochratoxin A (OTA)
Fusarium graminearium	Zearalenone (ZON)
Fusarium graminearium *Fusarium culmorum*	Deoxynivalenol (DON)
Fusarium moniliforme *Fusarium proliferatum*	Fumonisins
Alternaria alternata.	Alternaria alternariols, altenuenes, altertoxins, tenuazonic acid,

World Health Organization (WHO 2002) has characterized mycotoxins as significant source of food borne illnesses. These have severe acute and chronic effects on human beings and animals. Their toxic effects are well documented and they are classified as immunosuppressive, carcinogenic, hepatotoxic, nephrotoxic, teratogenic, neurotoxic and cytotoxic (Peracia *et al* 1999; JECFA 2001; CAST 2003).

In developing countries, grain quality always remains a matter of concern due to shortage of food, conventional agricultural practices and inadequate storage facility. Furthermore conducive environmental conditions make the food grains a good/ favorite substrate for fungal invasion and mycotoxin production.

In Pakistan the presence of pathogenic fungi (including *Fusarium graminarium, Aspergillus flavus)* on wheat crop has been reported too (Iftikhar *et al* 2003) but the mycotoxins contamination of wheat grains is relatively unexplored subject. There are sporadic data about the occurrence of mycotoxins in wheat giving emphasis to / on Aflatoxin B1 (Shah 1985). Isolated attempts were made to assess ochratoxin A in Wheat (Karim 1993). But so far data for the occurrence of other mycotoxins including *Fusarium* are not available. So this survey was designed to obtain information about the incidence and levels of commonly occurring mycotoxins in wheat.

MATERIALS AND METHODS

Sampling

Fifteen (15) wheat samples (1-2 kg) were collected from major wheat stores of government and private sector. The samples were ground and sub-sampled by Romer Series II mill for obtaining a homogenous and representative sample. The surplus samples were stored in sealed paper envelopes as file samples.

Sample Extraction, Cleanup and Thin Layer Chromatography

A 25g portion of well ground sample in mixture of Acetonitrile: water (84:16) was blended in Osterizer Blender (Osterizer Sunbeam – Oster Household Products, USA) at high speed for 3 minutes. For clean up MycoSep® column 226 (for aflatoxins and Zearalenone), MycoSep227® in combination with MultiSep® 216 (for A &B-Trichothecenes) and MycoSep229® (for Ochratoxin A) was used. These samples were then subjected to analysis for total Aflatoxins, Zearalenone, A & B Trichothecenes and Ochratoxin A according to the method described by-Hanif *et al* (2006).

Estimation of Mycotoxins

Toxin estimation in samples was made by comparing with mycotoxins standards that were procured from Biopure, Austria.

RESULTS AND DISCUSSIONS

Aflatoxin B1 was found in 33% samples with a range of 3-5 µg/kg. ZON was found in 20 % samples with a minimum and maximum content of 250µg/kg and 2200 ug/kg, respectively. DON also showed similar situation with 20% prevalence rate ranging from 1580--2800 µg /kg. One sample showed the presence of another Trichothecenes / fusarium toxin, Fusarenone-X with toxin level of 500 µg /kg. All other toxins of *Fusarium* group, *Aspergillus* group and OTA were not detected.

With regard to co-occurrence 13 % samples were concurrently contaminated with AfB1, ZON and DON while 20 % samples showed co-contamination of AfB1 with ZON and DON separately/each.

Mycotoxins are ubiquitous toxic secondary metabolites of fungi and cereals are their major substrate as they provide excellent growth conditions for fungi in the field, after harvest and during storage. The worldwide occurrence of aflatoxins in cereals is well documented. Among cereals corn is most frequently contaminated by aflatoxins where wheat like small sized grains are less susceptible (Galvano et *al* 2005). The results of present study partially confirmed the fact as low concentration (3-5 ug/kg) was found. However the high frequency of contamination with aflatoxins found here is at variance with previous studies and surveys (Curtui *et al* 1998; Shah 1985) who failed to find confirmable aflatoxins in wheat.

Fusaria can grow and produce toxins in cereals not only in the field but also after harvest if the moisture content of kernels is high enough. Thus, DON and ZON were formed to a considerable extent during ambient air drying of wheat (Langseth *et al* 1993).

Table 2: Occurrence of mycotoxins in wheat

Toxin	No. of Positive Samples	Positive Samples (%)	Range (µg /Kg)	Detection Limit (µg /Kg)
Aflatoxin B1	05	33.33	3-5	1.00
Aflatoxin B2	ND	ND	ND	0.50
Aflatoxin G1	ND	ND	ND	1.00
Aflatoxin G2	ND	ND	ND	0.50
Zearalenone	03	20.00	250-2200	125
Deoxynivalenol	03	20.00	1580-2800	100
3ac-Deoxynivalenol	ND	ND	ND	100
15ac-Deoxynivalenol	ND	ND	ND	100
Nivalenol	ND	ND	ND	500
Fusarenone-X	01	7.00	500	500
T-2toxin	ND	ND	ND	100
HT-2	ND	ND	ND	100
Neosolaniol	ND	ND	ND	500
Diacetoxyscirpenol	ND	ND	ND	250
Ochratoxin A	ND	ND	ND	2.00

ND-Not Detected

Numerous investigations on the natural occurrence of fusarium toxins in wheat have been carried out in different parts of the world (Bhat *et al.*, 1989; Luo *et al* 1990; Abouzied *et al* 1991 and Vrabcheva *et al* 1996). Generally DON and ZON were determined whereas NIV, 3ac-DON, 15ac-DON, T-2, HT-2, DAS and Fus-X were analyzed less frequently. The high levels of DON, in the present study, are consistent with results of other Asian countries as documented by Luo *et al* (1990) who analysed 30 wheat samples from two Chinese provinces for DON, NIV and ZON confirmed the high level of DON. Bhat (1989) also reported the occurrence of Trichothecenes in wheat in India.

Mycotoxin co-contamination is a growing issue/concern as mycotoxins in combination (either synergistic or additive) appear to exert greater negative impact on the health in comparison with their individual effects (Smith and Seden 1998). Natural co-occurrence of mycotoxins in wheat with special emphasis on *Fusarium* toxins (ZON & DON) has been reported in several countries (Abouzied *et al* 1991; Curtui *et al* 1998; Furlong *et al* 1995; Hagler *et al* 1984 and Vrabcheva *et al* 1996). Earlier Hagler *et al* (1984) reported simultaneous occurrence of AfB1, DON and ZON. According to him 67 % wheat samples were positive for both AfB1 and DON while 6 % samples contained AfB1, DON and ZON simultaneously. The present results were in accordance with him.

In summary/essence the data presented here demonstrated that there was low AfB1 level but had high levels of DON and ZON following Fus-X. As wheat is the major staple diet in Pakistan so, the present data suggested the need for regular screening of wheat grains and grains

based products for *Fusarium* toxins especially DON and ZON. Furthermore analytical data, obtained from food monitoring programme, in combination with available toxicological data be used for risk assessment that can serve as a basis for establishing particular regulatory limits to minimize the levels of mycotoxin contamination in the food / feed and thereby protect the human and animal health.

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Mycotoxin contamination in rice produced in Pakistan

Nafeesa Qudsia Hanif and Salma Khatoon

Romer Labs, Rawalpindi

ABSTRACT

Twenty samples were simultaneously collected from rice producing areas and were evaluated for total Aflatoxins, Zearalenone, Deoxynivalenol and T-2 toxin by thin layer chromatography. For Aflatoxin B1 (65 %), Aflatoxin B2 (5 %), Aflatoxin G1 (10 %) and Zearalenone (10 %), samples were found positive with a range of 4-18µg/kg, 0.5-3µg/kg, 1-22µg/kg and 261-2813µg/kg respectively. All contaminated samples were found to be at the elevated levels than the regulatory levels of the European Commission. These findings highlight the risk to the population exposed to contaminated rice grains. Attention must be paid by Ministry of Food and Agriculture and Ministry of Health to develop regulatory/advisory limits in food grains for mycotoxins in Pakistan.

Key Words: Pakistan, rice, mycotoxins, thin layer chromatography

INTRODUCTION

Rice (*Oryza sativa* L.) is the second major cash crop and is also one of the main export items of the country. It accounts for 6.10 percent of the total value added in the agriculture and 1.30 percent to the GDP (GOP 2006). The annual production of rice for the year 2006/07 is expected to reach 5.2 MMT and out of which 2.4 MMT (consisting 0.8 MMT of Basmati and 1.6 MMT of IRRI rice varieties) export volume is projected (Pakistan Grain and Feed Annual Report No. PK 6002 2006).

Rice is not a staple commodity in the Pakistani diet but its consumption is increasing slowly due to ability to mix well with most staple diets. However, the grain's nutritive value (as an energy source i.e. 2345 kcal/kg) (Singh and Panda 1988) and its high hygroscopicity make it an ideal substrate for the establishment and growth of fungal species, especially toxigenic fungi that produce mycotoxins like aflatoxins (Shotwell *et al* 1966). Factors like moisture content, water activity, temperature, period of storage, initial levels of contamination, toxigenic potential of fungal strains and nature of the substrate influence the production of mycotoxins (Lacey 1986). In addition, during storage fungi cause organoleptic changes that result in grain discoloration, mould odor, mustiness and reduced germinability by destroying embryo (Taligoola *et al* 2004). Furthermore, it will be unsafe for human consumption due to its ability to cause mycotoxicosis. Studies have demonstrated that mycotoxins are immunosuppressive, they are strongly suspected to interfere with immune processes in humans, thus increasing overall susceptibility to infective agents (Pfohl-Leszkowicz 2002).

In developing countries, consumers are more exposed to mycotoxins than in developed countries. This is due to several factors, e.g. shortage of food resources, conventional food handling procedures, improper/inadequate preservation technology and ineffective/no regulation and control of food quality. According to the United Nations Food and Agriculture Organization (FAO), approximately 25% of the world's grain supply is contaminated with mycotoxins (CAST 2003). Aflatoxin contamination of cereals is rarely a concern per se, although its frequent co-occurrence with various combinations of other mycotoxins can cause problems. Nevertheless, very recently Park *et al* (2004) reported that rice was the major contributor to the dietary intake of Aflatoxin B1in Korea and calculated that probable daily intake of Aflatoxin B1 for Koreans exceeds the estimated provisional maximum tolerable daily intake (0.58 to 3.94 ng/kg of body weight per day).

In Pakistan, weather conditions are conducive for fungal growth. Inadequate storage conditions facilitate the growth of storage fungi like *Aspergillus* and *Penicillium* species. Only skimpy

and patchy attempts have been made for mycotoxin analysis in rice other than aflatoxins and to some extent for ochratoxin A while rest of the commonly occurring mycotoxins were ignored. In view of above, present study was planned to observe the scenario of mycotoxins in rice in Pakistan.

MATERIALS AND METHODS

Sampling of rice grain

A total of 20 rice samples (1-2 kg) were collected from the rice producing areas and various major stores of government/private sector in Pakistan. The samples were ground and sub-sampled (50g) by Romer Series II mill for the purpose of obtaining a homogenous and representative sample. The surplus samples were stored in sealed envelopes as file samples.

Sample extraction and cleanup

A 25g portion of well ground sample in mixture of acetonitrile: water (84:16) was blended in Osterizer Blender (Osterizer Sunbeam – Oster Household Products, USA) at high speed for 3 minutes. For clean up MycoSep® column 226 (for Aflatoxins and Zearalenone) and MycoSep227® in combination with MultiSep® 216 (for Deoxynivalenol and T-2 toxin) were used. These samples were then subjected to analysis for total aflatoxins, Zearalenone, Deoxynivalenol and T-2 toxin according to the method described by Hanif *et al* (2006).

Determination and estimation of mycotoxins

The samples were spotted on the silica gel 60 TLC plates. The plates were developed according to the method as described above. For estimation of toxins under UV light standards used were procured from Biopure, Austria.

RESULTS

A total of 20 rice samples were analyzed for Aflatoxins, Zearalenone, Deoxynivalenol and T-2 toxin. Of these, 65 % were contaminated with Aflatoxin B1 and all contained toxin higher than the regulatory limits directed by EC (Pakistan has not established regulatory limits for mycotoxins up till now). These findings are in line with Toteja *et al* (2006) who found 17% samples positive for Aflatoxin B1 having levels above than 30µg/kg (Indian regulatory limit) but contrary with the findings of Shah *et al* (1985) who analyzed fourty-three samples and found no positive sample for Aflatoxin B1. Beside these, Aflatoxin B2 (5%) and Aflatoxin G1 (10%) were also detected with a range of 0.5-3 ppb and 1-22ppb respectively (Table 1). No sample was detected positive for Aflatoxin G2, Deoxynivalenol and T-2 toxin.

Table 1: Incidence and range of mycotoxins in Rice samples

Mycotoxins	No. of samples	Positive samples	Contamination detected (%)	Range of mycotoxin levels µg/kg	Detection Limits (µg/kg)	Action Levels (EC) No. 856/2005
Aflatoxin B1	20	13	65.0 %	4-18	1	2 for AfB1/4 for Total Aflatoxins (B1, B2, G1, G2)
Aflatoxin B2	20	1	5.0 %	0.5-3	0.5	
Aflatoxin G1	20	2	10.0 %	1-22	1	
Aflatoxin G2	20	ND	-	-	0.5	
Zearalenone	20	2	10.0 %	261-2813	125	1250
						200-250 for infants
Deoxynivalenol	20	ND	-	-	100	100
T-2 toxin	20	ND	-	-	125	Not legalized

ND: (not detected)

However, 10 % samples were found positive for Zearalenone with the range 261-2813 µg/kg which is much higher than the recommended levels (Table 1). There are no data available for the presence of field mycotoxins like Zearalenone in rice grain.

DISCUSSION

Mycotoxins are ubiquitously distributed. A number Mycotoxins are immunosuppressive and likely could be involved in human diseases. Humans are exposed to mycotoxins through several routed such as ingestion (the most prominent mean of exposure), contact and inhalation (CAST 2003). Aflatoxins are storage mycotoxins and known for causing acute aflatoxicosis in human but chronic forms of aflatoxicosis, especially carcinomas are more problematic. Aflatoxins have been declared by IARC (International Agency for Research on Cancer) as class 1 carcinogen. Similarly, Zearalenone is a non-steroidal estrogenic mycotoxin produced by several *Fusarium* species. Little information is available regarding the effects of Zearalenone on humans. However, ingestion of this estrogenic compound cause premature puberty occurred in 7 and 8 years old children in Peruto Rico (Painter 1997; Saenzde Rodriquez et *al* 1985).

The findings of present study highlight the risk to which populations exposed to rice grains contaminated with aflatoxins and Zearalenone. Rice is our major export item and is being exported to various countries of Europe and Middle East. So far in Pakistan no safety regulations have been established for mycotoxins in food grains for use as staple diet and for export. For population health concerns and our existence in the international market, it is very important to establish regulations for mycotoxin contamination.

CONCLUSION

A truly mycotoxin-free food supply for human consumption cannot be guaranteed. To identify and remove all naturally occurring mycotoxin contamination from foods and feeds is not possible. To avoid mycotoxins, attention must be paid by Ministry of Food and Agriculture and Ministry of Health to develop regulatory/advisory limits in food grains for mycotoxins in Pakistan.

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