PAK. J. FOOD SCI., 13(1-2): 1-4

Effect of Psyllium husk fiber on the quality of sweetened stirred yoghurt

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ABSTRACT

Yoghurt and Psyllium husk fiber possess nutritional and therapeutic values. This investigation was carried out to explore the possibility of using different level of Psyllium husk fiber and determine its effects on the quality of sweetened stirred yoghurt. Five yoghurt samples were prepared with three different levels, (0.2%, 0.3% and 0.4%) of Psyllium husk. It,was observed that addition of fiber reduced the changes in acidity, pH and syneresis but had no effect on fat, total solids and ash contents. During storage, the deterioration in samples without fiber was higher than with fiber. Sweetened stirred yoghurt fortified with 0.2% Psyllium husk fiber was better in quality and shelf life than all other treatments. Yoghurt prepared with 0.2% Psyllium husk fiber scored highest for flavor, body and texture, sensory acidity and appearance.

Keywords: Psyllium husk, sweetened, yoghurt, chemical analysis

INTRODUCTION

Yoghurt has long been believed to be a healthy food source and thus beneficial to the body in such a way that it seems to enhance the micro flora of the gut. The production of lactic acid by the microorganisms (*Lactobacillus bulgaricus* and *Streptococcus thermophillus*) in yoghurt decreases the pH of the gut. This increase in acidity affects the solubility and absorption of minerals, such as calcium and iron and creates environment less favorable for the common food born pathogens (Tamime and Robinson 1988).

Consumers are more concerned about the nutritional aspects of the food they eat. Many researchers have recognized the nutritional and therapeutic value of consuming fermented milk products because they contain significant levels of organic acids, among other substances, which may help in the cure of illness such as diarrhea and hypercholesterolemia (Deeth and Tamime 1981; Rubin and others 1982; Robinson and Dombrowski 1983).

The daily recommended intake of 25-30g dietary fiber (Labell 1990) also plays a major role in the prevention of constipation, hemorrhoids, hypercholesterolemia and colon cancer (Dreher 1987). It is therefore reasonable to expect a steady increase in the consumption of healthy, fiber-rich foods in future. Only a few studies of the development of fiber-fortified and flavored milk drinks have been carried out (Angelino 1993). Most of the fiber addition studies are carried out with the improvement of texture through the addition of stabilizers and emulsifiers such as pectin, Arabic gum, xanthan, gelatin and alginates (Wittinger and Smith 1986). The addition of oat fiber in yoghurt has already been studied with good results (Fernandez-Garcia and Mcgregor 1997).

Psyllium is a natural source of dietary fiber commonly consumed during diarrhea and constipation in Pakistan with milk, curd and water. It is made from a broad-leaved perennial herb called the 'Plantago ovata'. The outer husk and its seed has been treasured and utilized for its many therapeutic qualities. Isabgol contains mainly undigestible dietary fiber with negligible amount of calories. When it absorbs water, it characteristically swells several times and turns into jelly-like substance. This substance stimulates and lubricates the intestines. Hence it also helps to activate bowel movement (Leung and Foster 1996).

The disadvantage of higher doses of fiber is that some chemical compounds in fiber may interact with food components during processing. Interactions between ingredients may lead to changes in nutrient bioavailability and the texture and flavor of the final product. (Birch and Parker 1986)

The nutritional and therapeutic importance of yoghurt and Psyllium husk fiber forced to plan a study to develop Psyllium husk fiber (Ispaghol) fortified sweetened stirred yoghurt by using different levels of Psyllium husk powder. The objectives of investigation were to determine the effect of Psyllium husk fiber on shelf life, quality and consumer acceptance of yoghurt.

MATERIALS AND METHODS

The ingredients like milk of Milk pak brand (standardized to12.45% total solids), sugar (sucrose), Psyllium husk fiber of Marhaba Ispaghol *(Plantago ovata)* ground to fine powder and culture of Nestle plain yoghurt were obtained from local market and used for the sweetened stirred yogurt production following the formulations as given in table 1.

Formulation of Stirred Yoghurt

Table 1. Formulation of different stirred yoghurt treatments.

Treatments	Yoghurt (%)	Sugar (%)	Psyllium husk fiber (%)
T_1	100		
T_2	90	10	
T_3	89.8	10	0.20
T_4	89.7	10	0.30
T_5	89.6	10	0.40

Manufacture Of Stirred Yoghurt

Psyllium husk powder at different levels according to formulations were added in the milk and soaked for 5 minutes and then the added sugar was mixed thoroughly in a blender before pasteurization in water bath at 80-85°C for a period of 30 minutes. The mixtures were cooled to 42°C and inoculated with culture at the rate of 2.5% with continuously stirring for 5 minutes at 42°C. The inoculated mixtures were incubated for 3.5-4.5 hours at 42°C and cooled to 6-10°C when the pH reaches to 4.5-4.7. During cooling the yoghurts were stirred in a blender and filled in small cups of appropriate size before storing in a refrigerator at about 2-4°C.

ANALYSIS OF STIRRED YOGHURT

All the stirred yoghurts were evaluated for their physical, chemical and sensory characterized. Physical characteristic (syneresis) was determined by the method of Peri and others (1985) and chemical analysis (pH, acidity, total solids, ash, and fat) by the methods of AOAC (1995) after every five days intervals. To assess the sensory quality (flavor, body & texture, taste (sourness) and appearance) of sweetened stirred yoghurt fortified with psyllium husk fiber, the yoghurt samples were presented to a panel of five judges regularly after five days interval for a period of one month. The score card used for sensory evaluation was approved by American Dairy Science Association (Nelson and Trout, 1964).The data collected from chemical analysis and sensory evaluation was statically analyzed by applying the analysis of variance techniques of Steel and others (1997).

RESULTS AND DISCUSSION

Chemical Analysis Results

The effect of Psyllium husk addition on the quality of yoghurt was studied for total solids, fats, ash, acidity, pH and syneresis (Table 2), and effect of storage on these characteristic was also observed (Table 3). The results indicate that addition of fiber did not affect the characteristics of yoghurt except pH, acidity and syneresis. The total solids of control (T1) were less (13.11%) than the other treatments (22.81-23.51%) which is due to addition of sugar not fiber contents. As indicated in table 3, the total solids did not significantly decreased during storage (21.54-20.85%). The ash content no significantly increased from 0.78 to 0.81% in yoghurts from T1 to T5 and decreased (0.81-0.76%) during storage. Fat of all the samples varied from 3.46 to 3.52% no significantly but significant decreases from 3.69 to 3.19% in all samples were observed for one month of storage. The fiber addition decrease acidity (0.76-0.80%) in T3 to T5 than control and T2 (containing sucrose), which contain 1.26% and 1.22% acidity respectively. A no significant increase (0.79 to 0.87%) in acidity was found up to 15 days of storage however it increased significantly to 1.12% and 1.37% in yoghurt after 25 and 30 days of storage respectively. The pH of samples containing fiber (T3-T5) was no significant (4.36-4.40) but significantly higher than control and yoghurt containing sucrose having values 3.86 and 3.5 respectively. In contrary to acidity pH decreased during storage but significance was similar to acidity with days. Syneresis quantities were different for different samples. The highest was determined in T2 fallowed by T1, T5 and T4 (20.77, 18.56, 8.11 and 7.66ml respectively) while the lowest (7.29ml) was detected in T3 (containing 0.2% fiber).

The increases in total solids (T2-T5) were due to addition of sugar and decrease during storage might be due to change of lactose into lactic acid by the bacteria of yoghurt during fermentation (Tamime and Robinson, 1985). The decrease in fat during storage is due to the separation of whey proteins on the surface or sedimentation of casein proteins. The decrease in fat may also be due to deterioration of fat. A mild increase in ash content is due to addition of fiber because fiber contains an appreciable quantity of minerals. But the increase is not significant due lower level of fiber used. It was observed that Psyllium husk fiber stabilized the yoghurt samples and a decrease in acidity of fortified sampe was the result. The shelf life of yghurt containing fiber was 15 days more than the control and yoghurt containing sucrose only. Psyllium husk fiber contains cellulose, hemicellulose and gums etc which maintain the pH of yoghurt and less decrease is examined than the other samples during storage (Tamime and others, 1997). It is also observed that the pH of yoghurt prepared with 0.2% and 0.3% husk fiber were higher that 0.4% fiber containing yoghurt. The comparative studies indicate that least syneresis was found in yoghurt with 0.2% fiber than other samples. Which indicate that addition of fiber up to certain level has the stabilizing effect and above that level it start to lose its properties. Syneresis problem increases in all treatments gradually during storage period but fiber-fortified yoghurts have less syneresis problem due to its water binding capacity.

SENSORY EVALUATION

For the acceptance of consumers a sensory evaluation of yoghurts was carried out by a panel of judges who scored for different characteristics. The distribution of score was, 45 for flavor, 30 for body and texture, 10 for sourness and 15 for appearance. It was observed that highest score 36.37 for flavor, 24.17 for body and texture, 7.73 for sourness and 12.14 for appearance were given to T3 containing 0.2% fallowed by T4 containing 0.3% fiber. The minimum scores 31.20 and 17.8 of flavor and body & texture respectively were for T1, and 5.49 and 9.23 of sourness and appearance respectively for T2 (containing sucrose).

Fernandez-Garcia and others (1998) described that fiber addition improved the body and texture of unsweetened yoghurts but lowered overall scores for body and texture in yoghurts sweetened with sucrose. Fortification of stirred yoghurt with psyllium husk fiber produced the higher overall quality product. Psyllium husk fiber addition permitted the development of a good fermented product, with no significant decrease

Table 2. Effect of Treatments on Physico chemical Characteristics of Psyllium Husk Fiber Fortified Stirred Yoghurt

Treatments	Total solids (%)	Ash (%)	Fat (%)	Acidity (%)	pH	Synersis (mL)
T1	13.11b	0.78a	3.52a	1.26a	3.86c	18.56b
T2	22.81a	0.78a	3.49a	1.22a	3.95b	20.77a
T3	23.47a	0.80a	3.47a	0.76b	4.40a	7.29d
T4	23.43a	0.80a	3.48a	0.78b	4.40a	7.66c
T5	23.51a	0.81a	3.46a	0.80b	4.36a	8.11c

Table 3. Effect of Storage on Physico chemical Characteristics of Psyllium Husk Fiber ortified Stirred Yoghurt

Storage Days	Total solids (%)	Ash (%)	Fat (%)	Acidity (%)	pH	Synersis (mL)
0	21.54	0.81	3.69	0.79	4.43	1.18
5	21.51	0.81	3.64	0.18	4.41	3.42
10	21.44	0.80	3.57	0.84	4.36	8.47
15	21.35	0.80a	3.52	0.87	4.27	12.08
20	21.22	0.79	3.45	0.95	4.18	15.95
25	20.98	0.77	3.33	1.12	3.99	20.20
30	20.85	0.76	3.19	1.37	3.71	26.08

Table 4. Effect of Treatments on Sensory Characteristics of Psyllium Husk Fiber Fortified Stirred Yoghurt

Treatments	Flavor	Body & Texture	Sourness (Sensory acidity)	Appearance
T1	31.20c	17.80c	6.13c	10.11b
T2	32.80bc	18.66c	5.94c	9.23b
T3	36.37a	24.17a	7.73a	12.14a
T4	34.09ab	21.11b	7.14ab	10.51b
T5	31.49c	18.69c	6.30bc	9.37b

Table 5. Effect of Storage on Sensory Characteristics of Psyllium husk Fiber Fortified Stirred Yoghurt

Storage days	Flavor	Body & Texture	Sourness (Sensory acidity)	Appearance
0	40.56	24.96	8.36	12.92
5	38.64	23.04	8.08	12.44
10	35.92	21.38	7.48	11.32
15	34.00	20.40	6.78	10.56
20	32.04	18.86	6.14	9.56
25	27.88	16.92	5.42	8.40
30	23.28	15.04	4.28	6.72

T1 = Plain stirred yoghurt (Control)
T2 = Stirred yoghurt fortified with 10 % sucrose
T3 = Stirred yoghurt fortified with 0.2% psyllium husk fiber and 10% Sucrose
T4 = Stirred yoghurt fortified with 0.3% psyllium husk fiber and 10%
T5 = Stirred yoghurt fortified with 0.4% psyllium husk fiber and 10% Sucrose

in flavor quality and only a slight decrease in texture quality. Psyllium husk fiber addition to sweetened stirred yoghurt may prove to be an efficient method of increasing dietary fiber intake in a pleasant way.

CONCLUSION

Physico-chemical evaluation of sweetened stirred yoghurt fortified with 0.2% Psyllium husk fiber was of better quality and greater shelf life then all other treatments. It was observed that yoghurt prepared with 0.2% Psyllium husk fiber got maximum scores in connection with flavor, body and texture, sensory acidity and appearance on the basis of sensory evaluation of yoghurt samples under various treatments.

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PAK. J. FOOD SCI. 13(1-2): 5-9

Characterization and preservation of *streptococcus* spp. as yoghurt culture

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ABSTRACT

Yoghurt starter culture (*Streptococcus thermophillus*) isolated from indigenous source and Yoghurt was prepared from this isolated culture. Effect of different concentrations of single starter culture on quality of yoghurt during storage was observed. All the parameters i.e. total solids, pH and lactose showed decrease throughout the storage period while acidity increased. The investigation showed that 2% starter culture the best for the manufacturing of yoghurt.

Key Words: Yoghurt, Starter culture, *Streptococcus thermophillus*

INTRODUCTION

Successful production of yoghurt is directly related to the correct selection, preservation, handling and propagation of starter culture.

The yoghurt is produced under controlled conditions by following well-defined procedures. The basis of yoghurt manufacturing is lactic acid fermentation carried out by various types of bacteria under different incubation temperatures and periods. Fermented milk products (yoghurt) have been used for their possible therapeutic value in stomach and intestinal disorders.

The milk used for the preparation of yoghurt is pasteurized and then inoculated with starter culture. Starter culture consists of two bacteria i.e. *Streptococcus thermophillus* and *Lactobacillus bulgaricus*. *Streptococcus thermophillus* produces typical characteristics and yoghurt flavour. The acceptable limits of *Streptococcus thermophillus* are to be calculated for commercial production of yoghurt while establishing a good quality starter culture. Keeping in view this project was planned to find out the effect of different concentrations of single starter culture on the quality of yoghurt during storage.

MATERIALS AND METHODS

PROCUREMENT OF SAMPLES

Twenty samples of commercial curd were collected from different areas of Faisalabad. 10 mL curd sample (sterilized at 171°C for 30 min in hot-air-oven) was stored under refrigerated conditions for further processing.

Media preparation:

All media (Nutrient agar, Milk agar, Neutral red chalk lactose agar, Beta-disodium glycerol phosphate medium and Yeast lactose agar/broth) used for microbial growth were prepared according to Cappucino and Sherman (1996) and Harrigan and McCance (1976).

Isolation of *Streptococcus* spp

Isolation of the particular microorganisms was done according to method given by Harrigan and McCance (1976). Curd samples were diluted to 1:10 in sterilized normal saline solution and 1ml volume from each dilution was inoculated onto the surface of Milk agar (MA) and Nutrient agar (NA) and incubated at 37°C for 48 hours. The growth thus obtained was checked for morphological and cultural characteristics.

Morphological examination of culture

Morphological and cultural examination was carried out by using Gram's staining method as described by Cappucino and Sherman (1996).

Purification of isolates

All the colonies from Milk agar, showing frequent spherical, chain forming pattern and Gram positive (G+ve) character were separately studied and later on purified on Neutral red chalk lactose agar and Yeast lactose agar plates by applying streak plate techniques. Growth obtained after 48 hours at 37°C was then examined for morphological and cultural characteristics. Pure colonies of these cultural isolates were finally transferred on to the surface of Beta-disodium glycerophosphate agar plates. Incubation was made at 37°C for 48 hours and pure growth of Streptococcus thus obtained was transferred into Yeast lactose broth. Incubation was made at 37°C for 48 hours and then the culture isolates were stored at refrigeration temperature for further use.

Identification of pure culture

Pure culture thus isolated in the above process was identified up to species level on the basis of sugar fermentation tests, biochemical tests and enzyme activity tests as recommended by Harrigan and McCance (1976) and Cappuccino and Sherman (1996).

YOGHURT PREPARATION

(a) Procurement of Raw Materials

Milk. Milk Pak brand was procured from local market.

Skim Milk Powder. Skim milk powder namely 'skims' was procured from local market.

Stabilizers. Food grade stabilizer namely pectin at the rate of 0.5% was used and procured from local market.

Mono Culture. A monoculture of *Streptococcus thermophilus* thus obtained was used for yoghurt preparation.

Standardization. Milk was standardized to 3.5% fat and 13% solid not fat.

(c) Addition of Stabilizer.

Calculated amount of stabilizer as mentioned earlier was added in each sample at 75°C in water bath and blended for ten minutes for complete dispersion.

(d) Pasteurization.

After addition of stabilizer, the milk was pasteurized in water both at temperature of 75°C for a period of 30 minutes.

(e) Inoculation.

The standardized milk after treatment was cooled to 40°C and inoculated with pure single starter culture using different concentrations for each lot as showed in Table-1 and continuously stirred for ten minutes for thorough mixing of culture.

Table-1: Different concentrations of culture to be used for yoghurt preparation

Lot No.	% Culture
T_1	0.5
T_2	1.0
T_3	1.5
T_4	2.0
T_5	2.5
T_6	3.0
T_7	3.5

(f) Incubation

The inoculated milk was incubated at 40°C for 4 hours.

(g) Cooling

The yoghurt was cooled down at 4°C to check any further fermentation.

CHEMICAL ANALYSIS OF YOGHURT

The yoghurt samples were chemically analyzed for pH, acidity, total solids and lactose during storage of 4 days at intervals of 0, 24, 48, 72 and 96 hours in accordance with the methods explained in AOAC (1984).

STATISTICAL ANALYSIS

The data thus obtained was subjected to statistical analysis by complete randomized design (2-factor factorial) and comparison of means was carried out by Duncan's Multiple Range Test (Steel and others 1996).

RESULTS AND DISCUSSION

The research was conducted to study the isolation, effect of starter culture percentage and storage on the shelf life of plain set yoghurt. Different levels of culture were used and pH, acidity, total solids and lactose contents were considered as basic parameters to see the both effects.

Isolation of microorganisms

Three different types of colonies were observed on Nutrient agar and Milk agar plates. Most of the colonies were white, irregular, circular and pinpoints. The cultural and morphological characteristics were further resolved on the basis of microscopic examination. Majority of microorganisms were G+ve rods and cocci shaped bacteria. Out of total samples, 62% rods, 25% clusters and 13% cocci were observed on nutrient agar plates whereas 52% rods, 16% clusters and 32% cocci were examined on Milk agar plates respectively when all the twenty samples were considered. Results obtained were in accordance to the findings of Harrigan and McCance (1976) and Cappuccino and Sherman (1996).

Six samples (S_1, S_3, S_9, S_{12}, S_{14} and S_{18} were found to have maximum numbers of G +ve indicating *Streptococcus* spp. and were further proceeded for isolation and purification. The growth from these six samples was then especially transferred on to the surface of Neutral red chalk lactose agar and Yeast lactose agar plates for more specifications. Exuberant growth of *Streptococcus* spp. was obtained on Neutral red chalk lactose agar as compared to Yeast lactose agar showing the suitability of Neutral red chalk lactose agar for isolation of *Streptococcus* spp. as also recommended by Harrigan and McCance (1976) and Cappuccino and Sherman (1996). The results of colony and morphological characters observed on Neutral red chalk lactose agar and Yeast lactose agar were recorded.

Purification of isolates

The colonies for S_1 & S_2 showing G +ve chain forming cocci were further purified onto the surface of beta-di-sodium glycerophosphate (M17) agar plates and in Yeast lactose broth. Single types of colonies were obtained after 24 hours incubation at 40°C. Colonies found on M17 agar plates were white in colour, lens shape, small in size, pinpoint, smooth and 1.2- 2 mm in diameter, whereas there was turbidity and sedimentation in Yeast lactose broth. Medium M17

was found to be the most suitable selective medium for *Streptococcus* spp. as also confirmed by Shanker and Davies (1977).

Identification of Microorginisms

For the identification of microorganisms the purified culture of yoghurt samples (S_1 & S_{12}) were further analyzed on the basis of sugar fermentation, biochemical and enzyme activity tests. Results of sugar fermentation tests, biochemical tests and enzyme activity tests for culture isolates (from selected S_1 & S_{12}) are shown in Table-2 and Table-3. On the basis of these results it was confirmed that the culture isolated (from S_1 and S_{12}) was a pure culture of *Streptococcus thermophilus*

Table-2: Results indicating sugar fermentation tests recorded for the culture isolates from yoghurt sample (S_1 & S_{12})

Sugars	Acid production	Gas production
Lactose	+	–
Sucrose	+	-
Fructose	+	–
Glucose	+	-
Maltose	-	–
Glactose	-	-
Inulin	-	–
Glycerol	-	-
Manitol	-	–
Sorbitol	-	-
Dextrose	+	–

+ = Acid production, Gas production
- =No acid production, No gas production

Yoghurt preparation

Yoghurt was prepared by using different concentrations of culture isolated from indigenous source with a viable count of 10^8 /ml viable cells. The yoghurt prepared was then chemically analyzed.

Chemical analysis :

pH

The data for the effect of storage on pH under various starter culture treatments is presented in Table-4. It is indicated that pH of all the treatments of yoghurt samples decreased during storage. The minimum initial pH (4.16) in case of T_7 was remained minimum (4.05) and maximum pH (4.65) in case of T_1 was remained at higher level and found to be maximum (4.54) after storage of 96 hours. It was found that over all trend of pH decreased in all the samples with storage. The reason for decrease in pH was an increase in acidity due to the conversion of lactose into lactic acid during storage period.

The results are supported by the findings of Puhan and others (1974) who reported that pH decreases during storage. The studies could also be related to the conclusions of Bilal (1995) and Masood (1997) who stated that pH decreases during storage and acidity increases.

Statistical analysis of data showed that treatments, storage and their interaction has highly significant effects on pH of various yoghurt samples at the probability of 0.5%.

Table-3: Results indicating biochemical tests recorded for the culture isolates from yoghurt samples (S_1 & S_{12})

Experimental Procedure	Observation	Results
Litmus milk reaction	Acid, rapid reduction with curd	+ve
H_2S production	No black precipitation	-ve
Nitrate reduction	No change in colour	-ve
Indole production	Layer not red	-ve
Methyl red test	Bright red color	+ve
Voges Prospkauer test	Pink color	+ve
Citrate utilization	Green Color	- ve
Urease activity	Red colour	-ve
Catalase activity	No bubbling	-ve
Starch hydrolysis	No clear zone around the growth	-ve
Lipid hydrolysis	Medium retain opacity	-ve
Gelatin liquefaction	No liquefaction	-ve
Oxidase test	Light pink colouration on colonies	-ve
Casein hydrolysis	Loss of opacity	+ve

Acidity

The yoghurt prepared was subjected to analysis of acidity and it is evident from the data (Table-5) that there is gradual Increase in acidity during storage time. At '0' hour the acidity of yoghurt prepared from different concentrations of starter culture (T_1–T_7) ranged from 0.64-1.04% respectively increased with a range of 0.75-1.16% in (T_1-T_7) at 96 hours. Reason for increase in acidity, is reduction in pH during storage when the conditions are uncontrolled.

Similarly statistical analysis of acidity showed that culture, storage and their interaction explicit highly significant effects on acidity during storage. The results are in line with those of Bilal (1995); Masood

(1997); Puhan and others (1974); Salji and Ismail (1983) and Ahmad (1989) who reported significant increase in acidity during yoghurt storage.

Total solids

The data shown in Table-6 indicates that total solids decreased gradually during the storage. Maximum decrease in total solids was observed in treatment T_6 (1.3%) while minimum decrease was observed in T_4 and T_2 (0.7%).

It was evident from the results that decreased in total solids through out storage period is related to milk sugars i.e. lactose which is converted into lactic acid by fermentation bacteria in yoghurt. During the process some degradation of protein also occurred.

These findings are related to the studies of O'Neil *and others.* (1979) who observed variation in total solids, acidity and fat amongst different plain yoghurt samples. Results also confirm the findings of Verman and Sutherland (1994) and Tamime and Robinson (1985). Statistical analysis showed that results are highly significant for treatments, storage and their interaction during storage.

(d) Lactose:

The results are shown in Table-7 and it declares that there is a gradual and consistent decrease in lactose contents under various treatments of starter culture during storage period of 96 hours. It is obvious from the results that minimum decrease in lactose contents was observed in T_7 (3%) and maximum decrease was observed in T_4 (4%). The decrease in lactose might be due to the action of microorganisms, which lactose into lactic acid during fermentation. Statistical analysis showed that levels of starter culture, storage time and their interaction on lactose contents have highly significant effect during storage.

Table-4Effect of storage on pH of yoghurt prepared by different doses of culture

Treatment(T)	I_0	I_{24}	I_{48}	I_{72}	I_{96}	Means
T_1	4.65	4.64	4.58	4.56	4.54	4.59
T_2	4.61	4.59	4.55	4.52	4.46	4.54
T_3	4.59	4.56	4.52	4.51	4.44	4.52
T_4	4.45	4.42	4.41	4.38	4.37	4.40
T_5	4.41	4.40	4.38	4.36	4.34	4.37
T_6	4.25	4.22	4.20	4.18	4.15	4.28
T_7	4.16	4.13	4.11	4.09	4.05	4.10
Means	4.45	4.42	4.39	4.36	4.33	

Table-5 Effect of storage on acidity (%) of yoghurt prepared by different doses of culture

Treatment(T)	I_0	I_{24}	I_{48}	I_{72}	I_{96}	Means
T_1	0.64	0.68	0.70	0.73	0.75	0.70
T_2	0.67	0.72	0.75	0.78	0.80	0.74
T_3	0.72	0.74	0.76	0.78	0.80	0.76
T_4	0.77	0.82	0.85	0.88	0.90	0.84
T_5	0.83	0.85	0.88	0.92	0.94	0.88
T_6	0.98	1.0	1.03	1.05	1.08	1.02
T_7	1.04	1.07	1.10	1.13	1.16	1.10
Means	0.80	0.84	0.87	0.90	0.92	

Table-6 Effect of storage on total solids (%) of yoghurt prepared by different doses of culture

Treat-ment (T)	I_0	I_{24}	I_{48}	I_{72}	I_{96}	Means
T_1	16.54	16.52	16.50	16.43	16.43	16.49
T_2	16.60	16.58	16.56	16.51	16.50	16.55
T_3	16.50	16.47	16.43	16.41	16.38	16.43
T_4	15.20	16.17	16.13	16.10	16.10	16.14
T_5	15.85	15.82	15.80	15.77	15.74	15.80
T_6	15.74	15.70	15.63	15.60	15.53	15.64
T_7	15.80	16.17	15.74	15.70	15.68	15.74
Means	16.17	16.14	16.11	16.07	16.04	

Table-7 Effect of storage on lactose (%) of yoghurt prepared by different doses of culture

Treatment(T)	I_0	I_{24}	I_{48}	I_{72}	I_{96}	Means
T_1	4.90	4.85	4.80	4.77	4.75	4.81
T_2	4.85	4.81	4.77	4.74	4.70	4.77
T_3	4.80	4.76	4.72	4.68	4.65	4.72
T_4	4.70	4.66	4.62	4.55	4.50	4.60
T_5	4.65	4.61	4.57	4.52	4.48	4.56
T_6	4.60	4.57	4.50	4.47	4.44	4.51
T_7	4.40	4.37	4.32	4.30	4.27	4.33
Means	4.70	4.67	4.61	4.57	4.54	

CONCLUSIONS

The culture isolated from yoghurt was purified, and later on identified up to spp. level. In this way a pure culture of *Streptococcus thermophillus* was obtained. Yoghurt was prepared from this single starter culture and then stored for 96 hrs to observe the combined effect of culture percentage and storage on yoghurt quality. It was suggested on the basis of results obtained that a culture percentage of 2-2.5 could be the best inoculum size for yoghurt preparation from single starter culture *Streptococcus thermophillus.*

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PAK. J. FOOD SCI. 13(1-2): 11-14

Preparation of carbonated beverage from enzymatically clarified guava juice

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ABSTRACT

A carbonated beverage was prepared from enzymatically clarified guava juice. The beverage was subjected to chemical analysis and sensory evaluation at 7 days interval for a storage period of 90 days. During this period the level of ascorbic acid fell 36 mg/100mL to 1.5 mg/mL. Addition of sodium citrate in guava syrup helped in preserving the natural color and flavor of the drink.

Keywords: Enzymes, juice clarification, guava, carbonation, beverage

INTRODUCTION

Guava *(Psidium guajava Linn)* is a hardy plant which originated in tropical America It is distributed throughout the warmer regions of the globe. In the tropical countries guava is generally grown wild bearing sour fruit, which is utilized for processing into different products instead of being consumed as fresh fruit. In India and Pakistan, there are many varieties of guava grown as fleshy and juicy with agreeable sweet taste and are relished as fresh fruit Brekke and others (1970) reported the preparation of guava juice, puree for nectar, canned jelly and paste.

Guava is grown throughout Pakistan. The fruit is of great economic importance because of its low price and higher calorific value (70 k Cal /100 g, FAO 1982). Among the common fruits grown guava is an outstanding source of ascorbic acid and moderate in vitamin A, niacin, calcium and phosphorus (Kalra and Tandon 1984). The ascorbic acidic content of guava is higher than that of fresh orange juice 229 mg/100g (FAO 1982).

Guava plant is very tolerant to extreme climate and soil conditions (Chan and others 1971), and produces two crops a year, yet the fruit comes in the market during the specific and short period of time. During this period of the year there is glut of guava in the market which sells at a very low price. To avoid the wastage of surplus fruit attempts have been made to prepare different products. (Shah and others 1975) worked to produce carbonated beverage from guava juice obtained by pressing blanched, sliced fruit. (Amjad and Shah 1991) reported the preparation of different products like, chutney, marmalade and butter from guava fruit. Enzyme preparations are playing important role in the modern food processing (Joshi and others 1993). Pectolytic enzyme have been employed in the hydrolysis of pectin and clarification of the guava juice. (Sufi and others 1976) have been reported the clarification of guava juice by pectolytic enzyme.

Most of the aerated drinks available in the market, are prepared from artificially colored and flavored syrups, without natural fruit juices. Preliminary investigations have proved that some pure fruit juices could readily be converted into syrups suitable for the preparation of carbonated drinks with public preference (Hasan 1989). Carbonated fruit drinks not only have a pleasant and refreshing taste but they also improve the circulation of blood particularly in skin and increase the absorptive capacity of the mucous membrane in the stomach (Kohler 1973). The growth of microorganisms can be inhibited by carbon dioxide concentration greater than 5.0 volume /L without any detrimental effect on taste (Morris & Jacob 1959)

Carbonation is inhibitory or even germicidal to some microorganisms, and the acidity resulting from carbonation and the addition of acids e.g., citric, lactic, phosphoric, tartaric and malic, inhibits the growth of organisms not tolerant to acidity since moulds must have air, they cannot grown on carbonated beverages (William and others 1988)

This study was conducted to investigate the effect of different levels of pectolytic enzyme on the maximum yield and clarification of juice. Physico chemical properties and consumer acceptability of carbonated guava beverage during storage period of 90 days have been carried.

MATERIAL AND METHODS

Fully mature white flash variety of the guava fruit was purchased from local market. The fruit was sorted out, washed and sliced. To prevent the browning of the guava juice, sliced fruit was sprayed with ascorbic acid solution (70 mg/100g).It was passed through pulper fitted with 1:8 mm mesh stainless steel sieve and pulp was collected in stainless steel buckets.

Percentage of the fruit pulp was determined and analyzed. The parameters studied were T.S.S., pH, acidity and ascorbic acid, reducing and total sugars. Total soluble solid (TSS) were determined by Digital Refractometer ATAGO RX 1000.

Table I. Chemical composition of guava pulp

Parameter studied	Results
Moisture (%)	85.00
Acidity as citric acid (%)	0.280
pH	04.10
Protein (%)	01.00
Reducing sugars (%)	02.97
Total sugars (%)	04.30
Seeds (%)	03.00
Ash (%)	0.480
Ascorbic acid (mg/100g.)	80.40
Calcium (mg/100 g)	12.50
Iron (mg/100g))	1.100
Sodium (mg/100g)	1.420
Potassium (mg/100g)	20.00
T.S.S.(%)	11.50

CLARIFICATION OF GUAVA JUICE

Guava pulp was treated with different doses of pectolytic enzyme (Pectolase P.A. Grindsted, Denmark) in acidic pH and incubated at 40 °C to obtain clear juice. (Table 2)

DETERMINATION OF OPTIMUM AMOUNT OF ENZYME ADDED FOR MAXIMUM CLARIFICATION OF GUAVA JUICE

The guava pulp was divided into eight lots of 1 Kg each Enzyme pectinol was added to each lot ranging from 0.01 to 1.2 % respectively to the pulp to depectinize it and was incubated for two hours at a temperature of 40°C in acidic pH. The control without any added enzyme was also kept along with the samples. After two hours the juice was squeezed out through nylon cloth. Percentage of clarified juice in each lot was determined (Table 2).

PASTEURIZATION OF THE JUICE

The pressed juice was pasteurized to inactivate the enzyme at 80 °C for three minutes and clear juice was siphoned off.

Table 2. Effect of pectinol enzyme concentration on the clarification & yield of guava juice.

Pulp	1 Kg	1 Kg	1 Kg	1 Kg	1 Kg	1 Kg	1 Kg
Enzyme (%)	0.01	0.03	0.05	0.07	0.09	1	1.2
Yield of the juice (mL)	530	570	750	770	800	750	720
Clarity of the juice	Cloudy	Cloudy	Slightly opalescent	Highly Clear	Clear	Not clear	Not clear
TSS (%)	8.5	8.5	9	9	9	9	9
pH	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Percent juice yield	53	57	75	77	80	75	72

Table 3. Comparison of freshly extracted and enzymatically clarified juice

Parameters studied	Freshly extracted juice	Enzymatically clarified juice
pH	04.30	4.08
Acidity (%)	0.380	0.50
TSS (%)	09.00	11.5
Referactive Index (RI).	1.346	1.355
Total sugar (%)	06.30	11.00
Reducing sugar (%)	2.976	5.40
Ascorbic acid (mg/g)	190.4 /100	180/100
Ash (%)	0.48	0.59

The pH was determined by using a digital pH meter, total acidity by titrating juice against standard sodium hydroxide to pH 8.1 (AOAC. 1980). The result was expressed as percent citric acid. Ascorbic acid was determined by 2,6-dichlorophenol indophenol (Ruck, 1969). Total sugars, reducing sugars and trace elements were determined according to AOAC (1980)

FILTERATION OF THE JUICE

The pressed juice was slightly turbid and there was sedimentation on standing. This juice was filtered with the help of filter aid through filter press. Clear shining juice has been obtained. PH, acidity, TSS. and ascorbic acid of this juice was measured (Table 3)

PRESERVATION OF THE GUAVA JUICE

Experiments were conducted for the preservation of guava juice. Different preservative were used such as potassium meta bisulphate, sodium benzoate, potassium sorbet and mixed preservative sodium benzoate, potassium meta bisulphate (Table 4)

SYRUP PREPARATION

Clarified shining juice was sweetened with sugar syrup to raise the brix of the juice. Other additives were added and guava syrup was ready for carbonation.

CARBONATION OF GUAVA JUICE

Guava syrup was carbonated. Shelf life studies of the carbonated guava beverage have been carried out. Carbonated beverage was evaluated organolepticaly and chemically after one week interval and results are reported in (Table 5).

RESULTS AND DISCUSSION

Experiments were conducted to determine the optimum quantity of pectolytic enzyme to get maximum yield and clarity of juice. Different doses of enzyme had shown variable results (Table 2). The juice obtained from 0.01 - 0.03% enzyme was cloudy with 53-57% juice. Yield of the juice with 0.05% enzyme was 75% and it was opalescent. It was observed that maximum yield of the juice (80%) along with maximum clarity was obtained by 0.07% enzyme concentration. During the course of studies it was observed that in the summer season (RT 36-38 °C) a clear shining juice was obtained in about 12-16 hours, where the juice obtained in the winter was slightly turbid and it takes more time for complete clarification. It was observed that addition of pectolytic enzyme increased the yield of the juice. pH of the juice was not effected by processing conditions while the % titratable acidity was increased. A similar

Table 4. Effect of various preservatives on the sensory characteristics of clarified juice

Preservative	Flavor	Color	Acceptability
Potassium Metabisulphite	Acceptable	Good	Acceptable
Sodium Benzoate	Acceptable	Non acceptable	Non acceptable
Potassium Sorbate	Non acceptable	Non acceptable	Non acceptable
Potassium metabisulphite and Sodium Benzovate.	Non acceptable	Non acceptable	Non acceptable

Table 5. Effect of storage time on the pH, T.S.S. and acidity of the carbonated guava beverage.

Storage time (days)	Ascorbic acid (mg/100g)	pH	TSS (%)	Acidity (%)
0	36.00	3.58	14	0.315
7	35.40	3.57	14	0.328
14	34.70	3.56	14	0.360
21	34.00	3.56	14	0.476
28	33.00	3.34	14	0.480
35	30.10	3.26	14	0.480
42	25.00	3.22	14	0.480
49	19.90	3.06	14.5	0.490
56	14.00	3.06	14.5	0.490
63	08.90	3.06	14.5	0.490
70	05.70	3.04	14.5	0.490
77	04.00	3.04	14.5	0.490
84	02.70	3.01	14.5	0.510
90	01.50	3.01	14.5	0.500

increase in titratable acidity has been reported in black berry juice (Roommel and others 1992) and plum juices (Tung and others 1994). Carbonated guava juice was found to be the best in all sensory characteristics studied. It could be due to acceptable acid / sugar blend which is known to influence the taste perception of beverage Piggot (1988). As a very small amount of heat is involved to inactivate the enzyme, therefore the flavor was fully preserved. Flavor of the carbonated guava had the strongest effect on overall acceptability.

Carbonated guava beverage was analysed after one week interval, acidity, pH, TSS. and ascorbic acid were measured (Table 5). The beverage was found acceptable during the storage period it was organolyptically evaluated and liked by all the tasters. The pH of the carbonated beverage was 3.58. There was a gradual decrease in the pH during storage, period of 90 days (Table 5). This decline in pH is due to respective increase in acidity. The degradation of sugars during the storage is responsible for decrease in pH. The increase in acidity during storage has been reported by (Kalra and Tendon 1984) for guava and mango nectars. It was observed that the concentration of ascorbic acid fell from 36 mg/100 mL to 1.5 mg/100 ml in a period of 90 days (Table 5). There are several factors responsible for the destruction of ascorbic acid in food and food products. These include temperature, pH and oxidation. The rate of decrease was lower for the first 35 days than the last 55 days. This change in rate could possibly be attributed to presence of potassium metabisulphite, which is very good reducing agent and is oxidized in preference to ascorbic acid. Josyln and Braverman (1954) observed that the rate of oxidation of ascorbic acid increased after depletion of metabisulphite. It was observed that other preservatives (Table 4) were not suitable for the preservation of guava beverage. From organoleptic evaluation it was found that guava beverage was acceptable. The loss in the ascorbic acid after 35 days did not affect the acceptability of the beverage.

CONCLUSION

Application of pectolytic enzyme for the clarification of guava juice was found suitable for the production of carbonated guava beverage.

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PAK. J. FOOD SCI. 13(1-2): 15-19

Storage studies on wild Pakistani sun dried mushrooms

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ABSTRACT

Three wild species of mushrooms were collected from Murree and plain lands of District Faisalabad, Pakistan. The mushroom samples were divided into two lots, one was blanched and the other was kept as unblanched before drying in the sun. and dried mushrooms were subjected to physico-chemical analysis, residual SO_2 content, dehydration and rehydration ratios after 0, 7, 14 and 28 days. The results of all parameters differed significantly among varieties as well as treatments of unblanched and blanched mushrooms. There was a gradual decrease in protein, ash, fat, fiber and residual SO_2 content in sun dried mushrooms. Nutrient losses were more during storage in blanched mushrooms than unblanched. Sensory parameters revealed that color, flavor, taste and overall acceptability of unblanched mushroom soup prepared with sun dried mushrooms was better than blanched mushrooms.

Keywords: Mushrooms, sun drying, storage, chemical analysis

INTRODUCTION

Pakistan has a rich treasure of wild mushrooms, which is encountered in fields, forest, meadows and barren lands. Wild mushrooms may also emerge with their suitable natural conditions, especially humidity and temperature. Besides a large quantity of wild mushrooms collected during various periods, Pakistan is producing a sizeable quantity of cultivated mushrooms on commercial basis. Pakistan exported 97000 Kg mushrooms during the year 1999-2000 having value of 0.69 million US Dollars. GOP (2000).

Mushrooms contain 90% water and 10% dry matter. The dry matter contains 4% carbohydrates, 3.5% protein, 0.4% fat and 1.2% ash (Zafar 1986; Crisen and Sand 1978). Mushrooms are also rich source of minerals like calcium, phosphorus, iron, sodium, potassium, copper, magnesium, zinc, and manganese as well as vitamins like thiamine, riboflavin, nicotinic *and* ascorbic acid.

Mushrooms are highly perishable, they are crisp or firm at the time of harvest and tender but they subsequently soften and toughen during post harvest deteriorative (Beelman and others 1987) changes including wilting, ripening, browning, liquefaction, lose of moisture, texture, aroma and flavor. These changes are accelerated due to an increase in rate of respiration. Lack of organized marketing and poor storage facilities result in the wastage of mushrooms. The processing of mushrooms on the scientific lines is the only solution to reduce these losses. The mushrooms can be preserved by the methods as canning, refrigeration, freezing, chemicals, pickling and dehydration. On the basis of cost of drying, sun drying is the simple and cheapest method. Therefore there is a great need to introduce this useful method in villages and towns by taking advantage of solar energy at domestic level.

People of Pakistan are only aware of a mushroom known as *Podaxis pistillaris*, which is commonly found naturally in rainy season. Taste of this mushroom is liked by common people and sometimes the mushrooms are naturally dried in the sun and kept for further use. But the shelf life of these mushrooms is very short (only few hours at room temperature) as mushroom turn into black powdery mass after spore maturation. The present study was conducted to evaluate the physico-chemical characteristics of three sun-dried mushrooms commonly found in the rainy season.

MATERIALS AND METHODS

PROCUREMENT OF MUSHROOMS

Fresh mushroom cultivars viz: *Phellorina inequence*, *Pleurotus ostreatus* and *Podaxis pistillaris* were collected from hilly areas of Murree and plane lands of District Faisalabad, Pakistan. The mushrooms were identified according to the prevailing morphological nomenclature and classification guidelines (Bessy 1964). All species of fresh mushrooms were washed with tap water to remove contaminants followed by the processing operations.

BLANCHING

All three species of mushrooms were divided into 2 lots of 500 g each. One lot was blanched in boiling water for 2 minutes while the other was kept as such.

CHEMICAL TREATMENT

The blanched and unblanched samples from each species were treated with SO_2 fumes by burning 2-5 g

of sulfur for one Kg of fresh mushrooms, so as to keep the SO_2 content in the dried product in the range of 200-350 ppm.

SUN DRYING

The two lots from each species were then spread on plastic trays and left in open sun for drying. The trays were covered with muslin cloth to protect them from dust and flies. The mushrooms were turned occasionally until the product had dried to the moisture level of 5-8 %.

PHYSICO-CHEMICAL ANALYSIS

The fresh as well as dried samples were analyzed for physico-chemical characteristics as moisture, protein, ash, fat, and fiber after 0, 7, 14 and 28 days by following standard methods of (AOAC 1984). Residual sulphur dioxide content were determined by using the method of Monier-William's, (Luh and Woodroof, 1975). Dehydration and rehydration ratios were determined by using the method of (Pruthi and others 1978).

STATISTICAL STUDIES &SENSORY EVALUATION

Mushroom soup prepared from different unblanched and blanched sun dried mushrooms were evaluated by a panel of judges for sensory characteristics like color, flavor, taste and over all acceptability as described by (Larmond 1977). Finally the data obtained for each parameter was subjected to statistical analysis by using the techniques of (Steel and others 1996).

RESULTS AND DISCUSSIONS

The results of various physico-chemical analysis of wild fresh mushroom are given in Table 1. The results regarding various physico-chemical analysis, residual SO_2 determination, dehydration value and rehydration values for unblanched and blanched mushrooms is depicted in Table 2 and 3, respectively.

MOISTURE

The dried wild species shared highly significant differences from each other with respect to their moisture content. The blanched and unblanched dried mushrooms significantly differed from each other for their moisture content. The moisture content of blanched dried mushroom was less than unblanched dried mushrooms. The moisture content of dried samples increased gradually during storage in all samples. This increase may be attributed to the absorption of water by the product from the atmosphere during storage because of slight permeable nature of polyethylene film. The moisture content of each species is depended upon the initial moisture content of the fresh mushroom, soil and environmental conditions and specific characteristics of the species. The moisture content of dried mushroom was in the range of 5.01 to 7.67 % which is in comparison with early findings of (Achremowicz and others 1984).

FAT

The statistical analysis showed highly significant losses in fat contents during storage of dried mushrooms. The fat contents differed significantly during storage for 0, 7, 14 and 28 days. However, decrease in fat was not observed after 14 days of storage. Blanching also affected the fat .contents significantly. The fat content was less in blanched mushrooms than unblanched mushrooms. The loss in fat content in blanched mushrooms seems to be due to leaching of fat in the boiling water during blanching. The results agree with the findings of (Priestley 1984); (Al-Shabibi and others 1982; Coetzee and others 1982).

CRUDE FIBRE

The statistical analysis showed highly significant results for fiber contents during storage. The affect of blanching and unblanching conditions on fiber was highly significant. It was noticed that blanched mushrooms gave more fiber content than unblanched dried mushrooms. The decrease in fiber contents may be due to the hydrolyzation of fiber contents. The fiber contents ranged from 11.13 to 22.18 % among all the tested samples. (Achremowicz and others 1984) and (Chang and Hayes 1978) reported similar results in studies on mushrooms.

Table 1. Physico-chemical analysis of wild fresh mushrooms.

Parameter	*Phellorina inequence*	*Pleurotus ostreatus*	*Podaxis pistillaris*
Moisture %	87.82	89.20	90.10
Crude protein %	28.22	17.60	24.52
Crude fat %	0.85	1.94	4.27
Crude fiber %	21.12	12.20	13.31
Crude ash %	16.02	27.54	21.04

Table 2. Overall analysis of unblanched sun dried mushrooms

Parameter	Mushrooms Species											
	Phellorina inequence				*Pleurotus ostreatus*				*Podaxis pistillaris*			
	Storage (days)				Storage (days)				Storage (days)			
	0	7	14	28	0	7	14	28	0	7	14	28
Moisture %	6.35	6.41	6.47	7.51	7.46	7.52	7.57	7.64	7.52	7.57	7.61	7.67
Crude protein %	22.50	22.35	22.21	22.14	14.72	14.63	14.47	14.30	17.89	17.73	17.51	17.40
Crude fat %	0.41	0.33	0.24	0.10	1.22	1.11	0.86	0.79	2.97	2.83	2.79	2.64
Crude fiber %	20.12	20.10	20.07	19.97	11.22	11.19	11.17	11.13	12.19	12.17	12.13	11.98
Crude ash %	14.15	14.16	14.16	14.18	23.62	23.64	23.64	23.66	18.29	18.30	18.33	18.33
Res.SO_2 (ppm)	230.0	218.2	219.1	194.7	228.7	214.3	211.1	190.0	229.3	224.5	219.7	198.1
Dehydration ratio	10.41	10.39	10.41	10.40	11.63	11.65	11.64	11.64	10.90	10.92	10.92	10.91
Rehydration ratio	4.50	4.48	4.46	4.44	4.24	4.23	4.21	4.20	4.37	4.36	4.36	4.35

Table 3. Overall analysis of blanched sun dried mushrooms

Parameter	Mushrooms Species											
	Phellorina inequence				*Pleurotus ostreatus*				*Podaxis pistillaris*			
	Storage (days)				Storage (days)				Storage (days)			
	0	7	14	28	0	7	14	28	0	7	14	28
Moisture %	5.01	5.08	5.13	5.17	6.12	6.17	6.23	6.30	6.20	6.28	6.33	6.37
Crude protein %	21.37	21.25	21.19	21.02	13.97	13.89	13.80	13.71	16.89	16.87	16.71	16.70
Crude fat %	0.22	0.11	0.00	0.00	0.64	0.52	0.46	0.37	1.59	1.51	1.42	1.31
Crude fiber %	22.18	22.15	22.07	20.00	12.41	12.39	12.36	12.31	13.46	13.45	13.39	13.34
Crude ash %	10.13	10.13	10.14	10.15	17.16	17.18	17.18	17.19	13.36	13.37	13.38	13.38
Res.SO_2 (ppm)	257.7	241.3	230.4	190.1	260.5	256.4	224.1	189.1	253.4	233.1	217.3	195.7
Dehydration ratio	9.95	9.94	9.94	9.94	9.53	9.56	9.54	9.54	9.27	9.29	9.28	9.28
Rehydration ratio	2.32	2.32	2.31	2.30	2.37	2.37	2.36	2.35	2.22	2.21	2.20	2.20

ASH

The changes in ash contents due to unblanching and blanching conditions were highly significant. The blanched samples possess relatively less ash than unblanched samples. This may be due to the leaching of mineral contents during boiling which resulted in lowering ash contents. In these results ash content were in the range of 10.13 to 23.66 %, which is near to the ash contents reported by Khan (1984) working with similar species.

RESIDUAL SO_2

The analysis of variance showed highly significant results for residual SO_2 between unblanched and blanched sun dried mushrooms. Sulphur dioxide was more in blanched samples as compared to unblanched samples but after one month of storage period the SO_2 retention was reduced. The wild dried mushroom species differed significantly from each other for their dehydration and rehydration ratios. This difference was due to structural and textural differences between the species. The dehydration and rehydration ratios were affected due to unblanching and blanching conditions and showed significantly different results. Blanching treatment in combination with chemical treatments resulted in the lower dehydration ratio as the salt concentration goes on increasing during initial period of dehydration which raised the temperature and caused more dehydration as compared to unblanched samples. The dehydration ratio among different dried mushrooms varied from 9.28 to 11.64 which are near to the value of (Pruthi and others 1978). The rehydration ratio gradually decreases during storage. The rehydration ratios of unblanched samples were greater than blanched samples. This might be due to the removal of air spaces during blanching, which resulted in break down of elasticity of the cells on drying. The results regarding rehydration ratio are comparable with early findings of (Desphande and Tamhane 1980).

Table 4. Mean values for different parameters of mushroom soup prepared from unblanched dried mushrooms

Parameter	Mushrooms Species											
	Phellorina inequence				*Pleurotus ostreatus*				*Podaxis pistillaris*			
	Storage (days)				Storage (days)				Storage (days)			
	0	7	14	28	0	7	14	28	0	7	14	28
Color	8.0	7.9	7.6	7.2	7.8	7.6	7.4	7.2	8.0	7.8	7.6	7.2
Taste	7.8	7.6	7.5	7.2	7.4	7.0	7.6	7.3	8.0	7.8	7.6	7.3
Flavor	6.8	6.4	6.0	5.8	7.0	6.8	6.6	6.2	7.2	7.1	6.7	6.6
Overall acceptability	6.9	6.8	6.4	6.0	7.2	6.9	6.6	6.2	7.2	7.0	6.8	6.6

Table 5. Mean values for different parameters of mushroom soup prepared from blanched dried mushrooms

Parameter	Mushrooms species											
	Phellorina inequence				*Pleurotus ostreatus*				*Podaxis pistillaris*			
	Storage (days)				Storage (days)				Storage (days)			
	0	7	14	28	0	7	14	28	0	7	14	28
Color	6.7	6.6	6.3	5.8	6.9	6.6	6.4	6.0	6.2	6.0	5.6	5.2
Taste	7.1	6.9	6.6	6.2	6.9	6.7	6.3	6.0	7.4	7.2	6.7	6.6
Flavor	5.0	4.8	4.6	4.4	6.8	6.4	6.0	5.8	6.6	6.4	6.2	6.0
Overall acceptability	5.9	5.6	5.2	5.0	5.8	5.6	5.3	5.2	6.8	6.4	6.2	6.0

The soup prepared from three wild sun dried species differed significantly from each other for their color, flavor, taste and overall acceptability (Table 4, 5). However, the overall acceptability regarding two mushroom species viz. *Podaxis pistillaris* and *Pleurotus ostreatus* got maximum score for quality criterion (7.2) for unblanched sun dried mushrooms at zero day storage period. The scores for overall acceptability decreased gradually during storage. This change in overall acceptability was due to decrease in color, flavor, taste and texture of mushrooms during storage.

The blanched and unblanched mushrooms soup differed significantly from each other for overall acceptability. Soups prepared from unblanched mushrooms were liked more by the panel of judges than blanched mushrooms because of the fine taste, texture and color of mushrooms.

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PAK. J. FOOD SCI. 13(1-2): 21-27

Changes during storage in different branded yoghurts

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ABSTRACT

Physicochemical changes in three different commercial yoghurt brands were studied during twelve days storage at 10^0, 25^0 and 45^0C. A significant decrease in pH and an increase in titratable acidity occurred at all storage temperatures. At 10^0C, moisture, lactose, fat, casein nitrogen, non-casein nitrogen and nonprotein nitrogen remained unchanged. However, total nitrogen content remained constant. At 25^0 and 45^0C, moisture, lactose, fat, casein nitrogen and non protein nitrogen decreased to various extents. Results of sensory evaluation showed that all yoghurt brands stored at 10^0C were acceptable till the twelfth day. On the other hand, sensory quality characteristics were adversely affected at 25°C and 45°C, after twelve days.

Keywords: Yoghurts, commercial brands, quality, acidity, shelf life

INTRODUCTION

Yoghurt is an important and nutritious fermented milk product. Natural yoghurt is based entirely on milk. Stirred or fruit yoghurts usually have stabilizers incorporated to reduce whey separation during distribution (Wilbey and others 1986). Yoghurt provides a useful source of energy in diet. Natural yoghurt contains around 6.4g of carbohydrate /100g. The main components of yoghurt are carbohydrate, proteins, fat and minerals. Some yogurt preparations are also fortified with added vitamin A and D (Sanchez and others 2000). The principle source of lactic acid is milk which is available in variable quantities in yoghurt depending on the action of micro-organisms (Robinson and others 1994). Yoghurt may contain some 4 to 5% lactose (Tamime and others 1987). Protein content of yoghurt is often elevated by concentration or addition of skim milk solids and it becomes a more attractive source of protein than liquid milk (Zbikowski and others 1998). Protein in yoghurt is totally digestible. Yoghurt contained 3 - 4 % milk fat. Milk fat contained an extremely wide range of fatty acids (Yagin and others 1988). Most of these are present in the form of various glycerides, but over 400 individual fatty acids have been identified in cow's milk (Patten and Jensen 1974). Nutritional changes in carbohydrate and protein have been observed during storage of yoghurt (Kailasapathy and others 1996; Brazuelo and others 1995; Musaiger and others, 1998). However there is scarce information in the literature about the physicochemical changes during storage of yoghurt. Therefore present work was undertaken to investigate the physicochemical changes of yoghurt during storage at elevated temperatures.

MATERIALS AND METHODS

Three different branded yoghurt under the code names A, B, & C were collected from local market. All the yoghurt brands were available in disposable plastic jars of 500g weight. Fifteen samples of each brand of yoghurt were divided into three categories and stored at 10°, 25° and 45°C until their expiry date (12 days). All the yoghurt samples were analyzed after three days interval for pH, titratable acidity, lactose, fat total nitrogen, casein nitrogen, non casein nitrogen, non protein nitrogen and moisture. pH of yoghurt samples of each brand was determined by using a glass electrode pH meter (PYE Unicam England), whereas titratable acidity was measured by titration against 0.1 N sodium hydroxide solution using phenolphthalein as an indicator (AOAC 1990). Fat was determined by Gerber method using standard butyrometer (Pearson 1976). Lactose in yoghurt samples was determined spectrophotometrically at 370 nm using phenyl hydrazine solution (Wahba 1965). Whereas, total nitrogen, casein nitrogen, non casein nitrogen (soluble at pH 4.6), non protein nitrogen (soluble in 15% TCA) and moisture in yoghurt samples were determined according to standard methods of AOAC (1990).

Yoghurt samples after 12 days storage were evaluated for sensory qualities by a trained taste panel of ten persons. Samples were assessed for color, taste, flavor, texture and overall acceptability using a nine point Hendonic scale ranging from 1 for dislike extremely to 9 for like extremely.

Results were carried out in triplicate and standard deviations were calculated according to the methods of Steel and others (1996). Mutivariate Anova and Duncan's multiple range test was used to determine significant differences ($p<0.05$).

RESULTS AND DISCUSSION

Physicochemical changes in yoghurt samples of each brand occurred to various extents on storage at three different temperatures. The range of temperature

included in this study (10°, 25° and 45°C) covered the atmospheric temperatures of Pakistan.

A gradual decrease in pH and increase in titratable acidity was observed during storage of yoghurt brands A, B and C at different temperatures (10, 25 and 45°C), for a period of twelve days. pH of yoghurt brand 'A' at 10°C, 25°C and 45°C decreased significantly ($p<0.05$) from 3.95 to 3.60, 3.35 and 3.20, respectively. Similarly, increase in titratable acidity of yoghurt brand 'A' at 10°, 25° and 45°C was observed from 0.08 to 0.095, 0.128 and 0.140 %, respectively (Table 1). pH of yoghurt brand 'B' at 10°, 25° and 45°C was decreased from 4.01 to 3.82, 3.47 and 3.51, respectively. Similarly, increase in titratable acidity of yoghurt brand 'B' at 10°, 25° and 45°C was from 0.08 to 0.095, 0.128 and 0.145 %, respectively (Table 2). pH of yoghurt brand 'C' at 10°, 25° and 45°C decreased from 4.15 to 3.86, 3.52 and 3.35, respectively. Similarly, increase in titratable acidity of yoghurt brand 'C' at 10°, 25° and 45°C was from 0.08 – 0.10, 0.118 and 0.20 %, respectively (Table 3). The increase in titratable acidity during storage of yoghurt could be attributed to increase in the concentration of free fatty acids and lactic acids which resulted from the degradation of fat and lactose. Changes in calcium phosphate equilibrium might also be responsible for increased acidity and reduced pH of

Table-1. Storage effect on the nutritional composition of yoghurt brand 'A' at 10°, 25° and 45°C.

Storage temp.	Storage time (Days)	pH	Acidity %	Lactose g/100g	Fat %	T.N Mg/100g	C.N mg/100g	N.C.N Mg/100g	N.P.N Mg/100g	Moisture %
10°C	0	3.95 ±0.78	0.08 ±0.002	8.1 ±0.60	1.1 ±0.001	660 ±0.001	528 ±1.37	132 ±1.14	70 ±0.85	14.5 ±1.01
	3	3.85 ±0.72	0.08 ±0.002	8.1 ±0.60	1.1 ±0.001	660 ±0.001	528 ±1.37	132 ±1.14	70 ±0.85	14.5 ±1.01
	6	3.82 ±0.62	0.085 ±0.003	8.1 ±0.60	1.1 ±0.001	660 ±0.001	528 ±1.37	132 ±1.14	70 ±0.85	14.5 ±1.01
	9	3.65 ±0.67	0.095 ±0.005	8.1 ±0.60	1.1 ±0.001	660 ±0.001	528 ±1.37	132 ±1.14	70 ±0.85	14.5 ±1.01
	12	3.60 ±0.77	0.095 ±0.005	8.1 ±0.60	1.1 ±0.001	660 ±0.001	528 ±1.37	132 ±1.14	70 ±0.85	14.5 ±1.01
25°C	0	3.95 ±0.78	0.08 ±0.002	8.1 ±0.60	1.1 ±0.001	660 ±0.001	528 ±1.37	132 ±1.14	70 ±0.85	14.5 ±1.01
	3	3.70 ±0.68	0.10 ±0.001	8.1 ±0.60	1.1 ±0.001	660 ±0.001	527 ±1.35	133 ±1.15	70 ±0.85	14.5 ±1.01
	6	3.61 ±0.69	0.012 ±0.003	8.0 ±0.58	1.1 ±0.001	660 ±0.001	510 ±1.30	150 ±1.17	67 ±0.84	14.40 ±1.04
	9	3.41 ±0.71	0.125 ±0.002	7.7 ±0.55	1.1 ±0.001	660 ±0.001	490 ±1.25	170 ±1.13	61 ±0.81	14.35 ±1.05
	12	3.35 ±0.72	0.128 ±0.003	7.5 ±0.54	1.1 ±0.001	660 ±0.001	475 ±1.27	185 ±1.16	58 ±0.80	14.20 ±1.03
45°C	0	3.95 ±0.78	0.08 ±0.002	8.1 ±0.60	1.1 ±0.001	660 ±0.001	528 ±1.37	132 ±1.14	70 ±0.85	14.5 ±1.01
	3	3.67 ±0.81	0.11 ±0.001	7.8 ±0.67	1.02 ±0.001	660 ±0.001	510 ±1.35	150 ±1.15	70 ±0.85	14.0 ±1.00
	6	3.60 ±0.82	0.115 ±0.001	7.5 ±0.65	1.03 ±0.001	660 ±0.001	480 ±1.33	180 ±1.17	62 ±0.80	13.80 ±1.02
	9	3.47 ±0.77	0.135 ±0.003	7.1 ±0.66	1.05 ±0.001	660 ±0.001	440 ±1.38	220 ±1.16	58 ±0.78	13.70 ±1.05
	12	3.20 ±0.75	0.140 ±0.002	6.7 ±0.65	1.0 ±0.001	660 ±0.001	425 ±1.39	232 ±1.18	55 ±0.75	13.60 ±1.07

stored yoghurt brands as suggested by *Schmidt and Renner (1978)*. These results are consistent with the findings of earlier workers who found a small reduction in pH at higher temperature (Zadow and Chituta 1975). Storage temperature and time period significantly ($p < 0.05$) showed effect on the decrease in pH and increase in titratable acidity of all the branded yoghurts, according to multivariate ANOVA. However, decrease in pH and increase in titratable acidity was more pronounced at 45°C in all the yoghurt brands as compared to 25° and 10°C, at the end of storage period, according to Duncan's Multiple range test.

During storage of yoghurt brands A, B and C at different temperatures (10°, 25° and 45°C), decrease in lactose was observed to various extents. These changes were more pronounced during storage at 45°C than 25°C. Lactose of yoghurt brand 'A' at 25° and 45°C decreased from 8.1 to 7.5 and 6.7 mg / 100g, respectively. Lactose of yoghurt brand 'B' at 25° and 45°C decreased from 6.5 to 5.7 and 5.0 mg / 100g, respectively. Lactose of yoghurt brand 'C' at 25° and 45°C decreased from 7.4 to 7.0 and 5.2 mg /100g respectively. However, no change in lactose content was observed at 10°C in all the yoghurt brands. Storage temperature and time period insignificantly (p

Table-2. Storage effect on the nutritional composition of yoghurt brand 'B' at 10°, 25° and 45°C

Storage Temp.	Storage time (Days)	pH	Acidity %	Lactose g/100g	Fat %	T.N Mg/100g	C.N Mg/100g	N.C.N Mg/100g	N.P.N Mg/100g	Moisture %
10°C	0	4.01 ±0.80	0.08 ±0.002	6.5 ±0.45	1.1 ±0.001	560 ±1.15	320 ±1.41	240 ±1.35	160 ±1.01	13.58 ±0.90
	3	3.83 ±0.55	0.08 ±0.002	8.1 ±0.60	1.1 ±0.001	560 ±1.15	320 ±1.41	240 ±1.35	160 ±1.01	13.60 ±0.78
	6	3.96 ±0.65	0.09 ±0.004	8.1 ±0.60	1.1 ±0.001	560 ±1.15	320 ±1.40	240 ±1.36	160 ±1.02	13.60 ±0.78
	9	3.77 ±0.61	0.09 ±0.004	8.1 ±0.60	1.1 ±0.001	560 ±1.15	320 ±1.38	240 ±1.34	160 ±1.02	13.62 ±0.79
	12	3.82 ±0.59	0.095 ±0.005	8.1 ±0.60	1.1 ±0.001	560 ±1.15	320 ±1.36	240 ±1.31	160 ±1.03	13.64 ±0.75
25°C	0	4.01 ±0.80	0.08 ±0.002	6.5 ±0.45	1.0 ±0.001	560 ±1.15	320 ±1.41	240 ±1.35	160 ±1.01	13.58 ±0.90
	3	3.83 ±0.78	0.105 ±0.003	6.5 ±0.45	1.0 ±0.001	560 ±1.15	316 ±1.42	244 ±1.33	160 ±1.01	13.50 ±0.91
	6	3.55 ±0.75	0.0125 ±0.005	6.2 ±0.43	1.0 ±0.001	561 ±1.11	313 ±1.40	249 ±1.31	158 ±1.02	13.48 ±0.89
	9	3.57 ±0.73	0.125 ±0.005	6.0 ±0.40	1.0 ±0.001	562 ±1.17	303 ±1.45	259 ±1.37	155 ±1.05	12.00 ±0.85
	12	3.47 ±0.70	0.128 ±0.006	5.7 ±0.38	1.0 ±0.001	561 ±1.16	295 ±1.47	266 ±1.40	150 ±1.07	11.25 ±0.75
45°C	0	4.01 ±0.80	0.08 ±0.002	6.5 ±0.45	1.0 ±0.001	560 ±1.15	320 ±1.41	240 ±1.35	160 ±1.01	13.58 ±0.90
	3	3.50 ±0.81	0.13 ±0.001	6.3 ±0.43	1.0 ±0.001	560 ±1.15	313 ±1.40	249 ±1.25	154 ±1.05	13.00 ±0.85
	6	3.70 ±0.82	0.140 ±0.004	6.0 ±0.41	0.80 ±0.001	560 ±1.15	307 ±1.38	253 ±1.33	149 ±1.08	12.25 ±0.75
	9	3.52 ±0.85	0.145 ±0.005	5.6 ±0.45	0.80 ±0.001	560 ±1.14	298 ±1.39	264 ±1.31	144 ±1.05	11.75 ±0.77
	12	3.51 ±0.81	0.145 ±0.006	5.0 ±0.44	0.70 ±0.001	560 ±1.13	270 ±1.25	292 ±1.30	142 ±1.08	9.00 ±0.79

All the samples were analyzed in triplicate.
TN = Total Nitrogen, NCN = Non Casein Nitrogen CN = Casein Nitrogen, NPN = Non Protein Nitrogen

< 0.05) showed effect on the decrease in lactose content of all the branded yoghurts, according to multivariate ANOVA. However, decrease in lactose was more pronounced at 45°C in all the yoghurt brands as compared to 25° and 10°C, at the end of storage period, according to Duncan's Multiple range test. Decrease in lactose content was due to the formation of hydroxyl methyl furfural (HMF) during storage as reported by (Morales and others, 1997).

In yoghurt brands A, B and C, fat contents remained unchanged at 10° and 25°C. At 45° C, decrease in fat contents in yoghurt brands A, B and C was from 1.1 to 1.0%, 1.0 to 0.70% and 1.0 to 0.65 %, respectively. Decrease in fat content could be attributed to the decomposition of fat to fatty acids as a result of lipolysis process, which occurred at higher temperature (Robinson 1994).

It is apparent from the results that total nitrogen (TN) content at all the storage temperatures in yoghurt brands A, B and C did not change at all (Table 1, 2, 3). Yoghurt brands A, B and C at 10°C during twelve days storage period showed no change in casein contents. Yoghurt brands A, B and C at 25°C showed a decrease in casein nitrogen content which was from 528 to 475, 320 to 295 and 520 to 470 mg | 100g,

Table-3. Storage effect on the nutritional composition of yoghurt brand 'C' at 10°, 25° and 45°C.

Storage Temp.	Storage time (Days)	pH	Acidity %	Lactose g/100g	Fat %	T.N mg/100g	C.N mg/100g	N.C.N mg/100g	N.P.N mg/100g	Moisture %
10°C	0	4.15 ±0.80	0.08 ±0.002	7.4 ±0.55	1.0 ±0.001	680 ±1.1	520 ±1.35	160 ±1.50	80 ±1.25	15.30 ±0.91
	3	4.10 ±0.78	0.08 ±0.002	7.4 ±0.55	1.0 ±0.001	680 ±1.19	520 ±1.35	160 ±1.50	80 ±1.25	15.30 ±0.91
	6	4.10 ±0.78	0.085 ±0.003	7.4 ±0.55	1.0 ±0.001	680 ±1.19	520 ±1.35	160 ±1.50	80 ±1.25	15.30 ±0.91
	9	4.05 ±0.77	0.09 ±0.004	7.4 ±0.55	1.0 ±0.001	680 ±1.19	520 ±1.35	160 ±1.50	80 ±1.25	15.30 ±0.91
	12	3.86 ±0.75	0.10 ±0.005	7.4 ±0.55	1.0 ±0.001	680 ±1.19	520 ±1.35	160 ±1.50	80 ±1.25	15.30 ±0.91
25°C	0	4.15 ±0.80	0.08 ±0.002	7.4 ±0.55	1.0 ±0.001	680 ±1.19	520 ±1.35	160 ±1.50	80 ±1.25	15.30 ±0.91
	3	3.53 ±0.78	0.11 ±0.001	7.4 ±0.55	1.0 ±0.001	680 ±1.19	520 ±1.35	160 ±1.50	80 ±1.25	15.30 ±0.91
	6	3.45 ±0.75	0.0112 ±0.004	7.4 ±0.55	1.0 ±0.001	680 ±1.19	500 ±1.33	180 ±1.45	75 ±1.22	15.30 ±0.91
	9	3.40 ±0.77	0.115 ±0.003	7.2 ±0.54	1.0 ±0.001	680 ±1.19	490 ±1.31	190 ±1.47	75 ±1.31	15.10 ±0.89
	12	3.52 ±0.79	0.118 ±0.005	7.0 ±0.52	1.0 ±0.001	680 ±1.19	470 ±1.39	210 ±1.53	72 ±1.30	15.00 ±0.85
45°C	0	4.15 ±0.80	0.08 ±0.002	7.4 ±0.55	1.0 ±0.001	680 ±1.19	520 ±1.35	160 ±1.50	80 ±1.25	15.30 ±0.91
	3	3.50 ±0.81	0.11 ±0.001	7.2 ±0.45	0.80 ±0.001	680 ±1.19	500 ±1.30	168 ±1.49	173 ±1.21	15.00 ±0.81
	6	3.47 ±0.79	0.115 ±0.004	6.6 ±0.47	0.70 ±0.001	680 ±1.19	480 ±1.31	200 ±1.47	70 ±1.20	14.90 ±0.79
	9	3.40 ±0.75	0.115 ±0.005	6.0 ±0.43	0.70 ±0.001	680 ±1.19	450 ±1.33	230 ±1.45	61 ±1.15	14.85 ±0.92
	12	3.35 ±0.70	0.20 ±0.006	5.2 ±0.51	0.65 ±0.001	680 ±1.17	400 ±1.37	282 ±1.41	52 ±1.17	14.70 ±0.95

All the samples were analyzed in triplicate.
TN =Total Nitrogen, NCN = Non Casein Nitrogen CN = Casein Nitrogen, NPN = Non Protein Nitrogen

respectively. However, at 45°C decrease in casein nitrogen content in all the branded yoghurts were from 528 to 425, 320 to 270 and 520 to 400 mg / 100g, respectively. Non casein nitrogen (NCN) in yoghurt brands A, B and C at 10°C remained unchanged during twelve days storage period. Increase in NCN in yoghurt brand 'A' at 25° and 45°C was from 132 to 185 mg/100g and 132 to 232 mg/100g, respectively. Increase in NCN at 25° and 45°C in yoghurt brand 'B' was from 240 to 266 and 240 to 292 mg/100g, respectively. However, increase in NCN at 25° and 45°C in yoghurt brand 'C' was from 160 to 210 and 160 to 282 mg/100g, respectively. Increase in NCN was more pronounced at 45°C in all the yoghurt brands than 25° and 10°C. Non-protein nitrogen (NPN) in yoghurt brands A, B and C at 10°C remained unchanged during twelve days storage period. NPN at 25° and 45°C in yoghurt brand 'A' was decreased from 70 to 58 mg/100g and 70 to 55 mg/100g, respectively. NPN at 25° and 45°C in yoghurt brand 'B' was decreased from 160 to 150 mg/100g and 160 to 142 mg/100g, respectively. However, at 25°C and 45°C in yoghurt brand 'C' was decreased from 80 to 72 mg/100g and 80 to 52 mg/100g, respectively. Storage temperature and time period significantly ($p < 0.05$) affected the changes in nitrogenous components of all the branded yoghurts, according to multivariate ANOVA. However, these changes were more pronounced at 45°C in all the yoghurt brands as compared to 25° and 10°C, at the end of storage period, according to Duncan's multiple range test.

These changes in nitrogenous components of yoghurt could be attributed to proteolysis by a native milk proteases resistant to heat treatment or to reactivation of proteases during storage (Bengtsson and others, 1973; Bjorck, 1973; Snoeren and others, 1979).

Carzo and others (1988) observed the changes in carbohydrate and protein fractions of yoghurt during storage at room temperature. These changes included dissociation of casein |/whey protein complexes, on formational changes of casein molecules including break down of micelle structure, interaction of beta lacto-globulin and K-casein, disulphide (s - s) exchange reactions, phosphorylation of casein and interaction of casein and carbohydrates. These changes were accelerated by increased storage temperatures (Robinson 1994).

Moisture contents in yoghurt brands A,B & C remained unchanged at 10°C during twelve days storage period (Table 1, 2, 3). However, at 25°C decrease in moisture content in all of the branded yoghurts was from 14.5 to 14.20 % and 13.58 to 11.25 % and 15.30 to 15.00 %, respectively. However, decrease in moisture content was more pronounced at 45°C in yoghurt brands A, B and C which was from 14.5 to 13.60 %, 13.58 to 9.00 and 15.30 to 14.70, respectively. Storage temperature and time period significantly ($p < 0.05$) showed effect on the decrease in moisture content of all the branded yoghurts, according to multivariate ANOVA. However, decrease in moisture was more pronounced at 45°C in all the yoghurt brands as compared to 25° and 10°C, at the end of storage period, according to Duncan's Multiple range test.

Table 4 shows ±SEM and sensory attributes including color, taste, odor, texture and overall acceptability of yoghurt brands A, B and C, at 10°, 25° and 45°C, after a time period of twelve days storage. After twelve days of storage, color, taste, odor, texture and overall acceptability scores of all the branded yoghurts at 45°C were significantly ($p < 0.05$) less than the yoghurts (brands A, B and C) stored at 10° and 25°C. However, score rating for sensory qualities of all the branded yoghurts in this study at 10° and 25°C were not distinctly different from each other. These results coincides with the findings of earlier workers who reported undesirable changes in the sensory characteristics of yoghurt due to decomposition of fat and protein as a result of lipolysis and proteolysis process (Barrantes and others 1996 & Harasawa and others 1998). It has been reported in literature that the reaction products of maillard reaction might be responsible for adverse changes in sensory qualities during extended storage (PF. Fox 1985). Keeping in view about these facts, it is suggested that all the branded yoghurts cannot be stored at 45°C for longer period under hot climatic conditions during the months of summer.

stored yoghurt brands as suggested by *Schmidt and Renner (1978)*. These results are consistent with the findings of earlier workers who found a small reduction in pH at higher temperature (Zadow and Chituta 1975). Storage temperature and time period significantly ($p < 0.05$) showed effect on the decrease in pH and increase in titratable acidity of all the branded yoghurts, according to multivariate ANOVA. However, decrease in pH and increase in titratable acidity was more pronounced at 45°C in all the yoghurt brands as compared to 25° and 10°C, at the end of storage period, according to Duncan's Multiple range test.

During storage of yoghurt brands A, B and C at different temperatures (10°, 25° and 45°C), decrease in lactose was observed to various extents. These changes were more pronounced during storage at 45°C than 25°C. Lactose of yoghurt brand 'A' at 25° and 45°C decreased from 8.1 to 7.5 and 6.7 mg / 100g, respectively. Lactose of yoghurt brand 'B' at 25° and 45°C decreased from 6.5 to 5.7 and 5.0 mg / 100g, respectively. Lactose of yoghurt brand 'C' at 25° and 45°C decreased from 7.4 to 7.0 and 5.2 mg /100g respectively. However, no change in lactose content was observed at 10°C in all the yoghurt brands. Storage temperature and time period insignificantly (p

Table-2. Storage effect on the nutritional composition of yoghurt brand 'B' at 10°, 25° and 45°C

Storage Temp.	Storage time (Days)	pH	Acidity %	Lactose g/100g	Fat %	T.N Mg/100g	C.N Mg/100g	N.C.N Mg/100g	N.P.N Mg/100g	Moisture %
10°C	0	4.01 ±0.80	0.08 ±0.002	6.5 ±0.45	1.1 ±0.001	560 ±1.15	320 ±1.41	240 ±1.35	160 ±1.01	13.58 ±0.90
	3	3.83 ±0.55	0.08 ±0.002	8.1 ±0.60	1.1 ±0.001	560 ±1.15	320 ±1.41	240 ±1.35	160 ±1.01	13.60 ±0.78
	6	3.96 ±0.65	0.09 ±0.004	8.1 ±0.60	1.1 ±0.001	560 ±1.15	320 ±1.40	240 ±1.36	160 ±1.02	13.60 ±0.78
	9	3.77 ±0.61	0.09 ±0.004	8.1 ±0.60	1.1 ±0.001	560 ±1.15	320 ±1.38	240 ±1.34	160 ±1.02	13.62 ±0.79
	12	3.82 ±0.59	0.095 ±0.005	8.1 ±0.60	1.1 ±0.001	560 ±1.15	320 ±1.36	240 ±1.31	160 ±1.03	13.64 ±0.75
25°C	0	4.01 ±0.80	0.08 ±0.002	6.5 ±0.45	1.0 ±0.001	560 ±1.15	320 ±1.41	240 ±1.35	160 ±1.01	13.58 ±0.90
	3	3.83 ±0.78	0.105 ±0.003	6.5 ±0.45	1.0 ±0.001	560 ±1.15	316 ±1.42	244 ±1.33	160 ±1.01	13.50 ±0.91
	6	3.55 ±0.75	0.0125 ±0.005	6.2 ±0.43	1.0 ±0.001	561 ±1.11	313 ±1.40	249 ±1.31	158 ±1.02	13.48 ±0.89
	9	3.57 ±0.73	0.125 ±0.005	6.0 ±0.40	1.0 ±0.001	562 ±1.17	303 ±1.45	259 ±1.37	155 ±1.05	12.00 ±0.85
	12	3.47 ±0.70	0.128 ±0.006	5.7 ±0.38	1.0 ±0.001	561 ±1.16	295 ±1.47	266 ±1.40	150 ±1.07	11.25 ±0.75
45°C	0	4.01 ±0.80	0.08 ±0.002	6.5 ±0.45	1.0 ±0.001	560 ±1.15	320 ±1.41	240 ±1.35	160 ±1.01	13.58 ±0.90
	3	3.50 ±0.81	0.13 ±0.001	6.3 ±0.43	1.0 ±0.001	560 ±1.15	313 ±1.40	249 ±1.25	154 ±1.05	13.00 ±0.85
	6	3.70 ±0.82	0.140 ±0.004	6.0 ±0.41	0.80 ±0.001	560 ±1.15	307 ±1.38	253 ±1.33	149 ±1.08	12.25 ±0.75
	9	3.52 ±0.85	0.145 ±0.005	5.6 ±0.45	0.80 ±0.001	560 ±1.14	298 ±1.39	264 ±1.31	144 ±1.05	11.75 ±0.77
	12	3.51 ±0.81	0.145 ±0.006	5.0 ±0.44	0.70 ±0.001	560 ±1.13	270 ±1.25	292 ±1.30	142 ±1.08	9.00 ±0.79

All the samples were analyzed in triplicate.
TN = Total Nitrogen, NCN = Non Casein Nitrogen CN = Casein Nitrogen, NPN = Non Protein Nitrogen

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Pak. J. Food Sci., Vol. 13. No. 1-2, 2003

Effects of nitrogen fertilizer on the nitrate and nitrite content of vegetables

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ABSTRACT

Effects of nitrogenous fertilizer on the accumulation of nitrates and nitrites in various vegetables were determined. In experiments conducted in wooden crates (75 x 75 x 45 cm) fertilizer had greatest influence on nitrate accumulation. 25g/unit fertilizer level was the best level giving lower concentrations of nitrates and nitrites with increased crop yield in bitter gourd, pumpkin and cucumber. High doses of fertilizer tend to increase the concentration of nitrates and nitrite in these vegetables.

Key words: Vegetables, Urea Fertilizer, Nitrate and Nitrite.

INTRODUCTION

It has been observed in past that nitrate accumulated within plants to abnormally high concentrations. (Baker and others 1971). Nitrate accumulation usually has no toxic effect on plant tissues but it may be injurious to animals that consume these vegetation. Nitrate poisoning has been common among animals grazing on nitrate rich forage or feeding on nitrate rich fodder, hay or silage (Wright and Davison 1964).

Although no authenticated cases are known for nitrate poisoning of human adults who have eaten plants rich in nitrate, there have been reports of poisoning of very young babies (infants) who have been fed spinach with high nitrate content (Phillips 1968). Nitrite is known to be the toxic material involved in nitrate poisoning. Nitrate may be reduced to nitrite in stored vegetables or by the microflora of the gastrointestinal tract leading to the formation of N-nitroso compounds (Phillips 1971). Dietary exposure to nitrite and nitrosoamine leads to risk of nasopharyngeal carcinoma (Ward and others 2000).

Much of the work reported in the literature have shown increase in the accumulation of nitrate nitrogen in vegetable by nitrogen chemical fertilization (Bogommzov and others 1990; Schepers and others 1991; Miedozokrodzke and others 1993).

The objective of the present study was to determine the effect of nitrogen fertilizer on nitrate and nitrite accumulation in summer (April-May) season vegetables.

MATERIALS AND METHODS

Different vegetables namely bitter-gourd, Pumpkin and cucumber were grown during summer season. The seeds and urea fertilizer were procured from the Agriculture Department Lahore. The soil was collected in bulk from a depth of 0 to 20 cm from the fields of PCSIR Laboratories Complex, Lahore and placed in wooden crates (75 x 75 x 45 cm size) lined with polyethylene sheets to avoid leakage of soil and irrigation water. The soil was analyzed by USDA. method (1984) prior to the plantation of crops.

Fertilizer was applied at the time of seed sowing. Experimental crates were given following does of urea fertilizer: 0, 25, 50 and 75 grams/unit to each crop.

The experiment was laid out n a completely randomized design with four replicates of each treatment. Thinning of plants was carried out at an early seedling stages. The irrigation interval for vegetables was 7 to 8 days. Composite samples of vegetables selected at random were collected after two to three months (n=4 x 16 = 64) and analyzed for the following parameters.

(a) Dry matter (%)
(b) Nitrate (ppm)
(c) Nitrite (ppm)

Dry matter was determined by AOAC (1980). Nitrate and nitrite were analyzed by the nitroxyloenl distillation (Heisler and others 1973) method and diazotization (Schall and others 1986) methods respectively.

The data obtained were subjected to analysis of variance techniques, new Duncan's Multiple Range Tests, linear regression analysis and student "t" test. For the comparison of treatments single degree contrast were estimated.

RESULTS AND DISCUSSION

Plants responded to urea significantly in nitrate accumulation. The highest accumulation was observed in bitter gourd when applied at the rate of 75

grams unit (5013 ± 121 ppm). The lowest level of nitrate accumulation was also in bitter gourd that was upto 3558 ± 226ppm at the rate of 25 g per unit. Cucumber and Pumpkin showed maximum accumulation of nitrate at the rate of 50 grams per unit (1822 ± 161 and 2325 ± 225 ppm respectively).

range test for bitter gourd indicated that all treatment were significantly different while pumpkin and cucumber showed that treatment T_2 and T_3 were significantly different whereas T_3 and T_4 were non-significant. Similar types of findings were observed in crop yield and nitrite levels (Table I).

Table I. Influence of varying rates of nitrogen fertilizer on the accumulation of nitrate, nitrite content and yield in vegetables

Name of Vegetable	Rate grams/unit	Nitrate (ppm)	Nitrite (ppm)	Yield (g)
Bitter Gourd	T_1 (0)	4482 ± 335(c)	16 ± 2(c)	593 ± 74(c)
	T_2 (25)	3558 ± 226(d)	14 ± 2(c)	863 ± 83(a)
	T_3 (50)	4625 ± 139(b)	27 ± 2(b)	688 ± 53(b)
	T_4 (75)	5013 ± 121 (n)	36 ± 2(n)	600 ± 44(c)
Pumpkin	T_1 (0)	1801 ± 330(b)	14 ± 2(c)	1365 ± 131(c)
	T_2 (25)	1124 ± 188(c)	18 ± 1(b)	1770 ± 83(a)
	T_3 (50)	2335 ± 255(n)	25 ± 2(b)	1553 ± 100(b)
	T_4 (75)	1990 ± 139(b)	18 ± 2(b)	940 ± 94(d)
Cucumber	T_1 (0)	1565 ± 181(b)	14 ± 2(c)	558 ± 37(c)
	T_2 (25)	873 ± 128(c)	12 ± 2(d)	1013 ± 96(a)
	T_3 (50)	1822 ± 161(n)	25 ± 2(n)	753 ± 83(b)
	T_4 (75)	1518 ± 198(b)	18 ± 2(b)	533 ± 41(c)

All results are presented on dry weight basis.
± SD in a column followed by the name letter are not significantly at 5% probability level by Duncan's new Multiple Range Test.

Table II. Analysis of variance (orthogonal, contract) for nitrates levels of bitter gourd, pumpkin and cucumber

Source	d.f.	SS	MS	F-Ratio
Between	3	18262207.8	6087422.6	74.9
$T_1\ T_2$ Vs $T_3\ T_4$	1	5668462.8	5668462.8	69.7
$T_1\ T_4$ Vs $T_2\ T_3$	1	6890625.0	6890625.0	84.8
$T_1\ T_3$ Vs $T_2\ T_4$	1	5703120.0	570312.6	70.P2
Error	60	4874040.6	212334.0	...
Between	3	12472578.3	4157526.1	76.0
$T_1\ T_2$ Vs $T_3\ T_4$	1	2021208.2	2021208.2	36.9
$T_1\ T_4$ Vs $T_2\ T_3$	1	439569.0	439569.0	8.03
$T_1\ T_3$ Vs $T_2\ T_4$	1	9506826.0	9506826.0	173.7
Error	60	3282007.5	5470.1	
Between	3	7834067.2	2111355.7	*40.1
$T_1\ T_2$ Vs $T_3\ T_4$	1	525690.3	525690.3	8.0
$T_1\ T_4$ Vs $T_2\ T_3$	1	606451.6	606451.6	9.3
$T_1\ T_3$ Vs $T_2\ T_4$	1	6701925.3	6701925.3	103.1
Error	60	3899968.8	64999.4	

* Significant nt. 5% and 1% levels of probability.

The nitrite level of vegetable were generally low (Table -I). Maximum accumulation was observed in case of bitter gourd (36 ± 2ppm) at the highest rate of 75 grams/unit.

Analysis of variance showed that all means were statistically significant at 5% and 1% levels of probability (Tables II and III). Duncan's new multiple

The nitrate regression co-efficient of bitter gourd and pumpkin (10.50 and 8.30 respectively, Table. IV) indicated that with the unit change of fertilizer there was an increase of 10.5 and 8.30 nitrate levels. Variation in fertilizer for bitter gourd and Pumpkin were 26.5 and 22.3% (R^2 value=0.265 and 0.223 respectively). The co-efficient of correlation in bitter

gourd and pumpkin had significant positive correlations with the nitrate levels (r=0.515 and 0.473, p<0.05).

The nitrite levels of vegetables had significant positive correlation with the fertilizer levels (r=0.902, 0.573 and 0.746, p<.01). the values of regression co-efficient of nitrite were low when compared to nitrate. A significant (p<0.5) negative correlation was observed with the yield (r=0.524) in Pumpkin. Bitter gourd and cucumber have non-significant negative correlation (Table. IV).

(Table. V) shows the results of major parameters of soil tested before and after cultivation of vegetable crops. The decrease in pH from 7.6 to 7.2 was due to the addition of fertilizer and increase in percent saturation was probably explained as due to irrigation. Reduction in the potassium and phosphorus might have resulted by the uptake of crops. The increase in organic matter form 0.50 to 0.72 was probably due to decaying of dead plants, which liberate many nitrogenous compounds such as urea, ammonia, urates, etc. All of which contributes heavily thorough subsequent conversation to the nitrate content of the soil (Table -V).

Table III. Analysis of variance (orthogonal contrast) for nitrites levels of bitter gourd pumpkin and cucumber

Source	d.f.	SS	MS	F-Ratio
Between	3	5039.2	1679.7	*193.0
$T_1\ T_2$ Vs $T_3\ T_4$	1	4258.9	4258.9	180.3
$T_1\ T_4$ Vs $T_2\ T_3$	1	509.5	509.5	58.5
$T_1\ T_3$ Vs $T_2\ T_4$	1	270.6	270.6	31.1
Error	60	522.2	8.7	
Between	3	1067.9	356.0	*56.3
$T_1\ T_2$ Vs $T_3\ T_4$	1	382.3	382.3	60.5
$T_1\ T_4$ Vs $T_2\ T_3$	1	471.9	471.9	74.6
$T_1\ T_3$ Vs $T_2\ T_4$	1	213.5	213.5	33.7
Error	60	465.0	6.3	
Between	3	28.6.5	935.5	*120.6
$T_1\ T_2$ Vs $T_3\ T_4$	1	1676.2	1976.2	216.2
$T_1\ T_4$ Vs $T_2\ T_3$	1	9.8	9.8	1.27
$T_1\ T_3$ Vs $T_2\ T_4$	1	66.2	66.2	8.5
Error	60	465.0	7.7	

* Significant at 5% and 1% levels of probability.

Table. IV. Effect of fertilization on the accumulation of nitrate, nitrite content by linear regression analysis in vegetables

Name of Vegetables	Co-efficient of regression (b)			Co-efficient of determination $(R)^2$			Co-efficient of Co – relation (r)		
	NO_3	NO_2	Yield	NO_3	NO_2	Yield	NO_3	NO_2	Yield
Bitter Gourd	10.50	0.291	-0.637	0.265	0.813	0.021	0.515	0.902	-0.147
Pumpkin	8.30	0.087	-5.97	0.223	0.328	0.271	0.473	0.573	-0.524
Cucumber	1.69	0.180	-1.34	0.015	0.556	0.031	0.124	0.746	-0.182

*, ** Significant at 0.5 and 0.01 probability levels respectively.

Table. V. Analysis of soil collected from the field used for the cultivation of vegetables

Parameter Studied	Before Cultivation **	After Cultivation**
Soil pH	7.6 ± 0.05	7.2 + 0.05
Organic Matter (%)	0.50 ± 0.10	0.72 + 0.10
Available Phosphorus (ppm)	12.0 ± 1.0	106 + 0.5
Available Potassium (ppm)	115 ± 15.0	108 + 8.0
Saturation %	52 ± 6.3	55 + 4.0
Total Nitrogen %	0.07 ± 0.01	0.090 + 0.01
Nitrogen (ppm)	135 ± 10	160 + 14
Nitrite (ppm)	4.0 ± 1	5.5 + 0.5

The nitrate and nitrite contents of the soil were low with total nitrogen content of 0.075 to 0.090% (Table. V). Nitrate was absorbed rapidly by plant roots probably by a combination of active (metabolic) and passive (water flow) mechanisms. Soil characteristics were sufficiently close to ideal. Soil characteristics that affect crop yield are texture, structure permeability, infiltration, availability of nutrients (NPK etc.), water retention in the root zone, soil aeration and susceptibility to flooding. However, nutrient imbalance and deficiency in water might have limited the yield as well as nitrate and nitrite contents of a crop (Esingen 1984).

Significant different in nitrate nitrogen concentrations of vegetables were found when urea fertilizer was applied. Concentration of nitrate found in various species may be interrupted in terms of some well-established process within the plant (Greenwood and Hunt 1986).

Some of the nitrate absorbed by the root is stored within it. The reminder is transported in the xylem to the various leaves in amounts that depend partly on transpiration flow of water. Nitrate does not enter the phloem in more than trace amounts so that very little leaf nitrate is translocated to other parts of the plant (Greenwood and Hunt 1986). The effect of fertilizer practice on nitrate and nitrite concentration could be explained in terms of differences in the conditions of growth, light intensity, row spacing and irrigation water with different vegetables types (Table -I).

Our results indicate that 25 g/unit rate of fertilizer could decrease the nitrate concentration with substantial increase in the yield nitrogen utilization when compared to control. At 25 g/ unit fertilizer level dilution of fertilizer took place. Similar type of findings were reported by Ageev and others (1986) and Zugan and others (1997). The data is also partly in confirmation with the findings of Barker and others (1971).

AT 75 g per unit urea, fertilizer level of nitrate nitrogen concentration of all three vegetables was statistically similar to control. This indicates that at higher doses of fertilizer, the uptake of nitrogen is greatly reduced resulting in the decrease of plant yield (Table. I)

At high doses of fertilizer nitrate might have reduced to nitrite by bacterial action. Previous filed experiment of Rozek and others (1999) and Zhou and others (2000) have shown similar results. Cantiliffee 1973 also found that degree of nitrate accumulation in vegetables was affected by the nitrogen fertilizers.

Growers tend to fertilize crops heavily with nitrogen both pre-plant as well as sidedress. Under these conditions nitrate and nitrite might accumulate to at rather high level (Lapa and others 1990).

The rate of nitrate and nitrite accumulations varied from crop to crop.

In conclusion, it could be said that 25-grams/unit rate was the best level of nitrogen fertilizer where there was significant decrease of nitrate concentration in the vegetable with significant increase in the yield.

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Changes in *punica granatum* concentrate during storage at different temperatures

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ABSTRACT

Pomegranate juice was sweetened with the addition of sugar @ 20%. This was concentrated to 68 °Brix under vacuum (750-760 mm Hg) at 40-45°C. Sodium benzoate was added @ 1g per kg and stored at room temperature (15 to 30°C), refrigeration temperature (4 to 8°C) and at freezing temperature (-10 to -15°C) for 90 days. Results revealed losses in ascorbic acid (44 %), maximum increase in titratable acidity (37.5%) and decrease in pH. The product stored at freezing temperature exhibited slight decline in Brix (5.8 %), ascorbic acid and increase in acidity. Refrigeration temperature storage showed moderate changes in all parameters. Freezing temperature is recommended for storage of pomegranate concentrate.

Keywords: Pomegranate, concentrate, freezing, storage, quality

INTRODUCTION

Pomegranate (*Punica granatum*) is a common fruit in Pakistan, Afghanistan, Iran and Spain. It is native to Iran and is widely cultivated in tropical and subtropical areas. The fruit is delightedly refreshing in character. The ripe seeds are eaten fresh or processed into fruit juice. In Egypt pomegranate is fermented to produce a kind of wine (Mien and Ischak 1980).

This wider applications of pomegranate are in the form of dessert fruit or its juice is consumed for refreshing purposes. The fruit is nutritious and also considerably important for its medicinal value. The fruit is a source of vitamins, minerals, carbohydrates and amino acids. The seeds of pomegranate contain N, Mg and Fe, while its juice is found to have Ca, P, K, and Cu. The storage life of fruit is few days at ambient temperature. More over pomegranate is a seasonal fruit and available in the market for a very short duration from September to December, so there is a need to preserve its products to make it available during off-season.

Storage of fruit juice concentrates with suitable preservatives at appropriate temperature increases their keeping quality (Pruthi and others 1984) stored kinnow juice concentrate of 62° Brix with addition of SO_2 . Maximum loss of ascorbic acid was observed at room temperature, while minimum loss of 19.27 % occurred at lower temperature . Sandhu (1985) stored kinnow juice concentrate of 42° Brix at 2 to 5 °C and 10 to 38° C fortified with ascorbic acid and preserved with 700 ppm of SO_2 . The rate of loss of ascorbic acid is directly proportional to storage temperature. Sherafatian (1994) determined the effect of temperature during storage on keeping quality of pomegranate. Fruit quality was determined by a panel. It was concluded that the best storage temperature was 3°C.

MATERIALS AND METHODS

Pomegranate juice was extracted with fine pulper and drink was prepared by addition of sugar @ 20% by weight of juice and mixed thoroughly. The drink was concentrated to 68° Brix at 40 to 45°C under vacuum of 750- 760 mm Hg. Addition of sodium benzoate @ of 1 g per kg of the concentrate, was done. The concentrate was filled into the pre sterilized glass jars, divided into three lots.One lot was stored at room temperature (15 to 30°C) , second at refrigeration temperature (4 to 8°C) and third at freezing temperature (-10 to -15°C).

Chemical analysis for Brix , pH, titratable acidity and ascorbic acid contents of the drink concentrate were carried out at regular intervals of 15 days for 90 days storage by following the methods as described by (Redd and others 1986). Statistical analysis was done to compare the affect of storage temperatures according to (Steel and others 1996) using Randomised Complete Block design.

RESULTS AND DISCUSSION

BRIX

The total soluble solids (TSS) of fresh concentrate were 68°Brix which decreased to 67°Brix after 90 days storage at room temperature giving the mean value of T.S.S. 67.43°Brix during storage period. The mean value of Bix during storage was observed 66.27°Brix and 65.21°Brix for refrigeration and freezing temperatures respectively. The greatest loss of Brix at freezing temperature may be due to condensation of atmospheric vapours. The results were in matching with the findings of (El-Kassas and others 1995) who found that there was tendency for sugar contents to slightly decrease towards the end of the storage period for pomegranates.

Titratable Acidity. The acidity of the fresh concentrate was 1.28%. Storage of product at room temperature caused an increase of 37.5% resulting in final acidity of 1.76%. The average readings for titratable acidity at room temperature, refrigeration temperature and freezing temperature were 1.48 %, 1.49% and 1.46% respectively for 90 days of storage. Statistical analysis revealed non significant difference in temperatures for acidity of the product. Increase in acidity was observed by (Kalra and Revathi 1981) while working on storage of guava pulp.

Brix-acid ratio. A gradual decrease in Brix-acid ratio was observed in all samples stored at different temperatures. The values of Brix-acid ratio fell from 53.13 to 38.06 at room temperature, 37.79 at refrigeration and 38.09 at freezing temperatures. The mean values of Brix- acid ratio are given in table-1 for 90 days storage. The results were similar to that as reported by (Fellers 1986).

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Table .1 Effect of storage temperatures on physicochemical characteristics of pomegranate drink concentrate.

Storage Temperatures	T.S.S. (Brix)	Acidity (%)	Brix/Acid Ratio	pH	Ascorbic Acid (mg/100ml)
Room Temperture (15 to 30°C)	67.43a	1.48	46.14	3.57b	3.96b
Refrigeration (4 to 8°C)	66.29b	1.49	45.19	3.61a	4.57a
Freezing (-10 to -15°C)	62.21c	1.46	45.00	3.64a	4.83a

Note : The values under same heading with similar letter differ non significantly from each other.

pH value

The pH value of fresh concentrate was 3.65 which decreased to 3.51, 3.58 and 3.60 during storage at room temperature, refrigeration and freezing temperatures, respectively. Statistical analysis revealed highly significant effect of temperature on the pH value. The results resembled with the findings of (Artes and others 1996).

Ascorbic Acid

Ascorbic acid contents of fresh concentrate were 5.4 mg /100ml. Results revealed that maximum loss of 44% occurred at room temperature, 27% at refrigeration and 16% at freezing temperature storage. The final values for ascorbic acid after 90 days storage were 3.00, 3.90 and 4.50 at room temperature, refrigeration and freezing temperature respectively. The results showed highly significant effect of temperature on ascorbic acid values. The results were similar to the findings of Marcyaqnd others (1984) who observed the loss of ascorbic acid in orange concentrate.

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PAK. J. FOOD SCI. 13(1-2): 35-39

Studies on shelf life of non-cola carbonated beverages

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ABSTRACT

For beverage production usually municipal drinking water is used, sometimes without special treatment. The main problem is improper storage of drinks in shops sometimes for long periods. A comparative study was made to determine the shelf life of three non-cola carbonated beverages. Essential and toxic elemental analysis of Ca, Na, K, Mg, Zn, Mn, Co, Fe, Ni, Pb, and Cd was done by atomic absorption spectrophotometer and that of ions (fluoride, chloride. nitrite, nitrate and sulfate) by ion exchange chromatograph. Almost no change in the different parameters was observed in study. The levels of Na, K, Fe, Ni and Cd in sample 1, Na, Fe, Ni, Pb and Cd in sample 3 and Ni and Fe in sample 2 were found higher than permissible limits recommended by WHO. The microbiological study revealed the absence of Coliforms but presence of heavy bacterial load in samples after 3 months storage at room temperature.

Keywords: Carbonated beverages, microbiology, mineral elements, quality

INTRODUCTION

Thirst is fairly a safe guide to amount of water needed. The form in which this quantity is consumed ranges from water, soup, coffee, milk, soft drinks fruit juices and others.

Beverages are marketed the world over and are used by all people. These are impulse items i.e. they are bought when attractively displayed in abundance.

The ingredients of typical carbonated beverage are purified water saturated with carbon dioxide gas, sugar, flavoring, edible acids and coloring agents. They provide about 100 calories/100mL and being in the form of sugars, they convert almost instantly into energy. That is why soft drinks are particularly refreshing after physical activity (Woodroof and Philips 1981).

Commercially available carbonated soft drinks are widely used. At public places these are considered to be reliable source for drinking. With the passage of time the quality of these soft drinks deteriorates as they travel from source to end-user. Moreover stocks are improperly stored for long periods in shops. Particularly all beverages are deteriorate with age. The rate of loss of quality i.e. color, flavor aroma, fermentation, nutrition depends on the formulation, the type of container, temperature light and amount of handling (Anon 1963).

The simplest method of determining the shelf life of freshly bottled drink is the storage test. It entails withdrawing a representative quantity of bottles from each production batch and storing them for a given period at a certain temperature. These samples are subjected to regular checks for changing in appearance and in flavor and tests of contents (Lin 1976). Microbiological examination provides the rapid and reliable results concerning the biological shelf life of soft drinks.

If the water is not treated before beverage making, it may contain metals that also have adverse effects on human health due to their toxicity. There is a very little published data available on the study of parameters and microbial pollution of carbonated beverages in Pakistan. This study was conducted to determine the shelf life and to monitor the levels of mineral elements in locally available non-cola carbonated beverages.

MATERIALS AND METHODS

Sixteen samples of each three different brands (taken from local market) of non-cola carbonated beverages were taken from the production batch of each brand. These samples were stored at room temperature i.e. 25°C and subjected to analysis fortnightly for 105 days.

Physico-chemical Analysis

For the determination of shelf life, following characteristic parameters are studied fortnightly along with microbiology; appearance, taste, pH, acidity, pressure of CO_2 and inverted brix (concentration of all the dissolved materials). For the determination of these parameters standard methods of AOAC (1980) were followed. Sugars were estimated by the methods described by ICUMSA (Schneider 1979). For the determination of ascorbic acid 10 mL sample was taken and absorption was noted at 760 nm following the method described by Bajaj and Kaur (1981).

Elemental Analysis

For the determination of Na, K, Ca and Mg beverage samples were diluted to desired limit with deionized water, acidified with 100 µL of conc. nitric acid and analyzed against freshly prepared series of standard

of each element by atomic absorption spectrophotometer (Hitachi 170-10) using air-acetylene flame.

For the determination of trace elements, 25 mL samples were acidified with 250 µL conc. HNO_3 and subjected to analysis directly by atomic absorption spectrophotometer using air-acetylene flame. The concentration of trace elements viz. Fe, Cu, Cd, Ni, Pb, Zn and Mn was determined from the standard curve plotted for each element. The matrix of the standards and the sample solutions was the same.

Fluoride, chloride, sulphate, nitrite and nitrate were determined by directly injecting the sample solutions, after appropriate dilutions, in ion chromatograph (Shimadzu). Concentration of the ions (Table 3) analyzed was determined against standards of ions, respectively.

Microbiological Analysis

The samples were analyzed for microbiological study in order to determine biological shelf life. As finished beverage product normally needs no preparation and should be subjected to appropriate microbiological tests (Panezai 1976). Presence of viable bacteria, yeasts and molds was determined by direct plating or by pour plate method, under all possible aseptic measures, using nutrient agar media. The presence of coliforms was checked by lactose broth inoculation.

RESULTS AND DISCUSSION

Good taste of soft drink quenches thirst, encourages liquid intake and helps settle upset stomachs (Woodroof and Phillips, 1976). It contains enough carbonates to give a lift and replenish the energy.

The main ingredient of a sparkling carbonated beverage is 86-93% purified water. For beverages, water must be purified of undesirable materials and standardized for those that are desirable. This is the art and science of beverage making (Anon 1970).

Characteristic Parameter Study

All the samples collected had a normal sparkling appearance with a good taste throughout the period of study except sample 3, which gave a disagreeable taste after three months storage at room temperature due to fermentation that was further confirmed by coliform test (Table 2).

1. pH

Soft drinks are acid products having a pH of less than 7. pH has a considerable effect on flavor and taste of product. Drinks having pH lower than 4 are very safe from public health point of view as for many organisms the optimum pH lies between 6.5 and 7.5. Acidophilic organisms including yeasts, molds, lactic acid bacteria and acetic acid bacteria can even cause deleterious effects at pH as low as 2.9, growth of which is measured by rate of carbon dioxide production for dextrose (Albert 1976). Many manufacturers have long recognized the relationship between pH and shelf life and therefore keep the pH of their product as low as possible. pH of all three samples (Table 1) changed slightly from 3.29 to 4.08 for sample 1, from 3.15 to 3.54 for sample 2 and from 3.42 to 3.64 for sample 3 in 105 days storage at room temperature.

2. Carbon dioxide and pressure

Carbonation is dissolving CO_2 gas in water utilizing temperature and pressure. Carbonate in the form of CO_2 is added for effervescence in beverages. Correct carbonation of soft drink is important because of the pungent taste it gives to the beverage and the beneficial effect it has on the digestive system (Woodroof and Phillips 1976; Anon 1963). No change in the pressure (Table 1) of any sample (3.5;56lb at 33°C and 3.3; 53lb at 26°C, for sample 1 and 2, respectively) was observed during the storage period except that of sample 3 (3.3-3.6)that rose up after 90 days due to formation of few fermentation products.

3. Acidity

Acid is third in importance following water and sugar. It is not only the source of sourness but it also enhances palatability, increase thirst quenching effect by stimulating flow of saliva, modify sweetness of sugar and act as mild preservative. In soft drinks usually benzoic acid is used as preservative. Edible acids like citric acid are also used to give taste to product. Acidity remained unchanged during the whole storage period in all the samples of three brands of non-cola carbonated beverages. While the acidity value is high for sample **2,** (21.2-23.5 mL of 0.1N NaOH i.e. 135.68-150.4mg of citric acid) than sample **1,** (18.5-20.0 mL of 0.1N NaOH i.e. 118.4-128 mg of citric acid) and **3,** (16.3-19.4 mL of 0.1N NaOH i.e. 104.32-124.16 mg of citric acid).

4. Ascorbic acid

Ascorbic acid was also found in non-cola carbonated beverages at a value of 6 mg/100mL, 13 mg/100mL and 7 mg/100mL for sample 1, 2 and 3, respectively but only in fresh sample. Exposure to heat and air causes considerable loses of ascorbic acid. Copper and other metals, even present in traces, hasten destruction (Harrow and Abraham 1958). No amount of ascorbic acid was observed even after 15 days storage at room temperature.

Table-1. Characteristic parameter study (physico-chemical analysis) of non-cola carbonated beverages

Parameters	Sample1	Sample2	Sample3
pH	3.29-4.08	3.15-3.54	3.42-3.64
Acidity (mg citric acid)	118.4-128	135.68-150	104.32-124
Inverted brix	10.3	11.5	10.5
Ascorbic acid*(mg/100mL)	6	13	7
Total sugars (%)	10.8	9.5	8.8
CO_2 volume and pressure (56 lb;33°C)	3.5 (53 lb; 27°C)	3.3 (38 lb; 17°C)	3.3-3.6* 45 lb; 26°C)

*Rise in volume observed due to fermentation
*No amount was observed after15days storage at room temperature
Code No. Sample 1 L112626J42B/6
Sample 2 L1123K120
Sample 3 FEB122412343

Table-2: Total bacterial count (organism/mL) in non-cola carbonated beverages

Days	0	15	30	45	60	75	90
Sample 1	289	303	1232	1824	1878	over growth	over growth
Sample 2	118	1184	1336	2464	2664	over growth	over growth
Sample 3	206	696	1972	over growth	over growth		overgrowth*

*Positive coliforms test after 105 days

Table-3. Mineral elements and ions in non-cola carbonated beverages (mg/L)

Elements	WHO* Permissible Limits	Sample 1	Sample 2	Sample 3
Na	200	254.69	167.35	265.30
K	12	13.40	1.61	4.20
Ca	60	18.15	12.00	22.31
Mg	60	2.90	2.20	2.05
Cd	0.003	0.02	0.001	0.06
Mn	0.1	0.07	0.01	0.04
Pb	0.01	0.008	0.006	0.3
Fe	0.3	0.72	0.48	0.57
Cu	1.2	0.97	0.45	0.85
Zn	3.0	1.23	0.93	0.97
Ni	0.02	0.18	0.05	0.09
Fluoride	1.5	Nil	Nil	Nil
Chloride	250	132.03	128.60	165.40
Nitrite	3	112.90	97.05	130.00
Nitrate	50	12.90	19.50	31.07
Sulphate	400	84.27	69.31	122.63

* World Health Organization Standards for drinking water 1996

5. Sugar and inverted brix

Sample 2 had higher sugar contents (10.8%) than sample 1 (9.5%) and 3 (8.8%). The sugar content also remained unchanged during the storage period.

When acids act upon sucrose, it combines with water and converted into dextrose and laevulose. The distinct advantage of inversion is in the taste of finished product. Each sugar, in addition, in addition to its individual sweetness, has a distinct individual taste. By inverting the syrup this final taste is produced thereby ensuring a greater and continuing uniform taste in the finished product (Woodroof and Phillips 1981). As the sugar contents remained the same for all the samples of three brands of non-cola carbonated beverages, no change in inverted brix (the

total concentration of all the dissolved materials including sugars) was observed, the values of which were 10.3, 11.5 and 10.5 for samples 1, 2 and 3, respectively. Previously, it was reported (Birkhed 1984) that sugar content of beverages decreased by spontaneous hydrolysis of relatively low values when stored at room temperature for 5 months.

Microbiological Analysis

Microbiological standards for drinking water in most developing countries rely on the detection of total coliform and *E. coli* as markers for human pathogens (Kartz and others 1999). US Environmental Protection agency (USEPA) suggests maximum contamination level of total coliform and *E. coli* as < 5% (positive) and 0.00 (organisms/100mL), respectively.

Sand (969) reported that due to the physico-chemical properties in particular, their low pH values and low nitrogen and oxygen content soft drinks are, from a microbiological point of view, very selective media. Generally microorganisms do not survive in soft drinks.

Acidity is the single most important factor affecting microbiological spoilage, more generally pH is among physico-chemical factors of major concern affecting microbial growth (Casolari 1984).

Investigated data (Table 2) showed that in all samples heavy microbial load was observed with increase in storage time. Total viable count ranges from 289/mL to 1878/mL and 118/mL to 2464/mL for sample 1 and 2 after 60 days while for Sample 3 the value was 206/mL for fresh sample to 1972/mL after only 30 days. The data indicates variable extent of pollution in these samples. The high values of microorganisms in beverages create health problems.

The microbiological study revealed the absence of yeast, molds and coliform in all three samples except sample 3 which gave positive coliform test after 105 days storage at room temperature and taste of the sample was also affected. This off flavour was due to carbohydrate fermenting bacteria the growth of which was further confirmed by using differential medium.

Panezai (1976) reported that soft drinks are, in fact, more susceptible to microbial spoilage than other food products due to both intrinsic (which are not easily controllable e.g. pH, water activity, mineral content etc.) and extrinsic (controllable e.g. nature of raw materials, initial microbial load, packaging etc.) factors.

Mineral Elemental Study

Various local soft drink companies of Pakistan usually use municipal drinking water as raw material, without special treatment. This untreated water may contain toxic elements for which human body has almost zero tolerance limits (Tridev and Gurdeep 1992).

WHO standards for Na and K are 200 and 12 ppm, respectively. Both play an important role in establishing the electrolyte balance in human body. Except sample 2 (167.35 ppm), the other two samples contained high values of sodium (254.69 ppm for sample 1 and 265.30 ppm for sample 3) than WHO maximum tolerable limits, while sample 1 was observed to contain higher values of potassium (13.40 ppm) as well. This condition is alarming for the people having cardiac problems (Watto and others 2000).

Copper is not a significant constituent in natural waters, however it is introduced by dissolution from brass and copper pipes. In the present analysis all the samples had copper within the WHO permissible limits (1.0 ppm). Its presence is of physiological importance as supplement to iron for hemoglobin regeneration. Intake of iron from drinking water is insignificant for the body requirements.

In the present study the values of iron were found above the permissible limits (0.3 ppm) in all the samples. High concentration of iron may be attributed to local contamination due to corrosion in pipes. The permissible limit placed on this metal has no health significance but it renders water unsuitable for human hygiene.

Nickel compounds are toxic in conventional sense. Its excess may cause lung cancer. Its values were also found higher (> 0.02 ppm) in all samples, which is an alarming fact. Zinc also rarely occurs in natural waters and no significant health hazard was observed regarding high concentration of zinc. In all the samples the level of zinc was found within the recommended limits (3.0 ppm).

Lead and cadmium are toxic elements. The amount of lead and cadmium was found higher in sample 3 (0.3 and 0.06 ppm, respectively) while sample 1 contained high concentration of cadmium (0.02 ppm) only. This indicates pollution in these two brands sold in market.

Manganese is common in water supply. Chronic manganese poisoning affects the central nervous system. All the samples contained this element within the safe limits (0.1 ppm).

Among ions, fluoride was found absent in all the samples and the quantities of chloride, sulphate and nitrite also lie within the permissible limits. The values of nitrate were found alarmingly high which indicate organic pollution and can cause disease (Houghton and Mc-Donald 1976).

CONCLUSIONS

It was evident from the present study that the levels of K, Na, Fe, Ni, Pb, Cd and nitrate were above the permissible limits recommended by WHO. Moreover, all the samples have heavy bacterial load. This may be due to the use of untreated water. The local industries have old and rusted machinery and there may be no checks and balances for product quality maintenance. Moreover, comparative shelf life study revealed that among the three non-cola carbonated beverages, sample 2 is more suitable for drinking as it has lowest bacterial load and quantities of almost all mineral elements were found within permissible limits.

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PAK. J. FOOD SCI. 13(1-2): 41-44

Quality and grading of wheat produced in Faisalabad District

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ABSTRACT

Wheat grain samples collected from various locations in Faisalabad district were tested for physico-chemical characteristics. Wheat grains contained extraneous matter, which significantly varied from location to location within the District. The test weight and 1000 kernel weight were not affected due to the location but varieties. Particle size index of the grains varied due to the locations. However moisture content was found to be same in all locations. Gluten and protein content were found different depending upon the area and location. In general, investigations concluded that wheat grown in Faisalabad District had good quality, indicating desirable potential for flour milling and grain yield.

Keywords: Grain quality, grading, Faisalabad, physical tests, chemical analysis.

INTRODUCTION

Pakistan falls in the top ten of the wheat producers in the world. Wheat is the most important crop in the country by area as well as production. Wheat is the staple grain of Pakistan supplying 60% of the energy and protein in the average daily diet. Wheat quality is a complex term and is simply defined as "suitability of wheat" for a specific use. The quality of wheat depends upon the genetic factors but environmental conditions, growth locations, agronomic practices prevailing during different wheat growth stages greatly alter the wheat quality attributes. In majority of the wheat producing countries the grading of the grain is in vogue and has been under-revision in accordance with marketing requirements and problems.

USA is the leading country in wheat grading and characterization and had produced official grain standards which formed the basis for the grading system for many other countries. Hill 1991 pointed out that estimating the value of purchased through description or grade instead of personal, visual inspection requires a uniform set of grades and descriptive terminology, readily interpreted by buyers and sellers.

Ahmad and others (1992) carried out a survey in Sindh and Punjab provinces of Pakistan in April-May 1988 to assess the quality characteristics of wheat to the existing Fair Average Quality standards (FAQ). Fifty out of 824 samples were collected and their physical characteristics were and insect infestation were measured. The results indicated that the current FAQ standards did not describe of wheat produced.

Now in Pakistan this situation demands from scientists and technologists to give due consideration towards the importance of wheat quality of new wheat varieties in order to compete the world market.

Keeping in view the above stated issues and problems; present investigations were carried out to evaluate the wheat grown in Faisalabad district for their physico-chemical composition and grading quality.

MATERIAL AND METHODS

Collection of wheat samples

The detail of sampling procedure is given in table -1

Grading of wheat samples

Each sample was thoroughly mixed and the representative sample of 400 g was prepared. This sample was made to run through the U.S. sieve shaker model, RX-86-2 for grading. Three set of sieves U.S. No. 5, 8 and 12 were used in the present study.

Table-1. Wheat samples collected from various locations of Faisalabad District.

	2000-2001	2001-2002		
Locations	**Godown**	**Godown**	**Market**	**Field**
Darul-Ihsan	-	5	5	5
Jaranwala	-	5	5	5
Sumundri	-	5	5	5
Faisalabad	5	-	-	-
Tandlianwala	-	5	5	5
Kanjwani	-	5	5	5
Mamukanjan	5	5	5	5

Quality Evaluation

Physical tests such as test weight, thousand kernel weight, particle size index were determined using standard procedures of AACC (2000).

Chemical Tests

Moisture content, gluten (wet and dry), protein content, and fat acidity were determined using AACC-(2000) standard procedures.

Statistical Analysis

The data obtained from the experiment was analyzed according to the method described by Steel and others (1996).

RESULTS AND DISUSSIONS

Straw, Stones, Sticks, and Strings.

The results in Table -2 reveal that %age of straw, stones, sticks, etc. separable through US grading sieve # 5 was highest (0.995) in wheat grains collected from Jaranwala and lowest (0.757) in the samples from kanjwani.

Table 2: Effect of location on extraneous matter

Location	Straw, stones, sticks, and strings	Shriveled and broken grains	Dust, dirt, weeds
Darul Ihsan	0.821	1.06	0.46
Jaranwala	0.995	0.99	0.43
Samundri	0.590	1.34	0.22
Faisalabad	0.824	1.01	0.35
Tandilianwala	0.829	0.69	0.67
Kanjwani	0.757	0.98	0.50
Mamukanjan	0.789	0.79	0.38

In case of samples collected from market and of Food Department godowns, it was noted that the market samples contained more extraneous matter (0.854%) as compared to godown (0.731) and field (0.800) as seen in Table- 3

The results of other grains shriveled and broken grains given in table 2 and 3 , show that, they were significantly affected by the locations and type of samples. The highest %age of other grains was found in samples of Samundri and value was 1.34%.

Table 3: Effect of type of sample on the extraneous matter

Type of Sample	Straw, stones, sticks, strings,	Shriveled and broken grains	Dust, dirt, weeds
Godown	0.731	1.03	0.30
Market	0.854	0.81	0.41
Field	0.800	1.10	0.59

In case of samples collected from field, market, and godowns field samples contained maximum broken and shriveled grains and values were 1.10 for field, 1.03 for godowns nad 0.81 for market .The differences in other food grains in type of samples and differences in locations may be attributed to climatic conditions, harvesting and threshing operations. Planting time is also one of the most important factors.

Table 4: Effect of location on the physical characteristics of wheat

Location	Test wt Kg/hL	1000 kernel wt (g)	Particle size index (%)
Darul Ihsan	74.80	36.45	19.65
Jaranwala	75.00	36.64	15.90
Samundri.	75.40	35.55	16.70
Faisalabad	76.27	37.01	17.67
Tandilianwala	75.20	35.79	23.66
Kanjwani	75.80	36.39	20.81
Mamukanjan	75.80	36.73	20.91

Data given in table 2 & 3 showed that %age of these materials(collected in the bottom pan) significantly varied among the locations. All materials which passed through VS sieve # 12 was the highest i.e. 0.67 %In Tandlianwala and lowest 0.22% in Samundri.

Presence of dirt, dust, and weed seeds also varied in different types of samples. The percentage of dust, dirt, and weed seeds ranged from 0.02 to 1.42%, 0.02 to 1.13 % and 0.02 to 1.21 % among Govt. Godowns, markets and field samples respectively.

Foreign matter separated from the wheat sample included, seeds of barley, oat, bathu, pohli, jangli palak and lehli. The work on grading system was undertaken by many authors (Leonard and Edward 1967; Seibel 1969; Hill 1991 and Ahmad and others 1992).

The results of the present investigations revealed that in some of the physical parameters considered to important for wheat grading, there have been found variations due to differences in locations. In some cases results were significant between the samples within the locations. Dexter and Tipples (1987) and Gaines and others (1997) reported similar results in their studies.

Table 5: Effect of sampling on the physical characteristics of wheat

Type of sample	Test wt Kg/hL	1000 kernel wt (g)	Particle size (%)
Godown	75.51	36.55	18.35
Market	75.29	36.39	18.32
Field	75.37	36.16	21.31

2. 1000 kernel weight:

It is useful index for potential milling yield. Anjum (2002) reported that Pakistani wheats possess more than 30 g 1000 kernel weight. In the present study 1000 kernel wt. neither varied significantly in locations nor sampling places. But highest 1000 kernel wt. was recorded for Faisalabad (37.01g) and lowest for Samundri (35.55).

3. Partial size index:

Pakistani wheat have hardness level identical to American hard white wheat. The results of this study indicated that there is significant variation for particle size index among different locations and samples within each location also varied significantly from each other.

Data given in Tables 5 & 6 showed that particle size index ranges from 5.72 to 59.47%; 8.75 to 33.19 %; 33.07 to 45.32 % in godown, market and field samples respectively.

Table 6. Effect of location on the physico-chemical characteristics of wheat grains

Location	Moisture (%)	Wet gluten (%)	Dry gluten (%)	Protein (%)	Fat Acidity (%)
Darul Ihsan	10.42	27.70	9.21	10.13	0.071
Jaranwala	10.13	28.84	9.75	10.69	0.072
Samundri	9.81	29.29	10.11	11.03	0.064
Faisalabad	10.36	29.04	9.78	11.05	0.080
Tandilianwala	10.30	25.60	8.80	10.31	0.074
Kanjwani	10.09	27.99	9.36	10.40	0.072
Mamukanjan	10.20	26.65	8.84	10.42	0.070

Physical characteristics wheat grain samples:

1. Test weight

It is considered as the one of the important tool in all wheat grading systems. There is considerable decrease in the milling yield i.e. flour yield with the decrease in test weight. The results obtained from the study revealed that all samples exhibited test weight desirable limits and consequently wheat samples will give good flour yield.

The highest test weight in samples collected from Faisalabad district i.e. 76.27 kg/hl and the lowest from Darul-Islam 74.80 kg/hl. However the test wt. ranged from 75.29 – 75.51 kg/hl in samples of godowns, market and field.

Egidio and others (1993) who studied grain flour and dough characteristics of diploid wheat reported mean test weight as 77.8 kg/hL.

This indicates that Pakistan wheats are of medium hard to medium soft texture. In an other study by Ahmad and others (2001) have also been reported that Pakistani wheat falls in the category of medium hard to medium soft group on the basis of particle size index values.

Chemical characteristics

1. Moisture content

Moisture content present in the grain is important in two respects. One is that high moisture content in the grain increase wt. second, high moisture content effect of the grain. Wheat samples collected from different locations in Faisalabad district had different moisture content. Wheat grains collected from Darul-Islam had highest moisture content (10.43 % and Samundri had the lowest (9.85 %). However the moisture content differ significantly among the types of grain samples. The moisture content reported in this study matches with the findings of US Association (2001) for hard white wheat.

2. Wet gluten content

Results of analysis of variance are regarding wet gluten content are presented in table 5 & 6.Its evident from the results that wet gluten content varied significantly among locations. However within location gluten content showed non significant variations. The samples collected from Samundri had highest (29.29 %) wet gluten content and Tandlianwala had the lowest (25.60%). The moisture percentage ranged from 9.64 to 10.42 %.

3. Dry gluten content:

The dry gluten content exhibited significant variations depending upon the locations (Table-6) varied from 8.80-10.11%. The wheat grown in Tandlianwala had least dry gluten i.e. 8.80%. However, highest gluten content were found to be in samples of wheat grown in Sumumdri. The dry gluten content in types of wheat samples were non significant.

4. Fat acidity

Fat acidity percentage was almost similar in wheat samples of all locations except for Faisalabad. The results are shown in Table 6. The fat acidity in Faisalabad wheat sample was 0.080 slightly higher than 0.070 fat acidity value of all other samples. This parameter exhibited non significant variation in types of wheat samples.

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PAK. J. FOOD SCI. 13(1-2): 45-48

Sensory and physical evaluation of biscuits supplemented with soy flour

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ABSTRACT

In these studies wheat flour was replaced with soy flour at four different levels i.e. 5, 10, 15 and 20%. Biscuits prepared without soy flour were kept as control. Effect of soy flour on sensory characters of the biscuits was evaluated. It was found that quality score for all sensory parameters declined with increase in soy flour. Taste and texture of the biscuits showed significant declining trend as compared to color and flavor. Quality score for overall acceptability was not significantly different showing that biscuits with all levels of soy flour were equally acceptable. Protein and ash contents increased whereas moisture and fat remained almost unchanged Addition of soy flour restricted the spread of biscuits during baking. However, spread factor did not affect the acceptability of the biscuits.

Keywords: Biscuits, soy flour, parameters, sensory evaluations

INTRODUCTION

Proteins of plant origin are generally deficient in one or more of the essential amino acids. Proteins of wheat are low in lysine (Osborn and Mendel 1919; Khan and Eggum 1978a; Khan 1981). Biscuits are prepared from soft wheat of low extraction rate which is low in protein content. (Tahir 1974; Rehman and others 1988; FAO 1989). When wheat is supplemented with proteins that are relatively rich in lysine, then the amino acids profile becomes more balanced. The high quality of soy proteins is more important since the balance of essential amino acids in cereal proteins is often relatively poor and may, therefore, be significantly improved by the simple expedient of supplementation of soy proteins. The key to the supplementation of soy proteins as a nutritional supplement is its high lysine content as compared to cereal proteins. (Pyler 1988; Meyer 1987). It is also reported that the amino acid composition of soy protein meets or exceeds human requirements for high protein. (Young 1991). In Pakistan, cereals constitute the bulk of the average diet (Khan 1989). In contrast, legumes have been reported to meet the protein requirements of various age groups in the population (Khan and Eggum 1978b; Khan and Eggum 1979). In order to improve the nutritional quality and functional properties of wheat flour, various types of supplementations are used in different parts of the world. As reported by Marques and others (2000) these cheap vegetable protein sources can serve as an alternative to protein rich expensive foods to alleviate chronic protein malnutrition in under developed and developing countries.

Keeping in view the lack of lysine in the wheat flour and presence of high lysine in soy flour, the protein contents can be improved both in quality and quantity by supplementing soy flour into Wheat flour. This study was conducted to evaluate the effects of soy flour on the baking qualities of the biscuits and their level of acceptance.

MATERIALS AND METHODS

RAW MATERIALS

Wheat flour having low protein content (7%) was supplied by the Sihala Flour Mills, Islamabad.

Defatted soy flour (Soy protein concentrate)
Defatted soy flour was procured from the local market (Yousaf Ali & Sons, Nishter Road, Rawalpindi) and cleaned manually to remove all impurities.

Preparation of wheat-soy composite flour
Wheat flour was replaced by soy flour in four proportions 5, 10 15 and 20% and designated as T2, T3, T4 and T5 respectively, whereas T1 (without soy flour) was kept as control

PREPARATION OF BISCUITS

Biscuits were prepared according to the American Association of Cereal Chemists (AACC 1983) method No. 10.52 with a slight modification. Simple recipe was adopted using wheat flour/soy flour, shortening, sugar (sucrose), eggs and Baking powder (Pearce Duff, England). Mixer of Sanyo Food Factory was used instead of that mentioned in AACC (1983) method.

SENSORY EVALUATION

Biscuits were evaluated by a panel of judges according to the method described by Larmond (1977). The parameters studied were color, taste, flavor, texture and overall acceptability. The score card for the evaluation of the biscuits was provided along with instructions to each judge.

Physical analysis of biscuits

The AACC methods were adopted in order to determine the width, thickness and spread factor of biscuits.

Width (mm)

Six biscuits were placed edge to edge, their total width was measured and average with was determined by taking the mean value.

Thickness (mm)

Average thickness of the biscuits was measured by placing six biscuits one on top of another and measuring their height and taking average.

Spread factor (Sf) mm.

Spread factor was obtained with the help of following formula:

Sf = (Width/Thickness) x correction factor x 10

Correction factor at constant atmospheric pressure was 1.

CHEMICAL ANALYSIS

Moisture, ash, protein and fat contents in biscuits were determined according to AACC methods (AACC 1983).

STATISTICAL ANALYSIS

Data obtained was analyzed statistically as described by Steel and others (1996).

RESULTS AND DISCUSSION

SENSORY EVALUATION

Biscuits prepared from wheat flour and composite flours (wheat + soy flour) in various combinations were subjected to sensory evaluation for color, taste, flavor, texture and overall acceptability.

Color

The analysis of variance for the score of color (Table 1) of the biscuits prepared with different levels of soy flour revealed that there was no significant change in the color of the biscuits. Table 2 shows that control biscuits got the highest score (6.5), whereas at 20 percent level of soy flour, biscuits got the lowest score (4.5). Data also shows that there was a reduction in the quality score for color in the biscuits prepared with soy flour as the amount of soy flour was increased. This decreasing trend of quality score for color of the biscuits may be due to the high level of proteins present in the protein sources. As amino acids react with reducing sugars during baking and as a result Maillard reaction takes place. The color gets darker and when protein is further increased; more darkness takes place which results in the reduction of quality score for the color of the biscuits. Claughton and Pearce (1989) and Kailasapathy and others (1985) got similar effects. Hussain (1993) used gram flour to increase the protein content of biscuits and reported the similar results. Gandhi and others (2001) replaced wheat flour with defatted soy flour up to 40 % level and found that all the biscuits from various blends were acceptable with no significant differences among them.

Taste

Analysis of variance of the taste of the biscuits (Table 1) revealed that there was a significant decrease in the quality score for the taste of the biscuits. Table 2 shows that biscuits with 5 % soy flour got the highest score (6.5), and biscuits with 20 percent level of soy flour got the lowest score (4.5). The significant decreasing trend of taste may be due to the own taste of the soy flour which dominated when used in high amount.(Drobot and Stabikona 1976; Hussain 1993) The DMR (Ducan's Multiple Range Test) applied to treatments showed that only T1 and T3 differed non significantly while all other treatments were significantly different.(Table 1)

Flavor

Flavor of the biscuits was not significantly different (Table 1). All treatments were in the acceptable range. Control and biscuits with 5 percent soy flour got the highest score (6.5), and biscuits with 20 percent soy flour level got the lowest score(4.83). Hussain (1993) also reported a decreasing trend in the flavor of the biscuits enriched with gram flour. Similar results were reported by Mac Watters (1978) and Claughton and Pearce (1989) during the evaluation of biscuits.

Decreasing trend of quality score of the flavor of the biscuits may be due to proteins present in soy flour. This may also be due to the own flavor of soy flour. Drobot and Stabnikona. (1976) reported a same trend. Onweluzo and Iwezu (1998) also reported decreasing trend in the flavor score of the biscuits enriched with soy flour

Texture

Biscuit's texture analysis revealed that there was a significant effect on the texture of the biscuits when soy flour was added (Table 1). There was a decreasing trend in the quality score for the texture of the biscuits with an increase in the soy flour addition. Control and biscuits with 5 percent level got the

Table 1. Sensory characteristics of the biscuits prepared from composite flour of wheat-soy flour

Treatments	Sensory Characters (Range 1-9)				
	Color	Taste	Flavor	Texture	Overall acceptability
T1	6.5 +	6b	7.0	6.5 a	7.0
T2	5.1	6.5a	6.5	6.5a	7.3
T3	5.0	6.0b	6.0	5.5b	7.0
T4	5.0	5.0c	5.5	4.8c	7.0
T5	5.0	4.5d	4.8	4.5d	6.83

Significant at $p \leq 0.05$
+Means followed by different letters are significantly different ($p \leq 0.05$) as determined by Duncan's Multiple Range test

Table 2. Physical Analysis of the biscuits prepared from composite flour of wheat-soy flour

Control	T1	T2	T3	T4	T5
Width (mm)	57.00	56.67	56.42	56.25	56.13
Thickness (mm)	11.00	11.21	11.32	11.61	11.72
Spread Factor	51.81	50.55	49.84	48.44	47.89

Table 3. Chemical composition of the biscuits prepared with different levels of replacement of wheat flour with soy flour

Treatments	Moisture %	Ash %	Protein %	Fat %
T1	4.0	1.75	5.97	26.6
T2	4.15	1.85	7.28	26.67
T3	4.19	1.96	8.58	26.59
T4	4.27	2.07	9.87	26.63
T5	4.5	2.26	11.54	26.62

highest score (6.5), whereas biscuits with 20 percent level got the lowest score (4.5). However, all the biscuits were in the acceptable range.

The DMR (Ducan's Multiple Range Test) showed that in biscuits enriched with soy flour, only T1 and T2 differed non significantly (Table 1) whereas all other treatments were significantly different. The decreasing trend in the quality score for the texture of the biscuits was due to the proteins present in the soy flour (Onweluzo and Iwezu 1998). Biscuits are recommended to be made with soft wheat (low protein) to have a good texture. That is why due to high protein sources (soy flour), the protein contents raised due to which texture was affected (Mc Watters 1978). Hussain (1993) also reported a declining trend in texture of the biscuits enriched with gram flour.

Overall Acceptability

There was no significant difference among the treatments for the quality score. According to the data (Table 1), although all treatments were acceptable but T2 was best accepted by the panel. It was observed that overall acceptability was a totally different quality parameter and it was not affected with individual trends of taste, color, texture and flavor. Chakrabarti and others (2001) in their studies also showed that soy protein enriched biscuits were highly acceptable product

Physical Analysis

All the biscuits prepared with different levels of soy flour were analyzed for physical characteristics.

There width, thickness, and spread factor was measured. The data of these measurements (Table 2) shows that there was decrease in spread factor of the biscuits and an increase in the thickness of the biscuits as the level of protein sources are increased. This reduction in the spread factor of the biscuits may be due to the proteins present in the soy flour. This is because protein has more binding power and it binds water and restricts the spread of the biscuits.(Tsen 1976) Onweluzo and Iwezu (1998) reported a similar

observation that control biscuits showed a higher spread ratio than biscuits made with wheat-soybean blend.

CONCLUSION

It was found that soy flour is acceptable up to 20 % level without any adverse effects on the quality of the biscuits. Although, taste and texture changed significantly when protein was added but even then all the treatments remained within the acceptable range. Spread of the biscuits was also reduced with an increase in protein contents. As far as nutrition value is concerned, it was found that protein and ash contents of the biscuits increased with the addition of soy flour while moisture and fat contents remained almost unchanged. It is recommended that 20% defatted soy flour may be supplemented in wheat flour to make well accepted and nutritionally superior biscuits.

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PAK. J. FOOD SCI. 13(1-2): 49-52

Effect of carboxy methyl cellulose and carrageenan gum on the shelf life of bread

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ABSTRACT

Carboxymethyl cellulose (CMC) and carrageenan were incorporated @ 0.5 to 2 % and 0.1% respectively to enhance the shelf life of bread. Proximate analysis of flour showed 12.20% moisture, 0.50% ash, 12.38% crude protein, 0.94% crude fat, 0.54% crude fiber and 73.44% nitrogen free extract. Bread prepared by adding 0.1% carrageenan and 1% CMC contained 32.31% moisture, 8.89% crude protein, 1.87% ash, 1.07% crude fiber, 2.88% fat, and 52.98% nitrogen free extract. Using 0.1% carrageenan and 1% CMC in the bread showed that the first mold colony appeared after 72 hours. After 96 hours mould count was $2xl0^2$ CFU/g. It was concluded that good quality bread could be prepared with more shelf life with 0.1% carrageenan gum and 1% CMC.

Keywords: CMC, Carragenan, bread, shelf life

INTRODUCTION

Baking industry in Pakistan has made tremendous progress during the past decade. Bread is prepared at small scale and large scale by some modern bakery plants including Vita Bread Ltd, Dawn Bread Ltd, Rohi Bread Ltd. Wonder Bread Ltd, Bunny Bread Ltd. etc. Pan bread is the major product of these bakery plants (Chaudhary 1991). Unfortunately, the bread being marketed by some of these bakery plants lacks in quality and has short shelf life. Hence a substantial loss is borne by the producers from unsold loaves. Since, bread is an important part of our daily diet; therefore, ways and means should be explored to improve the quality and shelf life.

Some stabilizers produce about two days extension of shelf life of bakery and retain the sensory properties (Staszewska and Janik 1977) and improve water retention capacity, modify texture, volume and cell structure of the products Brummer (1977). The major type of microbial spoilage of baked bread is usually caused by molds and *Rizopus stolonifer* (Jay 1990). These stabilizers can be classified into seven categories such as proteins, natural plant exudates, plant seed gums, sea weed extracts, pectin, cellulose derivatives and microbial gums Ahmad (1995).

CMC is obtained by the action of mono chloro acetic acid on cellulose which is a non toxic solid. In bread industry, the term cellulose gum is used to designate purified CMC. Khundkar and Bhattacharjee (1965). Blends of xanthan gum with CMC have shown remarkable viscosity in aqueous solutions (Baird and others1982). When stabilizers are used in sour milk, it helps to impart body and textural attributes apart from enhancing their keeping quality (Gupta and others 2000).

The present study was undertaken to explore the effect of carboxy methyl cellulose and carrageenan gum to extend the shelf life and other quality characteristics of bread.

MATERIALS AND METHODS

Wheat flour, yeast, sugar, salt, shortening, preservatives and stabilizers i.e. CMC and carrageenan gum were purchased from the local market of Faisalabad.

Wheat flour was analyzed for moisture, crude fibre, crude fat, nitrogen free extracts, and total ash, according to the respective methods as described in AACC (2000). Breads were prepared from flour samples by using the following Recipe;

Flour	l00g
Active dry yeast	1.0
Sugar	4.0
Salt	1.0
Shortening	5.0
Gums	As per treatment
Water	Water absorption capacity
Preservatives	Calcium propionate @ 0.2% and Lactic acid @ 0.3%

The treatments used for preparation of bread are given as under:

Table 1. Levels of gums added in bread

Treatment	Carrageenan gum%	CMC%
T_1	0.1	0
T_2	0.1	0.5
T_3	0.1	1.0
T_4	0.1	1.5
T_5	0.1	2.0

Bread was prepared by following straight dough method as described in AACC (2000). Mixing was done in a Hobart mixer for five minutes, molding and panning was done manually after fermenting the dough for 105 minutes at 75% relative humidity and 30°C temperature. During baking process, the following conditions were maintained;

- Dry Mixing for 1 min and wet mixing for 6 min.
- Proofing time 50 min
- Wt. of dough ball 150 g
- Proofing temperature 35 °C
- Relative humidity 85%
- Baking temperature 230 °C and baking time 15 minutes.

Counting and identification of molds in bread after 0, 24, 48, 72 and 96 hours of storage were carried out by using serial dilutions and Agar Plate Technique on Sabouraud Agar Medium (Beneke 1962).

Sensory Evaluation

Bread loaves were evaluated by a panel of trained judges for external characteristics such as volume, color of crust, symmetry of form, evenness of bake, character of crust and internal characteristics such as grain, color of crumb, aroma, taste and texture. Scoring was done according to the procedure of Bread Score report of American Institute of Baking (Matz 1960).

Statistical Analysis

Data obtained for various parameters were statistically analyzed by using Analysis of Variance Techniques and the treatment means were compared by using Duncan's Multiple Range Test (DMR) (Steel and others 1996).

RESULTS AND DISCUSSION

The effect of CMC and carrageenan gum added to wheat flour was determined in respect of functional quality and extension in shelf life. The bread loaves were prepared by adding different levels of gums. Fermentation, proofing and baking conditions were kept constant. Commercial wheat flour was analysed and the results are presented in Table 2.The analysis showed that moisture, ash, crude protein, crude fat, crude fiber and nitrogen free extract of flour were 12.20, 0.50, 12.38, 0.97, 0.54 and 73.41 %, respectively where as moisture content of bread with additives ranged from 31.14 – 35.83, crude protein 8.76 – 8.6, ash 1.67 – 2.03, crude fiber 0.67 – 1.17, crude fat 2.40 – 3.77 and nitrogen free extract 51.70 – 73.41%. The results are agreed with the finding of Rahim (1999) who stated that wheat flour contained 12.59% moisture, 0.44% ash, 12.45% crude protein, 1.06% crude fat and 0.36% crude fibre. Brummer (1977) stated that Stabilizers improved water retention capacity, texture, volume and cell structure of the bakery products.

Table 2. Proximate composition of flour and bread containing different levels of gums.

Characteristics	Flour	T_0	T_1	T_2	T_3	T_4	T_5
Moisture (%)	12.2	30.8	31.6	32.6	33.3	33.7	33.9
Crude protein (%)	12.3	8.60	8.84	8.82	8.80	8.78	8.76
Ash (%)	0.50	1.67	1.72	1.80	1.87	1.96	2.03
Crude Fiber (%)	0.54	0.67	0.81	1.01	1.07	1.12	1.17
Crude Fat (%)	0.97	3.77	2.47	2.61	2.58	2.49	2.40
Nitrogen free extract (%)	73.4	54.4	54.5	53.0	52.3	51.9	51.7

In order to study the shelf life, microbiological count of bread was estimated after 0, 24, 48, 72 and 96 hrs. Results showed that mold colonies appeared after 72 hours of storage in the bread containing 0.1% carrageenan and 1% CMC. After 96 hours of storage, mold count was $2xl0^2$ CFU/g. Maximum numbers of colonies were observed in T^0 that contained $5X10^2$ CFU/g of bread after 96 hours of storage (Table 3).

The results showed that volume of bread was found to be highly significantly affected by the addition of gums. The gums at the level of 0.1% carrageenan

with 1% CMC (T^3) in wheat flour gave bread with higher score as compared to the breads prepared without or with gums at various proportions. In case of T^3 (containing 0.1% carrageenan and 1% CMC) the maximum volume was obtained and got a mean value of 6.17 at different storage intervals. The sample T^0 (control) obtained a mean value of 3.84 at same storage intervals (Table 4). The similar results were achieved by Akhtar (1993) who stated that bread of good quality can be prepared with guar gum upto 1 % level.

Golden brown color of bread crust provides good product appeal which was highly significantly affected by the addition of gums. T_3 (containing 0.1% carrageenan and 1% CMC) got maximum score (7.21) and minimum jn mean score (4.65) was obtained by T^5 (containing 0.1% carrageenan and 2% CMC). Bread containing gum at level of 0.1% carrageenan and 1% CMC (T_3), got maximum mean score 4.17 while bread without gums (To) got minimum mean score (1.66). The similar results were reported by Taboada and Santiesteban (2000) who stated that bread having 1% CMC gave better sensory characteristics.

Character of crust showed highly significant results according to mean squares table. Mean values of character of crust score have been shown in Table 4. Bread containing 0.1% carageenan and 1% CMC (T3) scored maximum points (3.82). Bread containing 0.1% carageenan and 2% CMC score minimum points, (1.86). Similar results were obtained in a study by Rao and others (1985).

Grain of bread was significantly affected by gums in the wheat flour at various levels. Maximum mean score (13.05) was obtained by bread containing 0.1% carageenan and 1% CMC (T^3), while minimum mean score (6.64) was obtained by the bread containing 0.1% carageenan and 2% CMC (T^5). Similar results were achieved by Menger and Ludewig (1977).

Bread samples were evaluated for color of crumb and the results showed the maximum means score (7.27) was obtained by bread containing 0.1% cargeenan and 1% CMC and minimum mean score (4.14) was obtained by bread without gums (T^0). Ahmad (1995) stated that the color of crumb was improved with the addition of gums in bread. The analysis of variance regarding color of crumb showed highly significant difference among the samples.

Similarly, highly significant difference was found between samples with respect to aroma of bread. Maximum mean score (8.77) was obtained by bread containing 0.1% carrageenan and 1 % CMC and minimum mean score (3.00) was obtained by bread without gums (T^0).

Maximum mean score (17.13) was obtained by T^3 and minimum mean score was obtained by T^0 (9.69) with respect to taste of bread. The results revealed that highly significant difference among different treatments has been found regarding taste.

Table 3. Mold count at different storage intervals.

	Storage Intervals (hours)				
Treatments	0	24	48	72	96
T^0	$2X10^2$	2×10^2	3×10^2	5×10^2	5×10^2
T^1	$2X10^2$	2×10^2	3×10^2	3×10^2	5×10^2
T^2		2×10^2	3×10^2	4×10^2	5×10^2
T^3				2×10^2	2×10^2
T^4				2×10^2	3×10^2
T^5				2×10^2	3×10^2

CONCLUSIONS

As regards sensory evaluation of bread, the treatment T^3 got maximum score followed by T_4 for the external

Table 4. Effect of different gums on external and internal characteristics of bread at different storage intervals (0, 24, 48, 72 and 96 hours).

Character	T_0	T_1	T_2	T_3	T_4	T_5
Volume	3.84e	5.40c	4.68d	6.17a	6.08a	5.89b
Color of crust	5.43c	6.79b	5.49e	7.21a	5.59c	4.65d
Symmetry of Form	1.66e	3.60b	2.64c	4.17a	3.63b	2.23d
Evenness of bake	1.69e	2. lice	2.65b	2.84a	1.85d	1.72e
Character of crust	2.16d	3.61b	3.0lc	3.82a	2.2d	1.86e
Grain	6.64f	11.1b	8.18e	13.05a	9.3d	9.61c
Color of crumb	4.14f	6.88b	4.70c	7.27a	6.23c	5.65d
Aroma	3.0f	4.65e	8.03d	8.77a	7.65b	7.15c
Taste	9.69f	ll.80e	13.44d	17.13a	16.54b	16.15c
Texture	7.29e	11.12c	9.98d	13.77a	10.13d	11.67b

and internal characteristics where as significantly lowest scores were obtained by the bread prepared from wheat flour having no gums (control). It was concluded that use of gums to a certain level in the bread improves the sensory characteristics and enhances its shelf life.

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PAK. J. FOOD. SCI. 13(1-2): 53-55

Antibacterial properties of essential oils from plant materials used in food and culinary preparations

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ABSTRACT

Antibacterial activity of essential oils from seven plant materials was studied against four strains of pathogenic bacteria. Essential oils from *Carum copticum* (Ajwain), *Ferula assofoetida* (Hing) and *Cuminum cyminum* (Zera White) exhibited maximum activity against *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The essential oils of *Myristica fragrans* (Jaifal), *Anethum sowa* (sowai) and *Zingiber officinale* (Ginger) also showed good but variable activity, while oil from *Illicium verum* (Star anise) showed poor activity; only *Escherichia coli* was sensitive to it.

Keywords: Essential oils, properties, antibacterial

INTRODUCTION

Plant materials are excellent sources of medicinal compounds to cure diseases. Plant extracts and essential oils are used in the Ayruvedic, Tibbi and Allopathic systems of medicine for the treatment of a number of human, animal and plant ailments. These are also used for the treatment of viral, fungal and bacterial diseases (Ahmad and others 1993; Siddique and others 1995). Bacteria invade the organism and multiply in it, feeding and living as parasites in the tissues. In many cases the poisonous waste products (toxins) by such parasites cause ill effects associated with a particular disease. In other cases these organisms upset the structure and functions of the body. Bacteria cause cholera, tuberculosis, diphtheria, anthrax, plague, lockjaw and numerous other diseases (Ahmad and others 1993)

Antibacterial activity of essential oils has been known for long time. This activity is being studied throughout the world as a substitute for existing antibiotics that have adverse side effects. Dornberger and others (1995) studied nearly seven hundred plant species and found that about 30% of these possess antibacterial properties. Several scientists (Kobert, 1908; Jordan 1911; Guenther 1948; Fleming, 1950; Johnson 1959; Kul'ski and others 1983; Dey 1984; Ilive and others 1984; Chalchat and others 1987; Malik and others 2002) have reported antibacterial activity of essential oils of different plants against various bacteria.

The essential oils are usually mixtures of different hydrocarbons and oxygenated compounds of different types (Malik and others 2002). The antibacterial activity of the essential oils is attributed, generally, to the presence of these oxygenated compounds (Ilive and others 1984; Onawunmi and others 1984). Many plant materials are used as condiments in food preparations. Their addition not only makes the food appetizing and palatable but also preserves and improves their keeping quality (Malik and others 2002). It is essential to understand the beneficial effects of such spices on human health. In the present investigation some condiments used commonly in culinary and other food preparations were selected to determine their antibacterial properties.

MATERIALS AND METHODS

The bacterial strains were provided by pathological laboratory, Mayo Hospital Lahore. These were indentified by carrying out biochemical tests. *Escherichia coli* and *Proteus mirabilis* were identified by entrotube II tests, *Staphylococcus aureus* by Mannitol Fermentation Test and Tube Coagulate Test, and *Pseudomonas aeruginosa* by Modified Oxydase Test.

The essential oils of plant materials from *Myristica fragrans* (jaifal), *Illicium verum* (star anise), *Anethum sowa* (sowai), *Zingibar officinale* (ginger), *Ferula assafoetida* (hing), *Carum copticum* (ajwain) and *Cuminum cyminum* (zera white) were extracted in PCSIR Laboriatories Complex, Lahore by dry steam distillation method using all glass assembly (Guenther 1948). To ensure complete aseptic working conditions, table-top and hands were cleansed with rectified spirit before starting work.

Bactonutrient broth was used for the preparation of inoculum. The ingredients of Bactonutrient agar were (a) beef extract 3 g, (b) peptone 5g, (c) agar 15g, and (d) distilled water 1000 mL. The pH was adjusted at 7.4-7.5. Broth was prepared by dissolving peptone 1 mg and sodium chloride 0.5 g in 100 mL distilled water and filtered. The pH was adjusted at 7.4-7.5.

Assay of antibacterial activity was carried out by Cavity or Well Method. Fleming (1950) demonstrated the Cavity method as an improvement of Cylinder Plate Method. Later Johnson (1959) also used the Cavity Method. In this method a cavity or well was made in the nutrient agar medium with the help of a cork borer of suitable diameter. The bacterial cultures were seeded by swabbing near a flame to avoid contamination. Essential oil (0.1mL) from each spice was poured with the help of a pipette into a cavity. The petri dishes were incubated for 24 hours at 37°C. Zones of inhibition were measured with the help of a scale to the nearest millimeter.

RESULTS AND DISCUSSION

In the present studies antibacterial properties of essential oils from seven different plants, viz. *Myistica fragrans* (Jaifal), *Illicium verum* (star anise), *Anethum sowa* (sowai), *Zingiber officinale* (ginger), *Ferula assafoetida* (hing), *Carum copticum* (ajwain) and *Cuminum cyminum* (zera white) have been studied.

Table 1. Antibacterial Activity of Essential Oils

Essential Oils	Conc. of essential oils (%)	Inhibition Zone (mm) of organism after 24 hrs 37° C			
		Staphylo-cocus aureus	Proteus mirabilis	Escheri-chia coli	Pseudo-monas aeruginosa
Myristaca fragrans	100	10	20	None	None
Illicium verum	100	None	None	10	None
Anethum sowa	100	10	None	12	None
Zingiber officinale	100	20	12	None	None
Ferula assafoetida	100	18	15	None	8
Carum copticum	100	35	35	40	25
Cuminum cyminum	100	25	16	20	None

Essential oils are mixtures of various compounds that have varying properties (Malik and others 2002). In the present study they act in such a way that bacterial activity is hindered and the medium in which they are nourishing becomes unfit for their reproduction and other life processes. Bacterial strains selected for these studies were the common human pathogens, i.e., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis*. These bacteria were identified by standard techniques using different biochemical reactions. Macronutrient agar medium was used for the stock culture and petri plate screening work. Essential oils from various plant materials when used against the bacteria gave variable activity against them. The zones of inhibition of *Myristica fragrans* with *Staphylococcus aureus* and *Proteus mirabilis* were 10 mm and 20 mm respectively. However, *Escherichia coli* and *Pseudomonas aeruginosa* were resistant.

Antibacterial activity of *Illicium verum* oil was poor and only *Escherichia coli* was sensitive to it. This was a clue to the fact that different bacterial strains respond differently. In case of *Anethum sowa* oil, zones of inhibition with *Staphylococcus aureus* and *Escherichia coli* were 10 mm and 12 mm respectively, while other two bacteria were found resistant. Zone of inhibition of *Zingiber officinale* oil with *Staphylococcus aureus* was 20 mm, while with *Proteus mirabilis* it was 12 mm; other two bacteria being resistant. Zone of inhibition of *Ferula assofoetida* oil with *Staphylococcus aureus* was 18 mm, with *Proteus mirobilis* it was 15 mm and with that of *Pseudomonas aeruginosa* it was 8 mm. *Escherichia coli* was resistant to Ferula oil. *Ferula assafoetida* showed good activity against bacteria probably due to sulphur compounds contained in it. *Carum coptcom* showed highest antibacterial activity. The zone of inhibition with *Staphylococcus aureus* was 35 mm, with *Proteus mirabilis* it was 25 mm, with *Escherichia coli* the zone of inhibition was 40 mm, and with *Pseudomonas aeruginosa* it was 25 mm.

It is reported that the essential oils can inhibit the growth and kill pathogenic bacteria like standard antibiotics in different ways (Ahmad and others 1993; Guenther 1948). They can precipitate bacterial proteins, including RNA and DNA. They can also kill the bacterial cell by deformation of its morphological characteristics. Mostly the oils cause destruction of selective permeability properties of cell membrane. Sometimes the essential oil forms a surface layer around the bacterial cell by adsorption. The process of respiration by absorption of nutrients and excretion of wastes are interrupted and the cell dies. Some components of the essential oils like aldehydes form charge transfer complexes with electron donors in the

bacterial cells and make them inactive. The activity of such compounds depends upon their molecular orbital energies.

The results from this study show that essential oils of these plants have antibacterial properties and are thus medicinally important for a number of infectious diseases. Their use in food and culinary preparations hinders bacterial growth and the resulting diseases. With the emerging world trends it is recommended to use such types of plant materials to reduce the chances of bacterial ailments. These trends should be encouraged also because adequate use of such food materials as alternative to pharmaceutical products leads to side-effects that are next to nothing.

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Towards Effective Oral Presentations[1]

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ABSTRACT

Oral presentations are important in the career of technical professionals. These are delivered by the students as part of their education programme, by scientists in conferences and workshops and by professionals in in-house meetings. The key to success is the manner in which a good presentation is delivered. The four steps essential for an effective oral presentation are analysis, preparation, rehearsal and presentation. Use of proper audio-visual aids is advantageous in expressing ideas, showing patterns and offering results that escape words.

INTRODUCTION

Scientific presentations are essential features in the life of technical professionals. Every technical person stands to gain from improving his or her communication skills (Huckin and Olson 1991). The most common ways to make others aware of the work being done is by publishing the results in a periodical, or by giving oral or poster presentations. Written and oral presentations are two different things. There are prescribed guidelines for writing a scientific paper (CBE 1994; Day 1988; Berry and Noyes 1993; Awan 2003b). The article must be written according to the format of the journal in which it is to be published. The references in these publications must be properly inserted at appropriate place in the text (Awan 2003a). The list of references must be prepared according to the format of the journal (Awan 2002).

Students, teachers, researchers, scientists, engineers, technologists, medical professionals and others make oral presentations from time to time. These may consist of delivering a seminar by a student, introducing a topic to an audience, or presenting survey reports in a gathering of experts (Huckin and Olson 1991). Scientists make oral presentations at conferences and workshops. Technical professionals participate in panel discussions and brief managers and each other on their own and others' activities and results. They are expected to prepare projects and reports and communicate them to their peers and superiors. They also analyze situations, phenomena, and problems, then propose courses of action or solutions and report on the progress of projects to other experts. Those aspiring for a postgraduate degree are required to put forward an oral defense of the research work pertaining to their thesis. Guidelines exist for delivering oral presentations (Haq 1987; Rupnow and others 2001; Awan 2003c), preparing visual aids (King and others 2001; King and Rupnow 1992) and posters for presentations (Rupnow and King 1995).

TALENT FOR PRESENTATION SKILLS

There are very few people having a natural talent for delivering outstanding presentations. On the other hand, foresight, hard work, and practice can carry most people into a high level of presentation skills. The standards for public speaking in the scientific and academic realms are relatively low, so a good presentation often is memorable. Much less attention is paid, especially to science students, to teach them skills necessary to develop a good oral presentation - even though these are the most common and most rapid ways to disseminate new findings. In addition, the skills needed to prepare an oral presentation can also be used in a variety of other settings such as preparing and conducting a job interview, or even addressing potential philanthropic sources.

OBJECTS OF ORAL PRESENTATIONS

The main purpose of an oral presentation is to provide information that the audience will then remember at a later date. Oral presentations give one an opportunity to get people interested in your work. It allows people to associate with the work and encourages discussion and feedback. The risk factor involved is that people only remember very little. The way that people would remember depends on the quality of the presentation. Detailed referencing of material or extensive review of data won't be remembered and may put the audience to sleep. For an extension worker the most important communication skill is delivering a message through word of mouth (Muhammad 2001).

ESSENTIALS OF AN ORAL PRESENTATION

In order to deliver a well-constructed and tidy presentation, and convey your viewpoint to the audience in a logical sequence, four vital steps have to be taken. These are analysis, preparation, rehearsal and presentation. An understanding of each would be helpful for a successful oral presentation.

1. Audience analysis

Whatever the speaking occasion, the speaker must have a goal. You will be more successful if you know what you want to accomplish and what your audience expects from you. Once you have decided that you can better select what and how to say, and in what order to say.

The audience attending an oral presentation is on the receiving end and normally consists of people with different interests. It is, therefore, important that their interests are analysed. The depth of the topic to which a speaker can go entirely depends upon the type of audience. The speaker must be aware of the competency of the audience in the specific topic. Because of the varied nature of the audience in some gatherings, it is essential to determine what the listeners need to know. Moreover, the amount of new information that can be absorbed by the audience must be determined and decided. To this end, it is important to decide what has to be put across to the listeners. A presentation delivered keeping in view the interest of the audience will be much appreciated.

2. Preparation

a. Understanding the psychology of audience - The listeners normally do not have a good memory. They can remember only 2 to 3 points. Hence, information must be selected that supports the main point and attracts the attention of the audience. Information retention by the audience is reduced as talk proceeds, so when series of points are to be made, these should be organized from the most to the least important. That way, the audience is more likely to remember the important points later. Even the less important points may become irrelevant to the focus of the talk as you practice.

b. Research for the presentation - The information to be provided in a presentation must appeal to the listeners. Lots of background research is needed. Material may be collected from different sources. Even if the information is not used in the presentation, it is useful to have as much knowledge as possible for the discussion and audience questions. Preparation must be completed well in advance of the event. No research and preparation should be left until the last minute.

c. Script writing - The preparation of an oral presentation begins with writing of the script. A complete write up may be needed. This should remain with the speaker during the talk. This is useful for students preparing to deliver a seminar as part of their educational programme. Furthermore, an outline should be prepared and kept ready for reference. Another choice would be to prepare cue or note cards, with one idea per card. The presentation must be structured and kept brief. Only main and supporting points need to be selected and arranged in a logical sequence.

i. Introduction - The introduction must define the topic, provide its significance and clarify the objectives. The listeners should be made clear about your talk, and what you are going to present. This selection should contain enough background and terminology to understand and appreciate the presentation. A road map or outline must be provided so that the audience remains alert during the presentation. The goal and expected contributions within the context should be emphasized. The emphasis on fundamentals will also depend on the audience background. There is a need to relate earlier works especially when an experimental work is reported. The objective(s) of the project must be stated clearly.

ii. Body of the script - The body of the presentation should consist of the description of the work done without going into excessive details. It should provide relevant material to support the topic. In student seminars, the presentation is based usually on some library work. It is more or less a review of literature meant to be educative for the peers. Hence, this presentation has a slightly different style. After having researched the topic, you definitely know more than your audience. Hence selection must be made as to what information will make sense to them, and what is most relevant to the point.

iii. Summary and conclusions - The psychology of the listener is to be very attentive at the beginning of a presentation, less attentive during the middle sections and then suddenly more attentive as it ends (Rupnow and others 2001). Hence, for the closing part of the presentation, a closing summary or conclusions must be prepared. The summary should consist of the highlights of the problem or the topic. It should be repetition of important points, reemphasizing conclusions and recommendations. With this the audience must be indicated that the talk is over.

d. Pattern of organization - When preparing the material, it is worth to select an appropriate pattern of organization. This must be developed according to the theme and the material organized into subtopics. The description should be appropriate to the topic. For example, if an experiment is to be described then it should have a standard descriptive pattern consisting of introduction, methodology, results, and discussion. One way to maintain interest in the listeners is to organize and present the material in a novel manner. This will help to keep your own interest in the topic, and will result in a talk, which is more fresh and exciting.

3. Rehearsal

This is an important but least practiced step in creating an effective oral presentation (Haq 1987). It helps to prepare the speaker for any potential problems with delivering the paper and eliminates embarrassing situations. Two important aspects are:

a. Practice - Practice is an essential component of effective presentation. It must be done alone, using audio- or videotape. Once having reached a point of satisfaction, it may be done in front of a few friends. This allows the speaker to spot and eradicate the flaws in a presentation. It also enables one to work on making smooth transitions from section to section and provides an idea of the length of presentation (Rupnow and others 2001). Time keeping is vital and is the responsibility of the presenter. It is very easy to misjudge timing unless a full-scale rehearsal has been made.

b. Presentation flow - Repetition of some points may become essential. Try varying the wording for repeating some points and devise ways to repeat without being repetitive. Smooth transitions must be created between sections and allow the listeners to know that you are changing. During practice where the flow of the presentation seems to break down use a phrase or two to act as filler. Briefly sum up the point you have just made, then signal the next point by a phrase like "the next step is" or "having said that," "the second reason is" or "in other words". Such phrases notify listeners that you are switching over. It will remind them of the key point you've just made, alert them to what to listen for next, and show them the connection between what you have said and what is coming ahead.

4. Presentation

a. Pre-presentation tasks - Having prepared the script, the note cards, and the visuals and rehearsed well, it is time to deliver the goods. Even before the presentation there are a few things to be done. If possible, take a tour of the room and ensure that the needed equipment is available and properly functioning. The slides or transparencies must be in proper order. Check to see that accessories such as chalk, eraser, marker, and especially a pointer, are present. If it is a laser pointer see whether the batteries are loaded.

Oral presentation is a physical activity – eat, drink and have some warm up activity. People will be looking at you. Make sure you are properly dressed and groomed. All speakers face some kind of nervousness. Control the nervousness by ensuring that the topic is well prepared. The notes must be ready and on note or cue cards, each properly numbered. In case you are the only presenter or the first one, make sure that the visual aids are functioning.

b. Presentation posture - Stand confidently on one side of the projection screen or blackboard, and closer to the audience. Do not stand at one point but walk purposefully in the room. Avoid standing behind a lectern or desk. During presentation face the audience, and use gestures.

c. Contact with audience - A good speaker should maintain an eye contact with the audience. Ensure that they are feeling comfortable during your talk. If you find them getting bored at a place, try skipping some points without sacrificing the contents and continuity of your talk. If you are good at telling jokes, there is no harm in telling one according to the situation so as to capture the attention of the audience.

There are several other ways to focus and hold your listeners' attention. The most important one is to tell them what they will gain from listening. Convince the listeners that the topic is important and that they stand to gain from it. You must concentrate full attention to the topic. It is best to stick to the outline. Visual aids should be used long enough for the audience to understand and appreciate your point.

d. Speaking style - Speaking style is important in a good presentation. Develop your own speaking style. Speak slowly and clearly, and loud enough so that every one could hear you. The speaker must remember the "3S" formula for good speaking: "*stand up*" so that you can be seen, "*speak up*" so that you can be heard and "*shut up*" so that you can be appreciated. Pauses attract and thus refocus the attention of audience.

One task of the speaker is to maintain the interest of the audience. Changes in voice can refocus attention. Raise voice pitch for more important words and allow dropping for less important ones. Avoid speaking too quickly and pause occasionally. When you begin a new point, use a higher pitch and volume and slow down for key points. Listeners will recognize a shift and listen to discover why you've changed. Reading from the manuscript should be avoided. However, there may be certain points that require reading. This should be given a lively accent - vary the tone of your voice and vary your intonations and rate of speech.

There is no need to rush the presentation by speaking too fast. Effort should be made not to go over the time allotted for the presentation.

e. Questions and answers - Prepare the topic well and allow your friends to ask typical questions during practice sessions (Rupnow and others 2001). Audience will raise questions so encourage them. Of course, you should not allow them to control the show. Answer questions with the iceberg technique: the tip only, details don't make headlines (Kroger 1987).

Restate any question that you don't quite understand or that may have been spoken too softly for some of the audience to hear. Allow as many audience interests as possible by restating narrow questions to include other concerns and phrasing answers to convey wider impact. Express appreciation for questions, even for hostile ones: "that's a good question" or "I'm glad you asked that, because I'm sure a lot of people are wondering the same thing". This will affirm the audience member's value and encourage others to contribute. Do not show any antagonism towards a questioner. Do not fake an answer. Where possible, put the question back on the audience. Ignore questions you do not wish to answer.

5 Visual Aids

Using visual aids can make a presentation more interesting and effective. When an audience can both hear and see what you are saying, they are more likely to retain the information. Visual aids not only focus attention, they reinforce your words. In an oral presentation the speaker faces numerous people with their eyes on him or her. Hence, he/she has no time to look through the notes. Visual aids are a great assistance to the speaker as well as to the audience. These serve to allow the speaker to remember all the important points and stay on the track. Moreover, people retain visual part of the information from graphs and tables far better than listening to someone explain the results, conclusions, etc. (Huckin and Olson 1991). The visual aids must be appropriate.

The choice of visual aids may be made from: -

a. Overhead projector (OHP)
b. Slides
c. Multimedia
d. Charts
e. Blackboard
f. Whiteboard
g. Handouts
h. Samples or objects

Each type has its own strengths and weaknesses. Handouts are useful when large amounts of data are to be conveyed, while for small amounts of data, transparencies, slides, flip charts, and white board can be employed. Similarly, transparencies or slides are handy for presenting graphs, lists, sketches, key words, etc. When some permanent features are to be displayed on different occasions, slides or assistance of computer software may be more beneficial. Samples can be shown for objects.

Computer-based presentation programmes such as Power Point is a wonderful tool. These and other similar programmes are good for organizing the presentation. They can be used to create visuals such as slides and transparencies and even project them during the presentation.

The speaker must be familiar with the equipment to be used. It must be checked ahead of time to ensure that it is in working order. In most cases overhead projector (OHP) is useful.

Preparation and use of transparencies

The fundamental guideline for using a visual that an entire audience will view simultaneously is to keep it simple. If it is overcrowded, uses too many colours, or contains too much data, it is difficult for an audience to grasp the point. Use single words or short phrases and avoid whole sentences, except for crucial quotations. King and others (2001) recommend 5 – 7 words per line and 5 -7 lines per visual. However, most suitable transparencies are designated as "8 x 8". This means a total of 8 lines in a transparency and maximum of 8 words in a line.

The transparencies are best prepared in dark or black ink or in a few colours. Slides and videos may be prepared in multicolours. Simple fonts such as serif (for example Times New Roman) and sans serif (for example Arial) provide good readability (King and Rupnow 1992). A font size of 20 point in Arial Black bold or above is recommended. Capital letters may be used in headings. Best use of OHP is to display a few headings rather than the whole text. These should also be the key points that will assist the speaker to remain on the track.

Direct reading from the transparency should be avoided. The information on the transparency should be used to supplement the points. Sketches, cartoons, maps, and diagrams are often helpful to the speaker to hold interest. When using OHP, only the relevant section should be shown and the rest gradually uncovered as the talk progresses. The equipment may be switched off when not in use for long.

SOME COMMON MISTAKES

While attending technical presentations, following common mistakes have been observed that need to be looked into.

a. Some speakers have a low voice or are too nervous to speak up before the audience. The voice must be loud enough to be heard in all parts of the room. In such cases the microphone can be placed nearer to the mouth.

b. Some speakers feel shy to face the audience and stand with their back towards them. This must be avoided.

c. Inexperienced speakers keep the laser pointer switched on all the time. This moves about and

distracts the audience. When not in use, switched off the laser pointer.

d. Sometimes there is a need to refer to a publication. Quite often speakers say "Ahmed two thousand worked" (as in the written form of a paper). In such cases it would be better to say, "in the year 2000 Ahmed did this experiment".

e. Similarly, while presenting data from a chart every figure is read. The audience can do that and probably better. It is best to just highlight a few important ones.

f. While narrating the results sometimes data are presented in a list form "first this happened". "Second we come to". This should be avoided and the data presented in a sequence without any interruption.

g. Some speakers have distracting habits such as chewing gum, biting nails, leaning against something, playing with marker, and so on. These should be avoided.

h. Using such phrases as "you know", "well", "ok", too often or making sounds such as "um," "uh," or "hun" also distracts the audience.

i. Another mistake often seen while presenting a paper is the transition between sections. The speakers simply read the manuscript prepared for a written presentation and move to each section in steps. For example "now materials and methods" or "now results and discussion" and so on. There should be smooth transition between the sections without mentioning the headings. After introducing the subject and talking about the objectives, one could continue by saying "to achieve these objectives, such and such raw material was used." "Moisture was determined by using standard method". Some useful verbal phrases for smooth transition from one topic to another are:

Hence a need was felt to .....

The next point is that .....

Now I'd like to explain how the analysis for.....

Of course, we must not forget that.....

However, it's important to realize that.....

Having described the methods, I would now move to the results .....

From the results obtained, it may be concluded .....

OK, to recap the main points.....

Clearly, from what we have seen this morning .....

This data taken together demonstrates that

If we've learnt anything this afternoon, we've learnt .....

I would like to leave you with this one key point .....

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[1] This paper is reproduced, with some amendments, from the book "Scientific Presentations" by the author.