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Intermediate moisture long term storage studies on mushrooms

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ABSTRACT

In the present study Button and oyster mushrooms were stored at 8-10% salt concentration and 20-40 % moisture content. These samples were prepared by steeping cut mushrooms in 10% brine solution over night containing potassium meta bi sulfite (0.1 - 0.5%), ascorbic acid (0.1-0.2%) and citric acid (0.1 - 0. 2%) followed by draining and drying. The dried mushrooms with 20, 30 and 40% moisture content were packed in polyethylene bags to check their shelf life at room and refrigerated temperature up to 60 days. Mushrooms were subjected to physico-chemical sensory analysis. It was found that moisture, fat, protein, ash, fiber, NFE, pH, acidity, drained weight, common salt and browning were significantly effected during storage intervals at 0, 20, 40 and 60 days. There was a progressive deterioration in all sensory parameters during storage. It was concluded mushrooms can be long term preserved with acceptable color, flavor and taste.
Keywords: Mushrooms, moisture, shelf life, storage

INTRODUCTION

There is world wide shortage of food and Pakistan is no exception to it. It is estimated that more than 50% of world population is deficient in protein. Supplementing the diets with mushrooms can bridge the gap of protein shortage. They are rich source of good quality protein, minerals and vitamins (Wahid, *et. al.*, 1986). On dry weight basis mushrooms have substantial protein ranging from 19.35% as compared to rice 7.3%, Wheat 3.2%, Soybean 39% & Milk 25.2% (Change and Miles 1991). Very recently commercial cultivation of different edible mushrooms has been stressed to meet the protein deficiency not only to utilize agricultural and industrial wastes but also to reduce pollution problem. It is a saprophytic plant that can easily be grown on agricultural waste material (Zafar, 1986).

Fresh mushrooms have an active enzyme system. Damage or rough handling releases these enzymes and browning commences. A bright color is a major requirement for the successful marketing of mushrooms. In fresh mushrooms the white the better. Frozen mushrooms must also be white. The requirement in the canned product is creamy white appearance. In freezing, the formation of ice disrupts the tissues and releases the enzymes. Despite the fact that the mushrooms are held at temperature below -20^0C, browning will slowly develop in the frozen product.

Today, preservation has utilized such fact as temperature, water activity, pH, gases, organic acid, salt, antibiotics, irradiation, packaging and various combinations of these factors (Wagner and Moberg 1989). There has been a recent upsurge of interest in mushroom as a source of biologically active camp of medicinal value including anti cancer, antiviral, immunopotentiating, hypocholestrolamic and hepto-protective agents. These new classes of camp termed as mushroom nutriceuticals are extracted from fungal mycelium or fruiting bodies and represent an imported component of the expending mushroom biotechnology industry (Change and Bus well 1996).

As regards composition of mushroom on average fresh basis, it contains typically 90% water, 4% Carbohydrates, 3.5 % Protein, 0.4% Fat, 1.2% Ash and 0.9% Crude fiber (Zafar, 1986), whereas fresh and air-dried mushrooms contain 90% moisture and 10-12% dry matter contents.

Besides Ca, P, Fe, Na, K, Co, Mg, Zn, Cd, Pb, Mn, mushrooms also contain vitamin B (Thiamine), B2 (Riboflavin), B3 (Pantothenic Acid), Nicotinic acid (Niacin), Vitamin C (ascorbic acid), E and K. While other vitamins such as A and D are present relatively in lesser amounts. They are the only kind of vegetables containing vitamin D2 precursor. Amount of minerals and vitamins are present in very less (1.0%) amount (Tewary, 1986).

MATERIALS AND METHODS

Fresh mushrooms were obtained from a local farm of Sheikhupura and Faisalabad and brought to place of research in Institute of Food Science and Technology, University of Agriculture, Faisalabad. Then the whole lot was divided into samples of equal weight.

Button and oyster mushrooms were washed with potable tap water to remove the bedding material and contaminants.

Both mushrooms were washed with washing solution containing 0.1 % citric acid and 0.3% potassium meta bisulphite to prevent browning.

Studies on preparation and storage of cultivated button (*Agaricus bisporus)* and oyster mushroom *(Pleorotus ostreatus*) at intermediate moisture content at different temperatures conducted. Study time lasted for 60 days.

The mushroom quarters were blanched for 1.5 and 2.5 minutes separately in boiling water. After cooling to room temperature they were dipped over night in 10% brine solution containing potassium meta bisulfite 0.1% along with 0.2% citric acid and 0.1% ascorbic acid. Mushrooms were dried in a cross flow drier at an initial temperature 80°C for one hour and subsequently at 25, 40 and 55°C to final moisture content 20 and 40%. They were packed in low-density polyethylene (LDPE) pouches of 300 mm gauge thickness with an outer cardboard container and were stored at room temperature at 37°C. The relative humidity was 42 - 74% during storage period. Each polyethylene pouch was contained about 100 g of intermediate moisture mushroom according to Bhatia *et al.*, 1982.

Table 1. Different chemical treatments used in the study.

Chemicals	Minimum %	Maximum %
Salt	8.0	10
Potassium meta bisulfite	0.1	0.2
Citric Acid	0.1	0.2
Ascorbic Acid	0.1	0.2
Sodium benzoate	0.1	0.2

All possible combinations of the above chemical doses were replicated three times made to find the best combination suitable for preservation of mushrooms with the storage intervals of 20 days while study period extended to 60 days.

The samples of mushrooms were analyzed for moisture, crude protein, crude fat, crude fiber, ash and NFE by the methods as given in AOAC (2000).

Acidity of brine was determined as described by Ruck (1969).

The pH of brine was estimated with the help of pH meter (HANNA Model No. B417). Common salt in the steeping solution was detected by salometer/brix hydrometer as described by Howard and Leonard (1982).

Drained weight of mushroom during steeping was determined by draining the mushroom over a muslin cloth.

The absorbance of brine filtrate (Fand *et al.*, 1974) was measured at 560-580 nm with the help of spectronic 20D and interpreted as browning index for mushroom (Mudahar and Bains, 1982).

To judge the quality of mushrooms during storage period, soups will be prepared from mushroom and cooking quality of mushroom will be evaluated for color, flavor, taste and overall acceptability at suitable intervals up to 60 days of storage as described by Land and Shepherd (1988) and marks were given on the basis of Hedonic Scale ranking method as given.

Table 2. Physico-chemical Analysis of processed Mushrooms

	Varieties of Mushrooms							
	Agaricus bisporus				*Pleurotus ostreatus*			
Storage days	0	20	40	60	0	20	40	60
Moisture %	30.510	28.410	25.247	24.617	25.413	23.7897	22.213	21.177
Protein %	22.740	20.503	18.580	18.950	20.547	17.950	16.520	17.840
Fat %	2.503	2.123	2.077	1.957	2.930	2.370	2.127	2.027
Ash %	10.050	9.050	8.200	8.033	11.167	10.683	9.733	8.433
Fiber %	23.497	23.323	23.807	23.727	25.237	24.793	23.920	23.537
NFE %	10.700	16.590	21.423	23.317	14.707	20.417	25.397	26.987
Acidity %	10.213	7.667	7.907	8.493	10.210	7.790	8.107	8.427
pH	3.397	3.180	3.147	3.107	3.413	3.333	3.307	3.247
Drain Weight	100.000	81.433	76.317	69.130	100.000	83.903	79.407	72.413
Common salt	80.157	70.763	69.403	69.350	90.347	79.207	75.170	74.217
Absorbance of salt or Browning	0.213	0.167	0.127	0.093	0.207	0.187	0.143	0.113

Data was analyzed using Analysis Of Variance Techniques as given by Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

The results of phsico-chemical analysis are shown in Table 2. Moisture, fat, protein, ash, fiber, NFE, pH, acidity, drain weight, common salt and browning effected significantly during storage of 0,20,40 and 60 days. There was a progressive deterioration in all sensory parameters during storage, which is also given in Table 3. The results of study are supported by the findings of Hafiz Abdul Majid (1993).

The color, flavor, taste and overall acceptability of processed mushroom at intermediate moisture content was estimated by making chicken mushroom soup under different treatments on scoring system as described by Larmond (1977) which are given in Table 3. The evaluations were done after 0, 2, 4, 6, days of storage by a Panel of 3 judges.

Table 3. Sensory evaluation of processed Mushrooms

Days	0	2	4	6
Color	7.50	6.50	5.50	4.50
Flavor	8.00	7.00	6.00	4.83
Taste	7.16	6.50	5.50	4.16
Overall acceptability	7.50	6.50	5.50	4.50

CONCLUSION

The present studies were under taken to investigate Intermediate and Long term moisture storage of two mushrooms varieties i.e. *Agaricus bisporus* and *Pleurotus ostreatus* to prolong their shelf life. Keeping in view the literature cited and experiments conducted it can safely be said that mushrooms, preserved at intermediate moisture contents with the help of above stated chemicals were safer for use even after 60 days of storage period having very similar characters of quality and freshness. Regarding color and texture visual observations showed that there were no much changes in preserved mushrooms at intermediate moisture contents that they were rejected. Sensory evaluation regarding texture, changed little bit and toughness was lost as a result of shriveling.

LITERATURE CITED

AOAC 2000. Official Methods of Analysis. Association of Official Analytical Chemists, 15 th Ed. Arlington.

Bhatia BS and Mudahar GS. 1982. Preparation and storage studies on some intermediate moisture vegetables (mushroom, cauliflower and carrots) J Food Sci Technol. Vol. 19:80-82.

Chang ST and Buswell JA. 1996. Mushroom nutriceuticals, world J. Microbiol and Biotechnol. 12(5):437-76.

Chang ST and Miles PG. 1991. Recent trends in world production of cultivated mushrooms. Mushroom Journal 504:15-18.

Fang TT Shau-MO and Chang H. 1974. Effect of Chemical Treatments before or after Blanching and Freezing methods on the Quality of Frozen Mushrooms. Mushroom Sci 9(1): 319-331.

Land DG and Shepherd R. 1988. Scaling and ranking methods. In: Sensory Analysis of Foods. JR. Piggot (Ed.). Elsevier applied Sci New York. P:155-185.

Larmond E. 1977. Laboratory methods for sensory evolution of food. Canadian dept Agri Pub 1637.

Mudahar GS and Bains GS. 1982. Pretreatment effect on quality of dehydrated (*agaricus bisporus*) mushroom. Indian Food Pecker, 36(2):19-21. [CF FSTA 15(11): J 1764, 1982].

Ruck JA. 1969. Chemical methods for analysis of fruits and vegetable products. Res Br Canadian Dept of Agri. Summerland B. C.

Steel RGD Dickey D and Torrie JH. 1996. Principles and procedure of statistics. A Biometrical Approach. 3rd ed McGraw Hill Book Co Inc New York.

Tewary RR. 1986. Mushroom cultivation. Extension Bulletin, Indian Inst. of Hort Res India.

Wahid M Sattar A and Khan S. 1988. Composition of wild and cultural mushrooms of pakistan. Mushrooms J Tropics 8:47-51.

Wanger MK and Moberg IJ. 1989. Present and future use of traditional antimicrobials 1: 143-147.

Zafar SI. 1986. Pakistan suited to mushroom cultivation. The Pakistan Times August 22.

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Preparation and evaluation of apple stirred Yogurt

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ABSTRACT

Apple stirred Yogurt was prepared by using 10, 12, 14, 16 and 18% apple puree and analyzed for physiochemical and sensory characteristics at 0, 5, 10 and 15 days of storage. The statistical analysis indicated highly significant effect of treatments and storage period on physical, chemical and sensory characteristics of apple stirred Yogurt. The addition of apple puree to stirred Yogurt contributed to increased acidity, total solids, SNF, synersis as well as sensory characteristics. However, a decrease in pH and fat contents were observed with the addition of apple puree. Acidity and synersis increased gradually during storage in all the treatments while pH, total solids, solids-not-fat and fat contents decreased during storage. There was a progressive deterioration in flavor, body and texture, sensory acidity and appearance of apple stirred Yogurt during 15 days of storage. The quality of apple stirred Yogurt remained acceptable up to 10 days and it was observed that addition of 16% apple puree was the best proportion in stirred Yogurt on the basis of acceptability when stored at 6°C.

Keywords: Yogurt, sensory quality, chemical analysis

INTRODUCTION

Yogurt is sour milk product prepared form milk by using selected micro-organism to develop not only characteristics flavor but also body and texture.

The most common inoculation material used by modern dairy plants is *S. thermophillus* and *L. bulgaricus* in the ratio of (1:1) using @ 2-2.5 % in fresh pasteurized milk and incubated at 42°C to desired acidity. *S. thermophillus* produce a weak acid (lactic acid) that make favorable condition for *L. bulgaricus* which in turn hydrolyze lactose & casein with the production of typical Yogurt flavor (Macrae *et al.* 1993).

Yogurt is more nutritive as compared to milk due to its vitamin contents, digestibility and source of calcium & phosphorus. Moreover, lactic acid micro-flora inhibits the growth of cancer cells (Foissy 1983) and has significant role in reducing blood cholesterol level and hence prevents coronary diseases (Foissy 1983; Gonc *et al.* 1996) and improve bone formation (Kaup 1988). Deeth and Tamime (1980) found that 100g of Yogurt contain 72 calories, 3.9g protein, 3.4g fat, 4.9g carbohydrate, 145mg calcium, 144mg phosphorus, and 47mg sodium and 186mg potassium.

Set Yogurt is packed immediately after inoculation with starter and is incubated in the packages. Stirred Yogurt is inoculated with starter and incubated in tanks. After this product is cooled and stirred before packaging. Fruit stirred Yogurt has definite advantage of sweetness, flavor and fruit contents (Hursit and Temiz, 1999). Fruit stirred Yogurt is more familiar due to its nutritive, digestibility, therapeutic properties against immune related diseases as compared to milk from which it is prepared (Meydani and Woel, 2000).

Numerous fruit Yogurts i.e. mahgo, falsa, strawberry and banana have been prepared but apple stirred Yogurt is more distinct of them because of its additional nutritional and medical benefits than other fruit Yogurts. Apple (*Pyrus malus*) belongs to family *rosaceae*, fruit of temperate climates. Its balanced nutrition is evident from this proverb "an apple in a day keeps the doctor away". According to Macrae *et al.* (1993) apple is a rich source of ascorbic acid (vitamin C), iron and also contains other vitamin in minute quantities such as vitamin B-complex (nicotinic acid, riboflavin, thiamin etc.).

Apple has also medicinal properties like, cholesterol reducing effect. In Europe it is used to treat infant intestinal disorders such as diarrhea and dysentery. Apple is said to be the king of fruits in temperate region because of its universal cultivation and worldwide consumption. 100g of apple provide 60 calories, a good source of vitamins and minerals i.e. potassium and iron (Mahmood *et al.* 1994).

Today people are more conscious about their health and nutrition. They have great expectation from food products they eat. Foods that are convenient with good taste, available at reasonable prices and possessing favorable nutrition are in great demand. Thus keeping in view the importance of apple and Yogurt the project was planned to determine the best proportion of apple puree in stirred Yogurt and to evaluate the quality of apple stirred Yogurt.

MATERIALS AND METHODS

Fresh milk and ripened golden apple were purchased from local market. Apples were washed, peeled, cut into small pieces, blanched, pasteurized with adding little water in open pan at 85^{0}C for 5 minutes and passed through muslin cloth to remove fibrous material. Several trials were conducted by using sugar, starter and pectin at different levels and following formulation/treatments were selected for final product (Table 1).

Table 1. Detail of treatments.

Treatments	Yogurt (%)	Sugar (%)	Apple puree (%)
T1	100	-	-
T2	85	5	10
T3	83	5	12
T4	81	5	14
T5	79	5	16
T6	77	5	18

Milk was standardized to 3.5 % fat, 11 % SNF and homogenized to improve texture and quality. Pectin (0.12%) was added and milk was pasteurized in water bath at 75^{0}C for 30 minutes. Milk was cooled to 42^{0}C, inoculated with starter culture (Nestle Yogurt pH 4.5) used @ 2.0 % level. Apple green color 0.001 % and flavor 4 ml/liter of Yogurt was added. The mix of each batch was continuously stirred for 10 minutes for thorough mixing of culture, incubated at 42^{0}C for 3-4 hours till pH reached at 4.6 and then cooled to 6^{0}C to prevent fermentation and reduction in pH.

Mixture of apple puree with sugar was added after incubation and thoroughly mixed to contain homogeneous mixture by stirring at 4-6^{0}C. Then the Yogurt was filled in polystyrene cups and stored at 4-6 ^{0}C. The product was analyzed for physiochemical and organoleptic characteristics at 0, 5, 10 and 15 days of storage.

Direct titration method (AOAC 1990) for acidity and Gerber method (David 1976) for fat determination were used. Digital pH meter Hanna 8416 was used to determine pH. SNF % was calculated according to Tamime and Robinson (1985) and Total solids were determined according to (AOAC 1990). Sensory evaluation was carried out according to Nelson and Trout (1964). Statistical analysis was done according to Steel *et al.* 1997).

RESULTS AND DISCUSSION

Statistical analysis showed highly significantly effect of treatments and storage days on acidity, pH, total solids, SNF, fat and synersis of apple stirred Yogurt. The detailed results are discussed below.

CHEMICAL ANALYSIS

Acidity: The acidity of apple stirred Yogurt increased with the addition of apple puree. The acidity further increased gradually during storage in all treatments. However the difference in treatments having 12 to 18 % apple pulp was non significant (Table 2). Yogurt is acidic dairy product with a natural keeping quality. The quality deteriorates quickly as the acidity increases with the passage of time and the Yogurt becomes bitter. Salji and Ismail (1983) also reported significant increase in acidity during Yogurt storage. The increase in acidity is due to conversion of lactose to lactic acid by lactic acid bacteria (Puhan *et al.* 1974).

pH: The addition of apple puree resulted in mild decrease in pH of apple stirred Yogurt. The pH decreased gradually in all treatments through out storage period of 15 days (Table 2). However, the differences between treatments containing 14 to 18% apple puree were non significant. The reason for decrease in pH was an increase in acidity due to conversion of lactose to lactic acid during storage period. Moon *et al.* (1993) also observed decrease in pH during storage of gel type Yogurt.

Total solids: The total solids of stirred Yogurt increased with the addition of apple puree. While during storage the total solids of Yogurt decreased in all treatments. T6 showed highest total solids followed by T5, T4, T3, T2 and T1 (Table 2). The present results also confirm the findings of Vernam and Sutherland (1994).

Solids-not-fat: The addition of apple puree increased SNF of apple stirred Yogurt. The SNF of apple stirred Yogurt samples irrespective of any treatment substantially decreased during storage (Table 3). The decrease in Yogurt SNF may be due to the reduction in the total solids as well as reduction of fat during refrigerated storage. These results are in accordance with the findings of Tamime and Robinson (1985).

Table 2. Effect of storage on acidity, pH and total solids of stirred apple yogurt.

	Acidity (%)					pH					Total solids				
	Storage days					Storage days					Storage days				
	0	5	10	15	Mean	0	5	10	15	Mean	0	5	10	15	Mean
T1	0.60	0.70	0.80	0.86	0.74 c	4.60	4.55	4.50	4.40	4.51a	14.50	14.46	14.40	14.30	14.41f
T2	1.00	1.05	1.10	1.15	1.07 b	4.40	4.35	4.20	4.06	4.25b	20.10	20.08	20.02	19.98	20.04e
T3	1.12	1.15	1.20	1.25	1.18 a	4.35	4.20	4.00	3.90	4.11c	22.30	22.25	22.20	22.12	22.22d
T4	1.15	1.17	1.20	1.22	1.18 a	4.30	4.15	3.95	3.85	4.06cd	26.20	26.15	26.10	26.03	26.12c
T5	1.18	1.20	1.22	1.25	1.21 a	4.28	4.10	4.00	3.90	4.07d	27.20	27.16	27.11	27.06	27.13b
T6	1.22	1.23	1.24	1.26	1.24 a	4.29	4.00	3.85	3.75	3.97d	28.70	28.64	28.58	28.51	28.61a
Mean	1.04 c	1.08 b	1.13ab	1.16 a		4.37a	4.22b	4.08c	4.00d		23.17a	23.12b	23.07c	23.00d	

Table 3. Effect of storage on SNF, fat and synersis of stirred apple yogurt.

	SNF (%)					Fat					Synersis				
	Storage days					Storage days					Storage days				
	0	5	10	15	Mean	0	5	10	15	Mean	0	5	10	15	Mean
T1	10.90	10.86	10.82	10.79	10.84f	3.60	3.60	3.58	3.51	3.57a	1.25	1.2	2.9	4.9	2.56de
T2	16.60	16.58	16.53	16.50	16.55e	3.50	3.50	3.49	3.48	3.49b	1.0	1.5	2.5	4.3	2.32e
T3	18.90	18.85	18.81	18.74	18.82d	3.40	3.40	3.39	3.38	3.39c	1.2	2.7	3.9	6.4	3.55cd
T4	22.90	22.86	22.81	22.75	22.83c	3.30	3.29	3.29	3.28	3.29e	1.4	2.9	4.1	7.5	3.97bc
T5	23.80	23.77	23.77	23.75	23.77b	3.40	3.39	3.34	3.31	3.36d	1.6	3.1	6.0	8.1	4.70ab
T6	25.60	25.54	25.49	25.43	25.51a	3.10	3.10	3.09	3.08	3.09f	2.2	3.7	6.4	9.1	5.35a
Mean	19.78a	19.74b	19.70c	19.66d		3.38a	3.38a	3.36a	3.34b		1.44d	2.52c	4.3b	6.72a	

Fat. The addition of apple puree caused a decrease in fat contents of apple stirred Yogurt. The fat contents decreased slightly during storage. However, the differences in fat contents of stirred apple Yogurt at 0, 5 and 10 days were non-significant (Table 3). The reduction in fat under the influence of storage appeared due to lipolytic activity of micro flora or due to acidic pH during storage. However, no rancidity was observed because of low storage temperature.

PHYSICAL ANALYSIS

Synersis: The addition of apple puree increased the synersis of apple stirred Yogurt which was further increased during storage (Table 3). The addition of sugar showed a decrease in synersis of Yogurt samples due to decrease in acidity. While during storage the synersis increased due to increase in acidity during storage. Balsubramanyan and Kulkarni (1991) also observed increase in synersis of Yogurt during storage. The increase in synersis might be due to rearrangement of protein network (Walstra *et al.* 1985) resulting from changes in pH, acidity and temperature during storage of Yogurt.

SENSORY EVALUATION

The statistical results indicated highly significant effect of treatments and storage period on sensory characteristics of apple stirred Yogurt. The addition of apple puree resulted in an increase in flavor, body and texture, sensory acidity and appearance. All these characteristics decreased gradually during storage (Table 4). The flavor scores of Yogurt samples containing sugar and apple puree remained acceptable up to 10 days storage period. T5 (5% sugar and 16 % apple puree) got maximum scores for flavor, body & texture, sensory acidity and appearance during storage periods of 15 days. However, T5 showed non-significant differences with T4 (5% sugar and 14% apple puree) and T6 (5% sugar and 18% apple puree) for flavor (Table 5). In case of appearance T6 got maximum score followed by T5, T4, T3 and T2. However, the differences between latter three treatments were non-significant (Table 5). Con *et al.* (1996) suggested that fruit Yogurt showed best storage for up to 7 days only without loss of desired flavor. Similarly, Giangiacomo *et al.* (1994) stated that addition of fruit cubes improved flavor scored of the fruit Yogurt compared to that of control.

Table 4. Comparison of means for sensory characteristics as influenced by storage days.

Days	Flavor (45)	Body & texture (30)	Sensory acidity (10)	Appearance (15)
0	40.00 a	24.33 a	7.08 a	12.42 a
5	37.00 b	21.83 b	6.50 b	11.83 b
10	33.67 c	19.83 c	5.83 c	11.00 c
15	28.67 d	16.83 d	4.92 d	9.17 d

Sugar concentration influences the flavor and overall rheological acceptability (Richter *et al.* 1979). Ming *et al.* (2000) also observed that quality parameters of the Yogurt were influenced by combination of ingredients. The decrease in flavor during storage may be due to proteolytic activity of bacteria and the production of higher acidity (Abrahamsen 1978) fat and protein degradation (Mottar *et al.* 1979).

Body and texture is influenced by many factors including acidity and total solids. Hardi and Slacanac (2000) found that texture depends upon many factors including starter culture, milk composition, milk viscosity, heat treatment, fermentation kinetics and homogenization.

RECOMMENDATIONS FOR FUTURE RESEARCH

Apple stirred Yogurt has more nutritional and therapeutic value than plain set Yogurt, having essential vitamins and minerals and it is nutritionally beneficial for growing children because of its good taste and flavor than plain Yogurt. Thus keeping in view the acceptability of apple stirred Yogurt more research work should be conducted with regard to its storage life.

Table 5. Comparison of means for sensory characteristics as influenced by treatments.

Treatments	Flavor (45)	Body & texture (30)	Sensory acidity (10)	Appearance (15)
T1	30.25 c	16.25 d	4.87 e	9.87 d
T2	30.75 c	21.25 b	5.37d	10.87 bc
T3	35.25 b	19.25 c	5.87 c	10.75 bc
T4	37.25 ab	21.75 b	6.62 b	11.37 b
T5	39.50 a	24.75 a	7.62 a	13.12 b
T6	36.00 ab	21.00 b	6.12 c	10.62 a

REFERENCES

Abrahamsen RK. 1978. The content of lactic acid acetaldehyde in Yogurt at different temperatures. International Dairy J 829-830. (FSTA 10(3): 11 P 2140; 1978).

AOAC. 1990. Official Methods of Analysis. The Association of Official Analytical Chemists, 16th ed. Arlington, Virginia.

Balasubranmanyam BV and Kulkarini S. 1991. Standardization and manufacture of high fat Yogurt with natural fruit pulp. J Food Sci Tech 28(6):389-90.

Con AH Caglar CA and Gokalp HY. 1996. Effects of different fruits and storage period on microbiological qualities of fruit flavored Yogurt produced in Turkey. J Food Protection. 59(4):402-406. (FSTA 28(10): 10 P155, 1996).

David P. 1976. The Chemical Analysis of Foods. 7th. Ed. Churchill Livingstone, London.

Deeth HC and Tamime AY. 1980. Yogurt Nutritive and Therapeutic Aspects. J Food Protection. 44(1):75-86. (FSTA 13(8): P1482, 1981).

Foissy H. 1883. Nutritional and Physiological aspects of Yogurt. Milchwirts Chaftliche Berichte aus den Bunde sanstalten wolfpassing und hotholz 75:137-142. (FSTA 16 (2): P404, 1994).

Giangiacomo RD. Torreggiani ML, Erba and Messina G. 1994. Use of osmodehydrofrozen fruit cubes in Yogurt. Italian J Food Sci 6(3):345-350. (FSTA 27(1): 1 P99, 1995).

Gonc S, AS Akalin and Kilic S. 1996. A review on the relationship between fermented dairy products and cholesterol. Gida. 21(2): 89-84 (FSTA 28(10): 10 P157, 1996).

Hardi J and Slacanac V. 2000. Examination of coagulation kinetics and rheological properties of fermented milk products; influence of starch culture, milk fat content, addition of insulin. Mljekarstvo. 50(3):217-226. (FSTA 33(2): P1364, 2001).

Hursit K and Temiz H. 1999. Composition of manufacturing methods of fruit flavored Yogurt. Ondokuzmayis-universitiesi, Ziraat Fakultesi Dergisi. 14(3):151-165.

Kaup SM. 1988. Bioavailability of calcium in Yogurt and its relationship to the hypcholesterolemic properties of Yogurt. Dissertation Abstracts International. Bulletin. No. 48(7): 1859 (FSTA 20(7): 7-73, 1988).

Macrae R, Robinson RK and Salder MJ. 1993. Encyclopedia of Food Science, Food Tech & Nutrition. Academic Press, New York.

Mahmood NM, Elina B and Robynn B. 1994. Horticulture. National Book Foundation, Islamabad, Pakistan.

Meydani SN and Woel HK. 2000. Immunologic effects of Yogurt. American J of Clin Nutr 71(4): 861-872. (FSTA 32(8): P11345. 2000).

Ming JU, Chen, Harper WJ and Lin Chin-wen. 2000. Study on the manufacture of tomato flavored Yogurt. Taiwanese J Agri Chem and Food Sci 38(2):133-140. (FSTA 33(1): P128, 2001).

Moon-Sukkim, Eun-Suk Ahn and Shin-Dong. 1993. Physicochemical properties of commercial Yogurt in Korea. Korean J. Food Sci. 25(4):340-344.

Mottar J, Waes G, Moermans R and Navdts M. 1979. Sensoric changes in UHT milk during uncooled storage: Milchwissen Schaft. 34(5):257-262. (FSTA 12 (2): P305, 1980).

Nelson JA and Trout GM. 1964. Judging Dairy Products. The Olsen Pub Co Milwaukee, Washington DC.

Puhan Z, Flueler O and M Banhegyi. 1974. Composition of Ikactic and bacterial flora and lactic acid configuration commercial Swiss Yogurt. International Dairy Congress. IE, 451-452 (FSTA 7(5): 895; 1975).

Ritchter RL, Watts CW, Gehrig TC, Cheshier K and Diu CW. 1979. The relationship of milk fat, milksolid not fat, and sugar to consumer acceptance of plain Yogurt. J. Dairy Sci. 62(1):205-206.

Salji TP and Ismail AA. 1983. Effect of initial acidity of plain Yogurt on acidity changes during refrigerated storage. J. Food Sci. 48(1):258-259. (FSTA 15(7): P997, 1984).

Steel RGD, Dickey D and Torrie JH. 1996. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Ed. McGraw Hill Book Co. Inc. New York.

Tamime AY and Robinson RK. 1885. Yogurt Science and Technology, Pergamon Press, Oxford.

Vernam AH and Sutherland JP. 1996. Milk Products-1 Chapman and Hall, New York.

Walstra P, Dijk HJ and Geurts TJ. 1985. The synersis of curd. General consideration and literature review. Milk and Dairy J. 39(4):209-246. (FSTA 18(9): 6 P79, 1986).

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Development, standardization and storage studies on grape fruit apple marmalade

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ABSTRACT

Grapefruit-apple marmalade with different proportions of grapefruit apple pulp (40:60, 45:55, 50:50, 55:45, 60:40) was prepared, packed in one pound glass jars and stored at ambient temperature. Marmalade samples were analyzed for total soluble solids, acidity, pH value, reducing & non reducing sugar and sensory attributes. There was an increase in the TSS, acidity and reducing sugars. Decreasing trend was recorded in pH value and non reducing sugars. Sensory evaluation scores remained in the acceptable range in all the samples but 50:50 grapefruit apple ranked highest. Slight bitter taste was observed in samples with high grapefruit juice.

Keywords: Marmalade, apple, grapefruit, chemical analysis

INTRODUCTION

In Pakistan the production of citrus fruit was 1897.7 thousand tonnes during the year 2000-2001(GOP 2002). Its consumption on commercial scale is limited due to bitterness and high acid content. No single product of grapefruit is available in the market. Most industries are crushing this fruit with the purpose of blending it with orange or lemon juice. Grape fruit juice consumption could be enhanced by utilizing it with juice of other fruits such as mango, apple, etc. to overcome its bitter acidic taste. This study therefore, was planned to develop and standardize grapefruit apple marmalade.

MATERIALS AND METHODS

Grapefruit and apple fruit were procured from the local market. After washing, juice was extracted using mechanical juice extractor. Marmalade was prepared using different proportions of grapefruit and apple juice (Table.1) by washing the fruit first followed by different preparatory operations.

The apples were sliced where as the juice sacs of grape fruit were mechanically pressed, thus releasing the juice. Juice was filtered and used in preparation of jam according to the treatment combinations.

Shreds of grapefruit were also added after removing the bitterness by boiling in water. Marmalade so prepared was packed in clean, dry glass jars and stored at ambient temperature to study the storage behavior.

TREATMENTS

Treatment	Grapefruit juice (%)	Apple juice(%).
T_1	40	60
T_2	45	55
T_3	50	50
T_4	55	45
T_5	60	40

PHYSICO CHEMICAL ANALYSIS

Physico chemical analysis for T.S.S., pH, acidity and reducing and non-reducing sugars were carried out at an interval of 15 days.

Total soluble solids

Total soluble solids of marmalade were determined by digital refractometer corrected at 20°C by the method given in AOAC (1999).

Acidity

Acidity of the marmalade sample was determined by titration method as given in AOAC(1999).

pH value.

pH value was measured by digital pH meter standardized with buffer solution.

Reducing and non reducing sugar

Reducing and non reducing sugars were determined by using Lane & Eynon method (Ruck 1963).

Sensory evaluation

Sensory evaluation of marmalade samples was carried out for color, flavor, taste and texture by using hedonic scale rating method by a panel of trained judges.

STATISTICAL ANALYSIS

The data obtained was statistically analyzed according to the methods described by Steel *et al* 1996.

RESULTS AND DISCUSSION

Total Soluble Solids (TSS)

Table-1 presents the effect of storage on the TSS of marmalade. The data show that there was a slight increase in TSS in all the samples during storage which may be due to acid hydrolysis of polysaccharides especially pectin and gums Luh and Woodroof (1975) also observed similar results. The results are also in line with the findings of Bindra et al (1974). The statistical analysis showed that results are non- significant for treatments, storage and their interactions.

pH value

Table-3 shows the effect of storage on the pH value of grapefruit; apple marmalade. Data revealed that there is decreasing trend in pH value of all the treatments during storage. Maximum decrease in pH value (3.19) was observed in T_3(50:50). There was a proportional decrease in pH value with the increased acidity during storage. Statistical analysis of data showed that results are significant for treatments and storage.

Reducing Sugar

Table-4 presents the effect of storage on reducing sugar of marmalade samples. The maximum increase was found in T_5 i.e. 31:36 followed by T_1 & T_4. The results agree with the findings of (El-Ashwash, et al, 1982); Sandhu et al, 1985, Javed, 1988 and Lodhi, 1989. Statistical analysis of the data showed that the results are highly significant for storage and treatments.

Table-1. Effect of storage on the total soluble solid(tss) of grapefruit-apple marmalade.

	STORAGE PERIOD IN DAYS					
Treatment	**0**	**15**	**30**	**45**	**60**	**Means**
T_1	70.0	70.2	70.3	70.5	70.5	70.30
T_2	70.0	70.1	70.3	70.5	70.7	70.32
T_3	70.1	70.2	70.4	70.6	70.7	70.40
T_4	70.0	70.2	70.4	70.6	70.7	70.38
T_5	70.1	70.3	70.5	70.6	70.8	70.46
Means	70.04	70.20	70.38	70.56	70.68	

Acidity

The effect of storage on the % acidity of marmalade is presented in Table-2 the date revealed that slight increase in titerable acidity of marmalade occurred. Maximum increase i.e. 0.87% was observed in T_5 containing in grapefruit: apple (60:40) and least increase in acidity 0.85% in T_1 containing 40:60 grapefruit:appe. The rise in acidity may be explained by the fact that the concentration of weekly-ionized acid and their salts increased during storage, resulting in increased acidity. The increase in acidity might also be due to formation of acid by degradation polysaccharads and oxidation of reducing sugars or by breakdown of pectic substances. Similar views were expressed by Hummel and Okey (1950) and Ahmad (1997). Statistical analysis of data for % acidity showed that treatments, storage and their interaction explicit highly significant effect on acidity during storage.

Non Reducing Sugars

The effect of storage on the non reducing sugars of grapefruit:apple marmalade is presented in Table-5. The data shows decreasing trend in non- reducing sugars in all the samples during storage. However maximum decrease was found in T_3 (50:50) followed by T_2, T_4 and T_5. Minimum decrease was recorded in T_1. The results are in accordance with the findings of Javed (1988) and Lodhi (1989). Statistical analysis of the data showed that treatments had non-significant effect on reducing sugars. However storage period had highly significant effect on reducing sugars.

Sensory evaluation

The sensory evaluation data presented in Table-6 indicated that all marmalade samples remained in the acceptable range.

Table-2. Effect of storage on the acidity(%) of grapefruit-apple marmalade.

STORAGE PERIOD IN DAYS

Treatment	0	15	30	45	60	Means
T_1	0.79	0.81	0.82	-0.84	0.85	0.822
T_2	0.78	0.80	0.81	0.83	0.85	0.814
T_3	0.79	0.81	0.82	0.85	0.87	0.828
T_4	0.80	0.82	0.83	0.85	0.86	0.832
T_5	0.78	0.80	0.82	0.85	0.87	0.824
Means	0.788	0.808	0.820	0.844	0.860	

Table-3. Effect of storage on the pH value of grapefruit-apple marmalade.

STORAGE PERIOD IN DAYS

Treatment	0	15	30	45	60	Means
T_1	3.25	3.23	3.22	3.20	3.19	3.218
T_2	3.24	3.22	3.20	3.18	3.17	3.202
T_3	3.23	3.20	3.18	3.15	3.13	3.178
T_4	3.25	3.23	3.22	3.20	3.18	3.216
T_5	3.26	3.24	3.22	3.19	3.17	3.216
Means	3.246	3.224	3.208	3.184	3.168	

Table-4. Effect of storage on the reducing sugars of grapefruit-apple marmalade.

STORAGE PERIOD IN DAYS

Treatment	0	15	30	45	60	Means
T_1	16.55	18.20	20.15	27.10	31.35	22.670
T_2	16.60	18.05	21.88	26.95	31.15	22.926
T_3	16.65	18.22	21.95	27.15	31.26	23.046
T_4	16.70	18.27	22.10	27.35	31.30	23.144
T_5	16.70	18.30	22.20	27.15	31.36	23.142
Means	16.640	18.188	21.656	27.140	31.284	

Table-5. Effect of storage on the non-reducing sugars of grapefruit-apple marmalade.

STORAGE PERIOD IN DAYS

Treatment	0	15	30	45	60	Means
T_1	49.50	47.95	45.90	38.95	34.60	43.38
T_2	49.45	48.10	45.10	39.25	34.84	43.348
T_3	49.41	47.90	44.98	38.90	34.85	43.208
T_4	49.35	47.85	44.65	38.80	34.76	43.082
T_5	49.34	47.80	44.60	38.92	34.70	43.072
Means	49.41	47.92	45.05	38.96	34.75	

Table-6. Effect of storage on the organoleptic characteristiccs of grapefruit-apple marmalade.

STORAGE PERIOD IN DAYS

COLOUR

Treatment	0	15	30	45	60	Means
T_1	8.8	8.6	8.4	8.2	8.0	8.40
T_2	9.0	8.8	8.6	8.4	8.0	8.56
T_3	9.0	8.8	8.6	8.4	8.2	8.60
T_4	8.8	8.8	8.6	8.2	8.0	8.48
T_5	8.6	8.4	8.2	8.0	7.8	8.20
Means	8.84	8.68	8.48	8.24	8.00	

FLAVOUR

Treatment	0	15	30	45	60	Means
T_1	8.8	8.6	8.2	8.0	7.8	8.28
T_2	8.8	8.6	8.4	8.2	8.0	8.40
T_3	9.0	8.8	8.6	8.4	8.2	8.60
T_4	8.8	8.4	8.2	8.0	7.8	8.24
T_5	8.6	8.2	8.0	7.8	7.6	8.23
Means	8.8	8.52	8.28	8.08	7.88	

TASTE

Treatment	0	15	30	45	60	Means
T_1	8.6	8.4	8.2	8.0	7.8	8.20
T_2	8.8	8.6	8.4	8.2	8.0	8.40
T_3	9.0	8.8	8.6	8.4	8.2	8.60
T_4	8.8	8.4	8.2	8.0	7.8	8.24
T_5	8.6	8.4	8.0	7.8	7.6	8.08
Means	8.76	8.52	8.28	8.08	7.88	

TEXTURE

Treatment	0	15	30	45	60	Means
T_1	8.8	8.6	8.4	8.0	7.8	8.32
T_2	8.8	8.6	8.4	8.2	8.0	8.40
T_3	9.0	8.8	8.6	8.4	8.2	8.60
T_4	8.6	8.4	8.2	8.0	7.8	8.20
T_5	8.6	8.2	8.0	7.6	7.4	7.96
Means	8.76	8.75	8.32	8.04	7.84	

REFERENCE

Ahmad A. 1997. Chemical preservation of tomato concentrate preserved by cold break method M.Sc. Thesis Dept University of Agriculture, Faisalabad.

GOP. 2002. Agricultural Statistics of Pakistan.

AOAC. 1999. Official method of analysis. Association of Official Analytical Chemists, 15 ed. Washigton DC.

Bindra U, Manjreker SP and Jain SC. 1973. Utilization of muskmelon. J Food Sci Technol 10(4):76-79. (Food Sci Technol Abst 6(5):J 639, 1974).

Cusina GB, Mark ZA, Perlova ND, Bogdanova ZN and Kotva KS. 1971. Dietetic juices made from cucurbitaceae fruits. U.K.R. Nauchno-issled. Inst Konservn Prom Odessa. USSR. (Chem Abst 76:57922 A, 1972).

El-Ashwash FA, Abd-El-Baki, NM El-Samaby, SK and Abd-El-Fadeel MG. 1982. Effect of storage on the characteristics of concentrated orange and lime juice. Agri Res Review 58(3): 275-88.

Gusina GB and Transtinskaya LO. 1974. Watermelon juice and pulp. Konsernaya, Ovoschchesubile nayaPromyshlennost. 3: 17-18. [Food Sci Technol Abst 6(12): 2020].

Hummel M and Okey R 1950. Relation of canned tomato products to storage losses of ascorbic acid JFood Res 15:405-414.

Javed A. 1988. Manufacture of different flavoured Kinnow juice based drinks M.Sc. Thesis Deptt Food Technol Univ Agric Faisalabad.

Larmond E. 1987. Methods for Sensory Evaluation of Foods. Deptt. of Agri Canada Pub No1637.

Luh BS and Wood Roof JG. 1975. Commercial Vegetable Processing. The Avi. Publishing Co Inc West Port, Connecticut.

Morgan RC.1986. Carotenoids as colorants and Vit A Precursors. J Food Sci 31:213-7

Myskin MM and Fokin ND. 1974. Frozen watermelon pulp. Konsernaya-I-Ovoshes-ushil naya. Promyshlennost. 1:10-12. Food Sci Technol Abst16(12) J 1771).

Sandhu KS, Bhatia BS and Shukla FC. 1985., Physico-chemical changes during storage of Kinnow, mandarin, orange and pine apple juice concentrates. J Food Sci Technol 22(5):342-5.

Steel RGD, Dickey D and Torrie JH. 1996 Principles and procedures of Statistics. A Biometrical Approach 3rd ed. McGraw Hill Book Co Inc New York.

Zaheer H. 1986. To study the acceptability of mixed fruit jam (apple and muskmelon) M.Sc. Thesis Deptt Food Technol Univ Agric Faisalabad.

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Water activity and storage stability of pine nuts

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ABSTRACT

In view of losses occurring due to lack of information on the storage of shelled pine nuts, sorption studies were carried out at 25°C and 37°C. It was found that shelled pine nuts have a typical sigmoid moisture sorption isotherm. Samples containing high moisture content (9.2% and above) deteriorate rapidly resulting in howing lump formation and microbial growth. Microbial growth did not appear in samples containing 6.2% moisture stored at 37°C even for 80 days storage. These investigations indicate that pine nut kernels should be dried to about 6.2% moisture and stored at water activity ranging from 0.57 to 0.68. Storage under these conditions was found to be safe even at 37°C.

Key words: Pine nut, absorption isotherm, water activity

INTRODUCTION

Pine nut (*Pinus girardiana*) is grown in N.W.F.P. and Baluchistan provinces of Pakistan. Like other nut foods it is rich in fat and protein. It is used in number of ways in bakeries and home cookery. In winter it is roasted, shelled and relished as a snack. However, recently , it has become an item of export particularly to the Gulf States. For this purpose unshelled pine nuts are half-roasted and then shelled. Kernels obtained in this way have 9-12 % moisture and are soft and pliable. White colour of raw kernels changes to somewhat dull white.

Exporters, sometime, incur losses because of microbial growth. Although it is known that pine nuts should be adequately dried to avoid spoilage due to microbial growth, there is little published literature on its moisture relations to microbial growth and storage stability. Beuchart (1978) investigated the relationship of water activity to moisture content in various tree nuts but pine nut was not included in that study.

Knowledge of sorption isotherm of a food product is important for predicting its quality and stability during storage (Labuza 1968). Therefore, the present studies were undertaken to investigate the moisture sorption characteristics of shelled pine nuts so that packaging and storage requirements could be defined.

MATERIALS AND METHODS

Freshly shelled pine nuts were obtained from local processor. Pine nuts were partially roasted prior to shelling. The moisture content was determined using a vacuum oven according to the method of AOAC (1990). For equilibration, samples were kept in airtight containers for 24 hours prior to determination of moisture. The samples were then dried in a cabinet drier at 60 °C for various lengths of time to obtain samples with moisture content varying from 5.3 to 13.5% (Table 1).

The water activity was determined by a modified graphical interpolation method of a Landrock and Proctor (1951) using "Conway water activity apparatus" (Sibata Sci. Tech. Ltd., Japan). About 2g samples, thinly sliced and uniformly spread in special aluminum dishes (inner dia 3 cm), were allowed to absorb or desorbs moisture for 3 hours in different RH environments created by saturated salt solutions at 25°C (±1°). Four salts were chosen for this purpose such that there was gain in case of two salts and loss in case of the other two. Gain/loss in weights was corrected for any sample weight differences so that they corresponded to exactly 2g samples. By plotting gain and loss in weight versus water activity (a_w) the exact water activity of the sample was determined, being the point of intersection of zero base line and the weight gain/loss plot. Sorption isotherm was then prepared from these water activities.

Representative samples were also stored in air-tight bottles at 25°C and 37°C to observe changes in samples such as lump formation, mould growth etc. to get an idea of the critical point as defined by Landrock and Proctor (1951).

RESULTS AND DISCUSSION

The interpolation isotherms of pine nut samples conditioned to various moisture levels is shown in Fig 1. Water activity of different samples as obtained from the interpolation curves when plotted against moisture content of the respective samples gave a typical sigmoid isotherm shown in (Fig 2). It is clear from the isotherm (Fig 1 & 2) that up to water activity of 0.7 the nuts pass through a wide span before their moisture increases by a small margin. After that this condition

is not maintained and there is a steep rise in the curve.

The results of storage showed that 7.1% is the approximate moisture level where the deteriorative process seems to begin (Table 1). Accordingly the point on the isotherm corresponding to this moisture content has been taken as the critical point 'C'. The critical point is the stage at which the product just begins to become unusable because of some physico-chemical change, and the point at 0.05 lower water activity is designated as danger point 'D'. The portion between these two points D and C is designated as the safety range (SR) as described by Landrock and Proctor (1951). Another point marked on the sorption isotherm is initial point '1'. This is usually the point corresponding to the moisture content at which the product is quite stable and has acceptable shelf life. In the present case the point corresponding to 5.3% has been arbitrarily taken as the initial point as the nuts could be easily dried to this level using the conventional method of air-drying. The portion of the isotherm between the points 1 and D may be defined as the safety margin (SM) as a helpful guide in the packaging of a material (Siddapa and Nanjundaswamy 1960). It is desirable that the packaging adopted should not permit the product to reach the danger point.

these recommendations. It was concluded from the graph that safe margin for storage of pine nuts is between 0.57 and 0.68 water activity. The shelled pine nuts having 9.2% moisture do not become moldy or show signs of lumping during storage at 25 °C for about 20 days. This safe period of storage can be appreciably increased by low temperature storage. Similarly sample containing 7.1% moisture has shelf life of about 60 days at 25 °C, which could also be enhanced at low temperature. Saleem *et al* (1997) observed that Dhakki dates might be stored at around its own water activity of 0.62 a_w, which would be the most promising one for the extended storage period.

The conditions stated above are for storage at 25 °C or below. At higher temperature water activity of a product increases at constant moisture content making the product more susceptible to microbial, nutritional and aesthetic degradation (Rockland and Nishi, 1980). However, storage of shelled pine nuts having 6.2% moisture at 37 °C did not develop any microbial growth or any other physico-chemical change even after 80 days (Table 1). This was perhaps because of the fact that lowering of the danger point by 0.05 a_w from the critical point had taken care of the susceptibility to deterioration at higher temperature storage.

Table 1. Microbial growth and lump formation in shelled pine nuts of different moisture content stored at 25°C and 37°C for 90 days).

Sample No.	Moisture (%) after drying	Storage temperature 25 °C		Storage temperature 37 °C	
		Growth after (days)	Lump formation after (days)	Growth after (days)	Lump formation after (days)
1	13.5 (No drying)	6	3	3	2
2	12.0	10	7	5	3
3	9.2	30	20	10	6
4	7.1	80*	60	60**	30
5	6.2	Nil	Nil	Nil	Nil
6	5.3	Nil	Nil	Nil	Nil

* Growth on few nuts only
** Scanty Growth

It is evident from the graph (Fig 2) that for safe storage, shelled pine nuts should be dried to below the danger point i.e. 6.2% moisture (water activity = 0.68). It may be mentioned here that according to US FDA (Federal Register, 1976) safe moisture level for tree nuts and peanuts has been defined as the water activity that does not exceed 0.70 a_w at 25 °C. Our results in the case of pine nuts agree closely with

REFERENCES

AOAC. 1990. Official methods of analysis. American Association of Official Analytical Chemists. 15th ed Arlington, USA.

Beuchart LR.1978. Relationship of water activity to moisture content in tree nuts. J Food Sci 43:754.

Federal Register 1976. Tree nuts and peanuts. Good manufacturing practice regulation. Part 128, 41(127): 27000.

Labuza TP. 1968. Sorption phenomena in foods. Food Technol. 22(3):15.

Landrock H and Proctor BE. 1951. A new graphical interpolation method for obtaining humidity equilibria data with special reference to its role in food packaging. Food Technol 5:332.

Rockland LB and Nishi SK. 1980. Influence of water activity on food quality and stability. Food Technol 34:40-42.

Saleem SA, Naeem M, Baloch WA and Baloch AK. 1997. Influence of water activity on the stability of "Dhakki" dates. Pak J Food Sci 7(1-2):1-6.

Siddapa GS and Nanjundaswamy AM. 1960 Equilibrium relative humidity (ERH) relationship of fruit juice and custard powders. Food Technol 14: 533.

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Effect of surfactants on maillard browning in food

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ABSTRACT

Effect of surfactants (emulsifiers) of CTABr (cationic), SDS (anionic) and Tween-20 (non-ionic) was studied on the absorbance (browning) of the reaction of glucose with different amino acids, pH.5.0 (acetate buffer 0.4 M), EDTA (0.2 mM), heated at 55°C. Absorbance of solutions was measured at 470 nm. Ratio of absorbance was obtained in fold of the reaction solutions (+surfactant/-surfactant). Maillard browning of glucose-glycine reaction reduced with CTABr to 0-44 fold (56%), with SDS to 0.60 fold (40%) and Tween-20 to 0.74 fold (36%). Effect of CTABr on decreasing Maillard browning of different amino acids was found in order: glycine=L-alanine>L-histidine>L-proline>L-glutamic acid>L-asparagine.

Key words: CTABr: Cetyltrimethylammomum bromide. SDS: Sodium dodecylsulfate. Tween-20: Polyoxyethylene (20) sorbitan mono-olaurate Y EDTA; Ethylenediaminetetra acetic acid

INTRODUCTION

Maillard browning reaction was first described by Louis Maillard (Maillard 1912) as the formation of coloured products (melanoidins) on heating a mixture of reducing sugars and amino acids. In this reaction proteins and amino acids provide amino component, while carbonyl compounds by reducing sugars (aldoses and ketoses). The flavours aromas and colours produced in food during frying, baking, roasting and storage may be desirable or undesirable. Maillard browning products are important contributors to the flavour of milk chocolate, caramels, toffees and fudges etc. Fujimaki *et al* (1986) investigated that Maillard products of glucose-lysine and cysteine are mutagenic and reduce the nutritional value, solubility and digestibility of proteins. Maillard browning not only occurs in food but also develops in pharmaceutical and cosmetic products. This reaction can reduce the effectiveness of these products and may produce compounds that can cause adverse or toxic effect buffer (0.4 M), pH 5.0, heated at 55°C. Absorbance of reaction solutions was measured at different time intervals (25-400 hr) at 470 nm.

MATERIALS AND METHODS

Experimental work was carried out in the Laboratory of Food Chemistry, Procter Department of Food Science, University of Leeds UK. All chemicals were of Analar grade, supplied by BDH, Sigma and Fluka. Measurements of pH were made using a Jenway-3020 pH meter. The meter was calibrated with buffers (potassium hydrogen phthalate and anhydrous disodium hydrogen orthophosphate/sodium dihydrogen orthophosphate). Progress in browning of the reaction solutions was assessed by measuring absorbance at 470 nm (1 cm cells) using spectrophotometer model No: CECIL CE-1020 1000-SERIES. This study was carried out in the following model systems.

RESULTS AND DISCUSSION

Model system-II

In this system different concentrations of surfactants (CTAB, SDS and Tween-20) were used. Model system containing solutions of glucose (0.125 M) and glycine (0.0625 M), CTABr (0-0.12 M), SDS (0-0.12 M), and Tween-20 (0-10% w/v), with EDTA (0.2 mM), pH 5.0 (acetate buffer 0.4 M), heated at 55°C. The absorbance of these solutions was measured at different time intervals (25-400 hr) at 470 nm

MODEL SYSTEM-I

Effect of CTABr on the Maillard browning of glucose-amino acids reactions

In this system, the reaction of glucose with different amino acids was studied in the presence of cationic surfactant of CTABr (0.04 M), pH 5.0 at 55°C. The amino acids used are: glycine (BDH), L. alanine (Acros), L. leucine (Sigma), L. histidine (Sigma), L.glutamic acid (Sigma), L. asparagine (Sigma), L. methionine (BDH), L. proline (Aldrich). Absorbance of the reaction solutions was measured at 470 nm. Ratio of absorbance (Maillard browning) in fold of the reaction solutions (+surfactants/ - surfactants) determined at a period of 400 hr.heating. Table 1 shows the effect of CTABr on the reduction of Maillard browning of the reaction solutions determined in fold and percent basis.

Table 1. Effect of CTABR (0.04 M) on the absorbance (Maillard browining) of glucose-amino acids reactions measured at 470 nm.

S.No	Amino acids	Absorbance ratio in fold	% Reduction Maillard Browning
1	Glycine	0.44	56
2	L-Alamine	0.44	56
3	L-Histidine	0.55	45
4	L-Proline	0.62	38
5	L-Glutamic acid	0.63	37
6	L-Asparagine	0.70	30

MODEL SYSTEM-II

Effect of various concentrations of CTABr and SDS on Maillard browning of Glucose-glycine reaction.

Maillard browning of glucose-glycine was studied with different concentrations of CTABr (0-0.12 M) an dSDS (0-0.12M), pH 5.0 at 55°C. Absorbance of the reaction solutions was measured at 470 nm. Ratio of absorbance (Maillard browning) in fold of the reaction solutions (+surfactants) determined at a period of 400 hr heating. Figure 1 shows the effect of CTABr and SDs on ratio of the absorbance of the reaction. Both these surfactants have decreased the browning to a maximum with CTABr (0.04M) to 0.44 fold (56%) and SDS (0.06 M) to 0.60 fold fold (40%)

DISCUSSION

The reactions of carbohydrates with ammo acids causing Maillard browning are well reviewed by Wedzicha (1984), which affect the quality of shelf-stable intermediate and high moisture foods. Most of the affects of the reaction such as golden brown colours or caramel aromas develop on heating, is an important contributor to many industrial products i.e the flavour of chocolate, toffees etc. Nevertheless, the Maillard browning reaction product (MRP) is sometimes undesirable (e.g. when dehydrated fruits & vegetables darken and develop off-flavours in storage or during sterilization of milk), and affects the nutritional value of food. MRP in foods can affect its protein bioavailability by derivatizing protein-bounds, dietary limiting amino acids such as lysine, arginine, histidine and possibly tryptophane and cysteine. Such reactions exhibit antinutritive effects by mechanisms involving complexation with micronutrients, destruction of vitamins and by acting as inhibitors of digestive enzymes. Hurrel (1990) reported that MRP might be the cause of observed urinary excretion of zinc, copper and iron. Ingredients commonly used in food and pharmaceuticals are lubricants, antioxidants, preservatives, colouring agents and flavouring agents. Kumar and Banker (1991) reported that great majority of drugs has amine functionality and in the presence of sugars or other carbonyl compounds often produce extensive discoloration (browning). Inhibition of browning is desirable in view of the losses caused in nutritive value together with the possible generation of off-flavours and diminished visual appeal of the product. Wedzicha (1988) reported that solubility of organic compounds sparingly soluble in water could be increased by the addition of surfactants. Previously we studied the influence of surfactants on the interaction of sorbic acid with thiols; DoTAB cationic surfactant increased while SDS anionic surfactant and Tween-80 non-ionic surfactant decreased the reaction rate (Wedzicha and Zeb 1990).

In this research, Maillard browning reactions of glucose-amino acids reduced significantly with surfactants of CTABr and SDS. CTABr. Inhibition of browning of different amino acids was found in order: Glvcme=L-Alanme>L-Histidine>L-Proline>L-Glutamic acid>L-Asparagine.

These results suggest that amino acids are easily penetrate in micellar phase, while most of the sugars molecules stay out side of micelles. This separation of glucose from glycine by micelles is possibly the cause of reducing the Maillard browning. MukerJee (1967) reported that further increase in chains length of micelles greater than 16 carbon atoms, has no appreciable effect on the CMC and this id possibly due to the coiling of the long hydrocarbon chains of surfactant in solution. Longer alkyi chain surfactants are known to be better catalysts or inhibitors of chemical reactions. For example, the catalytic effect of surfactants of sodium alkyi sulphate on the hydrolysis of methlyl orthobenzoate was found in the order: hexadecyi > tetradecyi > dodecyi > decyi > octyl (Dunlap and Cordes, 1968). However this catalytic effect is also depend on substrate structure. This can

be supported by the results of nitrosamine formation from amine-nitrate reactions studied in the presence of micelle-forming surfactant of DTAB, where longer hydrophobic alkyi side chains yield higher concentrations of the amine on the miceller phase (Okun and
Archer, 1977).

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REFERENCES

Dickinson E and Clements MC. 1995. Advances in Food Colloids. Blackie Academic & Professional. Pub. No. 247.

Dunlap RB and Cordes EH. 1968. Secondary valence force catalysis. Catalysis of hydrolysis of methyl orthobenzoate by sodium dodecyi sulphate. J Amer Chem Soc 90:4395.

Fujimaki M, Namiki M and Kato H. 1986. Ammo-Carbonyl reactions in food and biological systems, Elsevier Sci Pub. Elsevier Apllied Science Publishers, London. p:329-371.

Hurrel RF. 1990. Influence of the Maillard reaction on the nutritional value of foods in the Maillard reaction in food processing, human nutrition and physiology. Birkhauser Verlag, Boston, p:245-258.

Kumar V and Banker GS. 1991. Maillard reaction and drug stability. Maillard Reactions in Chemistry, Food and Health. Labuza et al. (1995). CRC Press, Boca Raton, FL.

Maillard LC. 1912. Action of amino acids on sugars. Fonnation of melanoidins in methodical way. Compt. Rend 154: 66.

Musthapha WAW, Hill SE, Blanshard MV and Derbyshire, W. 1998. Maillard reactions: do the properties of liquid matrices matter. Food Chem. 62(4): 441.

Okun JD and Archer MC. 1977. Kinetics of nitrosamine formation in the presence of micelle forming surfactants J National Cancer Institute 58: 409.

Wedzicha BL. 1984. Chemistry of sulphur dioxide in foods. Elsevier Applied Science Publishers LTD. London.

Wedzicha BL. 1988. Distribution of low molecular weight food additives, Advances in Food

Wedzicha BL and Zeb A. 1990.Catalysis of the reaction between sorbic acid and thiols by surfactants. International J Food Sci & Tech 25: 168.

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Effect of UHT and in-bottle sterilization temperatures on storage stability of milk

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ABSTRACT

Raw milk was pasteurized at 85°C for 16 seconds by a plate heat exchanger. Milk was standardized at 8.86 % milk solids not fat (MSNF) and toned at 3.5 % fat. The product was subjected to homogenization pressure of 190/40 bars, and treated for different UHT lime and temperatures, followed by filling in high-density polyethylene (HDPE) bottles. These filled bottles were capped with aluminum foil and then sterilized at different retort temperatures. The samples were stored at ambient temperature and monitored for quality parameters (sensory, pH, fat separation and sedimentation) on a weekly basis. It was found that out of six different treatment combinations for UHT and retort temperatures and time the best was 144°C for 16 seconds followed by 116° C for 1 minute and 45 seconds. The product was acceptable even after 24 weeks stored at ambient temperature.

Key words: Milk, shelf life, UHT, in-bottle sterilization temperature.

INTRODUCTION

Milk is the only food of young mammals during the first period of their life. The substances in milk provide both energy and the building materials for growth. Milk also contains antibodies, which protect the young against infection. The principal constituents in milk are water, fat, proteins, lactose, and mineral elements. Milk also contains trace amounts of other substances such as pigments, enzymes, vitamins and phospholipids as well as gases and white blood corpuscles (leucocytes) is highly perishable and is susceptible to spoilage (curdling) rapidly after milking. However, proper heat treatment for specified time followed by proper packaging and storage prolongs its life considerably. It has been observed that commercially processed milk shows some quality problems during its shelf life. Miralles *et al* (2000) studied the effect of refrigerated storage of raw milk, UHT treatment, and storage of UHT milk at room temperature on whey protein to total protein ratio. No significant differences (<0.05)among samples were found in any case.

Hardam *et al* (2000) studied the extent of fat separation during storage of UHT milk homogenised using conventional valve homogeniser and compared to milk emulsified using a microfluidiser. They found that only slight fat separation occurred in micro fluidised UHT milk samples after nine months storage at 25°C, compared to moderate fat separation after 2-3 months storage for milk subjected to conventional valve homogenization.

Morales *et al* (2000) worked on characterization of industrial processed milk by analyses of heat-induced changes. They revealed by using high performance liquid chromatography (HPLC) that in sterilized milk average amount of lactulose was 1120 mg/L and of hydroxy methyl furfural 22 µ mol/L, without any detectable presence of un denatured whey proteins.

Enright *et al* (1999) found that addition of plasmin to UHT milk after heating reduced the stability of the milk, increased proteolysis, and lead to early formation of sediment.

Keeping in view the commercial importance of the product and problems associated to its shelf life, the present study was aimed at determination of best time and temperature combination to minimize the quality issues during shelf life of the product.

MATERIALS AND METHODS

The study was conducted in a commercial dairy plane with commercial brands of UHT milk packed in High Density Polyethylene (HDPE) bottles.

Raw Milk Procurement

For trial purpose raw milk was procured from the farmers through routine collection system of the firm. Milk was analysed initially for quality parameters like fat%, MSNF%, pH, Salts % and Alcohol precipitate test (APT) etc.

Pasteurisation of Milk

The milk was pasteurised at 85° C for 16 seconds by using plate heat exchanger.

Table 1. Trials description along with temperature and time treatments.

Trial No	Bottle Size	UHT Temperature and Time	Retort temperature and time
1	500 ml	144°C for 16 second	116°C for 2 minutes
2	500 ml	144°C for 16 second	116°C for 1 minute 45 second
3	500 ml	144°C for 16 second	116°C for 2 minutes
4	500 ml	144°C for 2.5 second	116°C for 2 minutes
5	500 ml	144°C for 2.5 second	116°C for 2 minutes
6	500 ml	144°C for 2.5 second	116°C for 2 minutes

Batch Standardisation

Milk was standardised for milk solids not fat (MSNF) 8.9 % and toned for 3.5 % fat. A novel recipe for stabiliser was formulated which include permitted food additives namely Guar gum, Disodium phosphate, Glycerol mono steroid and Sodium hexa meta phosphate to enhance mouth feel and for buffering effect on pH.

Ultra Heat Treatment and Post Filling Sterilization Temperatures

The main batch was divided into six small batches. Each of this batch was treated as given in Table 1. The product was filled in 500 ml high density poly ethylene bottles and capped with aluminium foil by heat sealing.

Shelf Life Monitoring

The samples of all the treatments were checked at regular intervals of one week for Pat separation, sedimentation, pH and sensory characteristics. The details of these tests are given below.

Fat Separation

Few particles of fat float on undisturbed surface while opening the bottle = Slight
Fat particles of considerable size forming incomplete thin layer on the surfaces Moderate
Fat layer covering the whole surface of the milk = High

Sedimentation

Settled material up to 1 gram = slight
Settled material up to 3 gram but more than 1 gram = Moderate
Settled material more than 3 gram = Heavy

pH

The pH of the samples was checked at 20°C by using pH meter (WTW pH 330).

Sensory Evaluation

A panel consisting of 8 - 10 judges evaluated the samples for their sensory characteristics including smell and taste of the product.

RESULTS AND DISCUSSION

The results of six treatments for different times and temperatures combinations for UHT and retort have been discussed in detail below.

Treatment 1

The results of treatment number 1 are given in table No. 2. The product expired due to low pH and poor organoleptic after 7 weeks of its storage life at ambient temperature. There was very slight fat separation and slight sediment after 7 weeks. The colour of the milk changed to light brown after 7

Table 2. Results of weekly analyses for treatment number 1 (UHT 144 degree C for 16 second followed by retort temperature 116 degree C for 2 minutes)

Age (Weeks)	Colour	Organoleptic	pH at 20°C	Fat separation	Sediment
1	Off white	O.K	6.68	Nil	Nil
2	Off white	O.K	6.66	Nil	Traces
3	Off white	O.K	6.66	Nil	Traces
4	Off white	O.K	6.65	Traces	Traces
5	Off white	O.K	6.65	V. Slight	Traces
6	Off white	O.K	6.65	V. Slight	Traces
7	Off white	O.K	6.64	V. Slight	Traces
8	Brown	O.K	6.35	V. Slight	Slight

Table 3. Results of weekly analyses of treatment number 2 (UHT 144°C for 16 second followed by retort temperature 116°C for 1 minute 45 seconds).

Age (Weeks)	Colour	Organoleptic	pH at 20°C	Fat separation	Sediment
1	Off white	O.K	6.67	Nil	Traces
2	Off white	O.K	6.66	Nil	Traces
3	Off white	O.K	6.66	Nil	Traces
4	Off white	O.K	6.66	Nil	Traces
5	Off white	O.K	6.65	Nil	Traces
6	Off white	O.K	6.65	Nil	Traces
7	Off white	O.K	6.64	Nil	Traces
8	Off white	O.K	6.64	Nil	Traces
9	Light brown	O.K	6.64	Nil	Traces
10	Light brown	O.K	6.64	Nil	Traces
11	Light brown	O.K	6.64	Nil	Traces
12	Light brown	O.K	6.63	Nil	Traces
13	Light brown	O.K	6.68	Nil	Traces
14	Light brown	O.K	6.66	Nil	Traces
15	Light brown	O.K	6.71	Traces	Traces
16	Light brown	O.K	6.70	Traces	Traces
17	Light brown	O.K	6.68	Traces	Traces
18	Light brown	O.K	6.68	Traces	Traces
19	Light brown	O.K	6.68	Traces	V. Slight
20	Light brown	O.K	6.68	Traces	Slight
21	Light brown	O.K	6.67	Traces	Slight
22	Light brown	O.K	6.67	Slight	Slight
23	Light brown	O.K	6.67	Slight	Slight

weeks of its storage life. The results match with the findings of Celestino *et al* (1997), who found that during storage of UHT milk green red and blue-yellow colour components and the non-protein nitrogen increased while pH and colour lightness decreased during storage.

Treatment 2

The results of treatment number 2 are given in detail in table 3. The colour of product changed from off-white to light brown after eight weeks of storage at ambient temperature. Sediment and fat separation were slight and the product was quite acceptable even after 23 weeks of its shelf life.

Treatment 3

The detailed results of treatment number 3 are given in table 4. The product expired organoleptically just after 3 weeks of its shelf life at ambient temperature storage. The reason of changing taste may be due to

Table 4. Results of weekly analyses of treatment number 3 (UHT 144°C for 16 second followed by retort temperature 116°C for 1 minute 45 seconds).

Age (Weeks)	Colour	Organoleptic	pH at 20°C	Fat separation	Sediment
1	Off white	O.K	6.68	Nil	Nil
2	Off white	O.K	6.86	Nil	Traces
3	Off white	O.K	6.66	Trace	Slight
4	Off white	Not O.K	6.50	Slight	Slight

Table 5. Results of weekly analyses of treatment number 4 (UHT temperature 140°C for 2.5 seconds followed by retort temperature 116°C for 2 minutes).

Age (Weeks)	Colour	Organoleptic	pH at 20°C	Fat separation	Sediment
1	Off white	O.K	6.70	Nil	Nil
2	Off white	O.K	6.62	V. Slight	Traces
3	Off white	O.K	6.62	Slight	Slight
4	Off white	Not O.K	6.50	Slight	Slight

Table 6. Results of weekly analyses of treatment number 5 (UHT temperature 140°C for 2.5 seconds followed by retort temperature 116°C for 2 minutes).

Age (Weeks)	Colour	Organoleptic	pH at 20°C	Fat separation	Sediment
1	Off white	O.K	6.67	Nil	Nil
2	Off white	O.K	6.63	V. Slight	Traces
3	Off white	O.K	6.60	Slight	Slight
4	Off white	Not O.K	6.50	Slight	Slight

Table 7. Results of weekly analyses of treatment number 6 (UHT temperature 140°C for 2.5 seconds followed by retort temperature 116°C for 2 minutes).

Age (Weeks)	Colour	Organoleptic	pH at 20°C	Fat separation	Sediment
1	Off white	O.K	6.70	Nil	Nil
2	Off white	O.K	6.70	Nil	Traces
3	Off white	O.K	6.70	Nil	Traces
4	Off white	O.K	6.69	Nil	Traces
5	Off white	O.K	6.69	Nil	Traces
6	Off white	O.K	6.68	Nil	Traces
7	Off white	O.K	6.68	Nil	Traces
8	Off white	O.K	6.64	Nil	Traces
9	Off white	O.K	6.64	Nil	Traces
10	Off white	O.K	6.64	Nil	Traces
11	Light brown	O.K	6.64	Nil	Traces
12	Light brown	O.K	6.63	Nil	Traces
13	Light brown	O.K	6.68	Nil	Traces
14	Light brown	O.K	6.66	Nil	Traces
15	Light brown	Changed	6.60	Trace	Traces
16	Light brown	Not O.K.	6.59	Trace	V.Slight

lowering of the pH that dropped from 6.68 to 6.50. The change in sensory characteristics of the UHT milk may be due to some enzymatic action as described by Lopez-Fandino and Olano (1999). The fat separation and sediment were at slight level at this storage interval.

Treatment 4

The results of treatment number 4 are almost, same as that of treatment number 3 (table 5). The product was unacceptable after 3 weeks of storage at ambient temperature. It may be due to low pH similar to that of treatment number 3. The fat separation and sediment were slight at this storage interval.

Treatment 5

The detailed results of treatment number 5 are given, in table 6. The results are almost similar to those of treatment number 3 & 4. The product expired due to poor organoleptic and low pH after 3 weeks of its storage at ambient temperature. The fat separation and sediment were at slight level at this storage interval.

Treatment 6

The results of treatment number 6 (table 7) shows that this is the second best treatment. The product was acceptable till the 15 weeks of it storage life at ambient temperature. The colour of the milk changed from off-white to light brown after 10 weeks. At the end of 15 weeks the sediment and fat separation were at slight level, however the pH falls considerably during this period whereas after 15 weeks the product showed poor organoleptic.

CONCLUSION

The above six trials of UHT milk aseptically filled in high density poly ethylene bottles of 500 ml, and stored at ambient temperature, shows that treatment number 2 is the best to prolong the shelf life of the milk i.e., UHT 144 degree C for 16 seconds and retort temperature 116 for 1 minute and 45 second is recommended. The second best treatment was number 6, which recommends UHT 144 degree C for 10 seconds followed by retort temperature of 116 degree C for 2 minutes.

LITERATURE CITED

Celestino EL, Iyer M and Roginski H. 1997. Reconstituted UHT treated milk- effects of raw milk, powder quality and storage condition of UHT milk on its physico-chemical attributes and flavour. Intl Dairy 1(2-3):129-40.

Enright E Bland A Needs BC and Kelly AL. 1999. Proteolysis and physicochemical changes in milk on storage as affected by UHT treatment, plasmin activity and $KClO_3$ addition. Int Dairy J 9 (9):581-91.

Hardham JF Imisdn BW and French V. 2000. Effect of homogenization and micro fluidizations on the extent of fat separation during storage of UHT milk. Aus J Dairy Technol 55 (1):16-22

Lopez-Fandino R and Olano A. 1999. Selected indicators of the quality of thermal processed milk. J Food Sci Technol 5(2):121-37.

Miralles B, Bartolome B, Ramos M and Amigo L. 2000. Determination of whey protein to total protein ratio in UHT milk using fourth derivative spectroscopy. Intl Dairy J 10(3):191-7.

Morales FJ Romero C Jimenez-Perez S. 2000. Characterization of industrial processed milk by analysis of heat-induced changes. J Food Sci Technol 35(2):1s93-200.

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Potential of *Lactobacillus delbrueckii-02* for bacteriocin and antibiotic assay

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ABSTRACT

Lactobacillus delbrueckii-02 produces a bacteriocin which displays a narrow inhibiting spectrum with a bactericidal activity .The efficacy of this strain was found to be sensitive with regards to antibiotic assay as established against different antibiotics including ampicillin, cefozoline, chloramphenicol, nitrofurantoin and novobiocin, showing an inhibition zone of 28-35mm in diameter. The high degree of sensitivity provides potential for the exploitation of *Lactobacillus delbrueckii*-02 in establishing very well defined bacteriocin producer.
Keywords: Lactobacillus, antibiotic assay, bacteriocin

INTRODUCTION

The bacteriocins (Klaenhammer 1993; Bennik *et al* 1997a) are a group of antibacterial compounds produced by a large number of lactic acid producing bacteria (LAB) belonging to different genera (Stiles and Holzapfel 1997). These smaller weight peptides inhibit the growth of related genera and species of LAB as well as several other Gram positive pathogenic bacteria such as *Bacillus, Clostridium, Listeria, Staphylococcus* (Bennik *et al* 1997a). Therefore, bacteriocin of LAB are of particular interest because of their existing and potential applications as natural preservatives in foods (Holzapfel *et al* 1995., Delves-Broughton *et al* 1996 and Stiles 1996) and as a genetic markers in food grade cloning and expression systems (Allison and Klaenhammer 1996). Most of the bacteriocins from LAB have been isolated from species of the genus *Lactobacillus*, probably because of the diversity of its species and habitats. Bacteriocin production among strains of LAB is established primarily by carrying out assay against closely related LAB and a few important spoilage and pathogenic bacteria. The degree of inhibitory activity is dependent upon the nature of the indicator organism (Rogers and Montville 1991), the potentiality of using a similar type of indicator organism for both bacteriocin and antibiotic assay will enhance the degree of sensitivity and facilitate the antibiotic assay. In continuation of our work (Ahmed *et al* 2001) and against this back-ground, the prime objective of this study was to evaluate the native isolate of LAB with respect to some common food *borne* pathogenic bacteria and to check its sensitivity against majority of antibiotics.

MATERIALS AND METHODS

Antibiotics

Antibiotics used in the present study were obtained from Boehringer, Germany.

Bacterial Cultures and Their Cultivation

Locally isolated culture of *Lactobacillus delbrueckii* -02 was used through the present study (Ahmed *et al* 2001). Lactic acid bacteria were maintained at 4° C in MRS broth. The food pathogenic indicator strains as listed in table-1 were maintained on nutrient agar slants and were sub-cultured in Luria Bertani broth (LB).

Bacteriocin and Inhibitory Effect

The strain *Lactobacillus delbrueckii* –02 was grown in MRS broth for the production of bacteriocin. As solid media, assay culture agar (Difco) or MRS broth containing 1.5 % agar (Oxoid) were used. MRS was supplemented with 0.7% agar for a soft agar top layer. In order to establish the inhibitory effect of the bacteriocin, cell free supernatant fluid adjusted to pH 6.5 was incubated at 37° C for 12 hours in MRS broth. Then, it was spotted on to indicator lawns of several lactic acid bacteria and other Gram positive as well as Gram negative organisms (Table-1) to observe the possible inhibition against these strains. The lawns were prepared by propagating fresh bacterial cultures to an optimum density of 0.45 (600nm) and adding 200 uL of the cell suspension to 3.5ml of overlay agar (top layer). The over laid agar plates were incubated for 24 hours at 37° C.

RESULTS AND DISCUSSION

Inhibitory Effect

The cells of *Lactobacillus delbrueckii*-02 were removed after 12 hours of cultivation in MRS broth at 37° C. The pH of the cell free supernatant fluid was adjusted to 6.5 and the inhibitory activity towards different food *borneee* pathogens was evaluated. The results are presented in table 1. The inhibitory effect of *Lactobacillus delbrueckii*-2 was found to be quite narrow. However, it was investigated that this locally isolated strain gave good inhibition against two important food *borne* pathogens that is *Escherichia coli and Salmonella*. This strain produced a bacteriocin characterized by limited inhibitory effect, being active against some of the pathogenic organisms.

Table 1. Sensitivity of bacteriocin produced by *Lactobacillus delbrueckii*-02 against culture filterate of lactic acid bacteria and other bacterial strains

Indicator strains	Growth conditions	Inhibition
Llactobacillus casei	MRS, (37°C)	-ve
Llactobacillus acidophilus	MRS,(37°C)	+ve
Llactobacillus lactis	MRS, (37°C)	-ve
Bacillus cerus	LB, (37°C)	-ve
Bacillus subtilis	LB, (37°C)	-ve
Eescherichia coli	LB, (37°C)	+ve
Staphylococcus aureus	LB, (37°C)	-/+ve
Ssalmonella	LB, (37°C)	+ve
Pseudomonas aeroginosa	LB, (37°C)	-/+ve
klebsiella	LB, (37°C)	-/+ve

+ve = Clear zone of inhibition, -ve = No zone of inhibition,
-/+ = variable inhibition.

In general, bacteriocin from lactic acid bacteria was found to be only active towards Gram positive bacteria (Klaenhammer 1988). Gram negative bacterial cells may also exhibited inhibition by the activity of the bacteriocin when *Lactobacilli* were injured sub-lethally (Stevens *et al* 1991, Kalchayanand *et al* 1992). A narrow inhibiting effect, as observed during the present investigation with *Lactobacillus delbruecllii*-02, seems to be uncommon among the bacteriocin-producing isolates from the *Lactobacillus* group.

Antibiotic Effect

The effect of different antibiotics on the zone of growth inhibition of *Lactobacillus delbrueckii*-02 was studied in order to find out the extent of its sensitivity with respect to various common antibiotics. The result findings are presented in table 2, *Lactobacillus delbruecllii*-02 was found highly sensitive to majority of the antibiotics which were used during the present investigation, with the exception of cloxacillin at a concentration of 1ug.The maximum zone of inhibition was obtained by the use of chloramphenicol which was immediately followed by ampicillin, cefozoline, nitrofurantoin and novobiocin, as shown in table 2 at the concentration of 30ug, 10 units, 30ug, 30ug, and 30ug respectively. It was found that at a concentration of 5ug,ciprofloxacin showed an inhibitory zone of 27mm diameter and gave a sensitive reaction. It was found that antibiotics such as penicillin (10 units) and neomycin (30ug) showed a moderate reaction against *Lactobacillus delbrueckii*-02.

Table 2. Antibiotic sensitivity of *Lactobacillus delbrueckii*-02

Antibiotic	Concentration	Inhibition Zone (mm)	Sensitivity
Ampicillin	10 Units	32	S
Cefozoline	30 ug	32	S
Chloramphenicol	30 ug	35	S
Ciprofloxacin	5 ug	27	S
Erythromycin	15 ug	20	S
Gentamycin	10 ug	16	S
Nitrofurantoin	30 ug	30	S
Penicillin	10 Units	20	M
Cloxacillin	1 ug	-	R
Kanamycin	30 ug	17	S
Neomycin	30 ug	13	M
Novobiocin	30 ug	28	S
Polymixin-b	300 ug	10	S
Streptomycin	10 ug	18	S
Tetracycline	30 ug	25	S

R = resistant, S = sensitive, M = moderate.

Antibiotic resistance has been reported in many LAB by Reinbold and Reddy (1974) and Sozzi and Siniley (1980). Vescovo *et al* (1982) has shown that *Lactobacillus reuteri* had a chromosol-*bornee* resistance to different antibiotics such as streptomycin, neomycin, gentamycin, and kanamycin. Similarly, *Lactobacillus acidophilus* also had a same type of antibiotic resistance against penicillin, ampicillin, streptomycin, chloramphenicol and polymyxin-B. However, there are several other reports

which clearly indicated that LAB are usually found resistant to major classes of antibiotics such as *B* - lactam, cephalosporins, aminoglycosides, qunolines, imidazole, nitrofurantoin and fluroquinolines. These findings are found to be similar with the results obtained in case of *Lactobacillus delbrueckii*-02, which clearly established that it was susceptible to number of antibiotics. Thus the findings obtained during the present investigation clearly suggested that this strain of *Lactobacillus* could easily be exploited as an indicator organism in order to develop new antibiotic assays. The susceptibility of LAB against various types of antibiotics including bacteriocin was mainly due to their membrane composition (Montville and Bruno 1994, Bennik et al 1997b). While the differences in the degree of inhibition which were observed during the present investigation with respect to various antibiotics was possibly due to their different line of action on the cell wall components such as the cell wall protein DNA synthesis, DNA gyrase and RNA polymerase (Neu 1992)

REFERENCES

Ahmad R, Shah MA and Quratulain Syed. 2002. Cultural conditions for the production of bacteriocin by *Lactobacillus delbrueckii*-MA-01. Pak J Sci Res 54:(1-2) in process.

Allison GE and Klaenhammer JR. 1996. Functional analysis of the gene encoding immunity to lactacin F, laft, and its use as a *Lactobacillus* specific, food grade marker. App. Environ Microbial 62:4450-60.

Bennik MHJ, Verheul A, Abee T, Naaktge*borneee*-Stoffels G, Gorris GM, Smid EJ. 1997b. Interaction of niasin and pediocin PA-1 with closely related lactic acid bacteria that manifest over 100 fold differences in bacteriocin sensitivity. App Environ Microbial 63:3628-36.

Bennik MHJ, Smid EJ, and Gorris GM Lact. 1997a. Vegetable associated Pediococcas parvulus produces pediocin PA-1 App Environ Microbial 63:2074-6.

Delves-Broughton J Blackburn P Evans RJ and Hugenholtz J. 1996. Application of the bacteriocin, niacin. Antonie Van Leeuwenhoek 69:193-202.

De-Man JC, Rogosa M and Sharpe ME. 1960. A medium for the cultivation of *Lactobacilli.* J App Bacteriol 23:130-5.

Halami PM Chandrashekar A and Naud K. 2000. *Lactobacillus farciminis* MD, a newer strain with potential for bacteriocin and antibiotic assay. Let App Microbial 30:197-202.

Kalchayanand N, Hanlin MB and Ray B. 1992. Gram positive bacteria sensitivity to bacteriocin pediocin AcH and niacin. Let App Microbial15:239-45.

Klaenhammer TR. 1993. Genetics of Bacteriocins produced by lactic acid bacteria. FEMS Microbial. Review 12:39-86.

Klaenhammer TR. 1988. Bacteriocins of lactic acid bacteria. Biochem. 70: 337-49.

Montville TJ and Bruno EMC. 1994. Evidence that dissipation of proton motive force is a common mechanism of action for bacteriocins and other anti-microbial proteins. Int J Food Microbial 24: 53-74.

Neu HC. 1992. The crisis in antibiotic resistance. Sci. 257, 1064-78. Protective cultures, bacteriocins and food grade enzymes. Int J Food Microbial 24, 343-62.

Reinbold GW and Reddy MS. 1974. Sensitivity and resistant of dairy starter and associated microorganism to selected antibiotics. J Milk Food Tech 37:517-21.

Rogers AM and Montville TJ. 1991. Improved agar diffusion assay for niasin quantification. Food Biotechnol 5:161-8.

Sozzi T and Siniley MB. 1980. Antibiotic resistant of yoghurt starter cultures *Streptococcus thermophilus* and *Lactobacillus balgaricus.* App Environ Microbial. 40: 862-65.

Stevens KA, Sheldon BW, Klapes NA and Klaenhammer TR. 1991. Niasin treatment for the in activation of Salmonella species and other Gram negative bacteria. App Environ Microbial 57:3613-15.

Stiles ME and Holzapfel WH. 1997. Lactic acid bacteria in food and their current taxonomy. Int J Food microbial 36:1-29.

Stiles ME. 1996. Bio-preservation by lactic acid bacteria. Antonic Van Leeuwen hoek 70:331-45.

Vescovo M, Morelli Lact and Bottazzi V. 1982. Dug resistant plasmids in *Lactobacillus reuteri.* App. Environ. Microbial.43: 50-6.

Zamfir M, Callewaert Rcornea, PCVatafu I and De Vuyst L. 1999. Purification and characterization of a bacteriocin produced by *Lactobacillus acidophilus* IBB801. J App Microbial 87: 923-31.

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Influence of storage temperature on the microflora of cheddar cheese

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ABSTRACT

Eighteen samples of processed cheddar cheese were analysed for their respective bacterial load as psychrophiles, mesophiles, thermophiles, total and faecal coliforms and organoleptic attributes when stored at 10, 25 and 45^{0}C for the storage period of three months at the intervels of 15 days. These observations indicated that all the cheese samples were good in quality and within the recommended bacteriological limit.The psychrophiles slightly increased from $3.8x10^4$ to $7.8x10^5$ at 10^{0}C and $6.59x10^0$/ml at 25^0 C while at 45^0 C their number decreased to $5.8x10^0$ /ml during storage. Mesophiles grew gradually at all storage temperatures. Similarly, thermophilic count at l0^{0}C storage temperature was found to be low while it started increasing from $4.7x10^3$/ml to $8.9x10^3$ /ml ,$2.1x10^6$/ml at 37^0 C and 45^{0}C,respectively. So, the samples stored at 10^0 C were good in quality while the samples stored at 45^0 C were deteriorated due to significant increase of thermophiles. Sensory quality of cheese were acceptable after two months storage at 10^{0}C, 20^{0}C and 45^{0}C.After three months, taste, texture, odor, color and overall acceptability scores of cheese samples were significantly less then at 10^0 C and 25^0 C.

Keywords: Cheddar Cheese, bacteria, fermentation, storage, temperature

INTRODUCTION

Cheddar cheese is a popular variety cheese of produced in the world. It is produced from coagulation of sour milk by rennin. Lactic acid bacteria are used as starter in cheese making, *Streptococci, Leuconostocs* and *Lactobacilli* starters multiply during cheese making from about 10^7 CFU / mL in milk to 10^8 to 10^9 CFU / g of curd. Their growth is checked at salting stage. Low cooking temperatures are used, and acidity is developed during the manufacturing process before the curd is salted and pressed (Korolezuk *et al* 1991)

The changes which occur during the ripening of cheddar cheese are essentially the controlled degradation of milk carbohydrate, protein and butter fat. They are brought about by the action of enzymes from milk, rennet, starter and other microflora, and by chemical action such as oxidation, and yield a complex mixture of compounds which give mature cheese for the required balance of flavor and aroma (Meyrand *et al* 1999). Stimulation of *Lactobacillus* by *Streptococci* may play a role in ripening of cheese in which the two later species develop, die, disintegrate, and are followed by *Lactobacillus*. The quantity of soluble protein increases due to the activities of *Lactobacilli*. Besides protein breakdown, they are also responsible for the proper flavor development in ripened cheese. In addition to *strptococci* and *lactobacilli* various other types of microorganisms are more or less frequently found in cheese. Some of these play a part in ripening and others are inert and still others may cause defects in cheese. *Micrococci* and *E.cocci* are often found in cheese (Charalambides *et al* 1995).

The general changes that occur in cheeses during storage period involve the action of microorganisms which multiply rapidly, and cause deterioration of cheeses (Bouzas *et al* 1993). Microorganisms play chief role in development of defects in cheddar cheese. The most serious defect is lack of acid development in the curd during the process of cheese making. Too much starter and too much acid development in the milk before setting may also cause acid sour. The organisms responsible for the development of undesirable gas production with holes and swelling of the cheese are *A. aerogenes, Bacillus, yeasts,* and anaerobic sporeformers. Molds always present a problem in manufacture of cheddar cheese. It is extremely important to keep the curing room, floors, ceilings, and shelves free from molds (Mahanta 1984).

The present investigations were carried out to evaluate the microbial load in respect of psychrophiles, mesophiles, thermophiles, total and faecal coliforms at three different temperatures (10^{0}C, 25^{0}C and 45^{0}C), for three months storage period. The storage temperature were selected according to the temperature which prevail during different seasons in our country.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Eighteen samples of branded cheddar cheese were purchased from local market in disposable packs of 250g weight.

STORAGE CONDITIONS

All the samples of cheddar cheese were analyzed at three different temperatures (10°C, 25°C and 45°C) at the interval of fifteen days for a storage time period of three months.

MICROBIAL LOAD OF SAMPLES

Colony forming units (CFU) as psychorophiles, mesophiles and thermophiles were investigated in accordance with IDF (1994) by using plate count agar medium and incubation was done at 10°C, 37°C and 45°C respectively. Psychrophiles were incubated for 6 to 8 days at 10°C, mesophiles were incubated for 48 hours at 37°C and thermophiles were incubated for 24 hours at 45°C, for the interpretation of coliform, the standard Mc Cardy's MPN tubes were used by using Mac.conky broth.

ORGANOLEPTIC EVALUATION

All the samples of cheese after three months storage at 10°C, 37°C and 45°C temperatures 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 (Achi 1999).

STATISTICAL ANALYSIS

All the data were subjected to the standard deviation and analysis of variance (Multivariate Anova), and was calculated by the methods of Steel and Torrie (1984).

RESULTS AND DISCUSSION

Brand cheddar cheese was evaluated in respect of psychrophiles, mesophiles, thermophiles, total and faecal coliforms at three different temperatures (10°C, 25°C and 45°C) for a period of three months storage. Their bacterial count was monitered at the regular interval of fifteen days. The results of microbiological analysis of cheddar cheese at different incubation temperatures during three months storage are present here in table 1, 2 and 3.

Table 1. Effect of storage temperature on the bacterial count of 'cheddar cheese' samples.

Days	Psychrophiles (No./ml)	Mesophiles (No./ml)	Thermophiles (No./ml)	Total Coliforms (MPN/100ml)	Faecal Coliforms (MPN/100ml)
0	3.80×10^{4} ±0.011	2.37×10^{3} ±0.012	4.75×10^{3} ±0.013	-ve	-ve
15	1.73×10^{4} ±0.014	1.68×10^{4} ±0.016	2.7×10^{4} ±0.015	-ve	-ve
30	7.56×10^{4} ±0.015	2.11×10^{4} ±0.017	7.76×10^{4} ±0.019	-ve	-ve
45	3.70×10^{4} ±0.016	4.69×10^{4} ±0.015	2.56×10^{4} ±0.021	-ve	-ve
60	8.04×10^{5} ±0.019	5.68×10^{4} ±0.010	1.48×10^{4} ±0.013	-ve	-ve
75	7.53×10^{5} ±0.015	6.22×10^{4} ±0.019	2.10×10^{4} ±0.023	-ve	-ve
90	7.80×10^{5} ±0.018	5.48×10^{5} ±0.018	4.95×10^{4} ±0.020	-ve	-ve

MPN = Most Probable Number
All the samples were analyzed in triplicate.

Table 2. Effect of storage temperature (25°C) on bacterial count of cheddar cheese samples after three months.

Days	Psychrophiles (No/ml)	Mesophiles (No./ml)	Thermophiles (No./ml)	Total Coliforms (MPN/100ml)	Faecal Coliforms (MPN/100ml)
0	$3.80x10^4$ ±0.013	$2.37x10^3$ ±0.014	$4.75x10^3$ ±0.015	-ve	-ve
15	$4.1x10^4$ ±0.012	$1.89x10^3$ ±0.015	$1.1x10^4$ ±0.016	-ve	-ve
30	$3.21x10^5$ ±0.014	$3.25x10^3$ ±0.017	$3.85x10^4$ ±0.014	-ive	-ive
45	$4.59x10^5$ ±0.015	$5.68x10^3$ ±0.014	$7.0x10^4$ ±0.018	-ve	-ve
60	$5.15x10^5$ ±0.016	$6.24x10^3$ ±0.013	$7.26x10^4$ ±0.019	-ve	-ve
75	$4.29x10^5$ ±0.015	$1.32x10^4$ ±0.011	$8.24x10^4$ ±0.016	-ve	-ve
90	$6.59x10^5$ ±0.016	$6.16x10^5$ ±0.012	$8.99x10^4$ ±0.017	-ve	-ve

MPN = Most Probable Number

All the samples were analyzed in triplicate.

The psychrophiles decreased gradually at 10°C, 25°C and 45°C. During three months storage period, psychrophilic count was from 3.8 x 10^4 to 7.80 x 10^5 at 10°C, it ranged from 3.8 x 10^4 to 6.59 x 10^5 at 25°C and at 45°C it ranged from $3.8x10^4$ to 5.89 x 10^4. Where as, mesophiles grew gradually at 10°C, 25°C & 45°C. Mesophilic count 10°C during three months storage ranged between 2.37 x 10^4 to 5.48 x 10^5, at 25°C it ranged from 2.37 x 10^4 to 6.16 x 10^5 and at 45°C it ranged from 2.37 x 10^4 to 6.43 x 10^5. Similarly, thermophilic count at low temperature (10°C) during three months storage ranged between 4.75 x 10^3 to 4.95 x 10^4, at 25°C it ranged from 4.75 x 10^3 to 8.99 x 10^4 and at higher temperature (45°C) it ranged from 4.75 x 10^3 to 2.19 x 10^6.

Psychrophilic count was increased at 10°C and decreased at 45°C. Both mesophilic and thermophilic count was not significantly increased at 10°C. Where as, at 45°C a remarkable increase in thermophilic count was found and their count was ranged from 10^4/ml to 10^6/ml, as described above. At 45°C, both psychrophiles and mesophiles remained unaffected. However, their count was observed to change from 10^4/ml to 10^5/ml after three months storage. Coliforms, both faecal and non faecal were not detected throughout the storage period.

Both the storage temperature and time period significantly ($p<0.05$) effected the microbial load with respect to analysis of variance (multivariate anova). However, psychrophilic count was higher at 10°C, and lower at 45°C, Mesophililes grew gradually at all the incubation temperatures. Similarly, thermophilic count was lower at 10°C and higher at 45°C. Butter samples stored at 10°C were good in quality throughout the storage period. But the samples stored at 45°C were deteriorated due to the significant increase of themophilic count. The results of statistical analysis are present here in the table 5 and 6.

During manufacturing of cheese, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* and *Lactobacillus bulgaricus* increase in number and continue to multiply for a few days. Their extracellular enzymes contribute to proteolytic and lipolytic changes in the ripening of cheese. Bottazi et al., (1992) also reported that microflora of cheese was influenced by the season of production and the temperature of ripening. Digrak et al., (1996) were taken the microbiological quality of 21 cheeses samples, the average counts of coliform group bacteria, *Escherichia coli*, total microorganisms, *Staphylococcus aureus*, *Salmonella* sp., *Bacillus* sp., and psychrophillic bacteria in the samples of cheeses were recorded as, 8.26 x 10^2 CFU/g, 66.6%, 2.3 x 10^9 CFU/g, 5.5 x 10^2 CFU/g, 42.8 %, 2.18 x 10^5 CFU/g, 1.06 x 10^4 CFU/g, 90.4 %, 1.02 x 10^6 CFU/g, respectively.

Table 3. Effect of storage temperature (45°C) on bacterial count in Cheddar cheese samples after three months.

Days	Psychrophiles (No./ml)	Mesophiles (No./ml)	Thermophiles (No./ml)	Total Coliforms (MPN/100ml)	Faecal Coliforms (MPN/100ml) (MPN/100ml)
0	$3.80x10^4$ ±0.014	$2.37x10^4$ ±0.016	$4.75x10^3$ ±0.013	-ve	-ve
15	$1.61x10^4$ ±0.012	$2.88x10^4$ ±0.015	$4.87x10^3$ ±0.011	-ve	-ve
30	$2.04x10^4$ ±0.018	$4.40x10^4$ ±0.012	$4.91x10^4$ ±0.016	-ve	-ve
45	$5.41x10^4$ ±0.015	$6.42x10^4$ ±0.013	$7.51x10^5$ ±0.017	-ve	-ve
60	$4.46x10^4$ ±0.012	$6.76x10^4$ ±0.016	$2.22x10^5$ ±0.015	-ve	-ve
75	$4.84x10^4$ ±0.011	$6.86x10^4$ ±0.017	$4.22x10^5$ ±0.014	-ve	-ve
90	$5.89x10^4$ ±0.016	$6.43x10^5$ ±0.015	$2.19x10^6$ ±0.013	-ve	-ve

MPN = Most Probable Number

All the samples were analyzed in triplicate.

Table 4. Effect of storage temperatures(10°C, 25°C and 45°C) on the sensory organoleptic evaluation of Cheese after three months.

Temperature	Color	Taste	Odour	Texture	Overall* acceptability
10°C	8.5 ±0.26	8.45 ±0.25	8.55 ±0.27	8.7 ±0.25	8.55 ±0.24
25°C	7.85 ±0.19	7.95 ±0.17	8.0 ±0.19	7.85 ±0.15	7.91 ±0.13
45°C	6.1 ±0.15	4.95 ±0.12	6.2 ±0.11	5.0 ±0.21	5.56 ±0.17

All the samples were analyzed in triplicate.

***Overall acceptability using a nine point hendonic scale ranging from 1 for dislike extremely to 9 for like extremely.**

During storage of cheese at 37°C, faster microbial changes associated with the deterioration of flavor, increased hardness, adhesiveness, gumminess and chewiness were observed in this product reported by Thapa et al (1992). *Staphylococcus aureus* and *Esherichia coli* were not detected in cheddar cheese samples reported by Reddy et al (1995). Lopez-diaz et al (1995) investigated the bacteriological quality of cheese in a traditional way. The levels of main microbiological groups, including *Enterobacteria* and *Enterococci* were detected. Although the bacteriological quality was good (levels of *Enterobacteria* and *Enterococci* was lower than 3 and 5 log CFU/g, respectively). The high levels of these bacteria were faecal in origin and found in some samples which suggested the need of more strict hygienic and technological control of manufacturing processes.

Sensory quality characteristics of cheese samples were acceptable after two months storage at 10°C, 25°C and 45°C. Deterioration started in cheese samples at 45° C. After three months taste, texture, odor, color and overall acceptability score of cheese samples at 45° C were significantly ($p<.05$) less then the cheese sample stored at 10° C and 25° C just after two months (Table-4).However, score rating for sensory quality cheese sample stored at 10° C and 25°C were not distinctly different from each other.These results are consistent with the findings of other workers who reported desirable changes in Organoleptic characteristics UHT milk due to decomposition of milk fat and protein as a result of lipolysis and proteolysis process involved during storage (Garcia *et al*1999).

Torres *et al* (1995) studied the microbiological and sensory changes that occurred in relation to aging and storage conditions in cheddar cheese. Cheeses packaged 30 to 45 days were compared with traditionally ripened cheeses by Bertola *et al* (1995). Development of fruity, unclean and medicinal type of off-flavor during storage in cheese samples were

studied by Jha *et al* (1995). White soft cheese and Chihuahua type cheeses were analyzed by Diaz-Cinco *et al* (1998), which were stored at 5^0C and 25^0C and samples were taken at 4, 8 and 12 days for analysis. These results showed that the sanitary microbial counts were higher. The effect of time, temperature and extraction method on the level of 12% trichloroacetic acid solution and nitrogenous compounds (TCA-SN) in cheeses were investigated by Polychroniadou et al. (1999). Analysis of variance indicated significant differences due to all main effects ($p < 0.05$) but the differences induced by time and temperature were very small and of no practical interest. It was concluded that the cheese samples remained unaffected at 10^0C in terms of microbial load as well as flavor.

REFERENCES

Achi OK. 1999. Quality attributes of fermented jam flavour supplemented with processed soy flavour. Plant food Human Nutrition.54:151-158.

Banks JM, Hunter EA and Muir D. 1994. Sensory properties of cheddar cheese: effect of fat contents on maturation. Milchwissenschaft. 49(1):8–12.

Bottazzi V, Scolari GL, Cappa F, Battistotti B, Bosi F and Brambilla E. 1992. Lactic acid bacteria for Grana Cheese production. Part III. acidification rate and blowing Sci Tech Latt Casearia 43(2):71–93.

Bouzas J, Kannt CA, Bodyfelt FW and Torres JA. 1993. Time and temperature influence on chemical aging indicators for a commercial cheddar cheese. J Food Sci 58(6):1307–12.

Charalambides MN Williams JG and Chakrabarti SA. 1995. Study of the influence of aging on the mechanical properties of cheddar cheese. J Mater Sci 30(16): 3959–67.

Digrak Metin Yilmaz Okkes Celik Ozcelik and Sami. 1996. The microbiological quality and the analysis of fatty acid composition of the fresh white cheeses sold in Elazig. Turk J Biol. 20:221–230.

Diaz-Cinco Martha E Armenta Rodrigo Fraijo Oscar Acedo-Flix Evelia De Mejia Elvira Gonzalez, Lazano-Taylor and Jose. 1998. Differentiation of sanitary, chemical quality and amines production in Mexican white soft cheese and Chihuahua style cheese. Recent Res Dev Nutr Res 259–67.

Garcia MR Ramos M and Famidno R Protein distribution and stability of UHT milk during storage at room temperature. J Sci Fd Agric 79:1171-1178.

Green ML. In: Advances in the Biochemistry of cheese and fermented milk, Davies FL and Law BA (1984). Page 17-19. Elsevier Applied Science London and New York.

IDF. 1994. Milk and Milk products. Enumeration of presumptive *Escherichia Coli* count by (1) – Most Probable Number Technique, (2) – Most Probable Number Technique with the use of 4-methylumbelliferyl-ß-Gluconcside (MUG), (3) – Colony count technique at 44^0C using membranes. International Dairy Federation, Brussels Belgium.

Jha YK and Singh S. 1995. Effect of hydrogen peroxide treated milk on flavor development in buffalo cheddar cheese. Indian J. Dairy Sci. 48(12):696–698.

Mahanta KC. 1984. Dairy Microbiology. page 67–77. Omsons Publications. Gauhati New Delhi.

Korolezuk J and Mahaut M. 1991. Effect of whey proteins and heat treatment of milk on the consistency of UF fresh cheese. Milchwissenschaft. 46(7):435–437.

Lopez-Diaz TM, Santos JA, Gonzalez CJ, Moreno B and Garcia ML. 1995. Bacteriological qualit,y of a traditional Spanish blue cheese Milchwissenschaft 50(9):503–5.

Meyrand A, Vernozy-Rozand C, Gonthier A, Mazuy C, Ray-Gueniot S, Jaubert G and Perrin G. 1999. Main differences in behavior and eterotoxin production of Staphylococcus aureus in two different raw milk cheeses. Rev Med Vet 150(3):271–278.

Poly Chroniadou Anna Michaelidou Alexandra Paschaloudis and Nicolaos. 1999. Effect of time, temperature and extraction method on the tri chloro acetic acid soluble nitrogen of cheese. Int Dairy J 9(8):559–568

Reddy K , Anjan Marth and Elmer H. 1995. Microflora of cheddar cheese made with sodium chloride, potassium chloride or mixtures of two salts. J Food Prot 58 (1):54–61.

Steel RGD, Dickey D and Torrie JH. 1984. Principles and procedures of statistics.A biometrical approach. 3rd ed. McGraw Hill London. P:196.

Thapa TB and Gupta VK. 1992. Changes in sensoric and rheological characteristics during storage of processed cheese foods prepared with added whey protein concentrates. Indian J Dairy Sci 45(3):140–5.

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Gas chromatographic studies and composition of oils from different sunflower varieties

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ABSTRACT

Oils from three sunflower varieties (Triumph-573, Bemisal-205 and Pioneer- 6451) were extracted by solvent extraction method using n-hexane as solvent. Evaluations for various physical (colour, odour, melting point, refractive index, specific gravity) and chemical (acid value, saponification value, ester value, peroxide value, iodine value) characteristics were carried out after oil refining. Oil recovery percentage varied from 35.8-37%, melting point -10.9 to 11.9, specific gravity 0.8-0.92, refractive index 1.46-1.47, viscosity 61.26-85.36, acid value 2.23-3.02, saponification value 188-190.6, ester value 186.1-188.2, peroxide value 6.0-6.63, and iodine value 122.6-127.9.The fatty acid profile was analysed with GLC using a gas chromatograph which shows palmitic acid content 6.66-8.74, stearic acid 3.20-3.52, oleic acid 28.74-43.77 and linoleic acid 45.64-57.1%. It is concluded that sunflower oil is semidried in nature as indicated by linoleic acid content and can be used for edible purposes. Pioneer-6451 contains highest (57.71%) linoleic acid content.
Keywords: Sunflower oil, fatty acid profile, physico-chemical characteristics

INTRODUCTION

The main sources of fat in human diets are structural lipids and depot fats of foods from both animal and vegetable sources. However, animal fats tend to increase the level of cholesterol in the blood but no such cholesterol raising effect is obtained from plant sources (Berger 1992). World's total production of fats and oils is centered mainly 65% from vegetable sources, 31% is derived from animal and only 2% is got from the fish (Kirk and Othmer 1980).

The major oil seed crops in Pakistan include cotton seed, rapeseed/mustard, sunflower and canola. During 2002-03 local production of edible oil was 0.634 million tonnes which 0.971 million tonnes besides was imported 0.155 million tonnes recover was recovered from imported oil seeds. Total availability of edible oils during this period was 1.76 million tonnes (GOP 2003).

Sunflower (*Helianthus annus*) is the 2nd most important oil seed crop in Pakistan, growing on an area of 371,000 hectares and oil production was 99,000 tonnes (GOP 2003). Oil obtained from the kernels of sunflower is unique because it remains fluid at low temperature (Khan and Mondal 1969). Anatomical and morphological features are in accord with the percent yield and physico-chemical composition. Oil content and fatty acid composition of sunflower oil are affected by different growing conditions (Alpaslan and Gunduz 2000). Oil yield and oleic acid content increase while linoleic acid content decreased with increasing maturity temperature (Shahbaz and Hassan 2000).

The fatty acid composition and physico-chemical properties of a large number of sunflower varieties have been determined and found to vary with variety, environment, maturity, extraction techniques and seed purity either healthy or infected (Khan and Mondal 1969; Pierce, 1970; Lopez *et al* 1985; Lanfranco *et al* 1989; Sadaf 1994; Naz 1995; Alpaslan and Gunduz 2000; Shahbaz and Hassan 2000; Wan and Dowd, 2000; Ramirez *et al* 2001). The various findings, therefore, suggested that fatty acid composition and other properties might be different in oils from different varieties of sunflower.

Only few studies on the composition and fatty acid profile of sunflower oil have been made. The present project has been conducted to determine observe physical and chemical characteristics as well as fatty acid profile of three sunflower varieties.

MATERIALS AND METHODS

Sunflower varieties (Triumph-573, Bemisal-205 and Pioneer- 6451), were obtained from Oil Seed Section, Ayub Agricultural Research Institute, Faisalabad (Pakistan). After removing foreign matter and damaged seeds, oil was extracted from dry kernels by mechanical press (model no. 2950) and oil still remain in meal was extracted with n-hexane by soxhlet solvent extraction method (AACC, 2000). Physical parameters such as colour, odour, melting point, specific gravity, refractive index, viscosity and chemical parameters like acid value, saponification value, ester value, peroxide value and iodine value were determined according to their respective methods mentioned in AOCS (1998) and AOAC

(1990). Fatty acid profile was evaluated through Gas Liquid Chromatography (GLC). Fatty acid methyl esters were prepared by methylation method (Saleem, 2001) and GLC was carried out on 10% DEGS (diethylene glycol succinate) glass packed column using nitrogen as carrier gas by Perkin-Elmer Gas Chromatograph 3920 equipped with flame ionization detector FID. The peaks and area was acquired with Chromo Pac (C-R4A) Shimdazu, Japan.

The samples were injected by one micro litre syringe (SGE, Australia). Fatty acid methyl esters kit of Poly Science Corporation 6366 Gross Point Road, Niles,1L 60648 was taken as standard. From this kit methyl esters were analysed and peak of each fatty acid was marked. The gas chromatographic conditions were as under:

- Column length 2m
- Internal diameter of column 2mm
- Column temperature 200°c
- Injector temperature 250°c
- Nitrogen flow rate 30ml/min
- Hydrogen pressure 20psi
- Air pressure 50psi
- Volume of sample injected 0.4µL

The data obtained were analysed statistically by using the analysis of variance techniques (ANOVA) as described by Steel *et al.*, 1997.

RESULTS AND DISCUSSION

The physical characteristics like melting point, specific gravity, refractive index, viscosity and oil percentage of varieties (Triumph-573, Bemisal-205 and Pioneer-6451) were determined (Table I). Despite the general similarity of present oils, Pioneer-6451 sample was distinguished by slightly higher content of oil than the others. Then data was statistically analysed which showed highly significant differences for all physical parameters.

Table I. Physical characteristics of sunflower oil from different varieties.

Parameters	Triumph (573)	Bemisal (205)	Pioneer (6451)
Melting point (°C)	-10.9	-11.9	-12.1
Specific gravity	0.8844	0.9205	0.8643
Refractive index	1.4663	1.4715	1.4579
Viscosity (centipoises)	62.06	85.36	61.26
% Yield	36.6 %	35.8 %	37 %

Chemical analysis of oil from three varieties of sunflower (Table II) explicit that Pioneer-6451 has higher ester value which may be due to the presence of more triglycerides in oil as saponifiable matter. Bemisal-205 oil can be distinguished from others by higher peroxide value. This uniqueness was due to higher degree of unsaturation and poor keeping quality of oil. Iodine value of Bemisal-205 was also much higher which also indicate the more unsaturated fatty acids present. It may be due to higher temperature during the period seed is maturing. Statistically the difference between varieties for acid value and iodine value was highly significant while significant result was found for ester value and peroxide value where as saponification value showed non-significant results.

Table II. Chemical characteristics of sunflower varieties Triumph-573, Bemisal-205 and Pioneer-6451.

Parameters	Triumph (573)	Bemisal (205)	Pioneer (6451)
Acid value	2.23	2.7	3.02
Saponification value	188	190.4	190.6
Ester value	186.1	186.5	188.2
Peroxide value	6.0	8.1	6.63
Iodine value	122.9	127.9	12-122.06

The most important factor in the nutritional value of oils is the ratio of different fatty acids present in oil. The fatty acid composition (Table III and Fig. I, II, III)) showed that varieties (Triumph-573, Bemisal-205 and Pioneer-6451) contained palmitic acid (C16:0) 7.34, 6.66 and 8.74% respectively. An approximately symmetrical distribution of saturated fatty acids was found with the percentage of 3.20, 3.52 and 3.49 for stearic acid (C18:0) respectively in varieties (Triumph-573, Bemisal-205 and Pioneer-6451).

The percentage of oleic acid (C18:1), unsaturated fatty acid, was found greater than palmitic (C16:0) and stearic acid (C18:0) in all three varieties. The value of 28.74% oleic acid of pioneer 6451 does not agree well with values 39.30% and 43.77% for Triumph-573 and Bemisal-205. Factors such as growing year, location, storage and oil extraction methods may have influenced composition. The recognizable concentration of linoleic acid (C18:2) again reflects the greater abundance of unsaturated fatty acids in all three varieties. However Pioneer-6451 has maximum contents (57.71%) of linoleic acid.

Table III. Fatty acids profile of sunflower varieties

Variety	Palmitic acid C16:0	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid C18:2
Triumh-573	7.34 %	3.20 %	39.30 %	48.80 %
Bemisal-205	6.66 %	3.52 %	43.77 %	45.64 %
Pioneer-6451	8.74 %	3.49 %	28.74 %	57.71 %

It was observed in all three varieties of sunflower that unsaturated fatty acids (C18:1, C18:2) are present in greater amount than saturated fatty acids (C16:0, C18:0) (Table III). These data indicate that composition and quality studies on different sunflower varieties are actually observations on composite of several physiologically distinct seed types. The results of this study imply that quality factors used by the industry can be correlated to seed variety and physiological properties of the sunflower. It was suggested that these oils are semidrying as indicated from their linoleic acid content (57.71%) which is essential fatty acid, hence sunflower oil can be used for edible purposes.

REFERENCES

AACC. 2000. Official methods of analysis. American Association of Cereal Chemists. Am Assoc Cereal Chem Inc St. Paul, Minnesota, USA.

Alpaslan M and Gunduz H. 2000. The effect of growing conditions on oil content, fatty acid composition and tocopherol content of some sunflower varieties produced in Turkey. Nahrung, 44(6): 434-37.

AOAC.1990. Official Methods of Analysis. The Association of Official Analytical Chemists, Inc. 15 ed. Arlington, Virginia, USA.

AOCS. 1998. Official Methods and Recommended Practices. American Oil Chemists Society. 5th ed Champaign, Illinios, USA.

Berger KG. 1992. Food uses of palm oil. Malaysian Palm Oil Promotion Council Kuala Lumpr, Malaysia p. 3.

GOP (Government of Pakistan). 2003. Economic Survey of Pakistan. Economic Affairs Division Govt. Islamabad.

Khan NA and Mondal BK. 1969. Survey of oil seeds investigation on sunflower seed oil Pak J Sci Ind Res 63 (1):123-127.

Kirk RL and Othmer DF. 1980. Encyclopedia of Chemical Technology. 3rd ed John Wiley and Sons, New York.

Lanfranco C Zazzerini A and Tosi L. 1989. Changes in composition of sunflower oil extracted from Achenes of *Sclerotium bataticola* infected plants. J Agric. Food Chem 37 (1):36-38.

Lopez ZA Esteban FJ Ozomoza RM and Collado JG. 1985. Qualitative and quantitative aspects of lipids fraction in Spanish oleaginous seeds. Agrochinica 29(2-4):249-255. (Chem Abs 105(9): 77711k, 1986).

Naz F. 1995. Comparison of different physical and chemical aspects of oils and oil cake extracted from newly evolved sunflower varieties (Hysun 24, 33, 35). M.Sc Thesis. Deptt Chem Univ Agri Faisalabad.

Pierce R. 1970. Sunflower processing techniques. J Amer Oil Chem Soc 7(47):248A-250A.

Ramirez G Hough G and Contarini A. 2001. Influence of temperature and light exposure on sensory shelf life of a commercial sunflower oil. J Food Quality 24 (3):195-204. (FSTA 33. (11): 227, 2001).

Sadaf G. 1994. Studies of physical and chemical aspects of oil and oil cake extracted from recently evolved sunflower variety (G 358). M.Sc. Thesis Deptt Chem Univ Agric Faisalabad.

Saleem T. 2001. Composition of fatty acids of cooking oils available in the market. M.Sc. thesis. Deptt Chem Univ Agric Faisalabad, Pakistan.

Shahbaz A and Hassan F. 2000. Oil yield and fatty acid composition of spring sunflower. Pak J Biol Sci 3(12): 2063-64.

Steel RGD, Dickey D and Torrie JH. 1997. Principles and Procedures of Statistics. A biometrical approach, 3rd ed. McGraw Hill Book Co. Inc. New York.

Wan PJ and Dowd MK. 2000. Comparative study of the extraction and measurement of cottonseed free fatty acids. J Amer Oil Chem Soc 77(1): 23-27. (FSTA 32(6): 230, 2000).

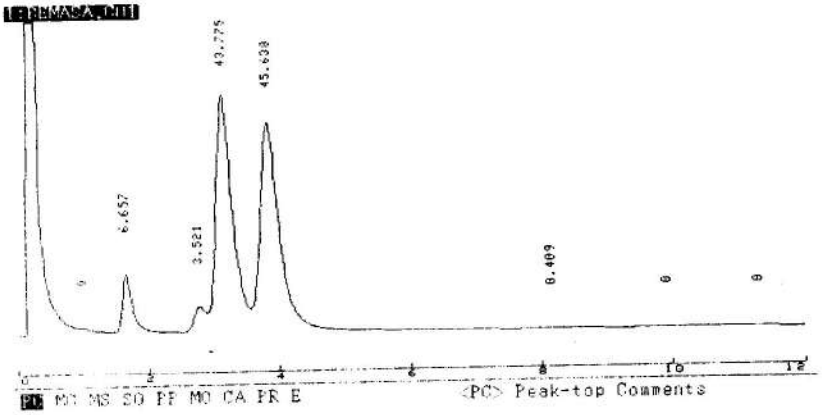

Fig I. Gas Chromatogram of Sunflower (Bemisal-205) Seed Oil showing the (Concentration of Fatty Acids)

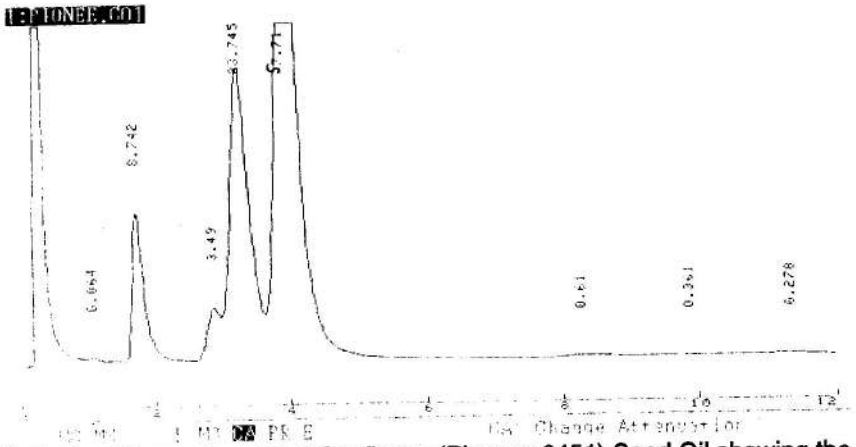

Fig II. Gas Chromatogram of Sunflower (Pioneer-6451) Seed Oil showing the (Concentration of Fatty Acids)

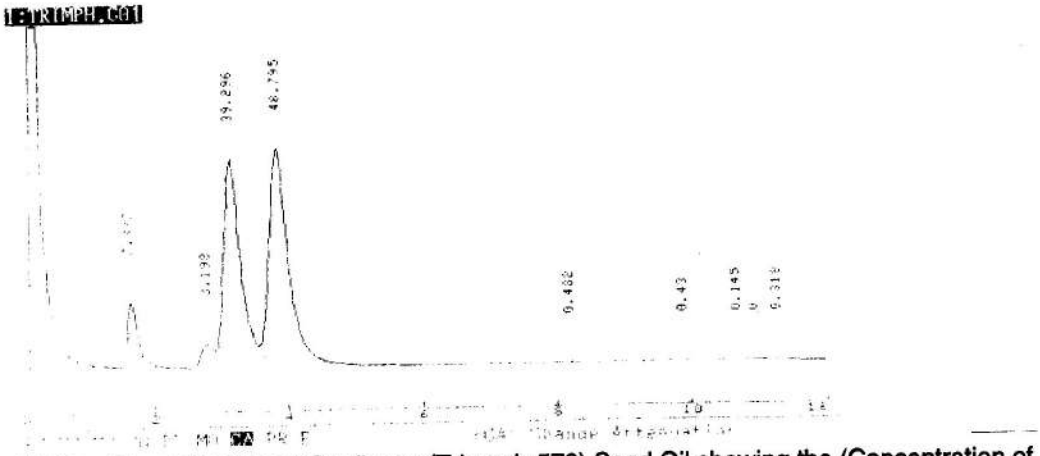

Fig III. Gas Chromatogram of Sunflower (Triumph-573) Seed Oil showing the (Concentration of Fatty Acids)

PAK. J. FOOD SCI., 13(3-4):45-46

Studies on suitability of olive varieties for pickling

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ABSTRACT

Four olive (*Olea europaea*) varieties i.e. Oltobratica, Leccino, Morailo and Caratino were evaluated in the present study. Unripe, green fruits at right maturity stage were selected. After washing, fruits were treated with 2% lye solution at ambient temperature for 48 hours for debittering. Then, washed thoroughly till lye free. The pickling process of olives was carried out at 35 - 40 °C by dipping the fruits in 15% brine solution for one week. Then 15% brine solution was replaced by a mixture of 10% brine + 4 % acetic acid solution. Samples so prepared were packed in glass jars and kept at ambient temperature for further studies from 180 days. Sensory evaluation i.e., color, flavor, taste and texture revealed that pickle prepared from Caratino variety ranked at the top followed by Ottobratica, Leccino and Morailo respectively.

Keywords: Olive, oil, pickling, quality

INTRODUCTION

Olive (*Olea europaea,* family Oleacdeae L.) is an evergreen xerophytic tree grown for its drupes, which yield oil and are also marketed as table or pickled olives. This fruit has been closely associated with religious, sociocultural, medicinal and nutritional needs of man Spain, Italy and Greece are leading producers of olive found abundantly in Northern Areas of Pakistan but they are not of much economic value. It has been observed that wild olive grows naturally in Murree Hills, Soan Valley, Swat, Malakand, Kurram Agency and Azad Jamu and Kashmir covgung an area of 0.25 million hectare. During its maturity, the olive fruit passes though successive shades of straw, pink and red, before finally turning purplish-black.

Apart from other factors affecting the propagation of olive fruit badly one of the major reason is the least attention to the processing of this fruit into various products. Taking into consideration the nutritional and medicinal values of this fruit the studies were planned to utilize the available olive varieties for the preparation of pickle through brining.

Table 1. Fruit Composition

Constituents	Green olive	Ripe olive
Moisture	78%	73-84
Protein	1.4	1.1
Fat	13	9-20
Carbohydrate	1.3	2.5-3.5
Ash	6.4	2.5
Sodium	323 mg/4 olives (16 g)	96 mg/4 olives (16 g)

Source: Food Science Sourcebook Part-1 by Herbert W.Ockerman

MATERIALS AND METHODS

Following four olive varieties were procured from Barani Agricultural Research Institute, Chakwal.

T1 = Leccino
T2 = Morailo
T3 = Caratino
T4 = Ottobratica

The fruits were selected at right stage of maturity with straw green colour. After washing thoroughly, the fruits were treated with 2 % lye (NaOH) solution (for 48 h) till bitterness of the fruit was removed. Then frequent water washings were applied to make the fruits lye free. The pickling process of the fruit was then carried out by dipping it in 15% brine (NaCl) solution and incubating at 35-40° C till a desirable pickled flavour developed in the fruits. After the completion of pickling the fruits of each variety were packed in glass jars containing 10% brine and 4% acetic acid solution. The samples were stored at ambient temperature for 180 days. The sensory evaluation-was carried out at 30 days intervals to observe the storage behaviour of the samples regarding taste, colour, flavour and texture. The sensory evaluation was carried out by a panel of 4 judges of Food Technology Section using nine point Hedonic scale rating.

RESULTS AND DISCUSSION

The aggregate scores regarding taste, colour, flavour and texture of the pickle from olive varieties are given in the table:

Table. 2. Effect of storage on the sensory evaluation of olive pickle

TASTE

Treatment	0	30	60	90	120	150	180
T_1	7.75	7.75	7.50	7.50	7.00	7.00	6.75
T_2	6.50	6.50	6.50	6.25	6.00	6.00	6.00
T_3	8.75	8.75	8.75	8.75	8.50	8.50	8.50
T_4	8.50	8.50	8.50	8.25	8.25	7.75	7.75

COLOUR

Treatment	0	30	60	90	120	150	180
T_1	7.75	7.75	7.50	7.50	7.00	7.00	7.00
T_2	6.75	6.75	6.75	6.25	6.00	6.00	6.00
T_3	8.75	8.75	8.75	8.50	8.50	8.50	8.50
T_4	8.50	8.50	8.50	8.25	7.75	7.75	7.50

FLAVOUR

Treatment	0	30	60	90	120	150	180
T_1	6.75	6.75	6.75	6.75	6.50	6.50	6.50
T_2	6.00	6.00	6.00	6.00	5.75	5.75	5.75
T_3	8.00	8.00	8.00	8.00	7.75	7.75	7.75
T_4	7.75	7.75	7.50	7.50	7.00	6.75	6.75

TEXTURE

Treatment	0	30	60	90	120	150	180
T_1	6.75	6.75	6.75	6.50	6.25	6.25	6.25
T_2	6.75	6.75	6.75	6.50	6.50	6.00	6.00
T_3	8.75	8.75	8.75	8.75	8.75	8.50	8.50
T_4	8.00	8.00	8.00	7.75	7.50	7.50	7.50

The results indicated in the table that the pickle prepared from Caratino variety ranked at the top followed by Ottobratica, Leccino and Morailo. Although a declining trned and significant changes (6) observed (figure 1, 2, 3, 4) in the organoleptic attributes of pickle from all the varieties but least changes were noted in the Caratino variety.

REFERENCES

Larmond E. 1987. Laboratory Methods for Sensory Evaluation of Foods. Research Branch Canada . Deptt AgriPub No. 1637

Steel RGD, Dickey D and Torrie JH. 1980. Principles and procedures of Statistics. A Biometrical approach. 3rd ed. McGraw Hill Book Co. Inc. New York.

Rama AM, Khan R and Munir M. 1988. Oilseed crops in Pakistan, Status, Constraints and Strategy. Pak Agric Res Council Publication Islamabad.p-69-71.

Ahmad S. 2002. Fruits and vegetables Export. Export Promotion Bureau, Govt Pakistan. Published in Brochure, Hortl. Foundation of Pakistan. Islamabad, Pakistan. p-107-109.

Godin VJ and Spensley PC. 1971. Oils and oilseeds. Tropical Products Institute England. Crop. Product Digest. P-100-101.

Wolff IA. 1973. Handbook of Processing and Waste Utilization in Agriculture. Plant Products Part-2, Vol.11:410-3 CRC Press, Florida.

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Chemical composition and cooking quality of some rice cultivars in Pakistan as affected by parboiling

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ABSTRACT

It was found that parboiling treatment significantly reduced moisture content. Change in protein was found as a function of rice cultivars and no effect on protein content was observed as a result of parboiling. Fat content in parboiled rice was found lower as compared non parboiled rice .The results obtained revealed that parboiling treatments reduced the fat content to a certain extent. The results for crude fiber indicated the change in content within varieties and not the parboiling treatments. Ash content varied significantly due to rice cultivars, parboiling treatments and interaction between cultivar and parboiling treatment. Iron content varied with variety, treatments and their interaction. It was concluded that the severity of parboiling treatment directly related to the iron concentration in the rice, i.e., more severe the parboiling more the iron content in the rice. No effect of parboiling was observed on copper, zinc, and magnesium. However calcium content was significantly affected by various parboiling treatments. Parboiling treatments increased sodium, potassium and manganese concentration in the rice cultivars.
Key words: Par boiling, rice cultivars, micronutrients, cooking quality

INTRODUCTION

In Pakistan rice it is the second largest staple food and a major foreign exchange earning crop. The chemical analysis of rice shows that most of the nutrients which are concentrated in the outer layer of the rice kernel are lost during milling. After soaking paddy in water and subsequent steaming, water soluble nutrients are retained to a greater extent in the kernel(Ali and Ojha 1976) The advantages claimed for parboiled rice comprise the improved nutrient availability, decreased susceptibility to insect attack during storage, decreased washing and cooking loss, more swelling when cooked to desirable softness, improved digestibility with high protein efficiency ratio and stabilization of the oil content in the bran. (Pillaiyar 1990). About 20% of the rice produced world wide and more than 50% rice produced in south Asia including Pakistan is parboiled (Chaudhry 1991). The rice kernel during parboiling undergoes a changes primarily chemical in nature which affects change in composition and properties of grain. The improved chemical composition of the milled parboiled rice is attributed to less removal of the material during milling process and diffusion of water soluble constituents, especially B vitamins (thiamin, riboflavin, niacin), free amino acids and various minerals (Ca, P and Fe) from outer layer into the interior of endosperm (Bandyopadhyay and Roy 1992). Luh and Mickus (1991) reported that changes during parboiling have been considered a major contributor to alter its physico-chemical and cooking qualities.

Parboiling reduces the amount of solids leached into cooking water and the extent to which the kernels solubilize during cooking. Therefore parboiling is carried out to improve chemical composition, storage stability and cooking attributes of rice. (Pomeranz, 1987)

MATERIAL AND METHODS

Four Pakistani rice cultivars i.e. Iri-6, Irri-9, Super Basmati and Basmati 2000 were obtained from Rice Research Institute, Kala Shah Kaku, Lahore an brought to the laboratory of Safa Rice Mills Ltd., Kasur for studies. The parboiling was carried out by using Laboratory Parboiling Apparatus (Gariboldi Engineering Co. Carugate-MI, Italy). Each cultivar was soaked, steamed and dried for accomplishment of the whole parboiling process. The parboiling was carried out at two levels /treatments i.e. (parboiling at 0.5 Kg/ cm^2 for 10 minutes) T_1 which was mild parboiling and T_2(parboiling at 1.0 Kg/ cm^2 for 10 minutes) which was severe parboiling and T_3(Non- parboiled) which was control. Moisture, crude protein, crude fat, crude fiber and ash contents in all the rice samples were determined by the method described in AACC(2000). Nitrogen free extract was calculated according to expression NFE (%) = 100- (moisture content % +, crude protein % + crude fat% +, crude fiber % + ash contents %). Mineral content i.e. Fe, Ca, Mg, Na, Zn, Cu, and Mn were determined by Atomic Absorption Spectrophotometer according to the method described in AOAC (1990). The samples were wet digested as reported by Richard (1969). In order to estimate the changes in cooking quality of the rice

cultivars as a function of treatments, water uptake ratio, equilibrium moisture contents, sedimentation volume tests were performed, as described by Bhattacharya (1979) and volume expansion ratio by the method given by Juliano (1971).The data were analyzed using the statistical techniques described by Steel *et al.* (1996).

RESULTS AND DISCUSSION

The moisture contents of various rice cultivars ranged from 10.72% to 10.92% when parboiled treatments were pooled (Table1). The moisture content was the highest in the non-parboiled rice while the moisture content was significantly lower in rice cultivars parboiled at 1.0 kg/cm^2 for 10 minutes. The result indicated that moisture contents varied from 10.28% to 11.28% among different parboiling treatments. These results indicating a reduction in moisture content by parboiling treatments are in conformity with those of reported by Houston and Kohler (1970). The results revealed that protein content was not significantly affected by different parboiling treatments. However rice cultivars significantly showed a change in protein content. The protein content varied from 6.95% to 7.06% among the rice cultivars subjected to various parboiling treatments (Table1). Non-significant results for protein contents may be attributed to the fact that the protein bodies are ruptured in the rice and solubility of protein decreased after parboiling because protein substances are separated and sink into the compact mass of gelatinized starch and hence become less susceptible to extraction. Similar findings have been given by Raghavandre Rao and Juliano (1971) and Benedito de Barber *et al.* (1977) who reported that the protein content of brown rice or milled rice seems to be unaffected by the parboiling treatment. Fat contents of rice cultivars varied significantly among treatments and cultivars but the interaction between rice cultivars and parboiling treatments did not show significant effect on this chemical constituent. The fat content was significantly lowest super Basmati than other cultivars. The fat contents ranged from 0.572% to 0.603% among rice cultivars but in parboiling treatments, variation was observed from 0.526 to 0.623% (Table2). The results indicated that parboiling treatments reduced the fat contents to a certain extent in the milled rice. These results are in line with those of Zecchinelli and Fossati (1983) and Bhattacharya and Ali (1985) who reported that parboiling disrupted the oil globules and tended to push the oil towards the outer periphery of the kernel. Fiber content was not significantly affected by different parboiling treatments. However fiber content varied significantly among various rice cultivars (Table 2). The interaction between rice cultivars and treatments was also found to be non-significant. These results are confirmed by the earlier findings of Houston and Kohler (1970) who found that fiber contents are not affected by parboiling treatment. Significant results were found for ash content due to the rice cultivars,

Table 1. Mean Values for the effect of parboiling on moisture, ash and protein contents of rice cultivars

Moisture(%)		Ash (%)		Protein(%)	
Cultivar Mean	Treatment Mean	Cultivars Mean	Treatment Mean	Cultivars Mean	Treatment Mean
10.92a	11.28a	0.573c	0.520c	6.97b	0.315a
10.72b	10.80b	0.600a	0.586b	6.95b	0.311a
10.78b	10.28c	0.577b	0.639a	7.06a	0.309a
10.72b	----	0.567c	----	7.03ab	----

The mean values with same letter in the column are non-significantly different (LSD:P=0.05)

Table 2. Mean values for the effect of parboiling on fat, fiber and NFE contents of rice cultivars

Fat(%)		Fiber(%)		NFE(%)	
Treatment Mean	Cultivar Mean	Treatment Mean	Cultivar Mean	Cultivar Mean	Treatment Mean
7.02a	0.586b	0.623a	0.319a	80.66d	80.07c
7.01a	0.603a	0.589b	0.312b	80.85a	80.69b
6.96a	0.577d	0.526c	0.311b	80.73c	81.47a
	0.572c	----	0.303c	80.77b	----

The mean values with same letter in the column are non-significantly different (LSD:P=0.05)

Table 3. Mean values for the effect of parboiling on iron, zinc and magnesium content of rice cultivars

Iron(mg/100g)		Zinc(mg/100g)		Magnesium(mg/100g)	
Cultivars Mean	Treatment Mean	Cultivars Mean	Treatment Mean	Cultivars Mean	Treatment Mean
1.008d	0.700c	1.196a	1.176a	26.64ab	25.86a
1.069c	1.354b	1.164b	1.173a	25.08c	26.77a
1.268a	1.383a	1.180ab	1.165a	26.05b	26.02a
1.238b	----	1.145c	----	27.10a	----

The mean values with same letter in the column are non- significantly different (LSD:P=0.05)s

parboiling treatment and interaction between cultivars and parboiling treatments (Table1). Bhattacharya and Ali (1985), Houston and Kohler (1970), Raghavandre Rao and Juliano (1970) reported similar results. Concentration of ash as a result of parboiling may be due to the diffusion of various water-soluble constituents from the outer layer into the interior of the grain during processing of parboiling. NFE varied significantly among different rice cultivars, parboiling treatment as well as interaction between cultivars and treatments (Table2). Houston and Kohler (1970) reported that NFE was increased due to the combined effect on the chemical constituents by parboiling treatments. Iron content was significantly affected by the rice cultivars, parboiling treatments and interaction between cultivars and parboiling treatments. Severity of parboiling treatment yielded significantly more iron concentration than the rice samples received mild parboiling treatment (Table3). The interactive effect of cultivar and treatment also showed a significant effect on iron content. The existing results are in accordance with the finding by Gariboldi(1984),Bhattacharya and Ali (1985) and Luh and Mickus(1991) who reported that water-soluble constituents diffused from outer layers into the endosperm during soaking, steaming and redistributed among various parts of endosperm. Zinc content was not influenced significantly by different parboiling treatment (Table3). However Zn content in rice cultivars varied significantly. Calcium, Sodium and Potassium contents varied significantly with parboiling treatment but magnesium content was not affected by parboiling treatment. Magnesium

Table 4. Mean values for the effect of parboiling on iron, zinc and magnesium content of rice cultivars

Calcium (mg/100g)		Sodium (mg/100g)		Potassium (mg/100g)	
Cultivars Mean	Treatment Mean	Cultivar Mean	Treatment Mean	Cultivars Mean	Treatment Mean
42.79c	24.41c	4.91c	4.67c	102.33b	102.80c
40.26d	52.55b	4.54d	5.07b	99.40c	106.90b
46.45a	53.46a	5.40a	5.42a	114.25a	111.60a
44.39b	----	5.36b	----	112.40a	----

The mean values with same letter in the column are non-significantly different (LSD:P=0.05)

Table 5. Treatment means for the effect of parboiling on the cooking quality of rice cultivars

% Water uptake		% EMC-S		mL Sedimentation vol.		Ratio Volume expansion	
Cultivars Mean	Treatment Mean	Cultivars Mean	Treatment Mean	Cultivars Mean	Treatment Mean	Cultivars Mean	Treatment Mean
18.90b	4.87c	64.66a	31.02c	7.54a	5.51c	3.79a	3.54
18.99a	10.27b	62.95b	62.64b	7.52a	6.82b	3.20c	3.50
16.78d	38.94a	54.59d	86.11a	6.51b	8.78a	3.12c	3.58
17.45c	----	57.50c	----	6.58b	----	3.54b	----

The mean values with same letter in the column are non- significantly different (LSD:P=0.05))

content significantly varied with cultivars like Ca, Na, and K. In case of magnesium the interactive effect of cultivars and treatments was also found to be non significant (Table2). These result are in line with those of Ocker *et al.* (1977) who concluded that Mg content are unaffected by parboiling treatment. Similarly interactive effect of treatments & cultivars was found to be significant in case of Ca, Na and K (Table4). Various parameters for assessing the cooking quality of parboiled rice. Water uptake ratio varied significantly among rice cultivars, treatments and interaction between cultivar and treatment. Severe parboiling treatment T_2 showed significantly highest effect in this character as compared to other parboiling treatments. The mean values for water uptake ranged from 16.78% to 18.99% among rice cultivars subjected to the parboiling treatment (Table5). These results resembled with those of Unni krishman and Bhattacharya (1987) who reported that water uptake at 60 ^{0}C to that at boiling temperature (W_{60}/W_{96}) gave an excellent differentiation of raw rice from parboiled rice produced under different conditions. Equilibrium Moisture contents (EMC-S) was significantly affected by parboiling treatment and rice cultivars. The interaction between the rice cultivars and parboiling treatment was also found to be significant (Table 3). Ali and Bhattacharya (1972) reported that such a change was caused by reduced starch retrogradation in parboiling process. The results showed that Parboiling treatment and rice cultivar are significantly affected sedimentation volume. T_2 gave significantly the highest value of sedimentation volume of rice cultivars (Table5). Volume expansion ratio of Irri-6 gave significantly highest value, Irri-9 and Super Basmati rice cultivars did not show significant difference with each other (Table5). Such results conform to some extent to the results of Tufail (1997) who also reported volume expansion ratio to be 3.20,3.64,3.08,3.08 and 2.35 from Super Basmati, Basmati 383, Basmati 370, Irri-6 and KS-282 respectively.

CONCLUSION

Parboiling carried out under a definite set of process conditions affected the various physicochemical and cooking quality parameters of rice. Mineral elements are available in relatively larger quantity due to retention of these constituents during soaking and steaming process of parboiling process. The rice cultivars parboiled at 1.0 kg/cm^2 for 10 minutes showed the significant variation with respect to moisture, fat, ash, NFE and mineral elements. The cooking quality attributes also varied significantly due to difference in treatments as well rice cultivars.

REFERENCES

AACC. 2000. Official Methods of Analysis. The American Association of Cereal Chemist, Inc St Paul Minnesota.

AOAC 1990. Official Methods of Analysis. The Association of the Official Analytical Chemists.15th ed Arlington, Virginia.

Ali N and Ojha TP. 1976. Parboiling in rice. Post Harvest Technology. Intern Develop. Research Center. Ottawa, Canada.

Ali SZ and Bhattacharya KR. 1972. Hydration and amylose-solubility behavior of parboiled rice. Lebensm WissTechnol 5:207-12.

Bandyopadhyay S and Roy NC. 1992. Rice process technology. Oxford and IBH Pub Co New Delhi.

Benedito de Barber C, Martinez J and Barber S. 1977. Effects of parboiling on the chemical composition and nutritional characteristics of rice bran. In: Rice by-products utilization. Vol IV Eds S Barber and E Tortosa Valencia.

Bhattacharya KR. 1979. Tests for parboiled rice. In: Proceedings of the workshop on chemical aspects of rice grain quality. IRRI. Los Banos Launa Philippine.

Bhattacharya KR and Ali SZ. 1985. Changes in rice during parboiling and properties of parboiled rice. In: Advances in cereal science and technology (ed Y. Pomeranz).

Chaudhry NH. 1991. Parboiling and consumer demand for parboiled rice in South Asia. In: Rice grain marketing and quality Issues. IRRI 1099, Manila.

Gariboldi F. 1984. Rice parboiling. FAO Agric Services. Bull.No.56.Food and Agricultural Organization of the United Nations Rome.

Houston DF and Kohler GO. 1970. Nutritional properties of rice. National Academy of Science, Washington, DC.

Juliano BO.1971. A simplified assay for milled rice amylose. J Cereal Sci 16(10):334-40.

Luh BS and Mickus RR. 1991. Parboiled Rice.In: Rice Utilization.Vol.II 2nd ed.B.S.Luh Van Nastrand Reinhold, New York.

Ocker HD, Bolling H and Elbaya AW. 1977. Effects of parboiling on vitamins and mineral contents of milled rice Rice report p:44-46.

Pillaiyar P. 1990. Rice parboiling research in India. Cereal Food World 35(2):225-7.

Pomeranz Y. 1987. Modren cereal science and technology VCH Pub Inc Weinheim, Germany.

Raghavandra Rao SN and Juliano BO.1970. Effects of parboiling on some physicochemical properties of rice. J Agric Food Chem 18(2):289-94.

Ricchard LA. 1969. Diagnosis and improvement of saline .and alkali soils. U.S. Deptt Agri Pub Washington DC.

Steel RGD Dicky D and Torrie JH. 1996. Principles and procedures of statistics; A Biometrical approach. 3rd ed. McGraw Hill Book Co. New York.

Tufail S.1997. Cooking and eating quality of some cultivars of rice. M.Sc. Thesis . Deptt. Food Tech University of Agriculture, Faislabad.

Krishnan KR and Bhattacharya KR. 1987. Properties of pressure parboiled rice as affected by variety. Cereal Chem 64(5):321-3.

Zecchinelli P and Fossati G. 1983. Comparison of crude lipids and total lipids in parboiled and non-parboiled rice. Rivesta dells Societa Italiana di scienza dell Alimentazione 12(3):173-6.(Rice Abst 8(3):397,1985).

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The effect of chickpea and guar gum addition to wheat flour on reduction of blood glucose and cholesterol in rats

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ABSTRACT

The Use of dietary fibers helps in reducing the blood cholesterol and glucose levels. In this experiment composite chickpea and guar gum flours were selected to study their effect on blood glucose and cholesterol levels. The experiment was run with four treatments, replacing wheat flour with chickpea at 5, 7.5 and 10% in three treatments, while replacing wheat flour with 5% chickpea and 1% guar gum in the fourth treatment. The effect of composite dietary flour on the sensory parameters was also studied and it was found that 5% chickpea and 1% guar gum gave best chapattis in sensoric attributes. Results from this study conclude in encouraging use of composite flours, especially those containing at least 5-7% dietary fiber, which significantly reduces the blood glucose and serum cholestrol.

Keywords: Chickpea, guar gum, composite flour, blood glucose, blood cholesterol

INTRODUCTION

The chickpea products contained 8.9-21.1% protein, 3.1-21.8% fat, 53.4-75.9% carbohydrate, 1.6-11.1% crude fiber, 1.2-5.9% ash. (Khan *et al* 1995). Crude fiber and protein increased from 0.36% to 0.55% and from 14% to 17.6%, respectively, when 15% chick-pea flour was added to the wheat flour for bread making. (Estevez *et al* 1987).

The feasibility of adding chick-pea flour substituting part of wheat flour in yeast-leavened bread-making in order to increase the protein value was studied. Chick-pea flour of commercial granulometry (150 mu) was prepared. Wheat flours of 74% and 78% extraction were then blended with 5%, 10% and 15% of chick-pea flour. Every flour and blend were subsequently analyzed to determine protein, ash, fiber, fat and maltose content, as well as sedimentation, farinograph and bread-making. Addition of chick-pea flour increased protein, fiber, ash and fat content in the blends, not causing a severe effect on quality, even at the 15% level of substitution. (Figuerola *et al* 1987).

Experimental bread made of wheat flour complemented with 5, 10 and 15% chick-pea flour was studied, using wheat bread as control. Samples were analyzed for their proximate chemical composition and amino acids content. Crude fiber and protein increased from 0.36% to 0.55% and from 14% to 17.6%, respectively, when 15% chick-pea flour was added. Bread nutritive value was significantly improved by adding chick-pea flour (Estevez et al. 1987).

Chickpea cakes were prepared according to the procedure of the traditional cake after replacing 0, 25, 50, 75 and 100% of wheat flour by chickpea flour. Ingestion of chickpea cakes did not alter the levels of serum proteins, globulins or albumins. Chickpea cakes had a lowering effect on the serum glucose levels of rats. Chickpea cakes had a cholesterol lowering effect either in the rat serum or organs: liver, kidney and heart. (Lasztity and Sharobeem 1992).

Duane (1997) concluded that legume consumption appears to lower serum cholesterol. Nine human subjects were studied on a metabolic ward during two randomly ordered 6-7 week periods: one during consumption of a control diet and the other during consumption of the same diet with 120 gm mixed legumes substituted for foods having equivalent calories, fat, protein, and carbohydrate. Mean serum LDL cholesterol was significantly lower during legume consumption (126 vs. 138 mg/dl).

Research has proved that ingestion of Fiber can be really helpful in reducing the glucose and cholesterol levels by reducing their absorption in the body (Shen-Hong *et al* 1998). Zulet *et al* (1999) reported that the chickpea was more effective than the control diet containing casein in the normalization of triglycerides as well as total and LDL-cholesterol levels. Wang and McIntosh (1996) studied the effect of feeding legumes containing peas (*Pisum sativum*) and chickpeas (*Cicer arietinum*) on plasma cholesterol. Plasma cholesterol concentrations were lower in rats fed legumes than in those fed casein.

A feeding trial was conducted in order to evaluate the potential effect on the lipid profile in a experimentally induced situation of hypercholesterolemia of a previously uninvestigated legume (Cicer arietinum L.). Significantly decreased concentrations of total

cholesterol (-54 percent) and triacylglycerols (-70 percent) as well as the levels of LDL (-54 percent) and VLDL (-70 percent) were seen in rats fed chickpeas. This suggests, for apparently the first time, that chickpea consumption may have a corrective effect in some alterations of the lipid profile (Zulet and Martinez 1995).Zulet *et al* (1999) reported the data concerning carbohydrate utilization and indicated the potential positive effects of chickpea in diabetes therapy and its role as biological active food supplements.

Lalor *et al* (1990) studied that guar gum can reduce fasting blood glucose from 11.4 +/- 3.7 mmol L-1 to 9.5 +/- 3.9 mmol L^{-1}. Reduction in low-density lipoprotein (LDL) cholesterol, possibly due to increased faecal loss, while high-density lipoprotein (HDL) cholesterol levels were unchanged has been reported following guar gum treatment. (Jenkins *et al* 1979: Smith and Holm 1982)

A scientific review concluded that comparable cholesterol lowering could be achieved with daily consumption of 8-36 g guar gum and 100-150 g dried beans or legumes. LDL cholesterol can be reduced by 5-10%. It is thought that soluble fiber lowers blood cholesterol by binding bile acids, which are made from cholesterol to digest dietary fats, and then excreting them (Glore *et al* 1994).

MATERIALS AND METHODS

Procurement of Wheat Flour, Chickpea and Guar gum

Wheat flour was collected from Aysha Flour Mills (Private) Limited, Faisalabad while Chickpea and guar gum were purchased from the local market of Faisalabad.

Fortification of Wheat Flour

Wheat flour was mixed with chickpea at levels of 5, 7.5 and 10% replacement while a fourth treatment was prepared by mixing 5% chickpea and 1% guar gum in wheat flour.

Proximate Analysis of flour samples

The control and composite flour samples were analyzed for moisture, ash, crude protein, crude fat, crude fiber and NFE according to their respective methods described in AACC (2000).

Preparation of Chapatti

Chapattis were prepared from various composite flours according to the conventional method followed at home.

Sensory evaluation of Chapattis

The chapattis from control and composite flours were tested by a panel of judges to evaluate for appearance, foldingability, chewinga-bility, texture, taste, flavor and color. The results were scored on Hedonic score system according to the method described by Larmond (1977). The best treatment of composite flour was selected for efficacy trials.

Biological Studies

The best-selected treatment along with wheat flour (control) was fed to rats. At the end of 28 days rats were decapitated to collect their blood. Plasma was procured to analyze the reduction in blood glucose and cholesterol.

Statistical Analysis

The data obtained for each parameter was subjected to statistical analysis to determine the level of significance according to the methods described by Steel *et al* (1997).

RESULTS AND DISCUSSION

Proximate Analysis of Flour samples

Proximate analysis of different flour samples was carried out. The results described in Table I illustrated that highest moisture contents were found in wheat flour (11.08%) while lowest value was seen in 10% chickpea composite flour (10.12%). The means regarding ash content revealed that 10% chickpea composite flour had the highest significant value (1.92%) while lowest mean was observed in wheat flour (1.64%). Protein contents were significantly highest in 10% chickpea composite flour (15.98%) while lowest in wheat flour (11.91%). Fat contents were significantly highest in 10% chickpea composite flour (1.92%) and lowest in wheat flour (1.64%). Fiber contents were also found to be significantly highest in 10% chickpea composite flour (1.03%) while lowest in wheat flour (0.59%). NFE contents were significantly highest in wheat flour (73.14%) while lowest in 10% chickpea composite flour (68.54%). Khan *et al* (1995) observed that the chickpeas products contained 8.9-21.1% protein, 3.1-21.8% fat, 53.4-75.9% carbohydrate, 1.6-11.1% crude fiber, 1.2-5.9% ash. Estevez *et al* (1987) reported that crude fiber and protein increased from 0.36% to 0.55% and from 14% to 17.6%, respectively, when 15% chick-pea flour was added to the wheat flour for bread making.

Table I. Proximate composition of different flour samples.

Treatments	Moisture %	Ash %	Crude Protein %	Crude Fat %	Crude Fiber %	NFE %
T1	11.08 a	1.64 d	11.91 e	1.64 d	0.59 e	73.14 a
T2	10.50 c	1.79 c	14.04 c	1.79 c	0.90 d	70.81 b
T3	10.28 d	1.88 b	14.98 b	1.88 b	0.95 b	69.88 d
T4	10.12 e	1.92 a	15.98 a	1.92 a	1.03 a	68.54 e
T5	10.73 b	1.68 d	13.78 d	1.68 d	0.93 c	71.05 c

T1 = wheat flour
T2 = wheat flour+ 5% chickpea
T3 = wheat flour+ 7.5% chickpea
T4 = wheat flour+ 10% chickpea
T5 = wheat flour+ 5% chickpea+ 1% guar gum

Sensory Evaluation of Chapattis

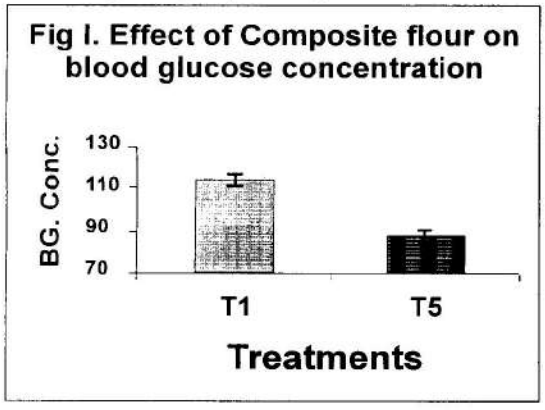
The chapattis prepared from different flour samples were evaluated for sensory evaluation including color, flavor, taste, texture, chewing ability, folding ability and appearance. The results are described in Table II. Composite flour of 5% chickpea and 1% guar gum gained maximum scores of 8, 7, 7, 8, 7, 7, and 8 for color, flavor, taste, texture, chewing ability, folding ability and appearance respectively. Wheat flour was declared to be second while composite flour containing 10% chickpea gained the lowest score.

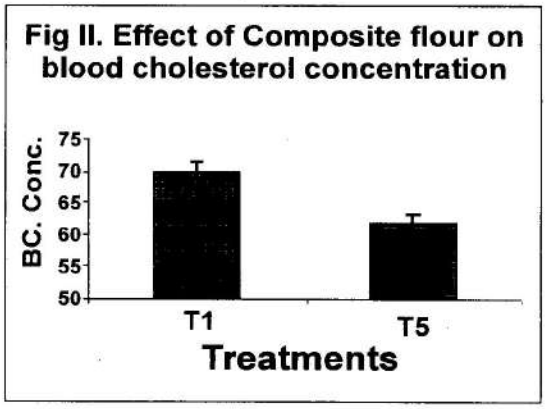
Table II. Sensory evaluation of chapattis

Treatments	Color	Flavor	Taste	Texture	Chewing ability	Folding ability	Appearance
T1	7	7	8	6	7	7	7
T2	6	7	7	6	6	7	7
T3	6	5	6	7	5	6	7
T4	5	5	6	6	6	5	6
T5	8	7	7	8	7	7	8

BIOLOGICAL STUDIES

The best selected treatment of composite flour (5% chickpea+1% guar gum) along with wheat flour (control) was fed to male albino rats for a period of 4 weeks. At the end of the experimental period rats were decapitated and blood was collected. Plasma was procured from the blood and used to analyze the blood glucose and blood cholesterol concentrations. The results (Fig I) revealed that the group fed wheat flour had significantly higher concentration of blood glucose and blood cholesterol i.e. 114 mg/dl and 70 mg/dl respectively while the group fed 5% chickpea composite flour had significantly lower blood glucose and blood cholesterol reduction i.e. 88 mg/dl and 62 mg/dl respectively. It was concluded from the results obtained that 5% chickpea and 1% guar gum composite flour caused a reduction of 22.08% in blood glucose and 11.43% in blood cholesterol concentration as compared to control.





Duane (1997) concluded that legume consumption appears to lower serum cholesterol. Lalor *et al* (1990) studied that guar gum can reduce fasting blood glucose from 11.4 +/- 3.7 mmol L-1 to 9.5 +/- 3.9 mmol L^{-1}. Chickpea cakes had a cholesterol lowering effect either in the rat serum or organs: liver, kidney and heart. (Lasztity and Sharobeem, 1992).

CONCLUSION

It is concluded from the investigation that composite flour of chickpea and guar gum is helpful in decreasing blood glucose and blood cholesterol concentration and ultimately in the reduction of the risk of developing diabetes and coronary heart diseases.

LITERATURE CITED

AACC. 2000. Approved methods of American Association of Cereal Chemists. The American Association of Cereal Chemists Inc St Paul Minnesota.

Duane WC. 1997. Effects of legume consumption on serum cholesterol, biliary lipids, and sterol metabolism in humans. Journal of Lipid Research Vol 38 1120-1128.

Estevez AM, Figuerola F, Vasquez M, Castillo E and Yanez E. 1987. Supplementation of wheat flour with chickpea (Cicer arietinum) flour. II. Chemical composition and biological quality of breads made with blends of the same Arch Latinoam Nutr 37(3):515-24.

Figuerola FE Estevez AM Castillo E. 1987. Supplementation of wheat flour with chickpea (Cicer arietinum) flour. I. Preparation of flours and their properties for bread making] (Arch Latinoam Nutr 37(2):378-87.

Glore SR, Van Treeck, D Knehans AW and Guild M 1994. Soluble fiber and serum lipids: a literature review. J Am Diet Assoc, 94:425–36.

Jenkins DJA, Leeds AR, Slavian B, Mann J and Jepson EM 1979. American Journal of Clinical Nutrition 32:16-18.

Lalor BC Bhatnagar D Winocour PH Ishola M Arrol S Brading M and Durrington PN. 1990. Placebo-controlled trial of the effects of guar gum and metformin on fasting blood glucose and serum lipids in obese, type 2 diabetic patients. Diabetes Med 7:242-245

Larmond E. 1977. Laboratory Methods of Sensory Evaluation of Foods. Research Branch, Canada. Department of Agriculture Pub: 1637.

Lásztity R Sharobeem SF. 1992. Biochemical Evaluation Of Chickpea Cakes As Administered To Albino Rats Per Pol Chem Eng 36(1):71-80.

Shen-Hong He-Lin Price RL Fernandez ML Shen H and He L. 1998. Dietary soluble fiber lowers plasma LDL cholesterol concentrations by altering lipoprotein metabolism in female guinea pigs. J Nut 1998 128(9):1434-1441.

Smith U and Holm G. 1982. Cholesterol lowering effects of guar gum Atherosclerosis 45:1-10.

Steel RJH Torrie and D Dickey. 1997. Principles and Procedures of Statistics. A Biometrical Approach, 3rd ed McGraw Hill Book Co Inc New York.

Zulet MA Macarulla MT Portillo MP Noel-Suberville C Higueret P and Martínez JA. 1999. Lipid and glucose utilization in hypercholesterolemic rats fed a diet containing heated chickpea (Cicer Aretinum L.): a potential functional food. International Journal for Vitamin and Nutrition Research, 69(6):403-411.

Zulet MA and Martinez JA. 1995. Corrective role of chickpea intake on a dietary-induced model of hypercholesterolemia. Plant Foods Hum Nutr 48(3):26

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Milling quality of rice

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ABSTRACT

Various factors such as variety/cultivars, cultural practices, environmental factors & the drying, storing & milling conditions affect milling quality of rice. Rice varieties/cultivars with white center gave the lowest milling recovery. In general cultivars with long grains & those having chalky grains gave lower head rice recovery. High milling recovery is generally associated with hardness & an absence of chalky spots in the endosperm. Varieties with high protein content have less breakage than those with low protein. High levels of N decreased the head rice recovery of long slender grain varieties. Head rice recovery of Supper Basmati was significantly higher in case of three splits of N fertilizer than those of two splits. Complete pudding before transplanting gave higher milling recovery. Early transplanting reduced the milling recovery of rice. Threshing 0-5 days after harvesting gave high head rice recovery. Drying at low temperature (40-50°C) is better than at high temperature (60-70°C). The quantity of total milled rice & head rice was higher in case of modern rubber roller, followed by disc sheller & steel huller.

Key words: Rice, milling quality, head rice, milled rice, chalkiness

INTRODUCTION

Rice means milled rice which includes cargo rice, white rice, and parboiled rice. Cargo rice means rice obtained from paddy of which only the husk has been removed (brown rice or husked rice). White rice means rice obtained from paddy which has been husked and milled white by removing its bran (aleurone) layers. Parboiled rice may be husked or milled rice, processed from paddy or husked rice that has been soaked in water to gain its equilibrium moisture content and subjected to a heat treatment so that the starch is fully gelatinized, followed by a drying process. The milling process generally consists of removing foreign matter from paddy, hulling, polishing and grading. Foreign matter means all material other than kernel, bran or paddy. It may include pieces of straw, dust, pebbles, etc. Hulling involves removing the husk from the paddy with minimum damage to the grain and separating the husk to produce brown rice. The process of removing the embryo and the outer bran layer from brown rice is known as whitening. While, polishing is a process of removing the subaleurone layer after whitening to give rice a shiny and pearly appearance.

In Pakistan milling process is characterized as extra well-milled, well-milled, reasonably well-milled, ordinarily milled and under-milled. Extra well-milled means the milling of paddy from which the husk, the germ, and the outer bran layer together with the inner ones have been completely removed to the extent that the appearance of the kernel is translucent except those varieties which have white abdomen as a distinguishing characteristics. Well-milled means the milling of paddy from which the husk, the germs and both of its outer and inner bran layers have been entirely removed to a degree that the translucence of the kernel is slightly lesser than that of extra well-milled rice. Reasonably well milled means the milling of paddy from which the husk, the greater parts of the germ and both of its outer and inner bran layers have been removed to a certain extent that the kernel presents its moderate translucence in a degree lesser than that of well milled rice. Ordinarily milled means the milling of paddy from which the husk and certain parts of the germ and the whole part of its outer bran layers have been removed with some parts of its inner bran layers remain intact. The degree of its translucence is moderate and being lesser than that of reasonably well-milled rice. Under-milled rice means milled rice which is not equal to the milling requirements for above mentioned categories of milling of rice.

Milled rice is divided into whole grain, head rice and brokens. Whole grain means the full kernel without any broken part. Head rice means the kernel that retains the length of $8/10^{th}$ or more of the average length of the unbroken kernel as specified for a particular class of rice. Brokens are divided into various categories such as ¾ broken, 1/2 broken, 1/4 broken and small broken. Three-fourth broken means the broken kernel that has $7.5/10^{th}$ length of the average length of the unbroken kernel as specified for that particular class of rice. One- half broken means the broken kernel that has $5/10^{th}$ length of the average

length of the unbroken kernel as specified for that particular class of rice. One-fourth broken means the broken kernel that has $2.5/10^{th}$ length of the average length of the unbroken kernel as specified for that particular class of rice. Small broken means the broken kernel that has $2/10^{th}$ or less length of the average length of the unbroken kernel as specified for that particular class of rice.

Milling ranges from hand pounding with the simple wooden morter and pestle to the modern mills. A milling operation should give maximum head rice recovery, ensure quality and reduce the processing cost. Milling brings changes in nutritional value and quality of rice primarily by removing bran, which contains comparatively larger amounts of protein , fat, vitamins, and minerals than the remaining endosperm. Milling also removes larger proportion of phytate and fibre which adversely affects the utilization of Zinc and certain other essential dietary minerals and trace elements. Moreover, milling drastically reduces the protein contents (about 86%) and mineral content (23%) in rice but increases true protein digestibility.

Milling recovery is one of the most important criteria of rice quality. A variety should possess a high turn out of whole grain rice and total milled rice (Webb,1985). Milling quality of rice may be defined as the ability of rice grain to stand milling and polishing without undue breakage so as to yield the greatest amount of total recovery and the highest proportion of head rice to brokens (Cruz and Khush, 2000).

Factors Affecting Milling Recovery Of Rice

Hulling, milling, and head rice recovery are three main quality indices influencing milling out turn. The hulling, milling and head rice varies from 70-80%, 68-73%, and 30-75%. Milling quality of rice is affected by many factors including variety, grain type, chalkiness, cultural practices, environmental factors, and the drying, storing and milling conditions (Wasserman and Calderwood,1972, Witte, 1972, Adair *et al*, 1973)

Variety/Cultivar:

Rice varieties with white centre gave the lowest milling recovery. The white centre is a characteristic which causes broken rice (Nwe *et al* 2001). Chalkiness is considered an undesirable characteristic in virtually all forms of rice. It detracts from general appearance and usually results in lower milling yield (Kushibuchi, 1973). Chalkiness can be classified into several types: white centre (core), white belly, milky white, opaque. White centres are chalky spots in the centre of grain, while white belly refers to chalkiness on the dorsal side of grain. Milky white grain has a chalky texture except in the peripheral part of the grain. Opaque or dead grain has an overall chalky texture caused by the interruption of final filling of the grain (Ikehashi and Khush, 1979). The formation of chalkiness is influenced by environmental factors, particularly those that interrupt normal grain filling such as infection by neck blast and drought stress during ripening and is also under genetic control (Tashiro and Ebata, 1975; Ikehashi and Khush, 1979).

Comparison of translucent and white belly grains revealed that milling recovery in japonica rice varieties was comparatively higher in translucent grains than those of white belly grains (Table-1) (Kanda et al., 1979). Head rice recovery is mainly dependent upon grain size, shape, and appearance. In general, cultivars with long or long bold grains and those having chalky grains give lower head rice recovery. Varieties having medium long, slender, and translucent grains give the best head rice recovery (Khush, *et al* 1979). High milling recovery is generally associated with hardness and an absence of chalky spots in the endosperm (Chang and Bardenas, 1965). Total milled rice and head rice decreased with increase in grain length. Hulling, milling and head rice recovery of Indian rice varieties varied from 73.0-78.9 %, 67.3-72.4% and 35.0-69.0%, respectively (Table-2) (Singh et al., 2000a; Singh et al., 2000b; Singh et al., 2000c and Singh *et al*, 2001). Milling recovery and head rice of US rice varieties were 68-74% and 56-68%, respectively (Table-3) (Webb et al., 1979). The hulling, milling and head rice recovery of IRRI rice varieties varied from 74-79%, 67-73% and 36-65%, respectively (Table-4) (Khush *et al*, 1979). In Pakistan the percentage of hulled, total milled rice and head rice of Basmati varieties varied from 77.6-82.0, 66.4-72.0 and 51.2-55.5, respectively. The corresponding figures for non- Basmati varieties varied from 77.6-79.7, 68.5-72.4 and 52.9-58.5 % respectively, (Table-5) (Coordinated Rice Programme, NARC, Islamabad).

Fertilizer Application:

Late fertilizer application at the time of flowering may improve milling and nutritional quality of rice grain (Perez *et al*, 1996). High level of N decreased the head rice recovery of long slender grain varieties – Kasturi,IET, Pakistan Basmati, and Basmati 370 (Ras, *et al* 1993). Head rice recovery of Supper Basmati variety was significantly higher in case of three splits of nitrogenous fertilizer than those of two splits (Ali *et al* 1992 C) (Table-6) (Ali *et al* 1992c).

Storage Time

Milling of Basmati paddy six months after storage was found to enhance the head rice with a marked improvement in quality characteristics (DRR, 1994a).

Puddling

Complete puddling (under 30 – day wet condition) before transplanting gave comparatively higher total and head rice recovery. Whereas dry land preparation followed by flooding and transplanting gave the lower value (Ali et al; 1992 a) (Table -7) (Ali et al 1992a).

Plant Density

Total milling recovery and head rice recovery decreased with increased plant densities.in two Basmati varieties (Karim et al 1992).

Transplanting Time

Early and late transplanting significantly influenced the milling recovery of rice (Ali et al., 1991). Late transplanting (August) of Basmati 370, Pusa Basmati-1, Haryana Basmati-1, and Kasturi improved hulling, milling and head rice recovery (Rao et al., 1996). Basmati 385 transplanted on July 16 gave maximum total rice recovery and head rice recovery, while Basmati 370 transplanted on July 1st gave better results (Table-8) (Ali et al., 1991).

Harvesting Time

Maximum head rice recovery was obtained when the rice crop was harvested at 35 days after 50% flowering when the moisture content ranged from 20-30%.The recovery reduced with delay in harvesting beyond this time (Chaudhary and Iqbal,1986). Harvesting 30 days after flowering gave high head rice recovery in Khao Dawk Mali 105 in Thailand (Nwe et al 2001). Harvesting 33- 39 days after 50% flowering gave significantly higher head rice recovery than 27-30 days or 42 days after 50% flowering (Ali et al; 1993) (Table-9) (Ali et al 1993).

Threshing Time

Threshing 0-5 days after harvesting gave high head rice recovery. Threshing 10-15 days after harvest produced more broken grains in Khao Dwak Mali 105 in Thailand (Nwe et al: 2001).

Drying Temperature

A low drying temperature (40-50 °C) is much better than a high (60-70 °C). Drying at lower temperatures reduces the broken kernels because of low moisture gradient. Rapid hydration and dehydration lead to cracking and cracked grains are apt to break during milling (Bhattacharya, K. R. 1980)

Harvesting Methods

Total milled rice and head rice recovery of both Basmati and non-Basmati rice varieties were significantly higher in case of manual threshing than harvesting and threshing with combine (Ali et al; 1993) (Table-10) (Ali et al 1992d).

Type of Mill

The quantity of total milled rice and head rice of Basmati 385 and KS-282 was the highest in case of modern rubber roller, followed by disc sheller and steel huller (Ali et al 1992b) (Table-11) (Ali et al 1992a).

Table-1. Comparison of translucent grain and white belly grain in Japonica rices

Variety	Grain translucency	Milling recovery (% wt of brown rice)	Broken (%)
Kinmaze	Translucent White	89.61 88.71	0.37 2.11
Fukuminori	Translucent White	89.76 89.47	1.03 1.75
Ginmasari	Translucent White	91.41 90.63	1.67 3.21

Source: Kanda et al., 1969.

Table-2. Milling recovery of Indian rice varieties

Variety	Hulling (%)	Milling (%)	Head rice (%)	Source
Taraori Basmati	75.5	68.5	45.5	Singh et al., (2000) a.
Basmati 370	76.8	69.0	46.0	
Haryana Bas.1	78.3	71.3	44.7	
Pusa Bas.1	76.3	67.7	44.2	
Jubraj	76.1	70.4	64.2	Singh et al . (2000) b.
Durgabhog	76.4	70.2	65.6	
Pimplibasa	75.5	70.2	64.0	
Mugajai	73.5	67.3	68.0	
Rangsuri	75.6	70.4	66.0	
Makarakanda	78.2	72.4	69.0	
Badshabhog	77.0	72.0	67.0	
Basmata	77.1	71.2	65.5	
Baiganbija	76.2	70.0	65.0	
Basmati 370	73.0	-	35.0	
Bamati (Kurnool)	74.5	-	47.0	
Basmati(Kota Rajasthan)	76.0	-	52.6	
Basmati(Amratsar)	76.0	-	55.0	
Kalanamak	75.0	-	64.0	
Rajanam	78.9	-	57.2	
Basmati 370	77.0	72.5	53.0	Singh 2000.
Type 3	80.0	75.0	59.0	
	78.9	67.7	49.9	
Karnal local	78.0	66.5	48.1	Singh et al , 2001.
Pusa Basmati 1	-	82.4	65.4	
Br 9	-	82.0	65.8	
Br 10	-	82.8	66.8	
Sugandh	-	66.3	55.0	
Pusa Basmati				

Table-3. Milling recovery of rough rice of US varieties.

Milled rice characteristics	Percentage of rough rice		
	Long	Medium	Short
Total milled rice	68-71	71-72	73-74
Head rice	56-61	65-68	63-68

Source: Webb et al. 1979.

Table-4. Milling characteristics of IRRI rice varieties.

Variety	Hulling (%)	Milled rice (%)	Head rice (%)
IR 5	78	68	40
IR 8	74	71	36
IR20	78	70	62
IR 22	78	71	63
IR 24	79	70	57
IR 26	76	69	63
IR 28	78	72	61
IR 29	75	71	63
IR 30	77	70	55
IR 32	77	67	64
IR 34	79	69	50
IR 36	79	71	57
IR 38	78	71	65
IR 40	79	73	63
IR 42	78	71	52

Source: Khush et al 1979.

Table 5. Milling recovery of rice varieties in Pakistan.

Variety	Husked rice (%)	Milled rice (%)	Head rice (%)
Basmati-370	77.9	70.0-70.4	53.0-56.2
Basmati-385	78.0	70.5-70.7	54.0-58.5
Basmati-198	78.2	70.5	53.8
Basmati-6129	77.6	69.0-69.8	47.7-51.2
Supper Basmati	78.3	69.8-70.7	53.7-57.1
Shaheen Basmati	78.3	66.4-68.4	53.7-58.8
Basmati-2000	82.0	72.0-72.3	55.5-55.8
Kashmir Basmati	-	68.0	52.0
IRRI 6	79.5	70.92	57.5
KS 282	79.7	70.0-72.4	52.5-58.5
DR 92	-	71.4-72.0	57.1-61.5
DR 82	78.0	68.6	55.1
DR 83	77.6	68.5	52.9
Shua 92	--	70.0	64.2
JP 5	--	72.8	60.0
Swat 1	--	70.5	60.2
Swat 2	--	69.5	62.2
Sada Hayat	--	68.1	63.2
Shadab	--	74.0	68.0
Pakhal	--	66.2	59.8
Khushboo 95	--	72.0	44.0

Source : Rice Programme, NARC, Islamabad.

Table-6. Effect of fertilizer application on milling recovery of rice in Pakistan.

Fertilizer application methods	Total milled rice (%)	Head rice(%)
Basal and 30 DAT*	70.2 a	48.3 b
Basal 30 and 60 DAT	70.7 a	53.7 a

Means in a column, followed by a common letter are not significantly different at 5% level by DMRT.
* Days after transplanting.
Source: Ali et al 1992c

Table 7. Effect of puddling on milling recovery of rice in Pakistan.

Method of land preparation	Total milled rice(%)	Head rice(%)
Complete puddling	71.6 a	57.0 a
Partial puddling	71.1 a	56.2 b
Without puddling	70.0 b	54.7 c

Means in a column, followed by a common letter are not significantly different at 5% level by DMRT.
Source: Ali et al. 1992a.

Table 8. Effect of transplanting time on milling recovery of rice in Pakistan.

Transplanting time	Total milling	Recovery(%)	Head rice(%)	
	Bas. 385	Bas. 370	Bas. 385	Bas. 370
Jun 1	66.1 e	67.2 c	48.3 d	49.2 c
Jun. 16	68.1 d	68.9 b	50.0 c	51.4 b
Jul. 1	70.1 b	70.6 a	54.0 b	53.4 a
Jul. 16	70.8 a	69.0 b	55.3 a	51.7 b
Aug.1	69.2 c	65.8 d	50.0 c	46.8 d

In a column, means followed by a common letter are not significantly different at 5% level by DMRT. Source : Ali et al 1991.

Table-9. Effect of different harvesting intervals after 50% flowering of Basmati-385 in Pakistan.

Harvesting interval(days)	Moisture(%)	Total milled rice(%)	Head rice (%)
27	27.8	68.02 c	49.62 d
30	25.3	69.13 b	52.75 bc
33	22.9	70.18 a	54.48 a
36	20.3	70.40 a	54.62 a
39	17.9	70.42 a	53.75 ab
42	15.5	70.28 a	51.87 c

In a column, means followed by a common letter are not significantly differently at 5% level by DMRT.
Source: Ali et al. 1993.

Table 10. Effect of harvesting method on milling recovery of rice in Pakistan.

Harvesting methods	Total milled rice		Head rice(%)	
	KS 282	Bas. 198	KS. 282	Bas. 198
Mechanical harvesting	69.8 b	68.7 b	54.2 b	50.0 b
Manual harvesting	71.5 a	70.0 a	58.1 a	55.0 a

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.
Source: Ali et al. 1992d.

Table 11. Effect of mill type on milling recovery of rice in Pakistan

Mill type	Total	Milled rice (%)	Head Rice (%)	
	Bas. 385	KS 282	Bas. 385	KS 282
Steel huller	66.3 c	67.6 c	49.7 c	51.8 c
Disc sheller	69.0 b	70.0 b	54.0 b	56.2 b
Rubber roller	70.0 a	71.7 a	57.0 a	59.6 a

Means in a column, followed by a common letter are not significantly different at 5% level by DMRT.
Source: Ali et al. 1992b.

REFERENCES

Adair CR, Bollich CN, Bowman DH, Jodon NE, Johnston TH, Webb BD and Atkins JC. 1973. Rice breeding and testing methods in the United States. P-22-75 In: Rice in the United States: Varieties and Production. US Dept Agric Handbook No. 289

Ali A ,Karim MA, Ali SS, Ali L, Hassan G, and Majid M. 1991. Relationship of transplanting time to grain quality in Basmati 385. IRRI. 16(5):11. International Rice Research Institute, Philippines.

Ali A, Karim MA, Ali L, Ali SS, Jamil M, Hassan G and Majid M. 1992a. Relation between rice grain quality and land preparation methods. IRRI 17(3):7. International rice Research Institute, Phillipines

Ali A, Karim MA, Ali L, Ali SS, Jamil M, Hassan G and Majid M 1992b. Milling recovery as influenced by different types of rice mills. IRRI 17(2):7. IRRI, Philippines.

Ali A, Karim MA, Hassan G, Ali L, Ali SS and Majid A. 1992c. Rice grain quality as influenced by split application of nitrogenous fertilizer. IRRI 17(3):7. International Rice Research Institute, Philippines.

Bal S. 1974. Measurement of milling quality of paddy. Rice Process Eng. Centre Publ. 742. Kharagpur West Bengal, India. p:8

Bhattacharya KR. 1980. Breakage of rice during milling: a review. Trop Sci 22(3):256-7.

Chang TT and Bardenas EA .1965. The morphology and varietal characteristics of the rice plant. IRRI, Tech Bull 4.

Chaudhry MN and Iqbal MS. 1986. Production technology of Basmati rice Prog Farm 6(1):17-25.

Dela CruzN. and GS Khush. 2000. pp. 15-28. In: Aromatic Rices. R.K. Singh US Singh and GS Khush. (eds.) Oxford & IBH Publishing Co Ltd New Delhi. India.

DRR (Directorate of Rice Research). 1994. Final report (1990-93), improvement of Basmati rices for increased productivity and export purposes.

Ikehashi H and Khush GS. 1979. Methodology of assessing appearance of the rice grain, including chalkiness and whiteness. P. 223-229. In: Proceedings of the Workshop on Chemical Aspects of Rice Grain Quality. IRRI, Los Banos, Laguna, Philippines.

Kanda M, Ikehshi H and Ito R. 1969. Evaluation of brown rice character through milling tests of samples partitioned according to grain trait (in Japanese). Agr Hort Technol Tokyo 24(4):24-7.

Karim MA, Ali A, Ali SS, Mahmood AA, Majid A and Akhtar T. 1992. Effect of plant density on rice grain quality. IRRI. 17(16):12. International Rice Research Institute, Philippines.

Khush GS, Paule CM and Cruz NM. 1979. Rice grain quality evaluation and improvement at IRRI. P:21-31. In: Proceedings of the Workshop on Chemical Aspects of Rice Grain Quality. IRRI, Los Banos, Laguna, Philippines.

Kushibuchi K. 1973. Introducing a testing instrument for determining the transparency of rice grain (in Japanese). Japan J. Breeding 23(23):35.

Nagato K, Kono Y. 1963. Grain texture of rice. 1. Relations among hardness distribution, grain shape and structure of endosperm tissue of rice kernel (in Japanese) Proc Crop Sci Japan 32:181-189.

New KT, Myint TT and Garcia AG. 2001. Breeding and cultivation of superior quality rices in Myanmar. pp. 115-127. In: RC Chaudhary and DV Tran (tech. eds.) and R. Duffy (ed.). Speciality Rice the World: Breeding, Production and Marketing. FAO, Rome Science Publishers Inc USA.

Perez CM, Juliano BO, Liboon SP, Alcantra JM and Cassman KG. 1996. Effect of late nitrogen fertilizer application on head rice yield, protein content, and grain quality of rice. Cereal Chem 73:556-560.

Rao KS Moorthy BTS Lodh SB and Sahoo K. 1993. Effect of graded level of nitrogen on yield and quality of different varieties of scented rice (*Oryza sativa*) in coastal Orisa Indian J Agric Sci 63:467-472.

Rao KS, Murty BTS, Dash AB and Lodh SB. 1996. Effect of time of transplanting on grain yield and quality traits of Basmati types scented rice (*Oryza sativa*) varieties in coastal Orissa. Indian J Agric Sci 66: 333-337.

Singh RK, Khush GS, Singh US, Singh AK and Sanjay Singh. 2000. Breeding aromatic rice for high yield, improved aroma and grain quality. P:71-105. In: RK Singh US Singh and GS Khush (eds.). Aromatic Rices. Oxford and IBH Publishing Co Pvt Ltd New Delhi.

Singh RK, Gautam PL, Sanjeev Saxena and S Singh. 2000. Scented rice germplasm: Conservation, evaluation and utilization. pp.107-134. In: RK Singh US Singh and GS Khush (eds.) Aromatic Rices. Oxford and IBH Publishing Co Pvt Ltd New Delhi.

Singh RK, Sahai VN, Sharma RN, Singh US, Singh S and Singh ON. 2001. Current status and future prospects of improving traditional aromatic rice varieties in India p: 79-87. In: Speciality Rices of the World: Breeding, production and marketing. FAO,Rome. Science Publishers, Inc Enfield(NH), USA.

Singh VP. 2000. The Basmati rice of India. p:135-153. In: RK Singh US Singh and GS Khush (eds.) Aromatic Rices. Oxford and IBH Publishing Co Pvt Ltd New Delhi.

Tashiro T and M Ebata. 1975. Studies on white belly rice kernel. 111. Effect of ripening conditions on occurrence of white belly kernel (in Japanese). Proc Crop Sci Soc Japan. 44: 86 92.

Wasserman T and DL Calderwood. 1972. Rough rice drying. PP.166-187 In: D.F.Houston (ed) Rice Chemistry And Technology. Am. Assoc. Cereal Chemists, Incorp St Paul Mimm.

Webb BD. 1985. Criteria of rice quality in the U.S. p:403-442. In: Rice Chemistry and Technology.

Webb, BO CN Bollich TH Johnston and WO Mcilrath. 1979. Components of rice quality: Their identification, methodology, and stage of application in United States breeding progrmmes. pp. 191-205. Proc. of the Workshop on Chemical Aspects of Rice Grain Quality. IRRI LosBanos Laguna Philippines.

Witte GC. 1972. Conventional rice milling in the United States. p:188-200. In: D.F. Houston, (ed.) Rice Chemistry and Technology. Am AssocCereal Chemists, Inc St Paul Minn.

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Effect of defatted rice bran on physico-chemical properties of bread

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ABSTRACT

Wheat flour was replaced with defatted rice bran at different levels like 5%, 10%, 15% and 20%. Prepared bread loaves were analyzed for chemical parameters and sensory evaluation at different storage intervals of 0, 24, 48, 72, 96 and 120 hours. Results revealed that protein, ash and fiber contents of bread improved, while moisture content decreased. Fat content showed non-significant effect with increasing levels of defatted rice bran. Maximum protein, ash and fiber contents were found in samples having 20% bran while minimum content were observed in control having 0% bran. Samples containing 5% scored highest in terms of external characteristics like volume, color of crust, symmetry of form, evenness of bake, character of crust and internal characteristics, i.e. grain, color of crumb, aroma, taste, and texture.

Key words: Defatted rice bran, fiber enriched bread, storage effect, sensory evaluation

INTRODUCTION

During 2000-2001, 4803 thousand tons rice was produced. Estimated bran yield from this production was about 240 thousand tons. (Anon 2001). Rice bran, as a co-product of the rice milling industry, is yet not to be efficiently utilized for human consumption. Despite its excellent nutrition, its hypoallergenicity and recently claimed nutraceutical properties, it is mainly utilized for animal feed or simply discharged. It is of interest to incorporate this healthy ingredient into our diet (Lima *et al* 2002). Rice bran, a good source of protein and fat, is at present underutilized as a food material. The potential of producing rice bran at the global level is 27.3 million ton. (Prakash 1996). Due to rice bran's over all composition, nutritional profile, functional characteristics, and apparent hypoallergenicity, it has many applications in a diet which is characterized by high in dietary fiber and low in saturated fat. It may be particularly beneficial to those individuals who are allergic to other cereal grains. However strong evidences are available that the consumption of rice bran may be beneficial in reducing the risk of cardiovascular disease and colon cancer (Marshall and James 1994). Defatted rice bran increases dough yield, contributes to an attractive tan crumb & crust, does not disturb fermentation or mixing tolerance of dough, causes baked products to remain fresher & more moist and adds significant amino acids, minerals, vitamins to baked goods (Lynn, 1969).

Rice bran was replaced with wheat flour at 15-30% in yeast bread and it was concluded that rice bran can be substituted successfully up to 15% replacement level without affecting loaf weight, height or volume (Sharp & Kitchens, 1990). Sharma and Chauhan (2002) substituted wheat flour with rice bran stabilized by dry heat and extrusion cooking at levels 5 to 20% in breads and cookies. Wheat flour replacement increased baking absorption, decreased loaf volume and overall quality scores of breads. Addition of rice bran to wheat flour increased the contents of proteins, lysine and dietary fiber in bread and cookies proportionately to the level of substitution. Bread volume and cookie spread decreased with blending of different types of rice bran however, the decrease was more pronounced with the defatted bran. Stabilized full fat rice bran up to 20% level and unstabilized full fat or stabilized defatted rice bran up to 10% was found suitable in various food products (Singh *et al* 1995).

Thus the object of present experiment is to study the physico-chemical characteristics of bread containing defatted rice bran and to find out the most appropriate level of defatted rice bran addition in bread for commercialization.

MATERIALS AND METHODS

A) i. PROCUREMENT OF RICE BRAN AND RAW MATERIAL

Rice bran was procured from Reem Rice Mills (Pvt.) Limited whereas flour, sugar, shortening, improver and yeast were purchased from local market of Faisalabad.

ii. STABILIZATION OF RICE BRAN

Rice bran was stabilized by heating at 125-135°C for 1-3 seconds (Randall 1985).

iii. PREPARATION OF DEFATTED RICE BRAN (DRB)

Rice bran was treated with hexane solvent to remove germ portion. Again it will be steamed for 5-10 minutes at 93-104°C to inactivate residual lipase activity (Yokochi, 1975).

iv. BREAD PREPARATION

The bread was prepared by Straight Dough Method as described in AACC (2000) with some modification. Defatted rice bran was incorporated in the dough during mixing at different levels as shown in the Table.1

Table 1. Different treatments used in the study

Treatments	Wheat flour	Defatted rice bran (DRB)
T_0	100%	0%
T_1	95%	5%
T_2	90%	10%
T_3	85%	15%
T_4	80%	20%

B) CHEMICAL ANALYSIS OF BREAD

Bread samples were analyzed for moisture, crude fat, crude fiber, crude protein, total ash, and NFE as described in AACC (2000).

C) SENSORY EVALUATION

Sensory evaluation of bread samples manufactured from different rice bran levels was carried out for the external characteristics i.e. volume, color of crust, symmetry of form, evenness of bake, character of crust and internal characteristics like grain, color of crumb, aroma, taste and texture at 0, 24, 48, 72, 96 and 120 hours of storage as described by Matz (1972).

D) STATISTICAL ANALYSIS

The data obtained for each parameter was statistically analyzed by Steel *et al* (1997) using analysis of variance.

RESULTS AND DISCUSSIONS

A. CHEMICAL ANALYSIS OF BREAD

Results demonstrated that various treatments of rice bran have significant effect on moisture, protein, fiber, ash and NFE contents whereas non-significant affect was observed for storage except moisture contents, which varied for different time intervals. Interaction between treatment and storage did not exhibit significant difference.

In case of moisture contents results showed that highest moisture content i.e. 35.0% was found in T_4 (20% defatted bran) followed by T_3 (15% bran) and minimum in T_2 (32.51%) containing 10% bran. Protein content is highly affected due to different levels of rice bran. Mean values showed that highest protein content was found 9.39 for T_4 (20% defatted bran) followed by 9.01, 8.31, 7.80 and 7.41 percent for T_3, T_2, T_1 and T_o respectively. The lowest protein content was observed in case of control. Means values for crude fat were 1.63%, 1.54%, 1.62%, 1.62% and 1.66% for T_0 (control), T_1 (5% bran), T_2 (10% bran), T_3 (15% bran) and T_4 (20% bran) respectively. Results pertaining to fiber contents showed that highest fiber content 3.00% was found for T_4 (20% defatted bran) followed by 2.51, 2.00, 1.61 and 1.43 percent for T_3, T_2, T_1 and T_o respectively. The lowest fiber content was observed in case of control. Mean values showed that the highest ash content was found 2.53 for T_4 (20% defatted bran) followed by 2.12, 1.67, 1.10 and 0.99 percent for T_3, T_2, T_1 and T_o respectively. The lowest fiber content was observed in case of control containing 0.99% ash. Ash contents were improved gradually from control to treatment T_4 by the level of substitution of defatted rice bran. Means for NFE content showed that T_o, T_1, T_2, T_3 and T_4 have values 88.55, 87.86, 86.40, 84.73 and 83.41% respectively. The highest value 88.54 was found in T_1 (100% flour) while lowest was 83.41 in case of T_4 (20% rice bran).

Table 2. Analysis of variance for chemical properties of Bread

S.O.V	df	Moisture	Protein	Fat	Fiber	Ash	NFE
Storage (S)	5	391.5**	$0.1^{N.S}$	$1.1^{N.S}$	$0.1^{N.S}$	$0.1^{N.S}$	$0.1^{N.S}$
Treatment (T)	4	28.4**	1383**	$1.3^{N.S}$	682 **	921 **	89.2**
S X T	20	$1.6^{N.S}$	$0.1^{N.S}$	$1.0^{N.S}$	$0.2^{N.S}$	$0.2^{N.S}$	$0.01^{N.S}$
Error	60						
Total	89						

Table 3. Effect of different treatments on chemical parameters of bread

Treatments	Moisture	Protein	Fat	Fiber	Ash	NFE
T_0	32.66c	7.41e	1.63	1.43e	0.99e	88.55a
T_1	32.96c	7.80d	1.54	1.61d	1.10d	87.86b
T_2	32.51c	8.31c	1.62	2.00c	1.67c	86.40c
T_3	34.00b	9.01b	1.62	2.51b	2.12b	84.73d
T_4	35.00a	9.39a	1.66	3.00a	2.53a	83.41e

T_0 = Control (0% defatted rice bran)
T_1 = 5% defatted rice bran
T_2 = 10% defatted rice bran
T_3 = 15% defatted rice bran
T_4 = 20% defatted rice bran

Table 4. Effect of storage on sensory attributes of bread

Storage	Moisture	Protein	Fat	Fiber	Ash	NFE
0 hour	40.36a	8.39	1.64	2.11	1.69	86.17
24 hours	36.36b	8.39	1.62	2.10	1.68	86.20
48 hours	33.39c	8.38	1.53	2.11	1.70	86.19
72 hours	31.39d	8.37	1.63	2.10	1.67	86.22
96 hours	30.23e	8.40	1.61	2.10	1.69	86.19
120 hours	28.82f	8.38	1.54	2.13	1.68	86.16

Moisture content of bread is affected during storage period of 0-120 hours. Fresh bread loaves (0 hour) contained maximum moisture percentage (40.36%) which gradually decreases after 24 (36.36%), 48 (33.39%), 72 (31.39%), 96 (30.23%) and 120 (28.82%) hours of storage respectively.

B. SENSORY EVALUATION OF BREAD

Results pertaining to sensory attributes expressed that different rice bran treatments and storage intervals significantly effect various sensory attributes like volume, color of crust, symmetry of form, evenness of bake, character of crust, aroma, grain, color of crumb, taste and texture of bread.Highest score for volume of bread was obtained by To (control) 6.26 followed by 5.97, 5.76, 5.20 and 4.80 for T1, T2, T3 and T4 respectively. Treatment T4 got the lowest scores. Maximum scores for color of crust were obtained by T1 (5% bran) 5.86 followed by 5.47, 5.13, 4.66 and 4.1 for To, T2, T3 and T4 respectively. Treatment T4 got the lowest scores. Mean values

Table 5. Analysis of variance for sensory properties of bread

SOV	df	Volume	Color of crust	Symmetry of form	Even-ness of bake	Charac-ter of crust	Aroma	Grain	Color of crumb	Taste	Texture
Storage (S)	5	91.3**	40.1**	14.5**	$2.2^{N.S}$	20.9**	145.6**	64.4**	143.3**	114.3**	146.5**
Treat (T)	4	19.7**	36.1**	33.0**	11.5**	32.6**	45.4**	80.0**	87.6**	136.8**	84.5**
S X T	20	$0.2^{N.S}$	$0.2^{N.S}$	$0.2^{N.S}$	$0.1^{N.S}$	$0.7^{N.S}$	$0.51^{N.S}$	$0.36^{N.S}$	$0.3^{N.S}$	$0.6^{N.S}$	$0.72^{N.S}$
Error	120										
Total	149										

expressed that T_1 (5% bran) obtained maximum scores for symmetry of form 3.33 followed by 2.83, 2.50, 2.23 and 1.2 for T_o, T_2, T_3 and T_4 respectively. Treatment T_4 got the lowest scores.

Table 6. Effect of different treatments on sensory attributes of bread

Treatments	Volume	Color of crust	Symmetry of form	Evenness of bake	Character of crust	Aroma	Grain	Color of crumb	Taste	Texture
T_0	6.26a	5.47b	2.83b	2.23ab	1.97b	5.18 c	10.63b	6.2b	13.36b	9.13b
T_1	5.97ab	5.86a	3.33a	2.48a	2.26a	5.91a	11.16a	6.63a	14.26a	9.86a
T_2	5.76b	5.13c	2.50c	1.95bc	1.82b	5.53b	9.73c	5.56c	11.97c	8.40c
T_3	5.20c	4.66d	2.23c	1.76c	1.45c	4.61d	8.56d	4.7d	9.76d	7.46d
T_4	4.80d	4.1e	1.67d	1.22d	1.18d	3.83e	7.76e	4.28e	8.4e	6.76e

T_1 (5% bran) obtained maximum scores for evenness of bake followed by T_o. Treatment T_4 got the lowest scores. Treatment T_1 (5% bran) obtained maximum scores for character of crust 2.26 followed by 1.97, 1.82, 1.45 and 1.18 for T_o, T_2, T_3 and T_4 respectively. Treatment T_4 got the lowest scores. Mean values showed that treatment T_1 (5% bran) obtained maximum scores for aroma i.e. 5.91 followed by 5.53, 5.18, 4.61 and 3.83 for T_2, T_o, T_3 and T_4 respectively. Treatment T_4 got the lowest scores for aroma of bread. Results expressed that treatment T_1 (5% bran) obtained highest scores for grain i.e. 11.16 followed Results obtained in the study expressed that treatment T_1 (5% bran) obtained highest scores regarding the texture of bread i.e. 9.86 followed by 9.13, 8.40, 7.46 and 6.76 for T_o, T_2, T_3 and T_4 respectively. Treatment T_4 was awarded the lowest scores for texture of bread.

Table 7. Effect of Storage on sensory attributes of bread

Storage	Volume	Color of crust	Symmetry of form	Evenness of bake	Character of crust	Aroma	Grain	Color of crumb	Taste	Texture
S_1	7.12a	6.12a	2.92a	2.32	2.28a	6.92a	11.2a	7.26a	15.16a	11.0a
S_2	6.60b	5.72b	2.96a	2.06	2.0b	6.44b	10.68b	6.64b	13.28b	9.56b
S_3	6.44b	5.16c	2.80a	1.94	1.8bc	5.56c	10.08c	5.8c	11.96c	8.68c
S_4	5.60c	4.88c	2.40b	1.80	1.66c	4.68d	9.48d	5.24d	10.84d	7.72d
S_5	4.12d	4.40d	2.16c	1.76	1.42d	3.76e	8.52e	4.32e	9.64e	6.88e
S_6	3.72d	4.0e	1.84c	1.70	1.26d	2.74f	7.48f	3.6f	8.44f	6.12f
Total	149									

During storage study, volume of bread decreases gradually from 7.12 (0 hour) to 3.72 (120 hours). Color of fresh bread loaves was 6.12, which decreases to 4.0 after 120 hours storage. Symmetry of bread also exhibits storage effect, which decreases from 2.92 (0 by 10.63, 9.73, 8.56 and 7.76 for T_o, T_2, T_3 and T_4 respectively. Treatment T_4 was awarded the lowest scores for grain of bread. Mean values showed that treatment T_1 (5% bran) obtained highest scores for color of crumb i.e. 6.63 followed by 6.2, 5.56, 4.7 and 4.28 for T_o, T_2, T_3 and T_4 respectively. Lowest scores for color of crumb of bread were found for Treatment T_4. Results showed that treatment T_1 (5% bran) obtained highest scores regarding the taste of bread i.e. 14.26 followed by 13.36, 11.97, 9.76 and 8.4 for T_o, T_2, T_3 and T_4 respectively. Treatment T_4 was awarded the lowest scores for taste of bread. hours) to 1.84 after 120 hours period. As crust of bread is concerned, fresh bread loaves (0-hour) obtained scores (2.28) followed by 2.0, 1.8, 1.66, 1.42 and 1.26 for 24, 48, 72, 96 and 120 hours of storage. Fresh bread has highest aroma 6.92 followed by 6.44, 5.56, 4.68, 3.76 and 2.74 for 24, 48, 72, 96 and 120 hours of storage. Bread loaves gained highest scores (11.2) for grain followed by 10.68, 10.08, 9.48, 8.52 and 7.48 for 24, 48, 72, 96 and 120 hours of storage. Color is also affected by storage from 7.26 at 0 hour followed by 6.64, 5.8, 5.24, 4.32 and 3.6 for 24, 48, 72, 96 and 120 hours of storage respectively. Fresh

bread loaves obtained highest scores (15.16) for taste followed by 13.28, 11.96, 10.84, 9.64 and 8.44 for 24, 48, 72, 96 and 120 hours of storage. Scores for taste of bread are gradually decreasing by increasing time interval due to chemical and bio-chemical changes taking place in bread. Texture of bread significantly decreases from 11.0 at 0 hour followed by 9.56, 8.68, 7.72, 6.88 and 6.12 for 24, 48, 72, 96 and 120 hours of storage.

CONCLUSION

Use of defatted rice bran has great potential for improving overall bread quality and nutritional profile of pan bread. It is therefore concluded that bread of excellent quality can be prepared by replacing the flour with defatted rice bran up to 5% level.

LITERATURE CITED

AACC. 2000. American Association of Cereal Chemists. Official Methods of Analysis. The American Association of Cereal Chemists Inc St Paul Minnesota.

GOP. 2001. Agriculture Statistics of Pakistan. Govt. Pak. Ministry Food. Agriculture and Livestock (Economic wing) Islamabad.

Lima I, Guraya H and Champagne E. 2002. The functional effectiveness of reprocessed rice bran as an ingredient in bakery products. Nahrung 46(2):112-7.

Lynn L. 1969. Edible rice bran foods. Cereal Chem.154-72.

Matz SA. 1972. Bakery Technology and Engineering. The AVI Pub Co Westport, Connecticut.

Marshall WE and James IW. 1994. Rice science and technology. Marcel Dekker. Inc New York.

Prakash J. 1996. Rice bran proteins: Properties and food uses. Crit Rev Food Sci Nutr 36(6): 537-52.

Randall JM, Sayre RN, Schultz WG Fong RY Mossman AP Tribelhorn RE and Saunders RM. 1985. Rice bran stabilization by extrusion cooking for extraction of vegetable oil J Food Sci 50:361.

Sharp CQ and Kitchen KJ. 1990. Using rice bran in yeast bread in a home baker. Cereal Foods World 35:1021

Sharma HR and Chauhan GS. 2002. Effect of stabilized rice bran-fenugreek blends on the quality of breads and cookies. J Food Sci and Nutr. 39(3):225-33.

Singh B, Sekhon KS Singh N. 1995. Suitability of full fat and defatted rice brain obtained from Indian rice for use in food products. Plant Foods Hum Nutr 47(3):191-200.

Steel RGD Dickey D and Torrie JH. 1997. Principles and Procedures of Statistics. A Biometrical Approach 3rd ed. McGraw Hill Book Co Inc New York

Yokochi K. 1977. Rice bran processing for production of rice bran oil and characteristics and uses of oil and deoiled bran. Proc Rice by-products utilization, International Conference, Valencia, Spain.

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Composition of common salt being marketed under the label "iodized salt"

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ABSTRACT

The study was carried out to assess the nutritional quality of eight refined iodized salt samples available in the local market. The samples were analyzed for moisture, sodium chloride and iodine contents. The sodium chloride content of around 60 % samples was below the standard values. The water insoluble matter including dust and stones in about 40 % samples were observed to be according to standard specifications, while rest of samples contained higher percentage of insoluble matter. Only two out of eight samples contained iodine as per specification (60-70 ppm), while iodine was not present at all in four samples. Moisture content in the samples was within the specified limits (not > 0.5 %). After one year storage at 45 °C a significant decrease in iodine content was observed, however non-significant change was noted on storage at 25°C.

Keywords: Common Salt, Iodized salt, composition, analysis

INTRODUCTION

Common salt iodized to make up the deficiency of iodine in human body in many parts of the world. Potassium iodide or potassium iodate are normally added salt for this purpose.

Iodine is unique among the required trace elements in that it is a constituent of the thyroid hormones, thyroxine and triiodothyroxine. Iodine deficiency disorders (IDD) is the term now used, instead of goiter (enlarged thyroid), to denote all the effects of iodine deficiency on growth and development (Hetzel 1990). Iodine deficiency in humans and farm animals is a prevalent deficiency disease and occurs in almost every country in the world.

In the thyroid iodine is trapped, concentrated, rapidly oxidized and converted to organic iodine by combining with tyrosine. Iodine is present in the thyroid as inorganic iodine, monoiodotyrosine, diiodotyrosine, triiodothyronine, tetraiodothyroxine (thyroxine T_4) and other iodinated organic compounds. Thyrogluboline, an iodinated glycoprotein in the thyroid, is the storage form of the hormones and represents 90 % of total thyroid iodine (Lee 1992).

Burgi *et al* (1999) established normal values for thyroid volume of school children who can be assumed to have had a sufficient iodine intake all their life time. They also investigated mean iodine concentration in the urine of school children. Remer *et al* (1999) reported low dietary iodine intake in vegetarian. They found potential danger of iodine deficiency disorders due to strict forms of vegetarian nutrition, especially when fruits and vegetables grown in soils with low iodine levels were ingested.

Vital *et al* (2000) demonstrated thyroid toxicity of iodide excess in animals fed with an iodide rich diet, in vitro iodide is cytotoxic, inhibits cell growth and induces morphological changes in thyroid cells of some species. Thyroid cells treated with iodide excess underwent apoptosis, as evidenced by morphological changes, plasma membrane, phosphatidyl serine exposure and DNA fragmentation. Jiang (1997) reported these content in the iodized salt was 0.03 – 0.05 % and the iodized salt was useful for prevention and control of lead poisoning. Aquaron (2000) carried out iodine determination by a very sensitive colorimetric and automatic method in natural sea and rock salts. This study confirmed the very low level of iodine (< 0.71 mg / Kg) in natural salts.

In view of importance of the iodine level worldwide and especially in Pakistan, studies were carried out to investigate the level of iodine in particular and general composition of samples labeled as iodized salt available in the market.

MATERIALS AND METHODS

Eight samples of common salt of different companies were collected from the local market in Peshawar to assess the quality of refined iodized salt. Sample of each company was randomly collected in triplicate. These were stored at 25 and 45 °C for one year. Moisture content was estimated by placing a ground sample (3-5g) at 105 °C in an oven for 6 hrs, iodine was determined by titration with 0.005 N thiosulphate solution using starch as indicator, sodium chloride in iodized salt was estimated by titration with 0.1 N silver nitrate solution using potassium chromate indicator. Insoluble matter, 9 stone and dust was calculated after dissolving the known weight of salt in distilled water as described in A.O.A.C. (199

Table 1. Chemical composition of iodized table salt

Sample	Chemical Composition (%) *					
	Moisture (%)	Sodium Chloride (%)	Iodine (ppm)	Total Water Insoluble Matter (%)	Stones (%)	Dust (%)
S-1	0.37	93.78	NP	5.85	1.78	4.07
S-2	0.34	92.70	NP	6.96	2.90	4.06
S-3	0.40	99.50	65.00	0.10	-	0.10
S-4	0.35	98.00	70.00	1.65	0.21	1.44
S-5	0.38	94.75	40.00	4.87	0.80	4.07
S-6	0.40	95.50	NP	4.10	1.08	3.02
S-7	0.35	94.82	NP	4.83	1.11	3.72
S-8	0.39	97.00	27.00	2.61	0.73	1.88

* Average of triplicate readings
NP : Not Present

Table-2 : Effect of one year storage on iodine contents of iodized salt

Sample	Iodine (ppm)				
	Initial Value	25°C.		45°C.	
		Value	% Loss	Value	% Loss
S-3	65	64	1.54	60	7.69
S-4	70	68	2.86	65	7.14
S-5	40	39	2.50	38	5.00
S-8	27	26	3.70	25	7.41

RESULTS AND DISCUSSION

Table-1 summarizes the chemical quality of commercially prepared refined iodized common salt. Moisture contents in these samples ranged from 0.34-0.41 % which were almost within the specified limit (not > 0.5 %). The contents of sodium chloride in about 62.5 % samples varied from 92.7 - 95.5% which were found below standard specification (98.0-99.5%). However, rest of the 37.5 % samples contained sodium chloride within the standard value. Similarly, water insoluble matter including stone and dust in 37.5 % samples were found according to specification whereas rest of the samples (62.5 %) contained higher amount of insoluble matter ranging from 4.10 to 6.96 %.

Iodine contents of iodized salt samples decreased to various extents on storage for one year at 25 & 45 °C. However, decrease in iodine contents was comparatively more at 45 °C than at 25 °C. Around 1.53 – 3.70 % at 25 °C. and 5.00 – 7.69 % at 45 °C., decrease in iodine contents was observed during one year storage of iodized salts (Table-2). It has been reported in literature that iodized salt did not undergo serious loss of iodine in the absence of sunlight (Educational Bureau, 1946). Volatization of iodine, by the action of sunlight, may be reduced by the addition of alkaline salt of sodium i.e. sodium bicarbonate. Potassium iodate is also added in common salt in order to overcome this problem (Hetzel and Meberly, 1986). The present investigation indicated that high temperature storage of iodized salt also adversely affects the value of iodine.

REFERENCES

AOAC. 1990. Official methods of analysis. Association of Official Analytical Chemists. American Association of Analytical Chemists 15th ed, Washington D.C.

Aquaron R. 2000. Iodine content of non-iodized salts and iodized salts obtained from the retail markets worldwide. World Salt Symp. to 935-940 (Eng) ed Geertman, Robm. Elsevier Science BV Amsterdam.

Burgi HP, Luc PJ, Vertongen F and Srbeeky M. 1999. Thyroid volumes and urinary iodine in Swiss school children, 17 years after improved prophylaxis of iodine deficiency. Eur J Endocrinal. 140(1):104-106.

Educational Bureau England. 1946. " Iodine Facts " No.332. Iodine Educational Bureau, p 215, Stone House Bishops Gate, London.

Hetzel, BS 1990. Proceedings. 7th Int. Symposium on trace elements in man and animals (TEMA-7) p-11. Dulironik. Yugoslavia.

Hetzel BS and Meberly GF. 1986. Trace elements in human and animal nutrition, 5th Academic Press, New York.

Jiang Y. 1997. Iodine containing salt against toxicity caused by lead Faming Zhuanli Shenqing Gongkai Shuomingshu CN1, 162, 408 (C1 A 23 L1/237) 22 Oct 1997).

Lee RM. 1992. Minerals in animal and human nutrition. P-226. Academic Press, New York.

Remer T Neubert A and Manz F. 1999. Increased risk of iodine deficiency with vegetarian nutrition. Br J Nutr 81(1):45-9.

Vital M, Dim JD, Ascoli F, Salzano S, Bogazzi F, Fenzi G, Martino E and Rossi G. 2000. Iodine excess induces apoptosis in thyroid cells through an independent mechanism involving oxidative stress. Endocrinology 141(2):598-605.

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Chemical, rheological and storage studies of iron fortified whole wheat flour

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ABSTRACT

Whole wheat flour was fortified with $FeSO_4$ and EDTA at various levels such as 0, 20, 40 and 60 ppm and folic acid @ 0, 1.5, 3.0 & 4.5 ppm respectively and stored for 42 days in cotton bags at ambient temperature. The chemical characteristics of whole wheat flour were affected non-significantly during storage and among treatments except moisture content were significantly affected. Physical dough properties were assessed with Farinograph and Amylograph. Physical dough characteristics as well as viscosity measurements of fortified flours revealed that flour of 40 ppm both the $FeSO_4$ and EDTA and 3.0 ppm of folic acid was more suitable especially for Iron deficiency anemic people.

Key words: Whole wheat flour, physicochemical, analysis, rheology.

INTRODUCTION

Iron is required as component of blood hemoglobins, which carry oxygen and transport carbon dioxide from tissue to the lungs as it is the part of muscle myoglobins, which store oxygen.

Iron deficiency anemia (IDA) is one of the most prevalent health problems with women and children in the developing world. The World Health Organization (WHO) estimates that approximately 1.48 billion individuals are afflicted worldwide (Anon., 2000). It is well estimated that IDA has a negative impact on the intellectual performance of children (Walter, 1995) and increases mortality and morbidity rates among pregnant mothers and infants (De Maeyer *et al.* 1989; Verster and Vander, 1995). Wheat is a principal source of calories, proteins and minerals. In Pakistan more than 70% of the total wheat production of 18.5 million tones (GOP, 2001) is milled into whole wheat flour in different flour mills. Unfortunately, wheat contains certain compounds (Phytates etc.) that bind iron, thus making it unavailable for the body. Since wheat is a staple food for the inhabitants of Pakistan, this results in iron deficiency in the population. To improve the iron level, the whole wheat flour was fortified with ferrous sulphate, EDTA and folic acid at different levels. The quality of flour may be affected by temperature, humidity, interactions of chemical constituents and packaging materials during storage.

The mandate of the present project was to determine the stability of iron fortified flour during storage and to evaluate the impact of fortificant on the chemical and rheological characteristics of flour samples.

MATERIALS AND METHODS

Whole wheat flour was procured from the local market and was fortified with $FeSO_4$ and EDTA at various levels such as 0, 20, 40 and 60 ppm of each and folic acid (using 0, 1.5, 3.0 and 4.5 ppm) designated as T_0, T_1, T_2 and T_3 respectively.

Flour and iron fortificant (premix) were mixed together with the help of mixer.. The fortified samples were stored at ambient temperature in cotton bags for 42 days.

Chemical characteristics such moisture, crude protein, crude fat, crude fiber and crude ash contents of whole wheat flour samples were determined according to the procedures given in AACC (2000). These parameters of each flour sample were determined weekly for 42 days.

Physical dough characteristics of whole wheat flour samples (Farinographic and Amylographic) were determined by following the methods given in AACC (2000).

The data obtained for each parameter was subjected to statistical analysis by using the Analysis of Variance Techniques (Steel *et al.*, 1996).

RESULTS AND DISCUSSION

Chemical Composition

Proximate analysis of fortified whole wheat flour samples were done weekly. It was observed that only the moisture content in flour samples was affected highly significantly while all other parameters were not effected by storage as well as treatments.

Moisture Content

The results regarding moisture (Table 1) indicates that there was a significant variation in the moisture content as a result of storage periods and treatments. The moisture content of wheat flour is influenced by

the milling techniques as well a storage conditions (Kirk and Sawyer 1991).

Where

S_0 = 0 day storage
S_1 = 1st week storage
S_2 = 2nd week storage
S_3 = 3rd week storage
S_4 = 4th week storage
S_5 = 5th week storage
S_6 = 6th week storage

DMR test shows that moisture content at the beginning of the experiment was 9.15%, which increased to 9.61% after 42 days of storage. Any change in relative humidity of room affects the moisture content of flour due to its hygroscopic nature.

Where

T_0 unfortified flour sample

T_1 Flour sampled fortified with 20 ppm each of $FeSO_4$ and EDTA and 1.5 ppm folic acid.

T_2 Flour sample fortified with 40 ppm each of FeSO4 and EDTA and 3.0 ppm folic acid.

T_3 Flour sample fortified with 60 ppm each of FeSO4 and EDTA and 4.5 ppm folic acid.

It is obvious from the results that the moisture content was significantly higher in unfortified flour where as the moisture content did not vary significantly among these fortified flour samples.

Table 1. Analysis of variance table showing the effect of storage on moisture content of flour

SOV	Df	SS	MS	F-Value
Storage period (S)	6	1.942	0.324	51.6042**
Treatments (T)	3	2.614	0.871	138.8923**
S x T	18	0.058	0.003	0.5143
Error	56	0.351	0.006	

Where

** = Highly Significant

Comparison of means of different storage periods

Storage periods	S_6	S_5	S_4	S_3	S_1	S_2	S_0
Means	9.61a	9.51b	9.44c	9.37d	9.29e	9.21f	9.15g

Table 2. Analysis of variance table showing the effect of storage on protein content of flour

SOV	Df	SS	MS	F-Value
Storage period (s)	6	0.402	0.067	1.3970 NS
Treatments (T)	3	0.167	0.056	1.1633 NS
S x T	18	0.086	0.005	0.0998
Error	56	2.683	0.048	

Where

NS = Non-Significant

Comparison of means of different storage periods

Storage periods	S_0	S_1	S_2	S_3	S_4	S_5	S_6
Means	10.44	10.44	10.40	10.34	10.30	10.28	10.24

Comparison of means of different treatments

Treatments	T_0	T_2	T_1	T_3
Means	9.67a	9.28b	9.26b	9.26b

Means sharing similar letters are statistically non-significant.

Protein Content

Analysis of Variance (Table 2) for protein content indicates that storage periods and treatments have a non-significant effect on flour samples.

These results describe that in the fresh flour samples, protein content was found to be 10.44% which decreased to 10.24% after 42 days storage. It was obvious from the results that protein was affected as a result of storage and showed a decreasing trend but the effect was non-significant.

Fat Content

The data regarding the fat content presented in Table 3 indicates that fat content was found statistically identical in flour sample stored for 42 days. The data also revealed that fortification did not show significant influence on fat content.

Table 3. Analysis of variance table showing the effect of storage on fat content of flour

SOV	df	SS	MS	F-Value
Storage period (S)	6	0.21	0.004	0.642 ^{NS}
Treatments (T)	3	0.003	0.001	0.1743 ^{NS}
S x T	18	0.007	0.00	0.0594
Error	56	0.353	0.006	

Where
NS = Non-Significant

Comparison of means of different storage periods

Storage periods	S_0	S_1	S_2	S_3	S_4	S_5	S_6
Means	2.29	2.28	2.27	2.26	2.25	2.25	2.24

It is indicated that fat decreased in wheat during storage period. The fat in fresh flour sample was 2.29%, which decreased to 2.24% during storage, but this decrease was non-significant.

Fiber Content

Crude fiber is an insoluble and combustible organic residue, which remains after the sample has been treated with dilute acid or alkali under prescribed conditions.

The data in table 4 shows that crude fiber was not affected as a result of storage period of 42 days and due to fortification.

Table 4. Analysis of variance table showing the effect of storage on fiber content of flour

SOV	df	SS	MS	F-Value
Storage period (S)	6	0.004	0.001	0.0157^{NS}
Treatments (T)	3	0.003	0.001	0.0234^{NS}
S x T	18	0.012	0.001	0.0162
Error	56	2.003	0.042	

Where
NS= Non-Significant.

Ash Content

The ash content in the foodstuff represents inorganic residues remained after the organic matter has been burnt away. The ash content obtained may not necessarily account for exactly the same composition as the mineral matter present in the original food. There may be some losses due to volatilization or some interaction between the constants (Krik and Sawyer, 1991). The data regarding the Analysis of Variance (Table 5) represents that, there was non-significant trend between storage intervals and different levels of iron fortification.

Table 5. Analysis of variance table showing the effect of storage on ash contents of flour

SOV	Df	SS	MS	F-Value
Storage period (S)	6	0.017	0.003	0.2559^{NS}
Treatments (T)	3	0.088	0.029	2.6124^{NS}
S x T	18	0.018	0.001	0.0904
Error	56	0.628	0.011	

Where
NS = Non-Significant

Comparison of means of different treatments storage periods

T_0	T_1	T_2	T_3
1.15	1.22	1.23	1.23
Means			

It was concluded that ash content in unfortified flour (T_0) was lower (1.15%) than the fortified flours (1.23%). However the fortified flours have exhibited non-significant differences with one another in this experiment. The presence of slightly higher levels of

Table 6: Farinographic characteristics of iron fortified and unfortified flour sample

Flour samples	WA (%)	AT (%)	DDT (Min)	DT (Min)	DS (Min)	T1 (BU)	SOD (BU)
T_0	73.0	2.0	3.0	4.0	2.0	80	90
T_1	73.0	2.5	3.5	4.5	2.0	70	90
T_2	73.2	2.0	3.5	5.0	3.0	65	100
T_3	73.4	2.0	3.0	5.5	3.5	80	100

ash in fortified flour might be attributed due to the addition of micronutrients (iron and folic acid) in the flour samples.

Rheological studies

Farinographic and Amylographic studies of fortified and unfortified flour samples were conducted in order to study the rheological characteristics of the flour samples.

The Farinogram shows a general profile of mixing behavior of the dough. The physical dough characteristics include water absorption, arrival time, dough development time, dough stability, departure time, tolerance index and softening of the dough.

Where
WA = Water Absorption
AT = Arrival Time
DDT = Dough Development Time
DT = Departure Time
DS = Dough Stability
T1 = Tolerance Index
SOD = Softening of Dough

The water absorption ranged from 73.0 to 73.4% among different treated wheat flours. The highest water absorption was recorded in T_3 with 60 ppm of iron levels and the lowest was found in T_0 i.e. unfortified flour sample.

The arrival time was in the range of 2.0 to 2.5 minutes. Maximum dough development time was observed in T_1 and T_2 (3.5 minutes) while the lowest in T_0 and T_3 samples. The highest departure time was observed in T_3 (5.5 minutes) and the lowest was obtained from the flour of T_0 (4.0 minutes). The dough stability varied from 2.0 to 3.5 minutes. The highest dough stability was exhibited by the farinograms of T_3 flour sample where as the lowest in T_0 and T_1 flour sample. The tolerance index ranged from 65-80 Brabender Units (BU). The dough softening varied from 90 to 100 BU.

The results pertaining to the physical dough properties obtained from different wheat flours samples are comparable with the early findings of Hinnai *et al* (2000) and Anjum *et al.* (2002).

The amylographs are related to the starch contents of the flour and its behavior during heating. Amylograph peak viscosities of whole wheat flour samples (fortified and unfortified) are given in Table 7.

Table 7. Amylographic characteristics of iron fortified and unfortified flour samples.

Treatments	Viscosity (BU)
T_0	2600
T_1	2560
T_2	2520
T_3	2500

Where BU = Brabender Units

Unfortified whole wheat flour (T_0) has peak viscosity 2600 BU, while T_1, T_2, and T_3 samples with 20, 40 and 60 ppm iron levels have peak viscosities of 2560, 2520 and 2500 BU, respectively. The decrease in peak viscosity may be attributed to oxidative enzymes and proteolytic activity during storage. Similar decline in peak viscosity of whole wheat flour at varying levels of iron fortification was observed by Hinnai *et al* (2000).

CONCLUSION

It was concluded that storage period of 42 days did not affect significantly all the chemical constituents of flour samples except moisture contents. The reheological studies also revealed that there was a non-significant effect on all the parameters pertaining to the evaluation of flour samples. However it was thus concluded that flour with 40 ppm iron +40 ppm of EDTA and 3.0 ppm of folic acid was more stable during storage.

REFERENCES

AACC. 2000. Approved Methods of the American Association of Cereal Chemists. The American Association of Cereal Chemists. Inc., St. Paul, Minnesota, USA.