Pak J Food Sci 2008, 18(1-4):1-8

Baking quality of wheat flour cookies supplemented with fiber from different sources

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ABSTRACT

Some bakery products are good source of dietary fiber and may serve as low caloric diet. Dietary fiber plays a very important role in the human diet. Dietary fiber, consisting of indigestible cellulose, hemicellulose, lignin & gum, provides a variety of health benefits. High fiber content in food products helps to overcome health problems such as high cholesterol, hypertension, diabetes, colon cancer and intestinal disorders. Fibers from wheat, corn, rice and Psyllium were selected to study their influence on baking quality of wheat flour cookies. Extracted wheat bran supplemented cookies were also compared with unextracted bran supplemented cookies. Sensory evaluation showed that cookies containing wheat bran (20%), corn bran (20%), rice bran (20%) and psyllium husk (10%) were highly accepted. Among extracted wheat bran supplemented cookies 8% substitution level was considered best. Calorific value also decreased gradually with increasing level of bran %age.

Key words: Cookies, fiber, baking quality

INTRODUCTION

Dietary fiber is considered as an important and essential component of diet and fiber obtained from various sources has been successfully incorporated in the food products with significant results. In recent years several types of breakfast cereals containing high amount of bran has been developed and marketed as it helps in weight control and play a key role in reducing colon cancer (Cornell and Hovelling, 1998). Enhancement of fiber content in snack foods, beverages, spices, sauces, frozen foods, meat products and other foods has also been investigated (Hesser 1994). Health benefits of dietary fiber indicate that dietary fiber may give protection against cardiovascular diseases, diabetes and obesity (Spiller, 2001; Anderson *et al* 1987; Burkitt 1988).

There are many sources of dietary fiber such as fruits, vegetables and less commonly used cereals such as barley, which are potential sources of dietary fiber supplements (Matz 1991). Amongst different sources of fiber, cereal bran is the most important and cheap source of fiber. The outer bran layers of cereal grains are rich in dietary fiber and contain high level of minerals. Wheat bran is about 14.5% of the kernel weight. It is rich in protein (~14%), carbohydrates (~27%), minerals (~5%), fat (~6%) and B-vitamins (Kent and Evers 1994) and an abundant source of dietary fiber (Burkitt 1988). Rice bran is rich in nutrients with 14-16% protein, 12-23% fat, and 8-10% crude fiber. It is also a good source of B vitamins and contains minerals such as iron, potassium, calcium, chlorine, magnesium, and manganese (Saunders, 1985) while corn bran contains 88.0% total dietary fiber with 67% of that total as hemicelluloses and 18% as cellulose. Psyllium husk has also been used to provide soluble fiber. Psyllium-enriched breakfast cereals have been included in diets aimed at reducing blood cholesterol levels (Cornell and Hovelling 1998; Olleros *et al* 1999).

Biscuits are ideal for their nutritive value, palatability, compactness and convenience (Malick and Sheikh 1976). Having low moisture content than cakes and bread, biscuits are generally safer from microbiological spoilage and have long shelf-life (Wade 1988). Several workers have used fiber sources such as wheat bran, oat bran, corn bran, barley bran and

psyllium husk, among others to prepare high fibre bread (Laurikainen *et al* 1998; Pomeranz *et al* 1977; Sidhu *et al* 1999; Wang *et al* 2002) and high fiber cookies (Sudha *et al* 2007, Artz *et al* 1990, Haque *et al* 2002).

The present investigation was planned to develop a product with high fiber content and low caloric value. The other objectives were to study the baking quality of cookies prepared from wheat flour supplemented with aqueous extracted and unextracted wheat bran and other fiber sources to investigate the effect of different levels of fiber supplementation on sensory characteristics of cookies.

MATERIALS AND METHODS

Collection of raw materials

Wheat and rice bran were obtained from respective flour mills. Corn bran was purchased from Rafhan Maize Products, Faisalabad and commercially available psyllium husk was procured from market. Wheat sample of variety Ufaq was procured from Wheat Research Institute, Faisalabad.

Physical analysis of wheat

The wheat sample was analyzed for different physical characteristics like grain size, thousand kernel weight and test weight according to the methods given in AACC (2000).

Chemical analysis of flour and fibers

Chemical analysis i.e. moisture, ash, crude protein, crude fiber and crude fat of bran samples was performed using methods 44-15A, 08-01, 46-12, 32-10 and 30-10, respectively (AACC 2000).

Aqueous extraction of wheat bran

Wheat bran was suspended in ten volumes of tap water (pH 6.7) in a flask and agitating at high speed on a horizontal flask shaker for 16 hours at room temperature (Roberts *et al* 1985). The resulting suspension was passed through a cheese-cloth and the separated bran was washed twice with five volumes of water which was later used as extracted bran. The extracted bran was dried in cabinet drier on a stainless steel tray at 70 °C for 4-5 hours.

All brans including extracted wheat bran and unextracted wheat bran, corn bran, rice bran and psyllium husk were grinded in a cyclone mill and the samples were stored in air-tight polythene bags.

Preparation of biscuits

Biscuits were prepared from flour with and without the addition of untreated and treated fibers according to the recipe given in AACC (2000).

Flour	225g
Sugar	130g
Vegetable ghee	64g
Baking powder	2.5g
Dextrose solution	33.0mL
Water (Distilled)	16 mL

Method:

The ingredients required for the preparation of cookies were weighed accurately. Vegetable ghee and sugar were creamed till foaming occurred. Mixture was mixed at low speed for 3 minutes and scraped down after each minute. Dextrose solution and distilled water were added and mixed for 1 minute at low speed. The flour and baking powder were added to the creamy mass and mixed to a homogenous mass for two minutes at low speed. Dough was scraped from the bowl and placed on lightly greased cookie sheet. Using gauge strips, rolled to the thickness with one forward rolling pin stroke and one return stroke. Dough was cut with cookie cutter and excess dough was discarded. The cookies were placed on baking trays at a proper distance and were backed at 185 °C for 10 minutes. After baking, the cookies were cooled at room temperature and packed in air tight polythene bags for further studies. In the formulation of cookies, wheat flour was replacement with different brans and psyllium husk at 10%, 20% and 30% level and in case of extracted wheat bran 4%, 8% & 12% flour was replaced with bran.

Extraction of cookies

Chemical Analysis of cookies

Cookies were analyzed for proximate composition according to the methods given in AACC (2000).

Physical analysis of cookies

The diameter, thickness, and spread factor for cookies was measured according to the methods described in AACC (2000).

Sensory evaluation

The cookies were evaluated by a panel of judges from the staff and postgraduate students of the Department for taste, color, flavour, texture, and overall acceptability according to the procedure described by Meilgaard *et al* (1991).

Gross Energy value

The amount of heat measured in calories that is released when a substance is completely oxidized in a bomb calorimeter is called the gross energy of a substance. Calorific Value (C.V) of the cookies was estimated by using Werke IKA C2000 Oxygen Bomb Calorimeter (Krishna and Ranjhan 1981).

Statistical Analysis

The data were analyzed statistically by the methods described by Steel *et al* (1996).

RESULTS AND DISCUSSION

Chemical composition of wheat flour

Wheat flour used for the preparation of cookies contained 10.5% moisture, 1.60% ash, 10.47% crude protein, 2.16% crude fat, 1.9% crude fiber and 73.62% nitrogen free extract (NFE). Results are in close agreement with the findings of Shafiq (1999) who reported that moisture content, crude protein, crude fat, crude fiber, ash and nitrogen free extract in wheat flour was 10.36%, 10.09 %, 1.24 %, 0.45 %, 0.57 % and 77.28% respectively. Various researchers observed that moisture content varied from 8.19-11.94 %.

Chemical composition of different fiber sources

Fiber from different sources was analyzed for moisture, ash, protein, fat and fiber content. The moisture content of corn bran was 8.5% and that of other fiber samples ranged between 5.2 and 14.5%. Ash content of corn bran was as high as 4.7% and that of all other fiber samples ranged between 2.0 and 4.7%. Crude fiber of corn bran was 11.7% and for all other fiber samples ranged between 3.5 to 10%. The highest fat content was found in rice bran as 18.6% and that of other fiber sources ranged between 0.3 and 6.5%. Similar results for different fiber sources were obtained by Sudha *et al* (2007) which indicated that the moisture content of rice bran was 10.56% and that of other bran samples ranged between 4.92% and 7.68%. Ash content of rice bran was as high as 10% and that of all other bran samples ranged between 4% and 5.7%. The fat content was in between 4–8.0% and protein content was 12% and 14%. The dietary fiber is ranged between 40% and 47.5% for all other bran samples. Moisture, protein, ash and fiber %age of corn bran found by Artz *et al* (1990) was 4.5%, 8.5%, 1% and 10% respectively.

Olerrus *et al* (1999) also observed the similar range after analyzing psyllium fiber and found that moisture, ash, crude protein, and crude fiber contents ranged between 7.88-8.96%, 1.43-1.79%, 10.11-11.77%, 1.74-2.93% and 0.98-1.42%, respectively.

The results regarding chemical composition of un-extracted wheat bran contained 15.5% moisture, 3.80% ash, 12% crude protein, 3.5 % crude fat, 5.6 % crude fiber and 60.1% nitrogen free extract (NFE) while extracted wheat bran contained 6.5% moisture, 2% ash, 7.2% crude protein, 6.5 % crude fat, 10 % crude fiber and 67.8% nitrogen free extract (NFE). The results are in line with the findings of Haque *et al* (2002)

Influence of fiber on physical characteristics of biscuits

Cookies prepared with 0%, 10%, 20%, and 30% addition of different fibers were evaluated for

various physical characteristics like diameter, thickness and spread factor. The results showed that incorporation of wheat bran decreased the spread of the biscuits from 41.79 to 41.30 mm without much change in the thickness of the cookies hence the spread ratio decreased. Incorporation of rice bran thickness of the cookies increased but reduced the spread of the biscuits from 40.26 for T_0 to 30.7 mm. Biscuits became harder as seen in the increasing breaking strength values especially at 30% levels of brans. Psyllium husk incorporation showed an increase in spread (40.2 – 30.7 mm) and thickness (10.33-10.90 mm). Sudha *et al* (2007) also showed that incorporation of different bran decreased the spread factor of cookies without much change in thickness. These results are in close agreement with Haque *et al* (2002) who observed that diameter of bran cookies first decreased and then increased with further increase in fiber. Reduction of cookies diameter was also observed by Sievert *et al* (1990).

Influence of fiber on sensory characteristics of cookies

Regarding the sensory evaluation, the scores for color of the cookies showed that the cookies became darker with increasing level of all the bran except for Psyllium husk incorporation where the percent whiteness decreased marginally. Wheat bran incorporation increased the darkness and reduced the surface smoothness. Above 30% incorporation of wheat bran in the formulation biscuits had dark crumb color and very hard texture. Taste and mouth feel of the biscuits were favorably affected at 20% level. Biscuits had a dry mouth feel at 20% level. Incorporation of rice and corn bran showed similar sensory characteristics at all levels. The crumb color darkened beyond 20%. At 10% incorporation, each bran samples did not affect the quality of biscuits. Quality of biscuits was acceptable at 20% for wheat bran and rice and corn bran and 10% for psyllium husk only. For taste the results indicated that with the increase of level of substitution of fiber in wheat flour cookies, the score for taste of cookies improved till 20% and exceeding this level the scores decreased. The scores for flavour of cookies were observed maximum at 20% wheat bran and also for corn, rice bran and psyllium husk.

Table 3. Influence of fibers on physical characteristics of cookies

Sample	Width (mm)	Thickness (mm)	Spread factor
Control	44.91	10.53	40.20
Wheat bran			
T_1	42.75	10.33	30.90
T_2	42.66	10.75	30.87
T_3	40.80	10.90	30.70
Corn bran			
T_1	41.50	10.16	40.00
T_2	40.95	10.08	30.90
T_3	40.25	10.0	40.02
Rice bran			
T_1	41.25	9.66	40.26
T_2	40.80	9.91	40.30
T_3	40.0	10.75	30.70
Psyllium husk			
T_1	41.66	10.25	30.66
T_2	40.81	10.00	40.08
T_3	40.83	9.33	40.30

Influence of fiber on the calorific value of cookies

As regarding the calorific value of fiber supplemented cookies the results indicated that the calorific value is reduced with addition of fiber. The increase in percentage of fiber progressively decreased the calorific value. Highest calorific value 5003 cal/g was observed in T_0 having wheat flour (100 %) while lowest calories 4938 cal/g was given by T_3 with wheat bran. In case of corn bran, rice bran and psyllium husk supplemented cookies the highest calorific values were 4970, 4933 and 4963 cal/g

respectively and lowest calorific values were 4875, 4930 and 4880 cal/g. For all the treatments and fiber sources T_3 had lowest values as compared to control as shown in Fig. 1. The results of this study are in close agreement with the findings of Anjum *et al* (2006) who reported that increasing levels bran in baked products (cakes) progressively decrease the calorific value of the product.

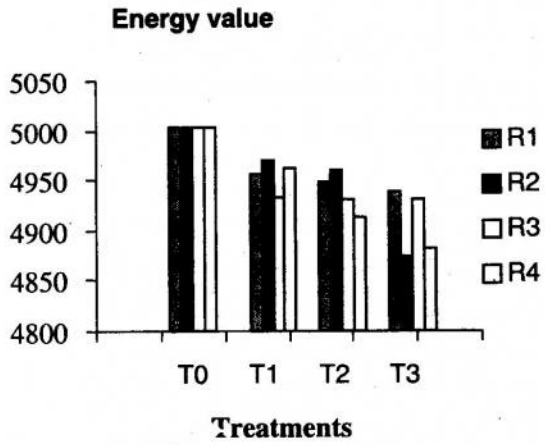


Fig.1. Effects of different fiber sources on the calorific value of cookies

Effects of extracted and unextracted wheat bran on the physical characteristics of Biscuits

Biscuits prepared with varying levels of replacement were evaluated for diameter, thickness and spread ratio. The results for analysis of variance are showed that all physical parameters affect significantly with the addition of extracted and unextracted bran as compared to control.

The mean values for diameter and thickness of all categories of biscuits made with different bran treatments. The results (Table 4) showed that up to 8% substitution level there was not much change in diameters, after which the values decreased with further increase of the substitution level from 42.83 to 41.60 in case of unextracted bran cookies and from 42.66 to 41.58 in case of extracted bran cookies. A reduction of cookie diameter with the increase of bran incorporation was also obtained by Sievert *et al* (1990). In general, Biscuits containing unextracted wheat bran were of larger diameter and thickness than biscuits containing extracted wheat bran.

Table 4. Effects of extracted and unextracted wheat bran on the physical characteristics of cookies

Treatments	Width (W) mm	Thickness (T) mm	Spread factor (W/T) × 10
T_0	44.91a	10.5f	4.27a
T_1	42.83b	10.83c	3.95b
T_2	41.33f	11.0b	3.75e
T_3	41.60d	10.67e	3.89c
T_4	42.66b	11.00b	3.87d
T_5	42.0c	11.50a	3.65f
T_6	41.58e	10.80d	3.85e

Note: The values having same letters have non-significant difference

T_0 = Cookies Sample without bran
T_1 = Cookies Sample with 4% wheat bran (Unextracted)
T_2 = Cookies Sample with 8% wheat bran (Unextracted)
T_3 = Cookies Sample with 12% wheat bran (Unextracted)
T_4 = Cookies Sample with 4% wheat bran (Aqueous extracted)
T_5 = Cookies Sample with 8% wheat bran (Aqueous extracted)
T_6 = Cookies Sample with 12% wheat bran (Aqueous extracted)

As shown in Table 4, the spread ratio is significantly influenced by the addition of extracted bran. In general, the flour without bran yielded cookies with higher spread ratio than that of fiber supplemented flour. Cookies with unextracted bran had higher spread ratio that ranged from 3.89-3.95 than that of cookies with extracted bran that ranged from 3.85-3.87. There is a slight decreasing trend in spread ratio

with increasing bran %age either extracted or unextracted.

These results are in close agreement with Haque *et al* (2002) who observed that diameter of bran cookies first decreased than increased with further increase in fiber. Reduction of cookie diameter was also observed by Sievert *et al* (1990).

Effects of flour replacement by extracted & unextracted bran on the sensory properties of cookies

Sensory evaluations on color, flavour and texture were judged by a panel of judges for all the biscuits containing different bran components. Table 5 shows the average scores of color, flavour, surface characteristics and texture for the cookies prepared with extracted and unextracted wheat bran. The scores for all the parameters were lower than that of the control biscuit. In general, up to about 8% substitution level yield higher scores which were close to the control for all the parameters studied. Substitution levels of 12% produced biscuit with relatively lower values for all the parameters. Based on this result, substitution level of 8% was chosen as best. Cookies containing extracted ground bran were mostly preferred and their score was closed to the control. The results are in close agreement with those of Haque *et al* (2002).

Table 5: Effects of extracted and unextracted wheat bran on the sensory evaluation of cookies

Treatments	Color	Crispness	Taste	Surface characteristics	Overall acceptability
T_0	6.15	7.0	6.37	7.87	6.9
T_1	7.0	7.25	7.25	7.0	7.5
T_2	7.62	7.5	7.37	6.75	8.2
T_3	5.6	6.87	6.62	6.1	7.8
T_4	5.5	6.75	5.5	6.75	6.9
T_5	6.25	6.25	6.12	6.125	5.9
T_6	5.25	3.5	5.87	5.16	5.6

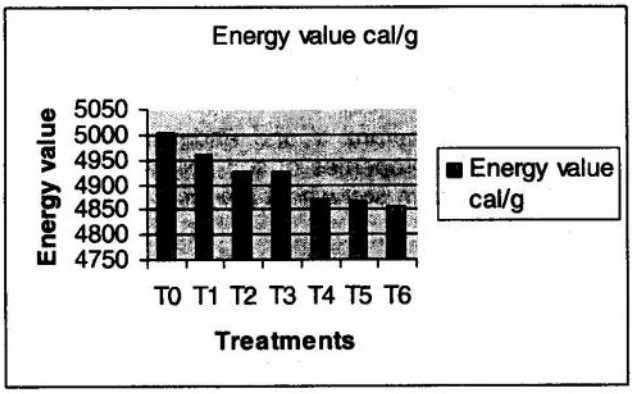
T_0 = Cookies Sample without bran
T_1 = Cookies Sample with 4% wheat bran (Unextracted)
T_2 = Cookies Sample with 8% wheat bran (Unextracted)
T_3 = Cookies Sample with 12% wheat bran (Unextracted)
T_4 = Cookies Sample with 4% wheat bran (Aqueous extracted)
T_5 = Cookies Sample with 8% wheat bran (Aqueous extracted)
T_6 = Cookies Sample with 12% wheat bran (Aqueous extracted)

Effects of flour replacement by extracted & unextracted wheat bran on the calorific value of cookies

It was evident from the results that the calorific value reduced by the use of extracted fiber. The increase in percentage of fiber progressively decreased the calorific value. Highest calorific value 5003 cal/g was observed in T_0 having sucrose (100 %) while lowest calorific value 4856 cal/g was given by T_3 having extracted wheat bran 12 %. In case of unextracted wheat bran supplemented cookies the calorific values were 4961, 4927 and 4926 cal/g for T_1, T_2 and T_3 respectively and calorific values for extracted wheat bran cookies were 4870, 4866 and 4856 cal/g. For all the treatments and fiber sources T3

had lowest values as compared to control. All the results show that due to increase in fiber content by wheat bran addition the calorific value of cookies decreased (fig. 2).

Fig. 2: Effects of extracted and unextracted wheat bran on the calorific value of cookies



T_0 = Cookies Sample without bran
T_1 = Cookies Sample with 4% wheat bran (Unextracted)
T_2 = Cookies Sample with 8% wheat bran (Unextracted)
T_3 = Cookies Sample with 12% wheat bran (Unextracted)
T_4 = Cookies Sample with 4% wheat bran (Aqueous extracted)
T_5 = Cookies Sample with 8% wheat bran (Aqueous extracted)
T_6 = Cookies Sample with 12% wheat bran (Aqueous extracted)

The results are in close agreement with the findings of Rauf (2004) who had observed decreasing trend in calorific value of cookies with treated and untreated wheat bran. He reported that calories decrease in both treated and untreated bran biscuits as the percentage of fiber increased.

The overall results showed that 30 % replacement of wheat flour with fiber in cookies gave better results. While in case of extracted and unextracted wheat bran supplemented Biscuits the best results were found by 8% incorporation of fiber. So the main objectives of this study are fulfilled.

CONCLUSION

Cookies containing wheat bran (20%), corn bran (20%), rice bran (20%) & psyllium husk (10%) were found highly acceptable. Among different sources wheat bran was considered best having pleasant flavour & taste but psyllium husk supplementary was not much liked by the judges. Cookies containing extracted wheat bran showed better nutrients as it contained more fiber, fat & carbohydrates. Extracted bran gave smooth texture to cookies but dark brown color was imparted. Overall extracted bran performs better in the production of cookies than parent bran itself.

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Pak J Food Sci 2008, 18(1-4):9-13

Iodine content of some fortified salt brands in Swat district as affected by different storage conditions

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ABSTRACT

Iodized salts collected from eight different factories of Swat, NWFP, Pakistan were assessed titrometrically for the stability of iodine under various storage conditions i.e. temperature, moisture and sunlight. The tested samples varied significantly in their fortified iodine content as well as in their response to various storage conditions. Storage under different temperature regimes resulted in significant reduction in iodine content, ranging from 2% at 30°C to 63% at 50°C. The effect of sunlight exposure and increased moisture content of the salt samples also resulted in reduction in iodine content. From these results it was concluded that iodine was adversely affected by various conditions during storage, hence after fortification with iodine in the factories, proper handling and storage of salt is required to extend the durability of this important element for its effectiveness.

Keywords: Salt, Fortified, iodine, moisture, temperature.

INTRODUCTION

Deficiency of iodine, which is among the body's essential micro nutrients, is both easy and inexpensive to prevent. Iodine is an essential element for normal growth and development in animals and humans.Iodine is one of the essential trace elements required for the synthesis of thyroid hormones, tetra-iodothyronine (T4) and tri-iodothyronine (T3), which are essential for normal growth and development. These are involved in body thermoregulation, oxidative phosphorylation, protein synthesis, fatty acid oxidation and for maintaining normal metabolic activities of brain and nervous tissues. Iodine is available to the body from both animal and plant sources. Dietary iodine is transformed in the gastro-intestinal (GI) tract into iodide, which is rapidly absorbed in the blood and subsequently distributed in the thyroidal and extra thyroidal body pool (Kapil *et al* 2003).

Iodine deficiency is one of the most widely prevalent nutritional disorders affecting six hundred fifty five million people with goiter and 11 million people with cretinism (Harr, 1997). Iodine deficiency results in several health problems and impaired growth ((WHO/UNICEF/ICCIDD 1996).Normally the human requirement of iodine is 150 µg per day. About 90 % of that comes from food and 10 % from the water (Goindi *et al* 1995). Deficiency of iodine occurs when the daily intake, especially of food and water does not fulfill the requirement of the body. Iodine deficiency was first recognized by McCarrison in Northern areas of Pakistan where 25.9% of the population was found to be affected by endemic goiter. This was followed by the survey in which 31% of the soldiers stationed at Gilgit had goiter (Rafiq1998).

Iodine requirement may be fulfilled through food in fortified salt, which is adequate for body requirement. However storage condition and cooking may affect the iodine content (Wang and Xinhaung 1998; Kelly 1953). The stability of iodine in iodized salt is determined by the moisture content of the salt, humidity of atmosphere, light, heat, impurities and acidity or

alkalinity (Kelly, 1953). Wang and Xinhaung (1998) reported greater loss of iodine for salt stored in plastic bags than in glass bottle. The coarse salt iodized with iodate at normal room temperature and humidity shows more iodine losses (up to 20% after 12 months) than the samples stabilized with calcium carbonate (no loss for 18 months) by (Rangnathna and Rao1986).

Thus in order to develop appropriate quality assurance system, the information about factors affecting the iodine content, degree of homogeneity and its stability during storage is required. The present study was aimed to access the factors affecting the stability of iodine in different brands of salt samples collected from the local factories of the area in order to make some precautionary measures to minimize the elimination of iodine from the iodine-fortified salts.

MATERIAL AND METHODS

Samples of iodine-fortified salts were obtained from the local factories of Swat available in different size packets. They were analyzed for iodine content by the titration method (AOAC 1990) at Postgraduate Laboratory of Agricultural Chemistry Department, NWFP Agricultural University, Peshawar. The salts were subjected to different ranges of temperature (30, 40 and 50^{0}C), to evaluate their effects on iodine reduction. Similarly the effects of sunlight exposure and moisture content (artificially increased) were checked at weakly intervals for three weeks.

To study the effect of temperature on iodine content, 1 g of each sample was taken in Petri dish and placed in oven at 30, 40 and 50 ^{0}C for 4 hours daily and were analyzed after one week each. The effect of sunlight was studied by placing the samples in Petri dishes under bright sunshine for duration of 4 hours per day. Iodine content was determined on weakly basis for three consecutive weeks. Similarly to assess the effect of increased moisture content of salt sample, the samples were taken in Petri dishes and were transferred to the cabin with dimensions of 4 × 3 feet having one shelf. A moistened towel was placed beneath the shelf and the door of cabin was closed. Iodine content was recorded after one-week interval along with total moisture absorbed was determined by oven drying method (AOAC 1990).

RESULTS AND DISCUSSION

Data on fortified iodine content of various salt brands is given in Table I. Perusal of the data on iodine content of various salt brands showed that iodine content of these were in the range of 45.5 mg kg^{-1} to 88.0 mg kg^{-1}. It was maximum in salt brand Pakistan National Refined Table Salt and the minimum content was found in salt brand of Utility Namak.

Table 1 Fortified iodine content (mg kg^{-1}) of various salt brands

Salt sample	Brand name	Initial iodine content (mg kg^{-1})
SS_1	Khalid iodized salt	46.6
SS_2	Habib Kohistan Qudrati Namak	48.7
SS_3	Kohistan Namak	59.2
SS_4	Mayar Namak	47.6
SS_5	Pakistan National Refined table salt	88.0
SS_6	Utility Namak	45.5
SS_7	Pakistan National iodized salt	55.0
SS_8	Pakistan National Refined table Salt	69.8
Mean		**57.5**

Effect of temperature

The effect of temperature on iodine stability in the samples, which were treated, with three different levels of temperature (30, 40 and 50^{0}C) showed the maximum loss i.e. 63% in SS_6 the brand sample of Utility Namak stored at 50^{0}C temperature. The minimum reduction (2%) occurred in sample SS_1 i.e., the salt sample of khalid iodized salt stored at 30^{0}C (Fig 1). Reduction observed in iodine content was statistically significant at ($p = 0.01$). The data was found to be closely related to Chauhan *et*

al. (1992) who also studied the effect of temperature on iodine content of fortified salt. Similar results were also observed by Arroyave *et al* (1956). The loss may be due to the fact that iodine is a volatile element and it is unstable to a high range of direct temperature, which converts it into elemental iodine, and is gradually lost from the salt, especially by the reaction with atmospheric oxygen (Diosady *et al* 1997).

Effect of sunlight

The effect of sunlight on reduction of iodine content in the salt samples was non-significant ($P>0.01$). However, maximum change (27%) was observed in salt sample SS_7 i.e., the brand sample of Pakistan National Iodized salt, stored for three weeks (Fig 2). Salt sample SS_4 i.e., the sample of Mayar Namak stored one weak, exhibited minimum reduction (4%) followed by SS_2 (7%) i.e., the salt sample of Habib Kohistan Qudrati Namak (Moaz Salt Factory, Swat), stored at the same period of time. This shows that storage under sunlight for prolonged period of time results in more loss in iodine content. Previously Holman (1960) studied the effect of light on the stability of iodine contents of iodized salt and suggested that light decrease iodine content during storage by enhancing different chemical reactions which results in increased electron transition.

Effect of moisture content

Iodine content of given samples was determined on weekly basis for three weeks. Moisture content of the container was 25% after one week, 12% after two weeks and 9% after three weeks. The effect of moisture level (artificially provided to the samples in vaporized cabin) was significant. However, the highest reduction (33.11%) was observed for salt sample SS_3 and SS_6 the brand sample of Kohistan Namak and Utility Namak, respectively, stored for three weeks. Minimum reduction in iodine content (7%) was recorded for SS_2 i.e., the sample of Habib Kohistan Qudrati Namak when stored for one week (Fig. 3). Similar results were shown by Chauhan *et al* (1992) who reported that moisture tends to dissolve the iodates, and hence enhance the evaporations of this element (Sooch *et al* 1973) thus resulting in reduced iodine content.

CONCLUSION AND RECOMMENDATION

From these results it was concluded that iodine could be affected by various conditions during storage, so after fortification with iodine in the factories, proper handling and storage of salt is required to extend the durability of this important element for its effectiveness.

Fig 1: Reduction in Iodine content (%) in different salt samples stored at three temperature regimes $T_1 = 30^0C$, $T_2 = 40^0C$ and $T_3 = 50^0C$) each for one weak.

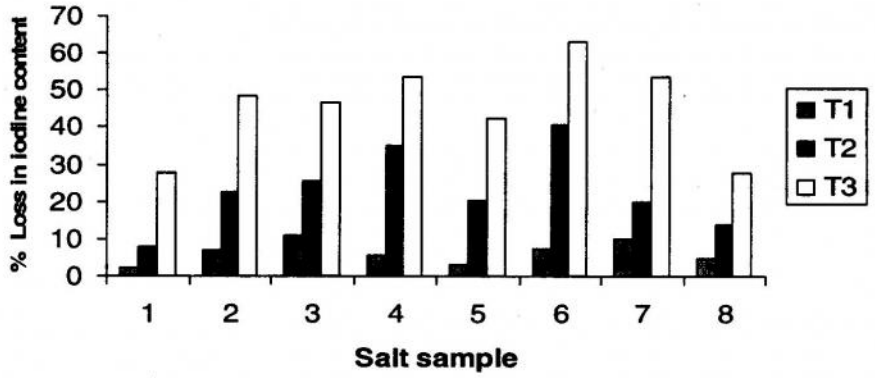


Fig 2: Reduction in Iodine content (%) in different salt samples stored under sunlight for three weeks.

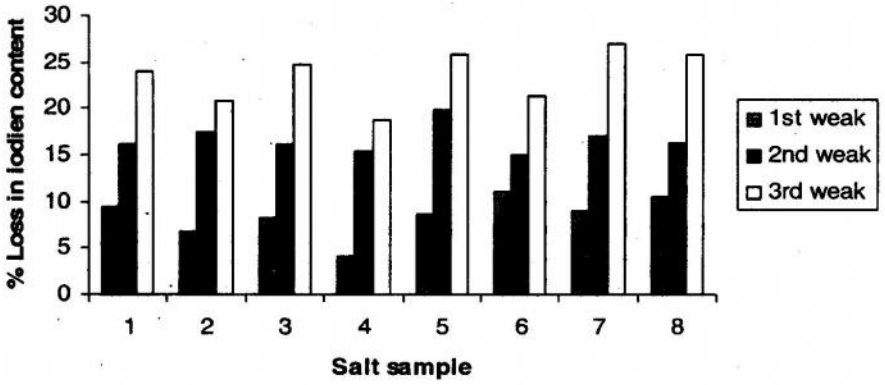
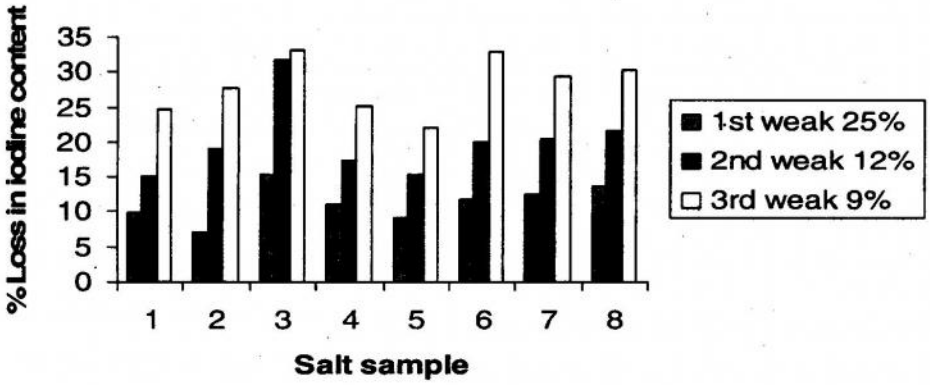


Fig 3: Reduction in iodine content (%) in different salt samples stored under increased moisture condition for three weeks



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Pak J Food Sci 2008, 18(1-4):14-20

Studies on the microbiological status of some ready-to-eat street foods sold in Peshawar city

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ABSTRACT:

To evaluate microbiological quality of ready-to-eat street foods, 33 samples were collected from three locations in Peshawar city. Standards methods were used to determine total plate count (TPC), total coliform bacteria (TC), fecal coliform bacteria (FC), *E. coli* 0157:H7, *Staphylococcus aureus* and Salmonella spp. The samples analyzed were beef, cooked chickpea, fried rice, samosa, pakorra, sandwich, burger, dehi bhalay, chicken soup, kheer and fried chips. Maximum and minimum TPC were found 8×10^9 cfu/g and 1×10^2 cfu/g, all the samples were contaminated with TC with a minimum count 21 MPN/g to high count >1100 MPN/g. FC were present in 61% (20 samples), whereas *E. coli* 0157:H7 were present in six samples. *Staphylococcus aureus* were present in 85 % (28 samples) with a high count of 32 cfu/g and low count of 2 cfu/g in the analyzed samples. Salmonella were present in 7 samples. It was concluded that the quality of ready-to-eat street food analyzed in this study was not satisfactory as the observed unhygienic food handling practices and unhygienic environmental conditions under which the vendors prepared and sold these food items.

Keywords: Microbial quality, street foods, disease, Peshawar city.

INTRODUCTION

In developing countries, drinks, meals and snacks sold by street vendors are widely consumed by millions of people. These street foods provide an affordable source of nutrients to many sectors of population, including the urban poor. Street foods include commercially produced snacks that are retailed by street food vendors, as well as food items made by vendors on-site or nearby kitchens (Ohiokpehai 2003). Ready-to-eat (RTE) foods refer to foods that do not require further significant preparation other than reheating or completion of a cooking process (FEHD 2001 and FSAI 2001). Vendors are usually congregate in over crowded areas where there are high numbers of potential customers. Such areas are usually provide limited access to basic sanitary facilities such as running water, garbage disposal and clean toilets. In these areas large amount of garbage accumulate and provide harborage for insects and animal pests (Bryan *et al* 1988, Dawson and Canet 1991, Bryan *et al* 1992a, Ekanem 1998, Mosupye and Von Holy 1999). Such conditions have given rise to many concerns regarding the sanitary standards of street-vending operations, especially because consumers are concerned about price of food rather than its safety and hygiene in many cases (Bryan *et al* 1988, Ekanem 1998). Microbiological studies carried out on street foods in several developing countries have reported high bacterial count in foods (Bryan *et al* 1988, Bryan *et al* 1992a, Bryan *et al* 1992b, Bryan *et al* 1992c, Bryan *et al* 1997, Umoh and Odoba 1999). Street-vended foods have also been implicated in several outbreaks of food born diseases. In Senegal more than 200 cases of food poisoning were reported and street foods made from dairy products were incriminated (Dawson and Canet 1991). In Cuba, 14 people died and 70 others were hospitalized for food poisoning after eating fried foods sold by a private vendor (CNN 1999). Fried and boiled rice were previously implicated in reported food borne disease outbreaks in the Philippines (Roque *et al* 1996 and Roces *et al* 2000). Characteristic symptoms of Salmonella food poisoning include diarrhea, nausea, abdominal pain, mild fever and chills, vomiting, headache and malaria (Forsythe 2006). Epidemiologist at central disease control (Center of Disease Control) estimate that approximately 73,000 cases of *E. coli* 0157:H7 infection occur

annually in the USA, with about 61 deaths (Jeffrey 2004).

The objective of present studies was to evaluate thedifferent samples of ready to eat street foods sold in Peshawar City for microbiological quality and tocreate awareness among the common people.

MATERIALS AND METHODS

Collection of samples

Approximately 200 g of each sample was packed from at least 3 shop in each locality in sterilized Duran glass bottle of capacity 500 mL, which were kept in ice during transportation to the laboratory for the microbiological analysis on the same day. The samples collection points were A (University Road), B (Khyber Bazaar) and C (Hashtnagri Area).

Microbiological Analysis

Total platecount (TPC), total coliforms bacteria (TC), fecal coliforms bacteria (FC), *Escherichia coli (E. coli* 0157: H7), *Staphylococcus aureus* and Salmonella were determined by the methods described by the FAO (Andrews 1992).

Pretreatment of samples

50 g of each samples were mixed with 450 mL, Buffer field's Phosphate buffer and blended for 2 minutes @ of 10,000 rpm in blender (Warring commercial USA) which is a dilution of 10^{-1}

Total plate count (TPC)

Serial dilutions (10^{-2},10^{-3},10^{-4},10^{-5},10^{-6} and 10^{-7}) were made by tranferring 1 mL of previous dilution to 9 mL of sterile diluent. Pipette 1 mL of each dilution into separate and duplicate petri dish using plate count agar as a culture medium and incubated for 48 h at 35 °C.

Total Coliform Bacteria (TC)

All the dilutions were shaken 25 times in 30 cm arc for 7 s. One mL portion for each dilution were transferred to 3 LT (Lauryl Tryptose Broth) tubes with inverted Durham tubes. These inoculated tubes were incubated for 48 ± 2 hrs at 35 ° C. After 24 ± 2 hrs, tubes were examined for gas i.e., displacement of medium in Durham tubes (Fermentation vials) or effervescence when tubes were gently agitated. Negative tubes were re-incubated for additional 24 hrs, after which, again examined for gas. Then a confirmed test on all presumptive positive (gassing) tubes were performed.

Each gassing LT tube was gently agitated and transferred a loopful of suspension to tubes of BGB broth. Then these BGB tubes were incubated for 48 ± 2 hrs at 35 ± 0.5 ° C after which they were examined for gas production and recorded the result.

Then MPN of total coliforms based on combination of confirmed gassing LT tubes for 03 consecutive dilutions were calculated, in our case after reference to the MPN rules and an appropriate MPN table, we reported results as the presumptive MPN of coliform bacteria per g or mL.

Confirmation of fecal coliforms (FC)

Each gassing LT tube was gently agitated and transferred loopful of each suspension to tube of EC medium (*E. coli* medium). These EC broth tubes were incubated for 48 ± 2 hrs 45.5 ± 0.2 ° C. After 24 ± 2 hrs, examined for gas production, if found negative, examined again at 48 ± 2 hrs.. MPN of fecal coliforms based on proportion of confirmed gassing EC medium tubes for 03 consecutive dilutions were noted from MPN table (FAO 1992).

Confirmed Test for E.coli

From each gassing EC medium tube, a loopful of suspension was streaked to L-EMB Agar. Plates were incubated for 18 – 24 hrs at 35 ° C. Plates for typical E. coli colonies i.e. dark centered with or without metallic sheen, were examined. Two typical E. coli colonies were picked from LEMB plate each, and transferred to PCA slants for morphological and biochemical tests. PCA slants were incubated for18-24 hrs at 35 ° C.

Gram staining was performed on each culture. For all cultures appearing as Gram-negative, short rods or cocci, further biochemical tests were performed. The biochemical test performed

were IMViC (Indole production, Voges-Proskauer , Methyl Red, Utilization of citrate. Production of gas in LT broth after the incubation of 48 ± 2 hrs. at 35°C is also a positive reaction for lactose fermentation.

Lastly these typical biochemically positive colonies were examined for their serological confirmation by kits (*E. coli* 0157: H 7 Latex Test Reagent kit, Pro-Lab, Canada) MPN of *E.coli* based on proportion of EC medium tubes in 3 successive dilutions which have been shown to contain *E. coli* were noted from MPN table (FAO 1992).

Table 1. Allowed Microbiological limits of ready-to-eat food

S #	Name of food	TPC (cfu/g)	*E. coli* O157:H7	*S. aureus* (cfu/g)	Salmonella spp
1	Beef	$<10^5$	Not detected in 25g	<20	Not detected in 25g
2	Rice	$<10^5$	Not detected in 25g	<20	Not detected in 25g
3	Samosa	$<10^4$	Not detected in 25g	<20	Not detected in 25g
4	Sandwich/ Burger	$<10^6$	Not detected in 25g	<20	Not detected in 25g
5	Fried chips	$<10^4$	Not detected in 25g	<20	Not detected in 25g

Source* = Microbiological guidelines for Ready-to-Eat food. (2002). Food and Environmental Hygiene Department 43/F, Queensway Government Offices, 66 Queensway, Hong Kong.

Staphylococcus aureus

One mL sample suspension was aseptically transferred to 3 plates of Baird Parker agar medium, distributing 1 mL of inoculum equitably to 3 plates (e.g.) 0.4 mL, 0.3 mL and 0.3 mL). Sterile glass spreader was used to spread inoculum over surface of agar plate. These plates were kept in the incubator upright for 1 hr so that inoculum is absorbed properly. Then plates were inverted and incubated for 45-48 hrs at 35° C. Suspected colonies were transferred into small tubes containing 0.2 – 0.3 mL BHI broth and emulsified thoroughly. From this BHI suspension, TSA slants were also inoculated and incubated for 18-24 hrs at 35°C then 0.5 mL reconstituted coagulase plasma with EDTA was added to BHI culture and mixed firmly. A complete clot that stayed in place on tilting or inverting of tube was considered positive for *Staphylococcus aureus*. Suspected colonies were also Gram stained and observed microscopically, catalase test was also performed and then serological confirmation was performed, using serological antisera (Pro-Lab Canada).

Salmonella spp

Twenty-five gm sample was aseptically weighed into sterile, wide-mouth, screw-capped jar (500 mL) and 225 mL sterile lactose broth was added. It was shifted to sterile blender and blended for 2 min at 10,000 – 12,000 rpm, and again transferred aseptically in the previous sterile container and let stand for 60 minutes at room temperature with jar securely capped. Mixed well and pH was determined with test paper when it was not correct, adjusted it to 6.8 ± 0.2. Incubated 24 ± 2 hrs at 35 °C.

Lid was tightening and it was gently shaken. Then one mL mixture was transferred to 10 mL SC broth and TT broth each and incubated for 24 hrs ± 2 hrs at 35°C. Then they were mixed well and streaked 3mm loopful incubated TT broth and SC broth on BSA, XLD and HEA. These six plates plus blank plates were incubated for 24 hrs ± 2 hrs at 35 °C.

Two or more colonies, typical or suspected to be salmonella from each selective agar were picked and inoculated into TSI agar and LI agar. TSI agar and LI agar slants were incubated at 35°C for 24 ± 2 hr and 48 ± 2 hr respectively. In case of negative result on BS agar plates, we incubated them for an additional 24 hrs.

All the cultures with alkaline but in LI agar regardless of TSI agar reaction, were submitted

for biochemical and serological test, considering them as potential Salmonella isolates. Otherwise, cultures with an acid butt in LI agar and an alkaline slant and acid butt in TS1 agar, were considered potential Salmonella isolates and submitted to biochemical and serological tests. Biochemical tests performed were lysine decarboxylase broth reaction, phenol red dulcitol broth, potassium cyanide (KCN) medium, Malonate broth and Indole test. Serological tests for polyvalent flagellar (H) test and polyvalent somatic (0) tests were performed for confirmation of Salmonella sp/25 g of sample.

RESULTS AND DISCUSSIONS

The results of microbiological analysis of ready-to-eat food sold in Peshawar City are presented in Table 2. Beef is very popular in Peshawar city. Total Plate count of beef samples were found highest of all the analyzed samples i.e., 7 x 10^8 cfu/g (Location C), 6 x 10^7cfu/g (Location B) and 4x10^6cfu/g (Location A). The highest TPC might be due to the incomplete cooking because these meat products are Bar-B-qued, and hence remains undercooked. Total coliform bacteria (TC) of these samples were found to be >1100 MPN /g (Location A), 460 MPN/g (Location B) and 1100 MPN/g (Location C). Fecal coliforms were also enumerated by MPN method and found as 240, 150 and 210 MPN/g at locations A, B & C respectively. *E. coli* 0157: H7 was positive only in a single sample of beef (Location C). *Staphylococcus.aureus* was found to be in numbers, 20 cfu/g and 12 cfu/g (Location A & B respectively). Salmonella was found to be positive only in beef samples of location C. Previously Wafa *et al.;* (1996) has also been reported presence of Salmonella in meat product.

As far as the results of chickpeas are concerned, total pate count were 3 x 10^8, 5 x 10^7 and 6 x 10^8 in numbers at locations A, B & C respectively. Total coliforms were also present at all the three locations and enumerated as 46, 240 and 110 MPN/g (locations A,B&C respectively). Fecal coliforms were also enumerated by MPN method and found in numbers as 15, 43 and 93 at locations A, B & C respectively. *E. coli* 0157: H7 was found to be positive in only one sample at location B. *Staphylococcus aureus* count was found as 4, 8 & 6 cfu/g at locations A, B & C respectively. Salmonella spp. was also found to be positive at only one location i.e, location A. In Peshawar, street venders serve fried rice with yogurt, thick sauce chutney, boiled egg and chopped onion or simply without adding these things according to will of the consumers. In these samples total plate count was 4 x 10^5, 3 x 10^4 and 5 x 10^5 cfu/g at location A, B & C respectively. Total coliforms were found to be positive, in numbers 75, 43 & 23 MPN/g at the three locations A, B & C respectively whereas fecal coliforms were present only at one location i.e. A in number 3 MPN/g. *E. coli* 0157: H7 and Salmonella spp. could not be detected in any of the analyzed samples Similar findings for the detection of *E.coli* were also reported by Saleem et al (1989).

As far as *Staphylococcus aureus* is considered, it was found to be positive in samples of locations A&B only, in numbers 3 and 4 cfu/g respectively. These results helped to conclude that among analyzed samples, fried rice samples were less contaminated than beaf and chick pea samples.

In samosa samples, total plate count was 4 x 10^6, 5x10^4 and 5 x 10^7 cfu/g at locations A, B & C respectively. Most probable number (MPN/g) for total coliforms in samosa samples were 1100, 1100 and 240, where as for fecal coliforms, MPN numbers (MPN/g) were 64, 150 and 120 for locations A, B & C respectively. *E coli* 0157: H7 and Salmonell spp. were found to be negative whereas *Staphylococcus aureus* was found to be positive for all these analyzed samosa samples i.e., 22, 16 and 11 cfu/g at locations A,B&C respectively.

The results of pakorra samples showed a high total plate count as compared to that of samosa and fried rice i.e., 3 x 10^6, 6 x 10^7 and 8 x 10^9 cfu/g. Total coliforms were found in a maximum number of 150 MPN/g, minimum count of 39 MPN/g and a moderate count of 75 MPN/g in samples of locations A, B & C respectively.

Fecal coliform was found in equal numbers in samples of locations A & C while negative in that of location B. *Staphylococcus aureus* was found to be positive in sample of location C (26 cfu/g) whereas, Salmonella spp. were detected only in samples of location A. *E. coli* were found to be negative in all analyzed samples of Pakora.

Street food sandwiches are normally made from salads, mayonnaise, eggs and thick sausages that are manually flaked or minced, which are then mixed with some dressing matrices without further cooking. All the samples of street vended sandwiches collected from these localities were found contaminated with all types of microbes tested.. Total plate counts for locations AB&C were 5 x 10^6, 3 x10^4 and 6 x 10^5 cfu/g respectively. Highest count of total coliforms was seen in samples of location A i.e., 210 MPN//g, minimum, at location B (64 MPN/g) and moderate at location C (75 MPN/g). Fecal coliforms were found to be positive in samples of only two locations, i.e., B & C which were 7 and 23 MPN/g respectively. *Staphylococcus aureus* was found to be positive at all the three locations A,B&C in numbers 18, 14 & 15 cfu/g respectively. *E. coli* 0157: H7 and Salmonella spp. were detected in the samples of location B and C.

Burgers are made of bread burns filled with fried and / or grilled beef patties, chicken, meat, onion, tomato and mayonnaise. The results of these samples showed total plate counts of 4 x 10^6, 4 x 10^4 and 2 x 10^3 cfu/g at the three locations A,B & C respectively. Total coliforms were enumerated as 64, 43 and 39 MPN/g at all the three locations whereas fecal coliforms were present in samples of only one location i.e. B (4 MPN/g). *Staphylococcus aureus* was present in samples of two locations A & C in numbers 2 and 13 cfu/g. *E. coli*: 0157: H7 and Salmonella were absent in all the analyzed samples. In another study carried out in Philippines, Patricia and Azana (2005) reported a similar finding of total plate count 10^5 cfu/g) in boiled rice, fried rice, sandwiches and burger samples.

The results of dahi bhalay showed a very higher contamination status which can be justified by the chances of cross contamination due to mixing of a lot of things with hands, like pakorras a bhalays, yogurt, chutneys, chopped onion and salads etc. The results of total plate count, total coliforms, fecal coliforms and *Staphylococcus aureus* in this type of ready to eat food were as (6 x10^7, 8 x 10^5 and 7 x 10^8), (1100, >1100 , and 1100 MPN/g), (210, 120 and 64 MPN/g) and (26, 32 and 17 MPN) in samples of location A,B & C respectively. *E.coli* 0157:H7 as well as Salmonella were also found to be positive in samples of location B.

Chicken soup "Yakhnee" is a traditional Peshawari food sold by street vendors which is made by chicken boiled in water. The total plate count in these samples were 5 x 10^3, 4 x 10^5 and 1 x 10^2 and total coliform count were 28, 39 and 210 MPN/g in the samples of locations A, B & C respectively. Fecal coliforms, *E.coli* 0157:H7 and Salmonella were absent in all the samples of all the locations, while, *Staphylococcus aureus* was present in numbers 9, 3 & 14 cfu/g in samples at locations A, B & C, showing contamination through handling.

Kheer samples showed a total plate count in numbers, 2 x 10^3, 3 x 10^2 and 7 x 10^5 cfu/g and total coliforms, in numbers 210, 75 and 39 MPN/g in samples of locations A, B & C respectively. Fecal coliforms were found in samples of two locations i.e., B & C in numbers 7 & 11 whereas *E. coli* 0157:H 7 was present in sample of only one location i.e., C. *Staphylococcus aureus* was enumerated as 3, 2 & 7 cfu/g in samples of all the three locations, i.e., A, B & C respectively whereas Salmonella was found to be negative in all collected kheer samples.

The analyzed samples of fried chips were found to be having total plate count in numbers 3 x 10^2, 4 x10^3 and 3x 10^4 cfu/g, total coliforms, in numbers, 150, 93 and 23 MPN/g and *Staphylococcus aureus* in numbers 22, 16 & 10cfu/g at locations A, B & C respectively. Salmonella was present in samples at location A whereas fecal coliforms as well as *E .coli* 0157:H7 were absent in all analyzed samples of fried chips.

CONCLUSION

Over all results of the study indicate that approximately all street vended food samples collected from different localities of Peshawar City showed contamination with fecal coliform and Staphylococcus aureus. E. coli was present in six samples and salmonella was present in seven samples out of thirty-three analyzed samples. It was concluded that microbiological quality of these street vended food samples was not satisfactory due to the absence of good manufacturing practice, inadequate hand washing of food handlers and cross contamination during food preparation etc.

Table 2. Microbiological analysis of ready-to-eat street food sold in Peshawar city.

Food product	Location	TPC (cfu/g)	TC (MPN/g)	FC (MPN/g)	*E.coli* 0157:H7	*S.aureus* (cfu/g)	Salmonella spp.
Beef	A	$4x\ 10^6$	>1100	240	-ve	20	-ve
	B	$6x\ 10^7$	460	150	-ve	12	-ve
	C	$7x\ 10^8$	1100	210	+ve	0	+ve
Chickpea	A	$3x\ 10^8$	46	15	-ve	4	+ve
	B	$5x\ 10^7$	240	43	+ve	8	-ve
	C	$6x\ 10^8$	1100	93	-ve	6	-ve
Fried Rice	A	$4x\ 10^5$	75	3	-ve	3	-ve
	B	$3x\ 10^4$	43	<3	-ve	4	-ve
	C	$5x\ 10^5$	23	<3	-ve	0	-ve
Samosa	A	$4x\ 10^6$	1100	64	-ve	22	-ve
	B	$5x\ 10^4$	1100	150	-ve	16	-ve
	C	$5x\ 10^7$	240	120	-ve	11	-ve
Pakorra	A	$3x\ 10^6$	150	3	-ve	0	+ve
	B	$6x\ 10^7$	39	<3	-ve	0	-ve
	C	$8x\ 10^9$	75	3	-ve	26	-ve
Sandwich	A	$5x\ 10^6$	210	<3	-ve	18	+ve
	B	$3x\ 10^4$	64	7	+ve	14	-ve
	C	$6x\ 10^5$	75	23	+ve	15	+ve
Burger	A	$4x\ 10^6$	43	<3	-ve	2	-ve
	B	$4x\ 10^4$	64	4	-ve	0	-ve
	C	$2x\ 10^3$	39	<3	-ve	13	-ve
Dehi Bhalay	A	$6x\ 10^7$	1100	210	-ve	26	-ve
	B	$8x\ 10^5$	>1100	120	+ve	32	+ve
	C	$7x\ 10^8$	1100	64	-ve	17	-ve
Chicken Soup	A	$5x\ 10^3$	28	<3	-ve	9	-ve
	B	$4x\ 10^5$	39	<3	-ve	3	-ve
	C	$1x\ 10^2$	210	<3	-ve	14	-ve
Kheer	A	$2x\ 10^3$	210	<3	-ve	3	-ve
	B	$3x10^2$	75	7	-ve	2	-ve
	C	$7x\ 10^5$	39	11	+ve	7	-ve
Fried chips	A	$3x\ 10^2$	150	<3	-ve	22	+ve
	B	$4x\ 10^3$	93	<3	-ve	16	-ve
	C	$3x\ 10^4$	23	<3	-ve	10	-ve

cfu = colony forming unit, MPN= most probable numbers
A = University road, B = Khyber bazaar, C= Hashtnagri area

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Pak J Food Sci 2008, 18(1-4): 21-24

Procedure for isolation and identification of natural food color (Anthocyanins) from *Reptonia bauxifolia*

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ABSTRACT

The natural food colors have gained popularity worldwide due to environment friendly nature and health benefits. The problem in the natural food colors is the availability of the sources. In this study we have identified a new source for the isolation of natural food color. *Reptonia bauxifolia* a wild plant abundantly available in Peshawar valley and tribal areas, was used as a source for the isolation of natural food colors. The red color pigment anthocyanin was isolated and purified by using thin layer and paper chromatography. The overall yield of anthocyanin from *Reptonia bauxifolia* was 0.15%.

Key Words: *Reptonia bauxifolia,* Anthocyanins, Natural food color

INTRODUCTION

Over the past decade the use of natural colors in food and beverages has increased at a much greater pace than that of synthetic colors. Natural food color is any dye obtained from vegetable, animal or mineral source that is capable of coloring food, drugs, cosmetics or any part of human body. Natural colors come from variety of sources such as seeds, fruits, vegetables, leaves, algae and insects. Commonly used natural colors are annatto (seed), turmeric, beet juice (root), red cabbage (vegetable) and spinach (leaf). The most important natural food colors are □-carotene (yellow), anthocyanins (red), cochineal (red) and annatto (yellow).

Anthocyanins are a group of natural polyphenolic pigments responsible for the red to blue color of a wide range of fruits and vegetables. They are probably the most important group of visible plant pigments besides chlorophyll (Konga *et al* 2003). Anthocyanins are members of a class of nearly universal, water-soluble, terrestrial plant pigments that can be classified chemically as both flavonoid and phenolic. They are found in most land plants, with the exception of the cacti and the group containing the beet. They contribute colors to flowers and other plant parts ranging from shades of red through crimson and blue to purple, including yellow and colorless. They are not found in animals, marine plants or in microorganisms. Anthocyanins are active components in several herbal medicines such as bilberry (*Vaccinium myrtillus*).

As against the synthetic food colors which have various toxic effects on the human body and environmental hazardous effects, the anthocyanins (the natural food color) are more beneficial for health and has various medicinal properties. Anthocyanins have received less attention than other flavonoids, despite their far-reaching effects. Anthocyanins have the strongest antioxidizing power through various mechanisms (Elliot *et al* 1992). Bilberry in even trace amounts effectively protects against LDL oxidation in test-tube studies (Bertuglia 1995). In the laboratory, they have been found to inhibit some human tumor cells (Meiers 2001). Anthocyanins effectively prevent inflammation and subsequent blood-vessel damage (Bertuglia 1995). Their anti-inflammatory ability has been shown to help dampen allergic reactions (Borissova 1994). It has been found that people taking 50 mg, but not 20 mg, black currant anthocyanins adapt better to the dark and have

less eye fatigue than those in the placebo group (Zadok 1999; Muth 2000).

Reptonia bauxifolia is a large shrub or small tree with auxiliary spines and spinescent branchlets. Flowers are whitish or greenish yellow. It grows abundantly in North Western hills of Pakistan, Afghanistan and Muscat up to 4700 ft. The fruit is called 'Gurgura' and finds no practical use. The pulp is sweet in taste while the hard seed is uneatable. The fruit is spherical in shape and dark-red in color and is available from July to September.. No investigational work has been reported in literature regarding the coloring matter of *Reptonia bauxifolia*. So the present research was conducted to isolate Anthocyanins from *Reptonia bauxifolia*.

MATERIALS AND METHODS

Materials

Hydrochloric acid, methanol, phenol, sulphuric acid, sample standard, and sugars were the products of E-Merck, Darmstadt, Germany. *Reptonia bauxifolia* fruit was procured from local market; it was washed thoroughly with running tap water and air dried.

Isolation of anthocyanins

The pigment was extracted with boiling ethanol containing 1% HCl. Boiling was done for five minutes and the mixture allowed to stand over night. The above mentioned procedure was repeated three times. The combined extracts were filtered and then added with sufficient amount of 1% aq solution of lead acetate with constant stirring. A dirty bluish-green precipitate settled down which was separated by centrifuge, washed several times with distilled water and finally with aqueous ethanol 1:1. The precipitate was dried and then suspended in minimum quantity of about 5% methanolic hydrochloric acid. The pigment was regenerated and lead chloride precipitate formed was removed by centrifugation. To this solution of pigment, excess of ether was added and pigment was precipitated as red amorphous powder.

Isolation of Anthocynadin

The anthocyanidin (sugar-free anthocyanin) was prepared readily by hydrolyzing the pigment with 20% HCl. About 5 mg of the pigment was taken in test tube and 0.5 mL 20% HCl was added. This was boiled for 3 minutes in a water bath and cooled. The excess acid was neutralized by adding sodium bicarbonate solution and concentrated carefully to near dryness. This was then extracted with ethanol, filtered and concentrated to 10 mL volume (Giesman 1962). The anthocyanidine was recrystallised from methanol containing 7% aq HCl and applied on Whatman Filter No. 1 with other standard sugars.

IDENTIFICATION OF THE COMPOUND

Anthocyanidin

The anthocyanidin obtained has a melting point above 325^{0}C. Upon shaking with cyclohexanol and toluene (1:5) it gives a red rose color. On shaking of the acidic solution in amyl alcohol with sodium acetate and then adding the ferric chloride solution subsequently gives reddish violet color.

Anthocyanin

The melting point of anthocyanin was found to be 205-206^{0}C with decomposition to a blackish mass. To a portion of its aq. solution, ferric chloride was added (Robinson and Robinson 1932). The color changed to blue with aq. sodium acetate solution, the color was reddish violet.

50 mg of the pigment was distributed between equal volumes of 0.5% HCl and amyl alcohol. On oxidation with H_2O_2 it gives sugar unit and the acid part. On estimation of sugar after hydrolysis and hydrogen peroxide oxidation gives the same ratio of sugar.

Table 1 . Rf values of Anthocyanidin

Solvent system	Ratio v/v	Rf value	Color	
			Visible light	Under UV
Acetic acid: HCl: Water	30:3:10	0.50	Magenta	Pink
Formic acid: HCl: Water	5:2:3	0.22	do	do
Butanol: Acetic acid: Water	4:1:5	0.68	do	do

Table 2. Rf values of Anthocyanins

Solvent system	Ratio v/v	Rf value	Color	
			Visible light	Under UV
Acetic acid: HCl: Water	82:3:15	0.26	Magenta	Dull Magenta
Butanol: HCl	1:1	0.25	do	do
Butanol: Acetic acid: Water	4:3:5	0.36	do	do
1% HCl aq.	97:3	0.07	do	do

RESULTS AND DISCUSSION

The pigment was isolated and purified using thin layer and paper chromatography. The overall yield of the anthocyanin was 0.15%. By varying the elution systems and then comparing the Rf values with those reported in literature and with co-TLC of authentic samples with the chromatographic methods have shown that the aglycone portion is cyanidin which was subsequently confirmed by various degradation and color reactions. The sugar moiety was found to be glucose. The hydrogen peroxide breaks the 3 glycosidic linkages, the sugar can be removed by treatment with hydrogen peroxide, and this shows that the sugar is at position 3. The sugar percentage obtained by hydrogen peroxide and by hydrochloric acid treatment is identical. This shows that only one sugar molecule is attached and that too is at 3 and not 3 and 5.

The results have further been confirmed by spectral analysis which clearly shows that the pigment under investigation is mono 3-glucose and not 3-5 diglucoside .

Crystallization of the pigment gave poor yield because slight impurities affect crystallization. The crystals were visible only under high resolving microscope. The pigment isolated gave Rf values absorption maxima, distribution number and color reactions, which are characteristics of cynidin 3 mono glycoside (Robinson and Robinson 1932).

CONCLUSION

The present work aims at the isolation of red natural food colors from the wildly grown *Reptonia bauxifolia.* The compound responsible for the color was determined to be the antocyanidin. The use of the plant in a more proper way will have a great impact on the value addition of the plant and as a result will increase the income of the grower.

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Pak J Food Sci 2008, 18(1-4): 25-29

Microbiological assessment of sugar cane juice sold in Peshawar City

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ABSTRACT

Sugar cane juice sold by street venders and hawkers are very popular in our country. The present study was aimed to examine the quality and safety of freshly squeezed sugar cane juice based on standards techniques. Forty samples of sugar cane juices were collected from selected area and analyzed for total viable count (TVC), total coliforms, E. coli, yeast and mould. All these parameters were detected on the operator hands (except yeast & mould) and ice used for cooling the juice. The results showed that in all the localities the street vended sugar cane juices remained hygienically poor as indicated through high bacterial load i.e. 4 x 102 – 3 x 107 cfu/mL. All samples were contaminated with coliforms bacteria ranged from 46 –1100 cfu/mL. Seventy five percent samples were contaminated with confirmed E. coli. All the examined samples were contaminated with yeast and mould. Total coliform was present in all analyzed samples of ice whereas confirmed E. coli were present in 37% samples and TVC of ice sample ranged from 1 x 102 – 2 x 105 cfu/mL.

Keywords: Sugar cane juice, ice, vendor's hands, microbial quality.

INTRODUCTION

Sugar cane is one of the most important crop of Pakistan. It is an important member of plant kingdom, which provides sugar to human body in the form of sucrose. Sugar cane belongs to grass family. Its juice is widely consumed as a refreshment as well as energetic drink, due to the high content of sugar, by most of the population. Sugar cane juice has therapeutic properties and is mostly consumed along with lemon juice to hepatitis patients. In many tropical countries the sugar cane juice is a common man's beverage and is sold at all public places, side shops recreational areas (benches, park) and busy market places (shopping malls, bus station etc). However in view of their ready consumption, quick methods of cleaning, handling and extraction, they could often prove to be a public health threats (Parish 1997, Sandeep *et al* 2001). The presence of indigenous, epiphytic micro flora on sugar cane plant was ascertained (Wolzogen 1923, Mayeux's1960). The cane arriving at the sugar cane extraction may contain an extensive micro flora on its surface (Geerlings 1924). Juice of sugarcane is normally obtained through squeezing sugarcane, therefore improper washing of sugarcane add bacteria in to extract leading to contamination. Therefore, sugar cane juice has high risk of pathogenic microbes (Jeffery 2004). Possible sources of such microbial contamination have been identified as unhygienic handling, raw material itself, inadequate cleaning of sugar cane press, knives, contact surface, clothes, vendor's hands, ice and air born contamination. Contaminated raw material used in these drinks have been known to be a source of infectious diseases such as nausea, abdominal cramps, vomiting, typhoid, diarrhea etc (Jeffery 2004; Annon 1998; Qureshi *et al* 1987). To ensure that food is microbiologically safe, both food handlers and food itself must be monitored on permanent bases (Coste *et al* 1995; Arias *et al* 1987). These unpasteurized juices have been a potential source of bacterial pathogens notably, Salmonella and E. coli (O157H7) (Ryu *et al* 1998; Ujlas *et al* 1998; Zhuaug *et al* 1995). Epidemiological data indicates that cross contamination during food preparation contribute notably to the occurrence of food born diseases (Kusumaningrum *et al* 2004). In 1991 cholera epidemic in Pune, India was attributed to contaminated sugar cane juice with added ice. In this case the ice was found contaminated with Vibrio Cholerae (Von Holy 1999).

The aim of the present work was to evaluate the quality of sugar cane juice, the microbes on vender's hands and ice used for cooling these juices and to evaluate the usefulness of these vended drinks by general public.

MATERIALS AND METHODS:

Collection of Samples

During the study, eight focal locations in the Peshawar city of NWFP and surrounding where maximum crowd of consumers using sugar cane juices were selected for the sample collection (Table 1). Samples of sugar cane juices and ice were collected from at least five shops in each location. All the samples were collected in sterile duran bottles of 500mL capacity properly sealed.

Table 1: Name and location of samples collected points.

Area	Location
A	Khyber Teaching Hospital Stop
B	Secondary Board Peshawar Stop
C	Khyber Bazar
D	Bhana Mari Chowk
E	Hashtnagari Stop
F	General Bus Stand
G	Sadder
H	Hayatabad

Sample Preparation

Each type of sample was analyzed for microbiological analysis. The samples were opened turn by turn in a laminar air flow chamber under all aseptic measures. The chamber provided sterile environment for the microbiological analysis.

Microbiological Analysis of Juices and Ice

Total Viable Count

Total Viable Count was determined by pour plate method as described by FAO (1992), and APHA (2005) serial dilution (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) of the juices and ice samples were made and aliquots of 1ml were added to each duplicate petri dish. Plate count agar ((PCA) was added to each petri dish and incubated at 35 °C for 48 hours ±2, after incubation colonies were counted by colony counter and result was expressed as cfu/mL.

Total Coliform Bacteria/Fecal Coliform Bacteria

The MPN of total coliforms bacteria were determined by multiple tube fermentation technique (APHA 2001). FAO (1992) and APHA (2005) 1ml from the previously prepared 10^{-1} 10^{-2} and 10^{-3} dilutions were inoculated into three replicate tubes containing 10 ml of LT Broth with inverted Durham tubes and incubated at 35 °C.± 0.5 °C for 24 and 48 ± 2 hrs after inoculation. Tubes were examined for evidence of gas production at the end of 24 hrs incubation. Gas production was measured by gas displacement in the inverted vial and also effervescence produced when the tube was gently shaken. Negative tubes were re-incubated for additional 24 hr and again examined for gas production. Positive tubes with gas formation and turbidity was sub-cultured into BGB (Brilliant Green Lactose bile broth and E.C. Broth having 10ml broth with inverted Durham tubes by means of 3mm loop. All BGB tubes were incubated at 35 °C and E.C. Broth tubes at 44.5 for 48 hrs. and examined for gas production . Total coliform and fecal coliform were calculated from MPN tables (APHA 2001) for ice as per 100ml and for sugar cane juice from MPN tables (FAO 1992) as per mL.

E. coli

EMB Agar was used for the enumeration of E. coli. All the tubes of E.C. broth showing gas were subcultured by streaking on EMB agar plates and incubated at 35 °C for 18-24 hrs. Positive plates contained typical colonies with green metallic sheen were inoculated on PCA slants (plate count agar) and incubated at 35 °C for 18-24 hrs. After 24 hrs incubation the growth was transferred into the following broths for identification for E. coli by biochemical tests (IMVic tests), Trypton broth, MRVP medium, Kosar's citrate broth, Lauryl sulfate tryptose (LST broth), Gram stain.

Yeast and Mold

Yeast and Mold was calculated following the FAO method described by Andrews (1992). Take 25 ml of juice and ice sample (melt at 5 ºC) and make a serial dilution (10^{-1} to 10^{-4}). One ml portion of each dilution was inoculated on Potato dextrose agar (PDA). Petri dishes were incubated at 22-25 ºC for 5 days and after incubation colonies were counted by colony counter and result was expressed as yeast and mould / g.

Bacteriological Analysis of Hands

The swab method was used for coliform determination on hands according to APHA 2001) Sterile nonabsorbent cotton swabs with the head firmly twisted rolled on a wooden. Applicator stick 12-15 cm long was used. Sterile swab was moistened in a sterile buffer peptone water. The excess buffer was removed by pressing the swab with the interior wall of the container by rotating motion. Rub the swab head slowly and thoroughly overall surface of hand three times reversing direction between strokes. Rinse the swab in known amount of sterile buffer after the area of hand has been swabbed. The swab was shacked vigorously making complete 50 cycling before analysis. Total plate count, total coliform, total fecal coliform, E. coli and yeast & mound were examined according to the FAO 1992 and APHA 2005 as described previously.

RESULTS AND DISCUSSION

Forty (40) samples of sugar cane juice collected from eight selected areas of Peshawar City NWFP (Table 1) were analyzed microbiologically for TVC, TC, FC and yeast/mould (Table 2)

The data presented in table2 shows heavy bacterial load ranging from $4 \times 10^{2} - 3 \times 10^{7}$ cfu/ml. Maximum viable count was found in area B ($3x10^{7}$)followed by A ($2x10^{7}$) Minimum TVC was found in area H. The higher TVC of this area are due to the heavy vehicular traffic, waste water lines and vegetable decomposing waste The lower TVC might be due to posh area of Peshawar city. Where hygienic conditions was maintained to some extent Data presented in table 2 also indicates that in all the samples total coliform bacteria were >110 MPN/mL except 46 MPN/mL which was found in area H. Fecal coliform showed variation the highest observed values were 110 and 46 MPN/ml in the area D and E respectively. The other area E such as GBA and H have 12, 20, 21 and 24 MPN/ml respectively. All the analyzed samples give positive result for E. coli except samples from area E and H. Yeast and mould count is significantly higher in area B as compared to other areas. The sugar cane juice was served along with ice to the consumers. Therefore the ice samples were also analyzed for microbial quality. The results are shown in table 3 TVC of ice samples were low as compared to sugar cane juice. The minimum TVC was 1 x10 2 cfu/mL (area H) and higher was $2x10^{5}$ cfu/mL (area A). The other counts were $3x10^{3}$, $2x10^{4}$, $5x10^{3}$,$4x10^{3}$,$2x10^{4}$ and $3x10^{3}$ cfu/mL. The maximum number of total coliform (110/100mL) were found in ice sample of area E followed by sample from area A which have 46 /100ml total coliform . The presence of coliform bacteria may also be due to the use of contaminated water in ice formation. Out of eight ice samples collected from different areas, three samples show positive indication of fecal coliform as well as E.coli as shown in table3. Samples collected from the operator hands from area ABCDEFGH by swab methods were also analyzed for total viable count TVC, total coliform (TC), fecal coliform (FC) and E. coli. The results are presented in table 4. TVC of all the operators ranged from 1 x 103 – 3 x 104 cfu/mL. All the operator hands were contaminated with fecal coliform. E. coli was not recovered from any operator hand.

CONCLUSION

It was concluded from the results of this study that all the sugar cane juices collected from eight focal areas of Peshawar City NWFP were highly contaminated with fecal coliform, E. Coli and yeast & mould. These contaminations might be due to the improper washing of sugarcane before juicing, operator hands and use of contaminated ice in these juices and also due to the heavy traffic and unhygienic environment in some area from where these samples have been collected.

Table 2: Area- wise Microbiological analysis of sugar cane juice.

Area	No. of samples collected from each area	TVC (cfu/mL)	TC (MPN/mL)	FC (MPN/mL)	E. coli	Y & M (g)
A	5	2×10^7	≥110	21	+ve	2×10^5
B	5	3×10^7	≥110	24	+ve	3×10^7
C	5	4×10^6	≥110	12	+ve	7×10^4
D	5	8×10^5	≥110	2.3	+ve	4×10^4
E	5	2×10^5	110	9.3	-ve	6×10^3
F	5	3×10^5	≥110	46	+ve	4×10^6
G	5	8×10^4	≥110	110	+ve	5×10^4
H	5	4×10^2	46	24	-ve	4×10^5

Table 3: Bacteriological analysis of ice used in sugar cane juice.

Area	No. of samples collected from each area	TVC (cfu/mL)	TC (MPN/100mL)	FC (MPN/100mL)	E. coli
A	5	2×10^5	46	≤0.3	-ve
B	5	3×10^3	12	2.0	+ve
C	5	2×10^4	24	≤0.3	-ve
D	5	5×10^3	21	≤0.3	-ve
E	5	4×10^3	110	2.8	+ve
F	5	2×10^4	7.5	≤0.3	-ve
G	5	3×10^3	9.3	1.5	+ve
H	5	1×10^2	15	≤0.3	-ve

Table 4: Bacteriological analysis of operators hands during juice preparation.

Area	No. of samples collected from each area	TVC (cfu/mL)	TC (MPN/100mL)	FC (MPN/100mL)	E. coli
A	5	3×10^2	21	2.3	-ve
B	5	2×10^2	24	3.9	-ve
C	5	2×10^3	15	2.3	-ve
D	5	4×10^2	46	2.3	-ve
E	5	1×10^4	12	4.3	-ve
F	5	2×10^3	7.5	1.5	-ve
G	5	3×10^4	6.4	1.1	-ve
H	5	1×10^3	12	2.3	-ve

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Pak J Food Sci 2008, 18(1-4): 30-35

Quality evaluation of renowned honey varieties available in local markets of Karachi

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ABSRACT

In order to evaluate quality and standard, the physicochemical properties of commercially available brands of honey samples from the markets of Karachi were studied. The samples were analyzed for pH, acidity, moisture, specific gravity, refractive index, ash, conductivity, brix, fructose (F), glucose (G), F/G ratio, reducing sugars and total sugars. The results were compared with Codex Alimentarius Commission (1993) and the studies conducted by other scientist. In honey samples pH ranged from 3.05 to 3.45, free acidity 27.8 to 52.0 mq/kg, moisture 18.9 to 21.8%, specific gravity 1.36 to 1.51, refractive index 1.4860 to 1.4936, ash 0.115 to 0.211%, conductivity 0.335 to 0.507 mS/cm, brix 78.9 to 81.8, fructose 29.0 to 38.98%, glucose 28.09 to 38.33%, F/G ratio 0.847 to 1.345, reducing sugars 64.24 to 75.98% and total sugars 69.80 to 80.29%. Two brands were found with somewhat high acidity whereas four brands had slightly high moisture content. A loose packed honey was found adulterated with 17% sucrose.

Keywords: Honey, quality, local market

INTRODUCTION

Codex Alimentarius Commission (1993) described honey as the natural sweet substance produced by honey bees from nectar of plants or from secretions of living parts of plants which honey bees collected and transformed by combining with specific substances of their own deposit, dehydrate, store and leave in the honey comb to ripen and mature. The honey is a most familiar, delicious and valuable natural food as it has the nutrients, which are necessary for maintaining proper health (Khalil *et al* 2001). It is not only considered an excellent adjuvant for the acceleration of wound healing (Beraman *et al* 1983) but also useful in treatment of burns, wounds, gastroenteritis, stomach and skin ulcers because of its antibacterial properties McCarthy 1995; Cooper and Molan 1999; Cooper 2001). Emarah *et al* (1997) has revealed that the bee honey is also used in the treatment of external eye diseases. Physicochemical characteristics of honey produced in different countries have been reported by many scientists (Siddiqui 1970; Doner 1977; Langridge 1977; Trasyvoulou 1986; Bonehi 1988; Singh *et al* 1988; Gupta 1992; Moteos -Nevado *et al,* 1994; Rodriguez-Otero *et al* 1994; Singh and Bath 1997; Mendes 1998; Khalil *et al* 2001; Kamal *et al* 2002; Anupama *et al* 2003; Al-Jedah *et al* 2003; Andrew *et al* 2004; Meda 2005) and has discussed the composition, quality and standard of honey using different parameters (pH, moisture, ash, conductivity, diastase activity, free acidity, glucose, fructose, reducing sugar etc.).

Honey under goes changes during storage leading to darkening and loss of aroma and the flavour (Samko *et al* 1992). Honey quality can also be affected by heating during the extracting, liquefying or clarifying process or by aging during storage (Mendes 1998). Commercially available honey brand /varieties differ in quality on account of various factors like geographical, seasonal and processing conditions; floral source, packaging and storage period (Anupama 2002). Adulteration of honey is possible, so its quality must be controlled analytically with the aims of guaranteeing the geninity and preserving consumers from commercial speculation. The reason for evaluating honey for quality control purposes is to verify the authenticity of the product and to reveal the possible adulterants as well as to

address processing and market needs (Krell 1996).

In Pakistan there is no effective legislation or policy for the quality control of honey. The aim of this study was to evaluate the quality as well as to verify the authenticity of some known branded and unbranded honey samples available in local markets of Karachi, by comparing with the Codex Alimentarius Commission (1993) and the studies conducted by other scientists.

MATERIALS AND METHODS

Commercially available varieties of 10 honey samples were randomly purchased from the local markets of Karachi City during the year 2006. Samples were kept at room temperature in their own original package and then analyzed for the parameters pH, free acidity, moisture, specific gravity, refractive index, ash, conductivity, Brix, and fructose (F), Glucose (G), F/G ratio, reducing sugar and total sugar by utilizing authentic methods, equipments and instruments.

To 5 g of homogenized honey sample, 50mL of distill water was added and mix. Then the pH was read directly from the pH meter (Hanna Instruments, Digital portable pH meter, model HI 8314). The instrument was calibrated with standard buffer solution of pH 4 and pH 7 prior to the measuring the pH of honey samples.

Free acidity was determined as milliequivalent/kg by the use of AOAC official method (2000). Brix and refractive index in honey was determined with Abbe Refractometer (Reichert Mark II Plus) reading taken at 20ºC and then obtaining the corresponding percent moisture by AOAC method (2000). Specific gravity of honey samples was determined by the method of Kalimuddin (1976). Ash percentage was measured by calcinations, in a furnace at 600ºC for 6-7 hrs to constant mass (AOAC 2000).

In order to determine the conductivity, dissolve 20.0 g anhydrous honey in distilled water. Transfer the solution quantitatively in 100 ml volumetric flask and make up to volume with distilled water. Then the conductivity was measured directly by conductivity meter (Jenway model 470 Portable Conductivity Meter) at 20 ºC. Instrument was calibrated by Potassium Chloride solution prior to measuring conductivity.

The chromatographic analysis for the determination of sugars (fructose, glucose, and sucrose) was carried out in Agilent 1100 series HPLC equipped with refractive index detector (Agilent RID G1362A). Chromatographic separation of sugars was achieved in Zorbax carbohydrates analysis column (4.6 x 150mm 5micron, Agilent serial no. USAR001360), using acetonitrile: water (75: 25) as mobile phase, at temperature 30ºC and flow rate of 2ml/min (Agilent Technologies 2002-2003).The sample preparation for honey sugar determination was easy, involving 1:200 dilution in deionized water furthermore filtration through Whatman No.1 filter paper and thereafter through 0.2 micron syringe filter.

Reducing sugars in honey samples were determined by AOAC (2000) method of reducing sugars in honey whereas the total sugar was determined by the anthrone method (Hansen and Moller 1975).

RESULTS AND DISCUSSION

pH

Mean pH value of honey samples was 3.24 that varied from 3.05 to 3.45 (Table 1), highest in MH and lowest in LPH and YFH among all brands. According to Bogdanov *et al* (1995) pH should be between 3.2 and 4.5. Three brands LPH, HiH, and YFH were found to have low pH value but no one brand was found with higher pH. This is mainly due to variation of different acids and minerals present in the honey.

Free Acidity

Free acidity is considered as important quality criteria. Fermentation of honey causes increase in acidity but acidity is also due to the presence of natural acids particularly, the gluconic acid in equilibrium with their lactones or esters and inorganic ions such as phosphorus and chloride (Echigo and Takenak, 1974). The average free acidity of the honey brands was 40.55 meq/kg

that varied from 27.8 to 52.0 meq/kg (Table.1). Slightly high free acidity value was observed in AKH (52.0 meq/kg) and SMA (51.4 meq/kg) whereas remaining brands had acidity within prescribed limits of (≤50 meq/kg) proposed by Codex Alimentarius Commission (1993).

Moisture

Moisture content of honey is one of the most important measurements as honey is hygroscopic and once it contains more than 20-21% moisture it is likely to be fermented by ubiquitous yeast (Hui 1992). The moisture content observed was18.9% to 21.8% (Table 1) and four brands (SJH, BHH, HiH, YEH) were found to be with slightly high moisture content more than 21%, recommended by Codex Alimentarious Commission (1993).

Refractive Index

The refractive index was recorded 1.4860 to 1.4936 (Table 1), highest in AKH and Lowest in BHH. These values were to great extend agreement with the values of Anupama *et al* (2003).

Specific Gravity

Specific gravity was observed 1.36 to 1.51 (Table 1), highest in pH and lowest in SMA, HH and YFH among the brands studied. To a large extend these values were not only agreement with the values of Andrew *et al* (2004) but also Kamal *et al* (2002), whereas specific gravity of three brands (SMA, HH, and YFH) is some how mimic to the values of Khalil *et al* (2001).

Ash content and conductivity

Ash content was observed between 0.115% and 0.211%, highest in AKH and lowest in SMA brand (Table 1). All brands had ash content below 0.6%, suggested by Codex Alimentarius Commission (1993) but values were lower than the values of Khalil *et al* (2001) and Meda *et al* (2005).

Conductivity is good criterion of botanical origin and this parameter is frequently used to determine the honey quality instead of ash content as conductivity mainly depends on ash and acid content (Vorwohl 1964). Conductivity in brands studied varied from 0.335 to 0.507 mS/cm, highest in AKH and lowest in LPH (Table 1). All brands were found to be with conductivity less than 0.8 mS/cm, suggested by Codex Alimentarious Commission (1993).

Brix

Mean Brix of studied varieties of honey was 80.07 that varied from 78.9 to 81.8, highest in AKH and lowest in BHH (Table 2). These values agreed with the recorded values of Anupama *et al* (2003) for Indian honey.

Sugars

Fructose (F), glucose (G), F/G ratios, reducing and total sugars of the brands studied is presented in Table 2. Fructose was observed between 29.0% and 38.98%, highest in SJH and lowest in LPH. These values were slightly higher than Al-Jedah *et al* (2003) but lower than the values of Surendra *et al* (2000). Glucose was examined between 28.09% and 38.33%, highest in HiH and lowest in AKH brand. The values of glucose in all bands were lower than the values recorded by Al-Jedah *et al* (2003) and Surendra *et al* (2000).

The F/G ratio was calculated between 0.847 and 1.345, highest in AKH and lowest in LPH. These values were agreement with the recorded values of Surendra *et al* (2000) whereas F/G ratio of AKH, SJH, SMA, BHH and YFH was agreement with the values of Mendes *et al* (1998). LPH, MH, HH and HiH samples were found to be with high glucose than fructose. Fructose level is almost always higher than glucose level. In fact although there were definitely some honey samples that contains more glucose than fructose. Some countries consider a honey adulterated if fructose is not major carbohydrate component (Hui 1992). Sucrose was observed between 1.54% and 17.0%, highest in LPH and lowest HiH.

Table.1 Physicochemical parameters of honey samples drawn from local market

Honey Brand/Code	pH	Free Acidity meq/kg	Moisture (%)	Specific Gravity	Refractive index	Ash %	Conductivity m S/cm
AKH	3.30±0.07	52.0±3.11	18.9±0.71	1.46±0.04	1.4936±0.004	0.211±0.009	0.507±0.03
SJH	3.35±0.09	38.6±2.02	21.6±0.74	1.43±0.07	1.4866±0.003	0.163±0.002	0.424±0.01
SMA	3.25±0.08	51.4±2.04	20.8±0:66	1.36±0.03	1.4885±0.003	0.115±0.002	0.340±0.00
LPH	3.05±0.09	42.3±2.08	19.3±0.42	1.44±0.05	1.4908±0.002	0.120±0.002	0.335±0.00
BHH	3.25±0.09	38.7±2.31	21.8±0.67	1.45±0.04	1.4860±0.004	0.116±0.001	0.342±0.01
PH	3.25±0.05	28.5±1.99	20.9±0.51	1.51±0.04	1.4884±0.001	0.162±0.002	0.422±0.01
MH	3.45±0.07	34.9±1.12	19.6±0.44	1.42±0.06	1.4916±0.003	0.142±0.002	0.387±0.01
HH	3.35±0.08	27.8±1.99	20.6 ±0.41	1.36±0.02	1.4911±0.002	0.161±0.001	0.420±0.00
HiH	3.10±0.05	43.1±1.41	21.7±0.86	1.43±0.04	1.4863±0.001	0.160±0.001	0.418±0.00
YFH	3.05±0.08	48.2±1.54	21.6±0.73	1.36±0.05	1.4867±0.001	0.207±0.030	0.495±0.09

* Al-Khalis Honey =**AKH**, S.J Herbal Products = **SJH**, S M Arif Honey = **SMA**, Loose Packed Honey=**LPH**, Bee Hive Honey (unbranded) = **BHH**, Paradise Honey = **PH**, Marhaba Honey = **MH**, Hamdard Honey = **HH**, Hijazy Honey = **HiH**, Young's French Honey = **YFH**
* Data represent mean & standard deviation of 3 individual samples.

Table.2 Physicochemical parameters of honey samples drawn from local market

Honey Brand/Code	Brix ^{0}	Fructose (F) (%)	Glucose (G) (%)	F/G Ratio	Sucrose (%)	Reducing Sugar (%)	Total Sugar (%)
AKH	81.8±2.35	37.76±0.17	28.09±0.17	1.345±0.01	2.95±0.06	66.85±0.17	69.80±1.22
SJH	79.2±1.99	38.98±0.14	32.58±0.27	1.196±0.04	2.08±0.07	72.56±0.21	73.54±1.43
SMA	79.9±1.39	35.75±0.18	32.36±0.23	1.105±0.02	1.73±0.05	69.11±0.21	69.84±1.17
LPH	80.7±2.14	29.00±0.11	34.24±0.31	0.847±0.03	17.0±0.40	64.24±0.21	80.29±0.97
BHH	78.9±3.04	35.15±0.20	33.37±0.40	1.047±0.06	3.03±0.10	69.52±0.30	69.92±1.44
PH	79.9±2.02	34.98±0.16	34.92±0.25	1.002±0.06	1.57±0.07	69.99±0.21	70.08±1.58
MH	81.1±2.79	35.61±0.19	35.85±0.19	0.993±0.02	1.81±0.06	71.96±0.19	71.89±2.04
HH	80.9±3.06	35.31±0.18	36.38±0.21	0.971±0.02	2.43±0.08	71.99±0.20	72.90±1.89
HiH	79.1±2.97	37.00±0.11	38.33±0.18	0.965±0.07	1.54±0.07	75.98±0.15	76.73±1.83
YFP	79.2±1.38	34.57±0.13	32.84±0.20	1.053±0.05	1.57±0.09	68.41±0.17	69.98±1.96

* Al-Khalis Honey =**AKH**, S.J Herbal Products = **SJH**, S M Arif Honey = **SMA**, Loose Packed Honey=**LPH**, Bee Hive Honey (unbranded) = **BHH**, Paradise Honey = **PH**, Marhaba Honey = **MH**, Hamdard Honey = **HH**, Hijazy Honey = **HiH**, Young's French Honey = **YFH**
* Data represent mean & standard deviation of 3 individual samples.

The sucrose value in nine brands was to some extend agreement with the values of Kamal *et al* (2002) but sucrose level in LPH is not only higher than the proposed value (≤ 5%) of Codex Alimentarious Commission (1993) but also than the recorded values of Surendra *et al* (2000), and Mincione and Leuzzi (1993), which indicates the sucrose tarnishing in any stage of processing or production of honey

Reducing sugar was varied from 64.24% to 75.98%, highest in HiH and lowest in LPH. These values were to a little extend agreement with the some values of Kamal *et al* (2002) and Al-Jedah *et al* (2003), but to a great extend with the values reported by Anupama *et al* (2003). All brands except LPH had reducing sugar level ≤65%, suggested by Codex Alimentarius Commission (1993) and European Honey Directive Standard (1974). The total sugar of the brand studied was estimated between 69.80% and 80.29%, highest in LPH and lowest in AKH. The values of total sugar were agreement with values recorded by Khalil *et al* (2001) and Kamal *et al* (2002) as well as to some extend with Al-Jedah *et al* (2003) monitored values.

CONCLUSION

The physicochemical characteristics of three (PH, MH, HH) of ten honey varieties analyzed in this study were agreed with Codex Alimentarius Commission and were found to be good quality with reference to employed parameters (pH, free acidity, moisture, specific gravity, refractive index, ash, conductivity, brix, fructose (F), glucose (G), F/G ratio, reducing sugar and total sugar) whereas one loose packed sample was found adulterated. In conclusion this study may help to choose good quality honey as well as to improve and maintain quality.

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Pak J Food Sci 2008, 18 (1-4): 36-43

Studies on polyphenol oxidase inhibitors to sulphite and effect of filtration for control of enzymatic browning in guava juice

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ABSTRACT

Studies were conducted to determine the efficacy of polyphenol oxidase inhibitors to controls enzymatic browning in guava juice compare to sulfite. Although sulfites are highly effective to control the browning but these have shown adverse effect on health particularly to those who have pulmonary disorder like asthma. Filtration with useful filter aid and juicing agents are found to be helpful in removing browning residue particulates fractions to extend the shelf life of the juice. Non-enzymatic browning can also be prevented by cold blanching.

Keywords: Guava, polyphenoloxidase, enzymatic browning

INTRODUCTION

Many food products undergo browning due to enzymatic or non enzymatic reactions that occur during processing and storage. Such reactions have an important bearing on food quality and therefore of great importance to the food industry. Sulfiting agents have been added to many foods since antiquity to prevent enzymatic and non enzymatic browning; control the growth of microorganisms, act as bleaching agents, anti oxidant, or reducing agents, and carry out various other technical functions (Taylor *et al.* 1986). Guava (*Psidium guajava L*) is tropical fruit with appealing taste, aroma, abundantly produced in Pakistan. Physical characteristics vary depending upon varieties and colour (i.e.) white, yellow, green or reddish white with or without seeds. It contains high amount of ascorbic acid in fresh raw fruit as well in juices (Khan *et al* 1991).

Raw fruit and juices are subjected to enzymatic changes during processing and storage that results in degradation of product, colour, flavour and consistency Mayer and (Harel 1979 and Vamos 1981). Conventionally such juices are preserved by heat pasteurization which kills pathogens and spoil microorganisms and at the same time inactivate degradative enzymes (Movily *et al* 1992, Shewfelt 1987 and Alvrect 1988). Such treatments may alter the flavour of heat sensitive juices, however by imparting cooked flavour notes and by the loss of low boiling volatiles. Guava is not an exceptional and it changes its colour on ripening, bruising, cutting or mishandling during transportation of the fruit. Guava juice is also prone to browning. Previously it has been observed that enzymatic browning in some raw apple juices could be prevented by polyphenol oxidase inhibitors and their chelating agents (Alizai MN 1993). It has also been reported that centrifugation or filtration with filter aids are useful tools in prevention of browning in many juices (Sims *et al* 1969, Goodwin and Morris 1991, Daeschel *et al* 1991 and Brown 1991). Their results suggested that capacity of the juice to brown, (i.e.) the enzyme polyphenol oxidase and/or its substrates are associated with particulate fractions that may be removed by clarification treatments. Clarified apple and pear juices were reported to be practically devoid of polyphenol oxidase activity. The objectives of our research on guava juice were to investigate the range of applicability of clarification treatment concept and to develop treatment suitable for juice production. The data presented in this paper deal specifically with treatment to control enzymatic browning of fresh juice by removal of particulate fraction along with using sulfite as parallel treatment to examine the efficacy of the treatment. We have also examined means of removing polyphenol oxidase activity from isolated juice particulates so that they might not be reactivated in the

clarify juice. Non-enzymatic browning of guava juice is highlighted in view of its prevention of browning by using orthodox methods (i.e.) addition of potassium metabisulphite with new concept of cold blanching of the juice.

MATERIAL AND METHODS

Juice preparation

Fresh guava fruit was purchased from local market and transported to PCSIR Laboratories Complex in hygienic condition. Fruit was thoroughly cleaned washed under portable water. Damaged, bruised parts were removed. Weighed the cutlings (Table-1.).

Table-1 Preparation of guava juice

1.	Total weight of fresh guava fruit	=	3 Kg.
2.	Weight of enzyme added (pictinol)	=	3 g
3.	Weight of cutlings	=	100 g
4.	Weight of cake	=	0.5 Kg
5.	Prepared juice in litres	=	2.1 Litres
6.	Brix of juice at 25°C	=	11.0
7.	RI	=	1.3481

Guava was cut into small pieces blended and pulp stored in steel basket for more than one hour at room temperature. One percent pictinol enzyme was added while stirring for one hour at room temperature. The pulp was left overnight at 4° C. Juice was extracted, passing through moslin cloth with slowly pressing the cloth and then twisting the cloth anticlock wise to get maximum juice. Juice was measured and cake weighed. Enzyme was added again to clarify the juice and stored overnight at 37°C. Juice was stirred thoroughly filtered with water suction pump using solid bed. Addition of 1-2% of enzyme was repeated two to three times until clear, transparent juice was obtained. Juice was kept for more than two minute, in heated water bath at 85°C to inactivate the enzyme. Cool and transparent clear juice was bottled and stored at 4°C in refrigerator for further investigation. Brix of the juice were noted at 25°C (Table-1).

Treatment with polyphenol oxidase (ppo) inhibitors

Various polyphenol oxidase inhibitors including their chelating and complexing agents were applied to investigate their efficacy independently. Juice in its original form was used as standard. Sodium metabisulphite was also used for comparative study. The following polyphenol oxidase inhibitors have been applied i.e. ascorbic acid, ascorbic acid 2 phosphate, ascorbic acid 3 phosphate, ascorbic acid polyphosphate and their complexing agents along with sucrets, cinnamates, citrates and benzoates. Experimental data of ascorbic acid, ascorbic acid 2 phosphate and citric acid is given in this paper. Procedure was followed as described by (Sapers 1989). 0.5 to 1.5% of polyphenol oxidase were applied and optic density (O.D) at 280 nm were noted for browning of the juice. Juice was kept at 25°C for different interval of time (Table-2).

Table-2: (OC) Optic density of clear juice noted at 280 nm with interval of time at 25°C (1% of the solution)

Treatment	Instantly	Two days	Four days	Eight days	Fifteen days
Ascorbic acid	0.11	0.30	0.32	0.41	0.54
Ascorbic – 2-phosphate	0.13	0.32	0.34	0.39	0.51
Citrates	0.16	0.36	0.38	0.39	0.42
Sucretes	0.13	0.26	0.30	0.30	0.32
Sulphite	0.14	0.21	0.22	0.24	0.26
Benzoates	0.13	0.28	0.30	0.29	0.36

Filtration of the juice

Few trails have been conducted to determine the effect of filtration on browning. The freshly prepared raw juice with or without addition of 0.1-0.2% celite analytical filter aid, bentonite and silica gel were filtered through Whatman No. 541 paper under suction. In one of the experiments

silica derived adsorbant as described by Sapers (1991) were also used. Juice was stirred thoroughly prior to filtration through Whatman No. 541 paper or by centrifugation.

Centrifugation

To determine the effect of centrifugation on the capacity of guava juice to brown. Portion of raw juice (25mL) were centrifuged in 50 mL centrifuge bottles. Centrifugation was done at speed of 1000 to 8000 rpm for 10 minutes at 4°C. Some of the trials were carried out with juice added to centrifuge tubes by pipette rather than graduated cylinder so that foam could be excluded.

Evaluation of capacity to brown

Portion of treated juices and untreated control, were stirred at 300 rpm for as long as 8 to 10 hours at room temperature on a multipoint stirrer to accelerate enzymatic browning if it were to occur. Samples were observed at frequent intervals for the onset of browning. The presence and absence of browning could be determined unambiguously by visual observation. No attempt was made to compare treatment on the basis of the extent of browning or time of on set since these were subjected to sample to sample variability. browning in treated sample after at least 6 hours storage at 20 °C Rather the success of the treatment in preventing browning was judged on a go, no go basis by the absence of with stirring, usually for one or more days without stirring. In some experiments, sample were stored at 4°C and 20°C without stirring and observation were made visually daily to daily bases to determine the presence or absence of the browning (Table-3). Polyphenol oxidase inhibitors treated juice samples optic density (O.D) at 280 nm were noted to see the browning of the juices in different interval of times (Table-4) at 4°C after filtration.

Preparation of concentrates

Two methods of concentrates preparations were applied, one hot concentration method, i.e. the evaporation method and other is cold osmosis method. In cold osmosis method bags of different pores were used which assists the exit of water and hence the juice become concentrated and at required thickness, brix noted and then stored at room temperature for evaluation of browning in the juice/concentrate. Guava concentrates were stored at 4°C and 25°C separately to see the effect of polyphenol oxidase inhibitors on different samples at different temperatures (Table-5).

Table-3 Visual observation of the guava juice with interval of temperature 25°c

	Stirring at 25 °C				Non Stirring at 25 °C				Non Stirring at 4 °C			
Treatment	8 days	15 days	1 Month	1½ month	8 days	15 days	1 Month	1½ months	8 days	15 days	1 Month	1 ½ months
Ascorbic acid	NS	NS	+	++	NS	NS	+	+	NS	NS	NS	+
Ascorbic acid 2-phosphate	NS	+	+	++	NS	NS	+	++	NS	NS	+	+
Citrates	NS	+	+	++	NS	+	++	+++	NS	NS	+	++
Sucrate (4-hexylresor-cincol) ki= 10 nm	NS	NS	NS	+	NS	NS	NS	+	NS	NS	NS	NS
Sulphite	NS	NS	NS	+	NS	NS	NS	NS	NS	NS	NS	+
Benzoate	NS	+	+	++	NS	+	++	++	NS	+	+	++
0.5% honey	NS	NS	+	+	NS	NS	NS	+	NS	NS	NS	+

(Browning)

NS= Not seen, + = mild ++ = Moderate +++ = severe

Table- 4 Optic density (od) at 280 nm (after gel filtration of juice at 4°c (1% of the solution)

Treatment	Instantly	4 days	8 days	15 days	20 days
Ascorbic acid	0.12	0.28	0.33	0.35	0.38
Ascorbic acid 2-phosphate	0.13	0.29	0.42	0.49	0.56
Citrates	0.14	0.26	0.32	0.36	0.43
Sucrete	0.14	0.24	0.28	0.36	0.32
Sulphite	0.13	0.21	0.27	0.31	0.34
Benzoate	0.14	0.26	0.34	0.39	0.45
0.5% honey	0.16	0.21	0.27	0.31	0.32
Sucrete at 4°C	0.15	0.18	0.18	0.20	0.21

Table-5 Visual observation of browning in guava concentrates at 25°C and 4°C

	At 25°C		At 4 °C	
Time	**Concentrate by evaporation**	**Concentration by cold osmosis**	**Concentrate by evaporation**	**Concentrate by cold osmosis**
Instantly	Yellowish gold colour (NS)	Yellowish gold colour (NS)	Yellowish gold colour (NS)	Yellowish gold colour (NS)
After 15 days	-do-	-do-	-do-	-do-
1 month	-do-	-do-	-do-	-do-
1 ½ month	Yellow (+)	-do-	-do-	-do-
2 months	Yellow (+)	Yellow (+)	-do-	-do-
3 months	Yellow (++)	Yellow (+)	-do-	-do-
3 ½ months	Yellow (+++)	Yellow (++)	Yellow (+)	-do-
4 months	Yellow (+++)	Yellow (+++)	Yellow (+)	-do-
5 months	Yellow (++++)	Yellow (+++)	Yellow (++)	Yellow (+)

(Browning) NS = Not Seen, + = Mild, ++ = Moderate, +++ = Severe, ++++ = very sever

RESULTS AND DISCUSSION

During preparation of the guava juice, 200 mL of the juice were separately collected prior to inactivation of the enzyme process so that parallel findings could be investigated. Polyphenol oxidase inhibitors treated samples were stored at room temperature. The visual observation along with O.D. at 280 nm with interval of time showed ascorbic acid (AA) is more effective than ascorbic acid-2-phosphate (AA2 P) and citric acid is more effective than Benzoate. Data presented in Table-4 indicated sucrates is more effective then all applied polyphenol oxidase inhibitors if pH of the juice remained low at approximately pH 5.0 or less.

Effect of filtration on browning

Clarified juices with addition of Celite Analytical Filter Aid (CAFA) and filtration indicated that such juices did not undergo enzymatic browning during subsequent storage. To confirm and

certify this observation, we investigated the ability of filtration with other filter aids and related products to eliminate the capacity of guava juice to brown (Table-6) CAFA proved to be the only material tested that appeared to prevent browning in apple pears juice (Saper 1991 and Barefoot *et al* 1989). Data presented here of guava juice confirmed the previously with reported results in literature for other species of fruit and their juices i.e. apple, pears etc. (Saper 1991, Barefoot *et al* 1989, Borgsttom 1954 and Bump1989).

Table- 6 Effect of filtration on enzymatic browning in guava juice without polyphenol oxidase inhibitor/ preservatives

Experiment	Treatment **	On set of browning (minutes at 25°C)
I	Unfiltered	200
	Filtered	240*
	Filtered with 1% CAFA	380 ***
	Filtered with 1% celite 545	240
	Filtered 0.5% Benzoate****	1800
II	Unfiltered control	80
	Filtered	200
	Filtered with 1% CAFA	310
	Filtered with 2% CAFA	440
	1% CAFA	180**
	2%CAFA	280***
	Filtered 0.5% Bentonite	1800
III	Filtered	50
	Filtered with 2% CAFA	310***
	2% CAFA	30
	Filtered 0.5% Bentonite ****	1680

* 1.Passed through Whatman No. 541 paper with suction.
** 2. CAFA= Celite Analytical Filter Aid.
*** 3. Browning after additional 48 hours at °4C.
**** 4.% Celite analytical filter aid additional prior to filtration through Whatman No. 541 paper with suction

The cold sterilization of fruit juices by filtration was first investigated and used in Europe more than fifty years ago (Charley 1934,1938). Juices prepared by this process retain the characteristic flavour for fresh fruit and are free of cooked taste, but may be unstable due to presence of active enzyme (Smock and Neubert 1950, Kim *et al* 1989). Moreover ultrafiltration has been used to clarify fruit juices (Kirk *et al*, Swientek 1986 and Thomas *et al* 1986). The potential use of such system for cold sterilization has been recognized (Koseoglue *et al* 1991).

The influence of ultrafiltration on juice quality has been investigated (Koseoglue *et al* 1991, Draks and Nelson 1977 and Heather *et al* 1977) however because these studies employed heat pasteurized products no conclusion could be drawn about the exclusion of polyphenol oxidase or other enzymes by the ultrafiltration system. Heatherbell *et al* (1977) observed haze and sediment formation during storage of unpasteurized juices that had been filtered through a 50,000 deltone MWCO membrane. They did not establish the fact whether small amount of enzyme or enzyme degradation products passing through the membrane were responsible for browning. (Kim 1989) reported high recovery of added pectines in apple juice retain taste under high temperature with short time ultrafiltration. (Koseoglue *et al* 1991) and employed ultrafiltration through 50,000 and 100,000 dalton MWCO membrane to remove degradative enzyme from raw citrus and available juices which were subsequently cold sterilized and combined with heat pasteurized pulp. They demonstrated the absence of pectin esterase activity in ultrafiltration permeates. Since the occurrence of enzymatic browning in fruit juice ultrafiltration permeates would have been obvious in many ultrafiltration studies reported in literature, it is highly unlikely that these permeates contain active polyphenol oxidase. Our findings suggest that filtration through such highly retentive membrane might not be required to remove polyphenol oxidase from raw juice these results are in agreement with those reported by (Sapers *et al* 1991) for apple and pear juices.

Effect of centrifugation on browning

It has been reported that browning could be eliminated by centrifugation at speeds of 1000 rpm or above (Koseoglue *et al* 1990). Subsequently we recognize the fact of excluding foam produced during juicing from the centrifuge tubes. The success of microfiltration with the juice indicate that failure of centrifugation to prevent browning is due to the presence of polyphenol oxidase bound to low density particulate rather than soluble polyphenol oxidase when care was taken to exclude foam, browning could be controlled by centrifugation at 4000 rpm and above (Mayor and Aitken 1980; Bolin and Huxsoll 1989). According to (Smock and Neubert 1950) centrifugation at 9000 rpm will produced a cloudy juice that will remain free from particulate during storage. They had suggested that high speed centrifugation could be used before filtration to reduce the load on filters. There is no indication in the literature that the effect of centrifugation on browning described herein was recognized previously, probably since centrifuged juices were subsequently pasteurized. We have adopted the procedures mentioned in literature (Huxsoll 1989 and Van Buren 1989) and found that browning in guava juice can be controlled for longer period by using centrifugation and filtration if at all were not successful eliminating the browning completely. In case of the treatment with SO_2 we observed that juice could be prevented from browning for more than four to five months at 4°C. Colour started changing after three months and completely changed after 4 to 5 months (Table-7).

Effect of polyphenol oxidase inhibitor on concentrates

Two methods were adopted for guava juice concentrate (see material and Methods) comparative study with or without polyphenol oxidase inhibitors applications were monitored and found that evaporation method concentrates change its colour quickly compare to cold osmosis procedure. It could be due to non-enzymatic browning, that coloration resulting from the reaction of carbonyl and free amino groups that lead to the formation of brown melanoid in pigments (i.e.) the classic maillard reaction (Walter and Feather 1963). Although non oxidative browning reaction between reducing sugars and amino acids or protein, are important in many products, browning may results from sugar degradation or from the oxidative degradation of ascorbic acid (Kacem *et al* 1987). Further reaction of the carbonyl compound formed via aldol condensation or reaction with amino groups to yield brown pigments (Loscher *et al* 1991). Sucretes found to be very effective in cold osmosis concentrated if pH were kept at pH 4.0 to 5.0. The shelf life of the concentrates made were found to more than 8 to 10 months.

CONCLUSION

In conclusion recent research related control of browning in guava fresh juice and their concentrates could be enhanced by removing particulate fraction by filtration using suitable filter aids and fining agents or by centrifugation. Specific filtration and centrifugation condition to prevent browning in guava raw juice depends upon commodity cultivars and ripeness. Non-browning, cloudy juice can be prepared by centrifugation to isolate particulates, heat treatment of particulate to inactivate polyphenol oxidase and recombination of the heated particulate with supernatant, treated by membrane filtration to remove soluble polyphenol oxidase. These approaches may be useful in production of unique minimally processed juices having the quality attributes of a fresh product though we were not completely successful controlling browning in concentrates.

Table-7 Effect of sodium meatabisulphite, ppo inhibitors, sucret (ph 4.00) on shelf life of guava juice

Treatment	1 months	2 months	3 months	4 months	5 months	6 months	8 months	10 months	12 months	14 months
Sulfite										
at 25 °C	NS	+	++	+++	++++	-	-	-	-	-
at 4°C	NS	NS	+	+++	++++	-	-	-	-	-
PPO Inhibitor										
at 25 °C	NS	NS	+	++	+++	++++	-	-	-	-
at 4°C	NS	NS	NS	+	++	+++	++++	-	-	-
Sucrete (pH 4.0)										
at 25 °C	NS	NS	NS	NS	NS	+	+	++	++	+++
at 4 ° C	NS	NS	NS	NS	NS	NS	NS	NS	+	+

Visual browning:- NS = Not seen, + = Mild,++ = Moderate, +++ = Severe, ++++ = Very Severe

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Pak J Food Sci 2008, 18(1-4): 44-49

Studies on the preparation and evaluation of sweet banana chips

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ABSTRACT

Sweet banana chips were made from fresh banana using osmotic dehydration that can be used as ready to eat snack food item. Shelf life of the product was monitored for six months with organoleptic, biochemical and microbiological evaluation. No change in color, flavor and texture was observed during storage. The microbiological studies revealed the absence of molds, coliforms and total bacterial counts were 10^3< to <10^5cfu/g values for the initial and the final study of shelf life respectively, indicating the satisfactory microbiological results throughout the storage of six months.

Key words: Banana, snack food, shelf life evaluation.

INTRODUCTION

Banana belongs to the family *Musaceae*. It is native to the tropical region of South East Asia, Malay Archipelago and Australia. It is also cultivated throughout the tropics and now it has become an important fruit crop of the world. Bananas are abundantly produced in Pakistan as well. It has many varieties. The two major varieties are *Cavendish dwarf* (Basrai) and *Cavendish giant* (William hybrid). It has a special value in human diet (Seymour, 1993) and is chiefly eaten raw as a dessert fruit, because in the ripe stage it is sweet and easily digestible. It is useful for patients with peptic ulcer, for treatment of infantile diarrhoea, celiac disease and colitis (Robinson, 1996). It is also a valuable fruit for the patients suffering from gout, arthritis, kidney disorders, blood pressure and heart problems. It is low in fats, cholesterol and salt (Stover *et al* 1987). Bananas are valuable source of vitamin B_6, vitamin C and potassium. It is a very good source of instant energy when taken in the form of drink and has mild laxative properties that help to ease constipation.

Despite of these beneficial properties there is very less utilization of banana in commercial products. Large quantity of this fruit is wasted due to poor transportation and storage facilities. Banana when ripened is a soft and delicate fruit with a post-harvest shelf life of 5-10 days (Surendranathan *et al* 2004). Therefore, keeping in view for maximum utilization of Banana and to increase its shelf life sweet banana chips were prepared that can be used as a ready to eat snack. Sensory evaluation was also done to see the efficacy of the product. The product captures attraction in local market as well as abroad due to its natural ingredients such as vitamins and minerals. The sweet banana chips (Table 1) are light in weight than their corresponding fresh product and can be available throughout the year. Shelf life of the dehydrated product was one year when stored in dry and cool conditions in a moisture proof packing material.

Table 1: Nutritional value of sweet banana chips

Parameters	
Energy (Kcal) per 100 g	361
Carbohydrates	87.83 g
Crude protein	2.37 g
Crude fat	Traces
Dietary Fiber	4.42 g
Crude ash	0.8 g

MATERIALS AND METHODS

Fresh bananas were purchased from local market of Karachi. The fruit was then peeled off and weighed. The weight of peel was taken separately. Bananas were cut into pieces in the form of discs of about ¼ inch thickness and dipped in a sulfite solution about 20 minutes. After this treatment bananas were blanched at a temperature of 80-90°C for 2-3 minutes into a solution containing food preservatives, which prevents the deterioration of the fruit in terms of color, texture and microbial growth. Blanching process also deactivates the enzymatic activity of the fruit thus preventing from enzymatic browning. The chips were then dipped in a sugar solution about 24 hrs at ambient temperature for osmotic dehydration. After 24 hours of osmotic treatment the chips were taken out and spread on wooden trays with perforated sieves. The trays were placed in thermostatically controlled cabinet hot air dryer at 55-60°C for 16–18 hours to reduce the moisture contents up to 5-6%. After drying, the product was cooled down and packed in polyethylene bags under hygienic condition and stored at low temperature. Physicochemical analysis such as Moisture, pH, Acidity, Brix, and Reducing sugars were tested along with microbiological tests to evaluate the product. Shelf life studies continued for more than six months..

Sensory Evaluation

Sensory characteristics of sweet banana chips were evaluated for color, flavor, texture and acceptability by a panel of ten judges from Food and Marine Research Resources Centre. The product was evaluated on a 9 point hedonic scale i.e. 9 (excellent), 8 (very good), 7 (moderately good), 6 (slightly good), 5 (neither good not bad), 4 (slightly bad), 3 (moderately bad), 2 (very bad) and 1 (extremely bad). The scores for each parameter was calculated as average scores awarded by the panel members. Over all quality scores were calculated as the mean scores of all the three separate quality parameters. The product was evaluated after fifteen days up to six months storage (Fig. I).

Physicochemical Analyses

The pH was estimated with the help of pH meter (HANNA, Model No. 8314). Moisture was estimated following the method of Lees (1975) (Figure II). Total acidity was determined by titrating against 0.1 N Sodium Hydroxide solution (Figure IV) and Total soluble solids were measured with Abbe's refractometer (I.S.O. 1993). 10% homogenized solution of the sample was taken for the determination of reducing sugars following the Lane and Eynon titrimetric method (AOAC, 2000). (Table 2 and Figure V).

Table 2: Physicochemical analyses of sweet banana chips

Sr.#	pH	Acidity (%)	Reducing Sugars (%)	Moisture (%)
1	4.05	0.042	48.11	6.66
2	4.15	0.039	49.07	6.54
3	4.16	0.040	48.95	6.31
4	4.19	0.036	49.18	6.28
5	4.18	0.037	50.08	6.22
6	4.20	0.042	52.16	6.31
7	4.22	0.040	53.27	6.37
8	4.16	0.030	54.42	6.48
9	4.12	0.039	56.99	6.79
10	4.04	0.042	59.58	6.44
11	4.21	0.040	55.67	6.14
12	4.15	0.044	57.60	5.22

Microbiological Assay

Presence of viable bacteria, yeasts and molds were determined by pour plate method (Anon 1984). Microbiological results showed that molds and coliforms were absent. Total bacterial counts for both level 1 and level 2 were $>10^3$ to $<10^5$cfu/g for the initial and at end of storage, respectively, which were within the permissible range for a sustainable preserved food product, whereas pathogen *L. monocytogenes* detected

within the marginal limit and which is also satisfactory up to the storage for six months shelf life (Table 3).

Table 3 Microbiological analyses of sweet banana chips during six months storage

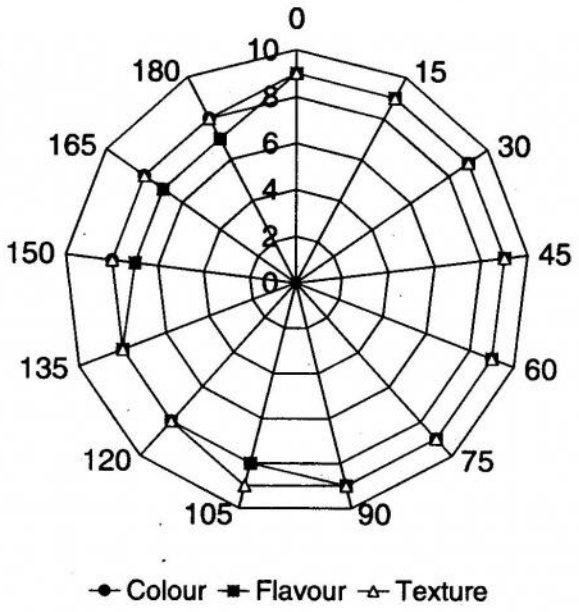
Test	Microbiological Quality (cfu per gram)
Standard Plate Count	
Level 1	$<10^3$
Level 2	$<10^5$
Indicators	
Escherichia coli	<3
Pathogens	
Clostridium perfringens	10^2
Campylobacter spp.	Not detected
Salmonella spp.	Not detected
Listeria monocytogenes	Not detected

RESULTS AND DISCUSSION

The results of physico-chemical analysis of the product are shown in Table 2. pH, acidity, reducing sugars and moisture showed no significant deterioration in all the sensory parameters during storage period of six months (Table 2). The color, flavor, texture and enzymatic browning during storage were least effected (McCord and Kilara, 1993; Ma *et al* 1992; Hall, 1989). The pH of the product remained within 4.0% to 4.2% (Table 3). This is mainly due to the addition of antioxidants and preservatives during processing. Lower pH is also one of the factors that increase the shelf life of the product (Dauthy 1995). As fruit ripens, it softens, its acidity declines and it produces certain volatile compounds that give its characteristics aroma (Chapman and Horvat, 1990). To maintain low pH and the addition of preservatives and antioxidants were the hurdles to formulate the preserved product. (Alzamora *et al* 1995, Argaiz *et al* 1995; de Daza *et al* 1995; Guerrero *et al* 1994). The choice of acidulant in the product depends mainly on the type of fruit, cost, sugar/acid ratio etc. Mostly acids which are added to adjust the acidity of fruit product are citric and phosphoric acids due to their low price and sensory compatibility (Argaiz *et al* 1995). Citric acid is the most widely used acid for the prevention of enzymatic browning because it inhibits polyphenol oxidase by reducing the pH and chelating the copper at the enzyme-active site. It was found that the moisture level of the product initially was 6.7% and remained within 5.2% during six months storage and this level retards the bacterial and yeast growth (Davies *et al* 1976). Water activity has a profound effect on the rate of many chemical reactions and microbial growth (Labuza, 1980). Most of traditional foods that remain stable, safe and tasty during long- term storage are intermediate moisture foods, in which lowering of water activity is one of the principal preservative factors or hurdles (Welti-Chanes *et al* 2000; Leitsner and Gould, 2002). Intermediate moisture foods have water activity from 0.60 to 0.90 and 10-50% water by weight (Davies *et al* 1976; Jayaraman, 1995). The safety against spoilage by microorganisms resistant to water activity (mainly moulds and yeasts, which can grow at as low as 0.60), and also against some bacterial species that are likely to grow when aw value of the intermediate moisture food is near the upper limit of water activities i.e. 0.90. Reducing sugars of the product ranged from 48 to 58% which is a high concentrated sugar product after osmotic infusion. Osmotic dehydration is a technique, which is effective at ambient temperature, minimal heat damage to color and flavor and high concentration of sugar surrounding fruit discs prevents discoloration. Osmotically dried banana retained more puffiness and a crisper texture then simple vacuum dried ones and the flavor lasted longer at ambient temperature. Osmotic dehydration can be used instead of air drying to obtain energy saving or quality improvement especially for fruits and vegetables sensitive to air drying.

Microbiological results showed absence of molds and coliforms. The total bacterial counts

Fig I: Sensory characteristics of sweet banana chips during storage



were $<10^3$ and $<10^5$cfu/g at initiation and end of study respectively, indicating that both the level 1 and level 2 SPC were within the permissible range for a sustainable preserved food product. Whereas *E. coli* and *L. monocytogenes* were within satisfactory and marginal ranges respectively, indicating the satisfactory shelf life of the product during storage of six months. In ready-to-eat food, *E. coli* is undesirable because it indicates poor hygienic conditions. Ideally *E. coli* should not be detected and as such a level of <3 cfu/g (the limit of the Most Probable Number test) has been given as the satisfactory criteria for this organism (Guidelines, 2001). Levels exceeding 100 per gram are unacceptable and indicate contamination, whereas higher levels of *L. monocytogenes* (10^2cfu per gram) indicate a failure with food handling controls and considered as a public health risk (Table 3). In traditional preservation methods of fruit products, the main function of heat treatment is to destroy the enzymes that could deteriorate vegetables and fruits. In minimal processing techniques, blanching of the product has another important role to reduce the initial microbial load by inactivating heat sensitive microorganisms and coliforms. Thus, blanching has found to reduce the microbial load from 60 to 99% (Alzamora *et al* 1995). Similarly, sulphites used in processing of the product although low in amounts mainly inhibits non-enzymatic browning and to prevent the growth of yeasts and moulds. Since bacterial growth was inhibited by the interaction of water activity, blanching inactivated pH and enzymes. The sulphites deplete in preserved fruit products, even faster and more completely than sorbate (Alzamora *et al* 1995; Guerrero *et al* 1996).

CONCLUSION

It is concluded from the study that *Sweet Banana Chips* can be successfully prepared and retained stable uptill six months in a good and acceptable condition. It could be utilized as ready to eat snack food throughout the year and incorporated in many sweet dishes such as pies, custards, ice cream and many other sweet mixes. They are light in weight than their corresponding fresh fruit and manufacturing of the product is feasible to industrialists/inventors/manufacturers and competes in the local market. Moreover, it would be of earning foreign exchange for the country.

Fig II: Changes in pH of sweet banana chips during storage

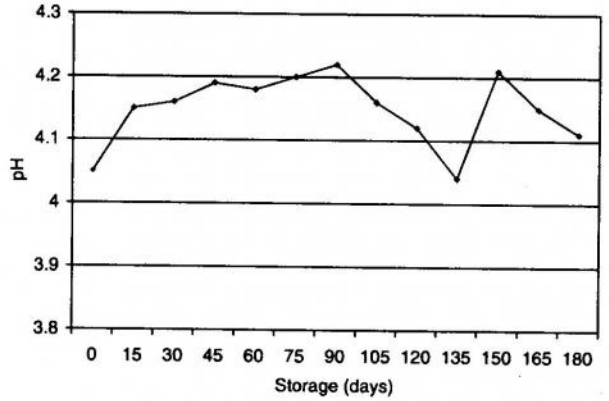


Fig III: Changes in moisture (%) in sweet banana chips during storage

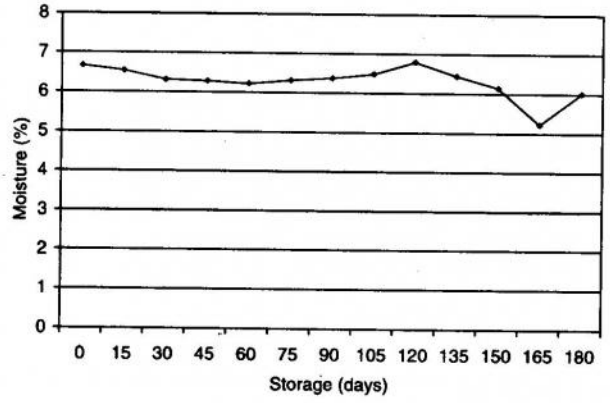


Fig IV: Changes in acidity (%) of sweet banana chips during storage

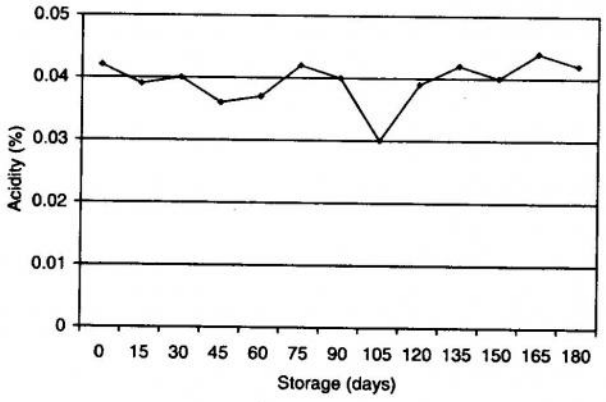
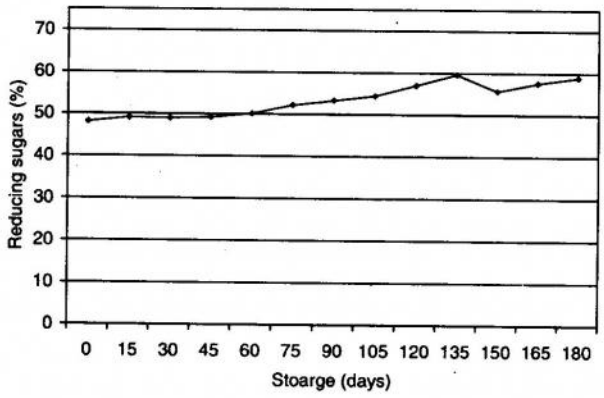


Fig V: Changes in reducing sugar (%) sweet banana chips during storage



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Pak J Food Sci 2008, 18(1-4): 50-54

Processing and shelf life studies of intermediate moisture mango slices

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ABSTRACT

Mango fruit belonging to genus *Mangifera* is the most delicious of all the tropical fruits and are available in bulk in Pakistan during summer season. Unfortunately, due to lack of storage facilities and fruit processing and preservation technology, a large amount of the fruit is wasted. Therefore, processing and product development of Mangoes is of extreme importance to minimize the fruit loss during the glut season. To preserve Mangoes and extend their shelf life they are processed through osmotic dehydration technique and can be utilized as a snack. The product Intermediate Moisture Mango Slices (IMF) was tested by using different biochemical parameters i.e. moisture, total soluble solids, brix, pH, acidity, reducing sugar etc. Shelf life of the product was monitored for more than six months. Microbiological studies were also satisfactory during six months of shelf life.

Key words: *Mangifera*, shelf life, snack, IMF

INTRODUCTION

Mango belongs to the genus *Mangifera and* family *Anacardiaceae,* consisting of numerous species of tropical fruit trees among flowering plants. Mango (*Mangifera indica* L.) is one of the important species of fruit. The fruit is relished for its succulence, exotic flavor and delicious taste. Mango is a rich source of carotenoids and provides high vitamin A content.

Pakistan stands 3rd among mango growing countries in the world. Mango has a prominent position among commercial fruits of Pakistan. It enjoys second position after citrus in Pakistan. The Pakistani mango is well known for its taste and quality. Mangoes are mainly eaten fresh, but are also used in preparing squash, jam, and other preserves and sometimes canned.

Pakistan is a significant exporter of mangoes and the third largest exporter in the world. Small quantities are also used for processing of various products. Mango production is mainly centered in two regions, the Punjab and the Sindh, producing 67% and 32% of the total production respectively. The principal varieties of mangoes are Sindhri, which dominates Sindh production, and Chaunsa, which dominates the production of Punjab. Commonly grown other varieties are Began Pali, Langra and Anwar Ratol.

Mango is an excellent nutritional source, containing many vitamins, minerals, and antioxidants, as well as enzymes such as magneferin and lactase which aid in digestion. Fresh mangoes are susceptible to deterioration during marketing and distribution channel from initial harvesting to final consumption. The major reasons causing deterioration are physical disorders, metabolic changes, pathological breakdowns and diseases (Tabassum 1992). Because of this perishability of mango fruit, it is highly utilized in commercial products like chutney, pickle, milk shakes, juice and squash etc. (Singh 1960)

For maximum utilization and enhancement of the shelf life of mango fruit, a process for the production of Intermediate Moisture Mango Slices has been developed which can be used as ready to eat (RTE) snack. Sensory studies were conducted to identify the efficacy of the product. Dehydrated Mango Slices offer ready to eat snack food with qualities resembling fresh fruit and will capture attraction in local market as well as abroad because of its good nutritional profile and can be eaten without any preparation. The product can be available throughout the year and can be kept for about six months at ambient temperature with moisture proof storage conditions.

MATERIALS AND METHODS

Fresh, ripe and healthy mangoes were purchased from local market of Karachi and transported to the laboratory under hygienic conditions. A batch of approximately 100 kg of raw mangoes *(Chaunsa)* was processed. Mangoes were peeled off and weighed and the weight of peel was taken separately. Mangoes were then sliced into pieces. The slices were blanched in water at 80-90°C for 1-2 minutes containing preservatives. This inhibits the deterioration of fruit in terms of texture, color and microbial growth. It also deactivates enzymatic activity. After blanching the slices were dipped in sugar solution for infusion (1:1.55 H_2O: sugar) containing food preservatives, ascorbic acid and citric acid.

After 24 hours of infusion, mango slices were taken out of the sugar syrup and washed in running water for 1-2 minutes. They were then placed on wooden trays on muslin cloth. The trays were then placed in cabinet dryer at 55-60 °C for 16 – 18 hours. After drying the product was cooled down and packed in polyethylene bags under hygienic conditions and stored at 12-15 °C. Biochemical analyses such as pH, Acidity, Brix, Moisture and Reducing sugar were performed along with microbiological evaluation. Shelf life studies continued for more than six months.

Sensory Analyses

Sensory quality of the product was evaluated by a panel consisting of ten assessors drawn from the laboratory staff of Food & Marine Research Resources Centre of PCSIR Labs. Complex, Karachi. The panelists were asked to assess the product for its color, flavor and texture. The product was evaluated on a 09 point score i.e. 9(excellent), 8(very good), 7(moderately good), 6(slightly good), 5(neither good not bad), 4(slightly bad), 3(moderately bad), 2(very bad) and 1(extremely bad). The panelists awarded an average score for each parameter. An over all quality score was calculated as the mean score of all the three separate quality parameters by 15 days interval of each month during six months of shelf life (Figure I)

Biochemical Analysis:

The pH was determined using electronic pH meter (HANNA Model No. 8314). A homogenized 10% solution of the sample was prepared for the determination of pH, whereas % Acidity was determined by titration method (AOAC, 2000). Total soluble solids were measured with Abbe's refractometer (I.S.O. 1993). A 10% of homogenized solution of the product was prepared to determine the reducing sugars following the Lane and Eynon titrimetric method (AOAC, 2000).

Microbiological Analysis

The processing of mango slices requires close supervision in order to control the quality of the product because the quality control is essential in terms of cleanliness and proper handling of the food product. The sample was examined for microbiological investigation by Total Bacterial Count (TBC) method (Diliello 1982). Then the sample was subjected to tests including, *E.Coli*, coagulase-positive staphylococci, *Listeria monocytogenes* detection, *Bacillus cereus*, campylobacter and salmonella detections, using the guidelines for the microbiological examination of ready-to-eat foods (Table 2).

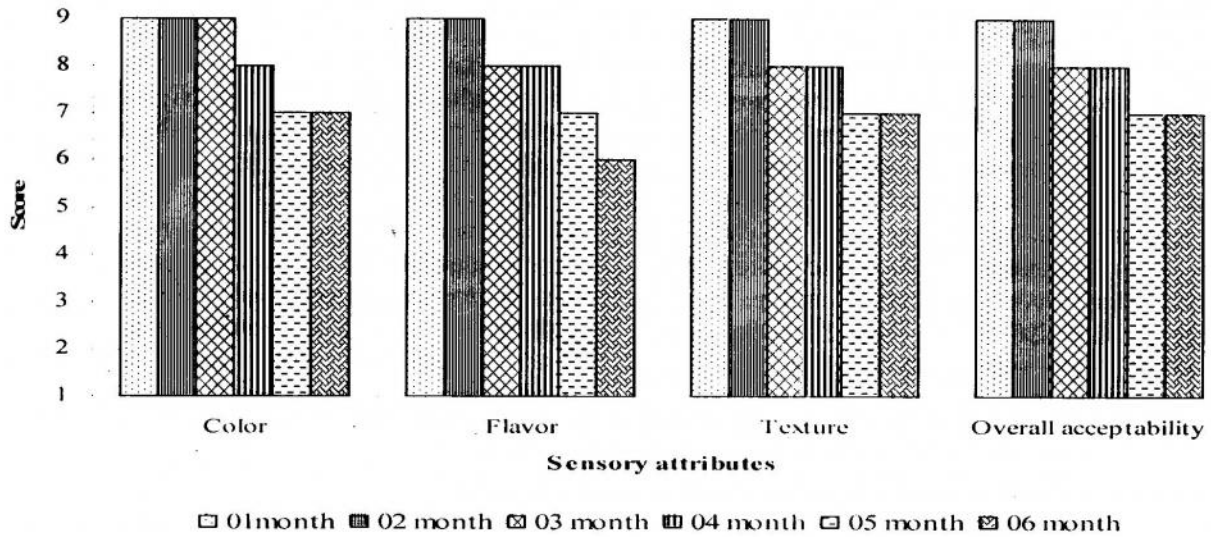
RESULTS AND DISCUSSION

The results of the product are depicted in Figure I during six months storage time. Color, flavor and texture were excellent and pH of the product was measure without any dilution (AOAC, 2000). The pH remains constant within 4.0 to 4.1 (Table 1). Low pH is also one of the factors that increase the shelf life of the product (Dauthy 1995), (Figure II) Mostly, citric and phosphoric acids are added to adjust the acidity of fruit products because of low price and sensory compatibility (Argaiz *et al* 1995) and the prevention of enzymatic browning was also done by using citric acid because it inhibits polyphenol oxidase by reducing the pH by chelating the copper at enzyme-active site (Figure III).

Table 1. Physicochemical analysis of mango slices for six months shelf life

Storage (months)	pH	Acidity (%)	Reducing sugar (%)	Moisture (%)
1	4.10	0.041	47.56	6.75
2	4.11	0.040	48.93	6.78
3	4.10	0.042	51.12	6.36
4	4.11	0.045	53.67	6.41
5	4.11	0.047	56.48	6.59
6	4.10	0.046	61.14	5.69
S.D	4.105 ± 0.005	0.043 ± 0.002	53.15 ± 4.62	6.43 ± 0.36

Fig. 1. Effect of storage on sensory scores for dehydrated mango slices during six months shelf life



It was found that the moisture level of the product remains within the range of 6-7% (Figure IV) during six months storage and this moisture level causes bacterial and yeast growth to cease (Davies *et al* 1976). Reducing sugar of the product ranges from 47 % to 48%, which is highly concentrated sugar product after osmotic infusion whereas osmotic dehydration is a technique, which is effective at ambient temperature, heat damage to color and flavor is minimized and the high concentration of the sugar surrounding the fruit prevents discoloration (Figure V). Osmotically dried Mango retained more puffiness texture then its raw form and at ambient temperature its flavor lasted longer.

Microbiological analyses showed the absence of molds and coliforms. The total bacterial counts were $<10^4$cfu/g and $<10^5$cfu/g for both the level 1 and level 2 SPC respectively, which is within satisfactory range for a sustainable preserved food product (Table 2).

Traditionally the preservation method for fruit products is mainly the function of heat treatment to destroy the enzymes that could deteriorate

Table 2. Microbiological analysis of dehydrated mango slices during six months storage

Standard Plate Count	Microbiological Quality (CFU per gram)
Level 1	$<10^4$
Level 2	$<10^5$
Indicators	
Escherichia coli	<3
Pathogens	
Clostridium perfringens	10^2
Campylobacter spp	Not detected
Salmonella spp	Not detected
Listeria monocytogenes	Not detected

the fruit but in minimal processing techniques, blanching of fruit has an important role to reduce the initial microbial load by inactivating heat sensitive microorganisms that are yeasts, moulds, coliforms and aerobic microorganisms. Similarly in processing of fruits, sulphites in low amounts inhibit the non-enzymatic browning and prevent the growth of yeasts and moulds. Sulphites deplete in preserved fruit products, even faster and more completely than sorbates (Alzamora *et al* 1995; Guerrero *et al* 1996).

CONCLUSION

It has been concluded that *Dehydrated Mango slices* prepared, remained stable during six months shelf life and can be utilized as ready to eat (RTE) throughout the year as compared to fresh fruit.

Fig 2. Changes in pH of dehydrated mango slices during six months of storage

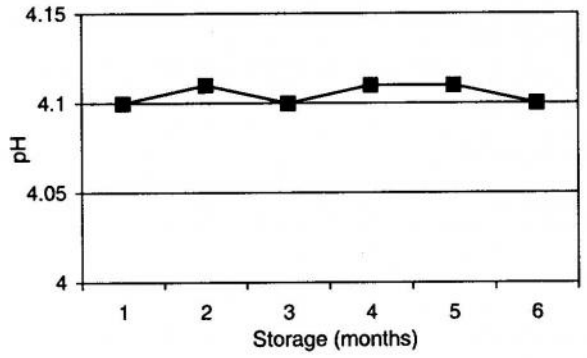


Fig 3 Effect of storage on % acidity of dehydrated mango slices during six months shelf life

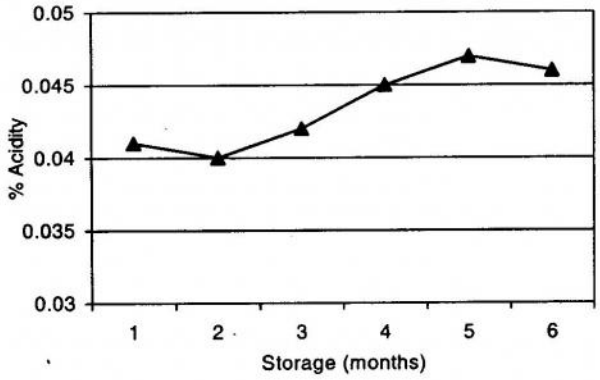


Fig 4. Changes in % moisture of dehydrated mango slices during six months storage

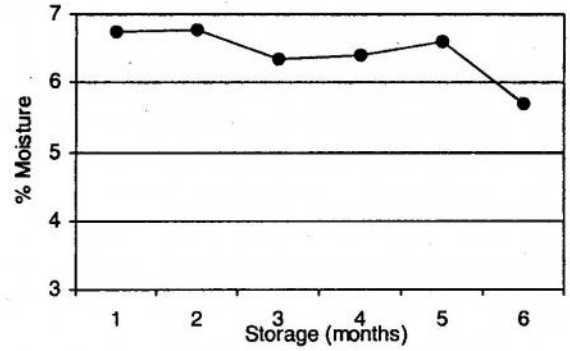
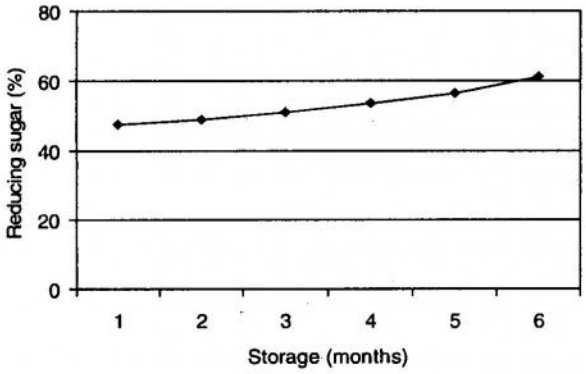


Fig. 5. Changes in % reducing sugar of dehydrated mango slices during six months of shelf life



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Pak J Food Sci 2008, 18(1-4): 55-62

Optimization of suitable stabilizer blend for mango milk drink

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ABSTRACT

Settling of solids and protein coagulation in milk based drinks on incorporation of fruit pulp has always been of a serious concern for industry. Use of stabilizers could be a solution of this problem. Addition of gelatin and pectin alone or in different proportions in mango milk drink was evaluated for ability to cater the settling issue in drinks. Changes in sensory attributes, pH, acidity, total soluble solids, sugars and ascorbic acid were monitored during storage to achieve the optimum level of stabilizers alone or in combination. The pH, ascorbic acid and non reducing sugar of samples decreased. It was found that better results for sensory evaluation were obtained for gelatin and pectin in blends at combination level of 4 and 1% respectively. The nominal shelf life determined using sensory changes and chemical analysis remained up to 15-20 days in the refrigerated conditions.

Key words: pectin, gelatin, mango milk drink, total sugars

INTRODUCTION

During the ages, man has developed a wide range of dairy products line through visual methods of trial and error. Milk and its sweetened and/or flavoured forms hold an important place among beverages because it contains a wide range of readily available nutrients required for normal growth of human beings, particularly for young ones. In Pakistan, the total production of milk during the years 2005-06 stood at 33.6 billion liters, out of which 27 billion litres were available for human consumption. Only 3-4% of produced milk is processed into the various dairy products (PDDC 2006). Milk is well known as a healthful source of protein and calcium but it is deprived of certain minerals especially iron (Ashourian *et al* 2007).

Mango (*Mangifera indica* L.) belongs to family *Anacardiaceae* and is one of the most popular fruit in Indo-Pak sub-continent due to its appealing flavor, taste, color, nutritive and therapeutic value. Mango ranks second in Pakistan after citrus with regard to production (GOP, 2008). It is cultivated through out the tropics. Mangoes have a short shelf life, so these can be preserved in the peak season for incorporation into food products i.e. milk drinks. Mango pulp could be incorporated into milk drink to enhance vitamin A, vitamin C and mineral contents as well as provide sweetness (Kumar and Mishra 2003). It contains a high proportion of sugar, mostly monosaccharide (Celik and Bakirci 2003). Addition of mango pulp in milk and milk products not only increases its nutrient content but also adds to its health promoting value (Kumar and Mishra 2004).

The difficulty in providing healthful product containing milk and fruits has generally been the lack of stability in such products. Stability with respect to the coagulation of milk and other proteins, however, is a significant concern in products having a lower pH. In addition, products are always subject to concerns regarding desirable taste and mouth feel. They can be stabilized with the addition of stabilizers and emulsifiers but it may cause chalky feel in consumer's mouth (Ashourian *et al* 2007).

Mango milk drink was prepared and preserved by adding the stabilizer alone or in blends with the objective to find the suitable blend of stabilizers and time dependent changes in physico-chemical and sensory quality were monitored.

MATERIALS AND METHODS

Mango fruit var. Chaunsa and UHT milk were purchased from the local market. Other ingredients such as pectin, sodium citrate, potassium sorbate and sugar were obtained from a scientific store.

Experimental Design

The formulation of the final product was obtained after conducting several trails by using sugar, pulp, milk concentration and stabilizers at different levels. The stabilizers were included in the experiment as following

Table 1. Description of treatments

Treatments	Pectin	Gelatin
T_0	-	-
T_1	4%	0%
T_2	3%	1%
T_3	2%	2%
T_4	1%	3%
T_5	0%	4%

Preparation of mango milk drink

The following formulation was used for the mango milk drink preparation.

Fresh ripe mangoes were washed under tap water to remove dust and dirt and to reduce the miscible load. The mangoes were softened by hand and with adding small amount of water, the pulp was obtained from mango pulper. All the dry ingredients such as stabilizer, potassium sorbate, sodium citrate, potassium sorbate and sugar were mixed to make a homogenous mixture. This mixture was dissolved in 10-20 mL of water by continuous agitation for the preparation of solution. Then this solution was used for the flavored fruit milk drink. After the mixing of this solution in the milk, 15 minutes were given to the milk for protein stabilization. Then mango pulp of 12^{0}Brix was added in the milk and homogenized twice for 3 minutes each.

The milk shake was filled in clean pre sterilized bottle which were semi sealed. The bottles were pasteurized at 80°C for 15 minutes in water bath and cooled to 10°C, and then caps were completely sealed. The milk shake was stored at refrigerated temperature 4°C for quality assessment for one month. A total of 6 treatments were prepared as shown in Table 2. The pasteurized mango fruit flavored milk was subjected to chemical and sensory evaluation on 0, 5, 10, 15 and 25 days of storage at refrigerated conditions.

Table 2. General formulation for mango milk drink

Sr. No.	Ingredients	Quantity (%)
1.	Sucrose	13.00%
2.	Mango pulp	12.00%
3.	Pasteurized milk	20.00%
4.	Stabilizer	0.400%
5.	Sodium citrate	0.020%
6.	Potassium sorbate	0.010%
7.	Water	54.00%

Chemical Analysis

Acidity

The titrable acidity was determined following the method given in AOAC (2000). According to this method 5 ml sample was titrated against 0.1N NaOH solution to a persistent pink color end point using phenolphthalein as indicator. The results were expressed as percent lactic acid.

pH

The product was taken in neat and clean 50 ml beaker and pH was directly recorded by using a pH meter as described in AOAC (2000).

Total Soluble Solids

Total soluble solids of pasteurized mango milkshake were directly recorded by hand refractometer equipped with a percent scale and the results were expressed as percent soluble solids (Brix^0).

Ascorbic acid

Titrimetric determination of ascorbic acid was done by method reported by Ruck (1969).

Sugars

Total sugars, reducing sugars and non-reducing sugars were determined by titration as outlined by Rehman *et al* (1995).

Sensory Evaluation

Seven panelists familiar with sensory evaluation techniques and regular consumers of flavored milk evaluated sensory quality changes of samples upon storage. Panelists were provided with evaluation Performa to evaluate the selected attributes i.e. color taste, flavor, and overall acceptability at 9 points hedonic scale (Meilgaard *et al* 1999).

Statistical analysis

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

RESULTS AND DISCUSSION

Sensory evaluation

Mean squares for analysis of variance for sensory evaluation are given in Table 3. The mean scores for sensory attributes are presented in Fig. 1. Sensory colour assessment of the mango milk drink indicated high scores for the color in the 2nd, 3rd and 4th evaluation. The maximum scores for colour declined after these storage periods (Table 3). As a matter of fact during 5 to 15 days storage, the colour of mango milk drink obtained maximum scores. Then there was degradation of fruit pigments and advancement in browning reducing the mean scores from 7.3 to 6.7. Destabilization of proteins during storage may contribute in this phenomenon.

The reason for the initial low score for color of the milk drink could possibly be the effect of high temperature on protein and lactose contents. The initial phase of heating has been reported to cause a slight shift in colour accompanied by an increase in brightness due to deterioration of whey proteins and breakdown of casein micelles (Hork and Kessler, 1981). Changes in mango pulp pigments seemed to have an effect on appearance. Decrease in pH could be another reason for change in colour (Worlsted *et al* 1970). It was found that pH was the only objective measurement having a high correlation with color quantity. Browning also has contribution in deterioration reaction accelerated by storage temperature (Lea 1948).

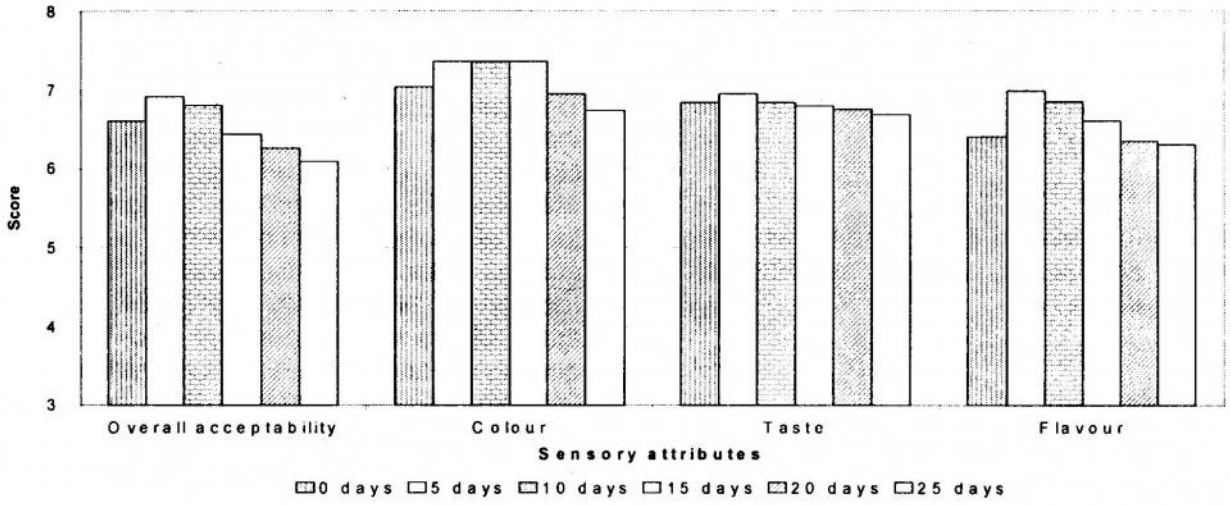
Statistical analysis of the data showed highly significant effect of storage time on taste of the samples (Table 3). While, there was less effect of the treatments on the taste of drink. The maximum scores were obtained at 2nd and 3rd week and then at the 5th week of all samples. Thereafter casein micelles destabilized and these were accompanied by other chemical changes, which rendered the product unacceptable. The reactions during storage due to enzyme action or oxidation activity could possibly explain this quality deterioration (Schroder, 1982). Marcy *et al* (1984) studied the effect of storage temperature and time on the quality of orange concentrate stored at low temperature and reported that as browning increased, the sensory panel scores for flavor significantly decreased with storage time.

Statistical analysis of data showed highly significant effect of storage time and significant effect of treatment on flavour of samples (Table 3). The maximum score was obtained by T5 (7.2) and minimum (6.35) war recorded for T3 at 25 days storage. From the Fig. 1, it is evident that product remained acceptable from flavour point of view even after 25 days storage. Fross (1969) stated that off flavor was produced by minute quantities of organic molecules which might gain access to the food by the chemical degradation of some of components of food. In dairy products the carbonyl compounds contribute greatly to flavor. They have been isolated in many flavor studies and found to be associated with both flavour and off-flavour production. Biryukova *et al* (1975) reported better flavor in UHT milk during storage for more than one month and attributed it to proteolysis. Sodium citrate is known to have a buffering effect on pasteurized mango milk drinks (Stringer *et al*1966).

Table 3. Mean squares for sensory attributes of mango milk drinks

Sources of variation	df	Overall acceptability	Colour	Taste	Flavour
Storage	5	0.567**	0.440**	0.930**	0.040**
Treatment	5	0.200**	0.121*	0.053*	0.021*
Error	25	0.009	0.006	0.008	0.006

Fig 1. Effect of storage on sensory scores for mango milk drinks



Highly significant differences for overall acceptability were obtained for the various treatments employed. Scores for this attribute of mango milk drinks are presented in Fig.1. According to data, treatment T5 and T1 got higher scores than other treatments. Duration of storage had a pronounced effect on all the sensory as well as chemical parameters. The product remained acceptable after 25 days under refrigerated storage conditions. Thereafter signs of spoilage appeared, the product had developed fermented taste and off flavor. The colour deterioration also took place with lowering in pH and then acidity became high. It appears that pectin and gelatin (alone) added in formulations tended to check coagulation of casein and separation of constituent thereby given a better quality product.

Charley (1957) stated that milk in bulk could be protected from curdling by applying preliminary low coating of pectin followed by the addition of fruit juices. Samuelsson and Holm (1966) have recommended the addition of sodium bicarbonate (0.5 g/L) for controlling sedimentation.

Chemical Analysis

pH

The pH of milk exhibits a greater dependence upon temperature than that of buffers such as phosphate, which is the principal buffer in milk at pH 6.0. Values of pH for all the mango milk drinks are presented in Table 4 and 5. At the first day of storage, the pH ranged between 6.78-6.81. These values decreased throughout the storage period ranging from 5.79-5.85 at the end of storage. When these results were subjected to statistical analysis, the effect of storage showed highly significant effect while the treatments have non significant effect. Webb *et al* (1974) reported tha

Table 4. Effect of treatments on pH, acidity, TSS and ascorbic acid contents of mango drink

Treatments	pH	Acidity (%)	TSS (%)	Ascorbic Acid (%)
T_0	6.31	0.22	18.77	3.92
T_1	6.25	0.20	18.72	3.99
T_2	6.29	0.21	18.74	3.97
T_3	6.24	0.21	18.65	3.97
T_4	6.24	0.21	18.72	3.97
T_5	6.26	0.21	18.79	3.94

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

Table 5. Effect of storage on pH, acidity, TSS and ascorbic acid contents of mango drink

Storage days	pH	Acidity (%)	TSS (%)	Ascorbic Acid (%)
0	6.81a	0.19a	17.39a	4.65a
5	6.45b	0.20b	17.77b	4.59a
10	6.32c	0.21b	18.13c	4.41b
15	6.18d	0.21b	18.83d	4.16c
20	6.00e	0.22ab	19.71e	3.60d
25	5.82f	0.23b	20.56f	2.35e

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

temperature could bring about insolubilization of salt constituent of milk, thereby increased the pH value. As the pH increased, the calcium and magnesium precipitated as colloidal phosphates and when the pH was lowered, colloidal calcium and magnesium phosphate were solubilized.

Acidity

The acidity of the formulation ranged from 0.183-0.201 at the time of preparation of milk drinks. All the samples showed an increase in acidity throughout the storage period ranging from 0.226-0.232 at the end of storage study (Table 5). This increase in acidity might be due to the thermal decomposition of lactose to organic acids, action of lactose with milk proteins, hydrolytic dephosphorylation of casein and displacement of calcium phosphate in equilibrium. The rate of acid formation had been found proportional to the lactose concentration and formation of formic acid and lactic acid in heated milk (Webb et al., 1974).

Wilkoboski (1954) observed the relationship between acidity and pH during lactic acid fermentation. Gorner et al. (1977) have observed gradual increase in titrable acidity and attributed to change in milk protein.

Total Soluble Solids

Data shows highly significant effect of storage and treatment on the total soluble solids of mango milk drink (Table 4 and 5). TSS increased at consistent level as the storage period proceeded.

According to Kanujosa and Luke (1967), the Brix of canned peaches increased gradually during storage. The increase might be due to the formation of water soluble pectin from protopectin during storage. Pectin is often also added to the products as stabilizer which could contribute towards the increase in TSS. Hasan (1986) reported that addition of thickeners i.e. carboxy methyl cellulose can cause an increase in TSS. It may be attributed to the pectin substance from protopectin and monosaccharides. Zia (1987) stated that TSS of guava, pomegranate and mango blend increased with the storage time.

Ascorbic Acid

Statistical analysis revealed that, storage has highly significant while treatments have non significant effect on ascorbic acid contents of mango milk drink (Table 4 and 5). Podgorka et al. (1983) studied the vitamin C contents declined with the time during storage of juice at 13°C but losses were very high when stored at room temperature. They also found 60-80 percent decrease in vitamin C at high temperature (80°C for 35-40 minutes) and 50-60 percent on flash pasteurization (120°C for 30 seconds). Decrease in pH and increase in acidity could possibly be the reason of loss in ascorbic acid. Webb et al. (1974) has stated that exposure of milk to light brought about oxidation of ascorbic acid.

Sugars

Statistical analysis showed highly significant effect of storage on all the sugars but non significant effect of treatments (Fig. 2, 3 and 4). Mcwilliam (1985) has reported that during boiling period some breakdown of sugar occurs, which depends upon the length of boiling time. Some of the sugar was changed by acid hydrolysis to invert sugars. The

storage duration, temperature and chemical reactions leading to the fall in pH which explain the changes observed in total sugar contents. Pruthi et al. (1984) reported an increase in reducing sugars from 13.6-23.8 percent at 25-30°C and decrease in non reducing sugar form 29.62-23.0 percent at room temperature during storage in juices of four commercial verities of oranges. Godara and Pareek (1985) also observed an increase in total sugars in date palm juice during storage at room temperature. Rehman (1989) found an increase in total and reducing sugars while decrease in non reducing sugar with the storage time. Palaniswany et al. (1974) observed an increase in reducing and total sugar during the storage of twelve months in the pulp and squash samples.

CONCLUSIONS

Use of stabilizers could be a solution of settling of solids in milk based drinks. Addition of gelatin and pectin at the combination level of 4% and 1%, respectively solved the problem to great extent. The optimum level of stabilizer combinations improved sensory attributes. The chemical analysis of the treated drinks supported the conclusion. The nominal shelf life determined using sensory changes and chemical analysis remained upto 15-20 days in the refrigerated storage conditions.

Fig. 2. Effect of storage on total sugars of mango milk drink

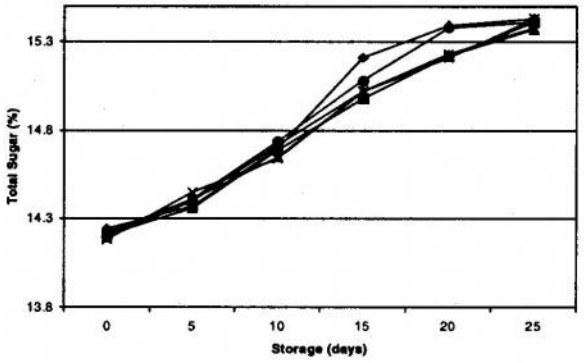


Fig. 3. Effect of storage on reducing sugar content of mango milk drink

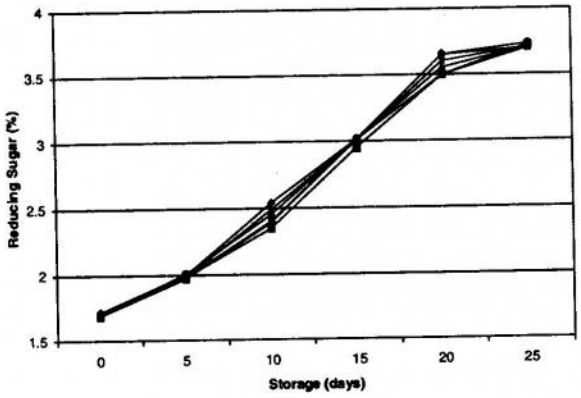
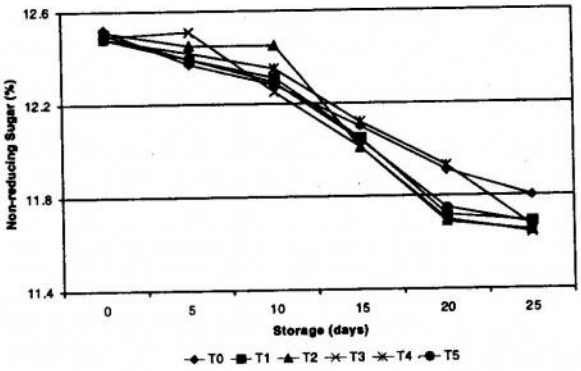


Fig. 4 Effect of storage on non-reducing sugar content of mango milk drink



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