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Effect of heat treatment and storage on the physico-chemical and microbiological characteristics of Kinnow pulp

Ibtesam Hussain, Faqir Muhammad Anjum, Imran Pasha, Muhammad Issa Khan and Ali Asghar
Institute of Food Science and Technology, University of Agriculture, Faisalabad.

ABSTRACT

Pasteurized and un-pasteurized Kinnow pulp was treated with chemical preservatives (potassium sorbate and sodium benzoate) and stored at room temperature. During processing of Kinnow fruit, pulp was taken from centrifuge and two lots were made. One lot was pasteurized and other kept unpasteurized. Each lot was treated with sodium benzoate and potassium sorbate each at concentration of 0.05%, 0.1% and 0.15% separately. The samples were fortnightly analyzed for Brix, acidity, Brix-acid ratio, ascorbic acid and pectin esterase enzyme activity, Total plate count and yeast and mould count. The sensoric attributes were judged after 90 days. A general loss in Brix, Brix-acid ratio, pH, ascorbic acid and pectin enzyme esterase activity was found with corresponding rise in acidity and micro flora in unpasteurized pulp samples treated with sodium benzoate or potassium sorbate. Pasteurized samples with chemical treatments remained unchanged for longer period. Un-pasteurized pulp samples showed serious flavour and colour changes after 15 days, while pasteurized samples showed better performance both for flavour and colour.

INTRODUCTION

Citrus is a collective generic term embracing a number of species and varieties of fruits known throughout the world due to their bright colour and characteristic flavour.The major citrus fruit cultivated is the tangerine like mandarin, Kinnow i.e., *Citrus reticulata* var. Blanco. The Kinnow fruit occupies top position with regard to its production (1.67 million tones) among fruits in the year 2004-05 in Pakistan (GOP 2005). It is a rich source of vitamin C which plays a significant role in human health. Its juice comprises 14-16% of bottom pulp which consists of crushed juice sacs, albedo fragments and other particles (Redd *et al* 1986). The composition of Kinnow pulp is similar to juice with 83.5% water. It contains sugars, citric acid, vitamins and a high quantity of carbohydrates. It contains 7.75 percent crude protein, 67.98 per cent crude fiber, 2 .2 percent calcium, 0.74 per cent potassium and 0.2 per cent sodium on dry weight basis (Ahmad and Gillani, 1991).

The Kinnow pulp is used in the formulations of ready-to-serve drinks, jams and jellies. It is also utilized as an ingredient in cattle feed since it is a cheaper source of energy than maize and does not impart any flavour to milk when fed to lactating animals (Ahmad and Gillani 1991). It is reported that the sugar content of discharge pulp and high microbial load results in the fermentation of the product (Wicker and Temelli 1988). The shelf-life of fruit juice and pulp is limited primarily by microbial, enzymatic and chemical reactions that adversely affect their nutritional quality, colour and flavour (Graumlich et *al* 1986).

This research study was conducted in collaboration with Sunflo Cit-Russ Ltd. at their processing plant in Sargodha to determine the effect of heat and chemical treatments on the physicochemical and microbiological characteristics of chemically pre-served Kinnow pulp during storage at room temperature.

MATERIALS AND METHODS

Fresh Kinnow fruit was procured from the local market, Sargodha and after grading and sorting, the fruit was brush washed and further transferred into a surge bin which controlled the flow of fruit. Juice was extracted by Food Machinery Corporation (FMC) extractors with standard pressure and sieves. FMC finisher screened out floating pulp, rags and seeds from the juice. After finisher, the juice was passed through a centrifuge which separated the pulp from the juice. Samples of this pulp were obtained and divided into two lots. One lot was pasteurized at 90°C for one minute after adding different doses of sodium benzoate (0.05%, 0. 1% and 0.15%) and potassium sorbate (0.05%, 0.1% and 0.15%) separately. Similarly, other lot was treated chemically in the same way but no heat treatment was given. All the treated samples were kept in sealed glass bottles and stored at room temperature (20-30°C).

The samples were analyzed fortnightly for Brix, acidity, Brix-acid ratio, pH, ascorbic acid and pectin esterase enzyme activity. Brix was measured by Abbe's Refractometer with automatic temperature compensation having 0.01 degree accuracy as

described by Ranganna (1991). Titerable acidity, which is a measure of citric acid content of the sample, was determined according to the method of Fox (1983).

Hanna HI 9020 Microprocessor pH meter was used to determine pH of the samples (AOAC 1990). The pulp sample were titrated with iodine solution to light pink colour end point using starch solution as an indicator to determine the ascorbic acid (Redd et *al.* 1986). Pectin esterase enzyme activity was determined by Kimball's method (1991).

The Kinnow pulp samples were tested for total plate counts and yeast and mould counts according to the method described by Cappuccino and Sherman (1996).

Sensory analysis was carried out by following the method of Bielig *et al* (1987).

RESULTS AND DISCUSSION

The data in Tables 1 and 2 show a significant decrease in Brix, Brix-acid ratio, pH, ascorbic acid and ↑ EU with a corresponding rise in acidity and microflora in all Kinnow pulp, pasteurized or un-pasteurized, during storage.

In case of un-pasteurized pulp the treatments with sodium benzoate as preservative resulted in higher brix value, brix acid ratio, pH, ascorbic acid concentration and more flavor retention than that treated with potassium sorbate as preservative. Addition of sodium benzoate to pulp lowered the value of acidity than potassium sorbate. Both preservatives had similar effect on the yeast and molds count as they reduce the growth of these microorganisms while the same effect was found on the color of the pulp.

Pasteurized Kinnow pulp exhibited similar trend with regard to preservative treatments. The pasteurized pulp had higher values for brix, brix acid ratio, ascorbic acid concentration, pectin esterase unit (PEU) and flavor of the Kinnow pulp, while the values for acidity and TPC showed a reduction in the pasteurized pulp as compared to the unpasturized pulp. In case of unpasteurized Kinnow pulp (Table 1), the samples without any preservative deteriorated adversely after fortnight at room temperature as there was no preventive to control the microbial growth. These results substantiate the earlier finding of the Arafat, (1995) and Kaanane et *al.* (1984). The pasteurized samples with no preservative treatment spoiled physico-chemically and microbiologically after few days as microbial count increases from 5 cfu/ml to 7386 cfu/ml. Pasteurization of Kinnow pulp significantly reduce the microbial load which results in the extension of shelf life of the pulp (Table 2).Sodium benzoate successfully preserved the Kinnow pulp at room temperature. In contrast, Mehta and Bajaj (1983) observed a slight increase in the Brix of juices of three citrus varieties preserved by sodium benzoate during storage at room temperature.

The best sample with regard to storage stability upto 90 days was the once treated with 0.15% sodium benzoate and pasteurized. It showed good stability for Brix, acidity, pH and ascorbic acid content. For instance, ascorbic acid content decreased from 28.60 to 25.37 mg/100 ml only after 90 days of storage at room temperature. Microbial count was reduced by the combination of heat treatment and chemical preservatives. Wicker and Temelli (1988) reported that high temperature and the sugar contents of the discharge pulp and high microbial load resulted in the fermentation of the product.

Heat inactivation of pectin esterase for 19 seconds at 80°C in juice pulp was effective. Residual activity decreased very little on subsequent heating for upto 180 seconds at 80°C after 90 days and remained under control throughout the storage period. Moreover treated sample obtained maximum scores for flavour (9/10) and colour (4/4) during subjective evaluation (Tables 1 & 2).On the other hand potassium sorbate in any concentration of 0.05%, 0.10% or 0.15% was unable to preserve the Kinnow pulp effectively (Table 2). The total plate count in case of un-pasteurized Kinnow pulp was much higher as compared to pulp preserved with sodium benzoate.

Ascorbic acid losses during the storage of pulp were higher in case of potassium sorbate than the pulp containing sodium benzoate. It is obvious from the study that pasteurization alone is not sufficient to control deterioration for a longer storage period as the spore of the microorganisms could germinate under favorable condition. Secondly, sodium benzoate in concentration of 0.15% can be used to preserve the Kinnow pulp at room temperature without any deleterious effect on the quality characteristics

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Table 1. **Effect of heat and chemical preservative treatments on the physico-chemical characteristics of chemically preserved kinnow pulp.**

CHARACTERS	UNPASTEURIZED						
	CONTROL	SODIUM BENZOATE (%)			POTASSIUM SORBATE (%)		
		0.05	0.1	0.15	0.05	0.1	0.15
BRIX	10.01	10.36	13.89	13.95	11.49	12.88	12.96
ACIDITY (%)	1.91	0.91	0.92	0.92	1.80	1.81	1.62
B/A RATIO	6.24	11.54	15.12	15.09	7.36	8.00	8.68
pH	3.41	3.83	3.68	3.67	3.60	3.62	3.62
ASCORBIC ACID (%)	12.58	14.89	16.45	27.20	8.55	9.86	10.13
PEU	0.83	0.75	1.03	1.03	0.94	0.99	0.97
TPC (cfu/mL)	140035.00	849.05	552.33	493.30	21444.00	35014.40	14161.00
YEAST & MOLD cfu/mL)	2496.70	2.86	2.86	1.90	60.00	24.30	17.14
FLAVOUR	3.14	7.71	8.14	9.57	4.71	5.00	6.00
COLOUR	2.43	4.00	4.00	4.00	4.00	4.00	4.00

Table 2. **Effect of heat and chemical preservative treatments on the physico-chemical Characteristics of chemically preserved kinnow pulp**

CHARACTERS	PASTEURIZED						
	CONTROL	SODIUM BENZOATE (%)			POTASSIUM SORBATE (%)		
		0.05	0.1	0.15	0.05	0.1	0.15
BRIX	13.05	13.55	13.50	13.73	12.70	12.89	13.30
ACIDITY (%)	0.98	0.83	0.85	0.87	1.30	1.15	1.03
B/A RATIO	14.51	19.12	16.04	15.79	10.28	11.22	13.32
pH	3.74	3.73	3.73	3.74	3.70	3.74	3.77
ASCORBIC ACID (%)	13.73	23.58	25.50	26.94	11.21	11.63	11.82
PEU	12.58	14.89	16.45	27.20	8.55	9.86	10.13
TPC (cfu/mL)	13001.05	31.81	40.57	7.76	290.50	231.40	181.20
YEAST & MOLD cfu/mL)	32.86	0.00	0.00	0.00	0.00	0.00	0.00
FLAVOUR	5.43	8.28	9.57	9.85	77.14	7.85	8.43
COLOUR	3.00	4.00	4.00	4.00	4.00	4.00	4.00

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Effect of different improvers on the rheological properties of selected wheat cultivars

Bahzad Afzal, Faqir Muhammad Anjum, Shahzad Hussain, Muhammad Issa Khan and Ali Asghar
Institute of Food Science and Technology, University of Agriculture, Faisalabad

ABSTRACT

Two bread improvers, Eka-300 and Majimax, were incorporated in the flour of five Pakistani wheat varieties to study their effects on the dough rheology. The mixographic characteristics of the treated flour showed that mixing time increased with increase in improver concentration. The highest mixing time was observed with 0.2% EKA-300 (T_4) in all wheat varieties. The peak height varied from 47 to 55%. Farinographic studies of different flour treatments revealed that addition of improver increased water absorption capacity, dough development time, departure time and dough stability but decreased the arrival time in all treatments. Ideal rheological characteristics were obtained with the addition of EKA-300 improver at the rate of 0.2%.

INTRODUCTION

In Pakistan wheat is mainly used for the preparation of unleavened flat bread, which is locally known as 'chapatti' or 'roti'. The consumption of wheat in the other bakery products i.e. bread, cakes, biscuits has also increased rapidly during the last two decades. Wheat is chosen among the cereal grains due to presence of special nitrogenous compound gluten, which possess unique properties of forming cohesive and elastic dough (Pyler, 1988). Wheat is not only a staple food but also a source of calories, protein and also of considerable amount of dietary fiber. Whole wheat flour consists of 85-86% dry matter, 12.72% protein, 2.71% fat, 1.75% ash and 2.62% crude fiber (Ahmad 1997).

Dough rheology characterization, which relates to dough handling properties and the tendency of the dough to contract, is an important parameter in the evaluation of wheat quality. Several methods are used to characterize the rheological properties of dough, including Farinographic and Extensographic methods. The rheological characteristics of wheat flour dough form the basis for understanding dough handling properties in baking. It also predicts the quality of bakery products. Farinograph shows a general profile of the mixing behaviour (Pyler 1988). There is a possibility to improve the performance of flour (fermentation, dough properties, oxidation, pH control and emulsification) by the use of different additives like fungal amylase, proteolytic enzyme, L-ascorbic acid, azodicarbonamide, potassium bromate, monocalcium phosphate, lecithine, benzol peroxide and chlorine dioxide (Anonymous 1987).

Bread improvers play important role in both dough and finished loaf volume, aid dough development, improve gas retention capacity of the dough, enhance dough tolerance to mechanical shock, speed up dough maturity , reduce rate of crumb firming/bread staling, improve crumb structure, texture of the loaf and loaf volume (GOP 2003). Present study was designed to investigate the effect of commercially available bread improvers on the rheological characteristics of bread dough.

MATERIALS AND METHODS

Procurement of raw materials

Five wheat (*Triticum aestivum*) varieties/lines namely Auqab-2000, SH-2000, Inqulab-91, 95153, and AS-2002 were collected from Postgraduate Agriculture Research Station (PARS), University of Agriculture, Faisalabad, Pakistan. Two bread improvers namely Eka-300 and Majimax were provided by Vita Industries (Pvt.) Ltd. Faisalabad, Pakistan.

Milling of wheat varieties

Milling of different wheat samples was performed on Brabender Quadrumate Senior Mill following the instructions of Williams *et al* (1986). Before milling, the samples were conditioned to 15% moisture for 12 hours.

Treatments of Wheat flour

Different treatments of wheat flour with improver were performed as depicted in Table 1.

Table 1 Treatments used in study of the rheological properties

Treatments	Improver	Level (%)
T_1	Control	-
T_2	EKA-300	0.1
T_3	EKA-300	0.15
T_4	EKA-300	0.2
T_5	Majimax	0.1
T_6	Majimax	0.15
T_7	Majimax	0.2

Mixographic studies

The mixographic studies were carried by running straight grade flour sample through mixograph equipped with 10 g bowl capacity to obtain information such as dough development time and peak height percentage from the mixograph. The mixographic data were interpreted for mixing time and peak height by following the procedure described in AACC (2000).

Farinographic studies

Each treatment was run through Brabender Farinograph equipped with 50 g, capacity bowl to determine various dough characteristics according to the procedure of AACC (2000). The physical dough properties such as water absorption, arrival time, dough development time/peak time, departure time, dough stability, tolerance index and softening of the dough were recorded.

Statistical analysis

The data for each parameter were subjected to statistical analysis to determine the level of significance between quality parameters of different straight grade flours by using completely randomized design (Steel *et al* 1997).

RESULTS AND DISCUSSION

Wheat flours containing different levels of improvers were subjected to rheological studies and significant results obtained for the mixographic and farinographic parameters are presented in Tables 2 and 3.

Mixographic Studies

The physical dough characteristics such as mixing time and peak height percent derived from mixograms of different wheat varieties flour supplemented with different level of improvers are shown in Table 2.

Mixing Time

The mixographic data indicated that mixing time ranged from 3.5 to 5.2 minutes between various treatments of Auqab-2000. The maximum mixing time was observed in treatment with 0.2% EKA-300 (T_4), while minimum mixing time was found in T_1 (control). The results of wheat variety SH-2002 showed that maximum mixing time was observed in treatment with 0.2% EKA-300 (T_4) followed by treatments with 0.15% EKA-300 (T_3) and 0.1% EKA-300 (T_2). The minimum mixing time was recorded in treatment T_1 (control). In the wheat variety Inqualb-91 the highest mixing time was obtained by the treatment T_4. The mixing time ranged from 3.5 to 4.6 minutes. The lowest mixing time was for the treatment T_1 (control). For wheat variety 95153, the mixing time ranged from 3.4 to 4.7 minutes and the treatment T_4 got the maximum mixing time while the control minimum. For wheat variety AS-2002 the mixing time was maximum for treatment T_4 whereas the treatments T_3 and T_6 (containing 0.15% Majixmax) exhibited the same mixing time with control showing minimum value for mixing time. An earlier study reported that wheat flour with long mixing time is suitable for good quality bread production (Farooq, 1984) The results regarding the mixing time obtained in the present study are in conformity with the earlier findings of Ayaz (1998) who displayed that with increase in gluten contents, mixing time is also increased.

Peak height percentage

The peak height percentage indicates the strength and water absorption capacity of the flour. However, the mixogram is not as sensitive to absorption as the farinograph, so that the peak height percentage reflects only an approximate indication of the gluten strength.

Table 2 Effect of different treatments on the Mixographic characteristics of wheat varieties

Treat-ments	Auqab-2000	SH-2002		Inqulab-91		95153		AS-2002		
	Mixing time (Min)	Peak height (%)	Mixing time (Min)	Peak height (%)	Mixing time (Min)	Peak height (%)	Mixing time (Min)	Peak height (%)	Mixing time (Min)	Peak height (%)
T_1	3.5	56	3.5	48	3.5	49	3.4	50	2.5	47
T_2	4.6	53	4.6	52	4.3	51	4.5	51	2.6	48
T_3	5	54	4.9	52	4.5	51	4.6	52	2.7	50
T_4	5.2	55	5	54	4.6	52	4.7	52	3	48
T_5	4	54	3.7	54	3.8	53	3.6	51	2.6	48
T_6	4.2	55	3.8	52	3.9	50	3.7	52	2.7	50
T_7	4.5	53	4	53	4.1	51	4.2	52	3	47

T_1 = Control
T_2 = 0.1% EKA-300
T_3 = 0.15% EKA-300
T_4 = 0.2% EKA-300
T_5 = 0.1% Majimax
T_6 = 0.15% Majimax
T_7 = 0.2% Majimax

The mixographic peak height ranged from 53 to 56% between the different treatments of wheat variety Auqab-2000 where the maximum peak height was observed for the control sample. Wheat variety SH-2002 showed the maximum peak height for the treatments T_4 with 0.2% EKA-300 and T_5 with 0.1% Majimax, and the minimum peak height was observed for the control. The mixographic studies of wheat variety Inqualb-91 displayed that the highest peak height percentage for the treatment having 0.1% majimax (T_5). The peak height percentage ranged from 49 to 53%. For wheat variety 95153 for which the peak height ranged from 50 to 52%. The treatments T_4 and T_3 attained the maximum peak height and control got minimum peak height percentage. The results of wheat variety AS-2002 showed the highest peak height for treatment T_3. The control and T_7 with 0.2% majimax showed minimum values for peak height. Hence it can be concluded that all the wheat varieties showed a significant response to the dough improvers. However the treatment T_4 attained the maximum values as compared to control in all the wheat varieties. The results of the present study are comparable with those of Rafiq (1997) and Ayaz (1998).

Farinographic Studies

Farinograph is a sensitive device, which measures the water absorption and mixing behavior of flour during mixing. It provides informations about the absorption and the amount of water required for dough to reach a definite consistency and secondly it provides a general profile of the mixing behavior of the dough. Pyler (1988) has reported that flour from strong wheat varieties has the ability to absorb and retain larger quantities of water. The effect of different improvers with special reference to farinographic characteristics of different wheat varieties is shown in Table 3.

Water Absorption

It is obvious from the data (Table 3) that water absorption capacity ranged from 57.2 to 60.6% in different treatments of flour from different wheat varieties. The maximum water absorption occurred in the flour from wheat variety Auqab-2000 and minimum in wheat variety AS-2002. The results showed that the addition of improver affected the water absorption capacity of flour from all the wheat varieties. The maximum water absorption was observed in the flour added with 0.2% EKA-300 in all wheat varieties. The water absorption of Majimax improver was lower than the EKA-300 improver. The minimum water absorption was found in treatment T_1 (control) in all wheat varieties. The addition of ascorbic acid as improver increased water absorption capacity of all wheat varieties. Flour dough containing salts of 6-acly esters of L-ascorbic acid and D-iso ascorbic acid could tolerate more water than the flour dough containing no additive. The addition of emulsifiers (monoglyceride, diacetyl tartaric esters, and stearoyl lactylate) improved the mixing tolerance and elasticity of dough as compared to the control (Chen and Chang 2001).

Arrival Time

The arrival time is a measurement of the rate at which water is taken up by the flour. Table 3 presented the arrival time of different wheat varieties. The arrival time of wheat variety Auqab-2000 ranged from 1.95-2.40 min, SH-2002, 2.40-2.40 min, Inqulab-91, 2.10-2.55 min, 95153, 2.30-2.70 min and AS-2002, 1.70-3.00 min. The maximum arrival time was found in wheat variety 95153 (2.70 min) and the minimum arrival time was found for wheat variety AS-2002 (1.70 min).varieties. The arrival time in all varieties was decreased with the addition of improvers. The maximum arrival time was found in treatment T_1 (control) in all wheat varieties. The minimum arrival time was observed in T_4 (having 0.2% EKA-300) in all wheat varieties.

Departure Time

The departure time varied from 9.08 to 14.20 min. among different wheat varieties. The highest departure time was recorded for the flour of wheat variety Auqab-2000 14.20 min. (T_4) and lowest time was found for the flour of variety AS-2002 9.08 min (T_5). The departure time increased with increase in the level of improvers. Maximum departure time was observed in T_4 (containing 0.2% EKA) in different wheat varieties and minimum departure time was found for T_1 (control) in all wheat varieties. The results of present study substantiate findings of Farooq (1999) and Mehdi (1999).

Dough Development Time

The dough development time ranged from 4.35 to 7.10 minutes among different wheat varieties. The addition of different improvers increased the dough development time in all wheat varieties. The flour of wheat variety AS-2002 exhibited the lowest dough development time T_1 (4.35 min). The maximum dough development time was observed in the flour of wheat variety Auqab-2000 T_4 (7.10 min) followed by wheat varieties 95153 and SH-2002. The highest dough development time was obtained by treatment with 0.2% EKA-300 (T_4) and the lowest dough development time was observed in control samples. Dough development time is an indication of protein quality and strength of flour (Pyler, 1988). The results

Table 3 Effect of different treatments on the Farinographic characteristics of wheat varieties

Variety	Treatment	W.A. (%)	A.T. (min)	D.T. (min)	D.D.T (min)	D.S. (min)	M.T.I (BU)	SOD (BU)
Auqab-2000	T_1	60.1	2.25	13.50	6.15	11.25	38	80
	T_2	60.4	2.00	14.05	6.57	12.05	40	90
	T_3	60.4	2.00	14.15	7.05	12.15	42	92
	T_4	60.6	1.58	14.20	7.10	12.22	42	94
	T_5	60.2	2.20	14.10	6.50	11.50	40	80
	T_6	60.3	2.18	14.12	6.52	11.54	38	82
	T_7	60.4	2.12	14.15	6.55	12.03	36	85
SH-2002	T_1	58.4	2.32	11.35	5.52	9.03	28	75
	T_2	58.8	2.20	12.20	6.05	10.00	30	80
	T_3	59.0	2.15	12.28	6.13	10.13	30	76
	T_4	59.1	1.55	12.30	6.35	10.35	32	85
	T_5	58.6	2.30	11.40	5.55	9.10	26	79
	T_6	58.6	2.15	11.30	5.58	9.15	29	86
	T_7	58.8	2.12	11.50	6.00	9.38	31	83
Inqalab-91	T_1	59.2	2.30	12.35	5.35	10.05	20	90
	T_2	59.4	2.28	12.42	5.50	10.14	25	100
	T_3	59.5	2.27	13.00	6.15	10.33	25	75
	T_4	59.7	2.10	13.05	6.20	10.55	30	80
	T_5	59.2	2.30	12.40	5.58	10.10	28	95
	T_6	59.3	2.27	12.40	6.00	10.13	27	92
	T_7	59.5	2.25	12.45	6.05	10.20	30	90
95153	T_1	57.9	2.40	12.00	6.30	9.20	50	105
	T_2	58.0	2.20	13.00	6.58	10.40	52	90
	T_3	58.1	2.20	13.20	6.59	11.00	46	90
	T_4	58.3	2.15	13.20	7.00	11.05	48	95
	T_5	58.0	2.30	12.25	6.35	9.55	46	90
	T_6	58.2	2.28	12.26	6.40	9.58	45	90
	T_7	58.2	2.25	13.35	6.50	11.10	45	85
AS-2002	T_1	57.2	2.55	10.00	4.35	7.05	35	100
	T_2	57.4	2.15	10.00	4.40	7.45	42	90
	T_3	57.5	1.50	9.40	5.00	7.50	45	105
	T_4	57.8	1.40	9.40	5.05	8.00	55	110
	T_5	57.4	2.00	9.08	4.40	7.08	50	118
	T_6	57.4	2.00	9.10	4.44	7.10	50	120
	T_7	57.6	2.00	9.25	5.00	7.25	55	110

are comparable with the findings of Mumtaz (1997) who reported that the addition of soy flour contributed to higher dough development time. Mehdi (1999) also got similar results with the addition of improver in the wheat flour.

Dough Stability

The dough stability varied from 7.05 to 12.22 minutes among flour of different wheat varieties. An overall increasing trend of dough stability was observed with the addition of dough improving agents. The highest dough stability was exhibited by the wheat variety Auqab-2000 i.e. 12.22 minutes (T_4), while the lowest 7.05 min (T_1) reflects by wheat variety AS-2002. The dough stability is actually tolerance of the flour to over or under mixing. It is a primary index of flour quality and is one of the most significant determinations made by farinograph (Pyler 1988). Dough stability also gives a good indication of mixing tolerance of flour (Anonymous 1987). Higher dough stability of flour might be due to the addition of enzymatically active soy flour in to the flour as improvers as reported by Mumtaz (1997). The dough stability increased with the addition of improvers (Mehdi 1999).

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Assessment of nutritional value and color in whole grain wheat breads (brown breads)

Nusrat Ejaz, Alim-un-Nisa*, Shamma Firdous and Naheed Abdullah
PCSIR Laboratories Complex, Lahore-54600
***** Corresponding Author**

ABSTRACT

Eleven varieties of brown breads prepared by local bakeries were investigated for their chemical composition, nutritive values and added color. .Carbohydrates in the bread samples average from 53.16 – 58.06%. Protein contents varied from 6.5 – 8.30%, moisture ranged from 28.0 – 34.0%, crude fiber averaged 0.56 – 0.67%, ash content ranged from 2.08 – 2.3%, fat contents varied from 2.95 – 3.61%and the total food energy ranged from 271.0 – 296.67 K.cal/100 g. Among vitamins thiamine and riboflavin ranged from 0.087 – 0.160 and 0.05 – 0.073 mg/100 g respectively. The mineral contents i.e. Ca &Fe varied from 99.89 – 113.93 Ca and 1.88 – 2.26 Fe mg/100 g respectively. The breads contributed 26.0 – 33.0, 2.95 – 3.61, 212.64 – 232.16 of the total food energy from protein, fat, and available carbohydrates respectively. Only two types of breads contained added colors.

INTODUCTION

Bread has been considered as a basic food throughout the recorded history of civilized man. Bread is a good source of energy and therefore can help to solve one of the major problems of malnutrition, namely, lack of calories (Mickelsen, 1975).

Brown breads usually contain a mixture of about 75% white flour and 25% whole grain flour. It is made by mixing dough from flour, water, yeast and salt allowing the dough to rest at a temperature of about 80°F (Kent 1983). Diets rich in fibre may reduce the risk of heart disease in part by lowering the bad type of cholesterol (Whole Grain Bureau 2005). This may help to improve and maintain a healthy blood sugar level by showing the absorption of simple sugar (Whole Grain Bureau, 2005). Whole grains are higher in dietary fibre and nutrients than refined grains and play an important role in lowering cancer risk (Diane 2001).

Nutritive value of various breads in Saudi Arabia were measured chemically by proximate, minerals and vitamin analysis and it was found that wheat bread provided 45 and 61% of energy and protein requirements respectively at national level per person per day (Kanhal *et al.* 1999). It was concluded that bread from the industrial origin have high mineral contents than traditional bakery. Carbohydrate contents, dietary fibre, minerals, various amino acids and nutritive value of various breads were evaluated in Saudi Arabia (Mousa *et al.* 1992).

White bread sold in Zaria (Nigeria) was analyzed for the chemical composition and nutritive value. Samples were rich in iron, phosphorus, moisture and fat, while moderate in protein and low in calcium (Abede *et al.* 1992). Nine varieties of bread in various cities of USA were analysed and found that protein averaged between 7.6-10.4% and insoluble dietary fibre in whole wheat bread averaged 5.6% (Ranhotra *et al.* 1984).

The nutritional composition of white and brown bread samples was determined for available, calcium , carbohydrates, dietary fiber, fatty acids, riboflavin, vitamins B_6 , iodine, moisture, fat and protein etc (Sivell and Wenlock 1983).

The present study was undertaken to evaluate the nutritional value as well as additive color identification in the brown breads available in the markets of Lahore city.

MATERIALS AND METHODS

Raw materials collection

Brown breads were collected from different markets of Lahore city. Five samples of each brand were picked up to get a representative sample of each brand and stored in the refrigerator prior to analysis.

Chemical analysis

Protein, ash, fat, carbohydrate, crude fiber, iron, calcium, vitamins B_1 and B_2 were determined by the methods of the AOAC (AOAC 2005). Additive color was extracted by wool extraction method (FDA 2002).

Statistical analysis

The data obtained was statistically analyzed by using the method as described by Steel *et al.*(1997).

RESULTS AND DISCUSSION

The results of proximate analysis of different brands of breads are reported in Table 1.

Table 1: Proximate composition and energy values of brown breads

Sr. #	Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Crude Fibre (%)	Available Carbohydrate (%)	Total Energy (Kcal/100g)
1.	Dawn brown bread	30.99 c	8.20 abc	3.61 ab	2.3 a	0.64 ab	54.9 bc	284.8
2.	Shezan brown bread	33.85 ab	8.25 ab	3.2 cd	2.2 a	0.67 a	53.27 a	271.0
3.	Heidies brown bread	28.99 d	8.00 abc	3.35 bc	2.28 a	0.58 ab	57.38 a	291.67
4.	Gourmet brown bread	28.0 ce	8.30 a	3.45 ab	2.21 a	0.66 a	58.04 a	296.67
5.	Bunny's brown bread	33.08 b	8.15 abc	2.95 c	2.19 a	0.65 a	53.63 c	278.77
6.	Sun brown bread	33.50 ab	7.88 abc	3.0 d	2.09 a	0.59 ab	53.53 c	272.64
7.	Sona brown bread	32.95 b	7.75 c	3.55 a	2.18 a	0.61 ab	53.57 c	277.23
8.	Mary Gold brown bread	34.0 a	6.99 d	3.45 ab	2.25 a	0.62 ab	53.31 c	272.5
9.	Wonder brown bread	33.52 ab	7.99 abc	3.25 cd	2.08 a	0.60 b	53.16 c	273.83
10.	Shahab brown bread	33.04 b	6.5 d	3.37 abc	2.22 a	0.63 ab	54.87 b	275.21
11.	Broadway brown bread	33.20 b	7.9 bc	2.99 e	2.08 a	0.56 b	53.83 c	273.79

LSD – 0.05 Protein – highly significant
Fat - highly significant Crude fiber. - significant level
Available carbohydrate - highly significant Significant difference (P=0.05) as determined by DMR test

Protien content ranged from 6.5 – 8.3%.Protein content of Gourmet brown breads was high as compared to the other bread types. Actually the protein relates to the quality of the flour used. This gives breads correct strength and texture (Kent 1983). Similarly the fat content of Dawn brown breads was much higher (3.61%) than the other bread types. This is due to the addition of fat to the bread baking. Sona breads also had relatively high fat content. It was found that considerable amount of fats are used in baking, probably to make breads tender and easy to slice loaves.The ash content of bread samples varied from 2.08% - 2.30%. Dawn breads had the highest ash contents (Minerals) and Heidies breads had relatively high ash content 2.28%. It is well known that ash content of the breads depends on the inclusion of other ingredients such as non fat dry milk and salt in bread formula (Mousa *et al.*1992). It was observed that Shezan breads had the highest fibre contents (0.67%) followed by Gourmet breads (0.66%), while other breads varied from 0.56% to 0.65%. Brown bread is actually called as brown bread for its fibre content and due to this reason they are becoming famous as aid to fight against heart diseases.

Table 2: Vitamins and mineral contents of brown bread samples

Sr. No	Samples	Thiamine mg/100 g	Riboflavin mg/100 g	Calcium mg/100 g	Iron mg/100 g
1	Dawn brown bread	0.156 a	0.067 abc	113.93 a	1.99 cd
2	Shezan brown bread	0.120 c	0.072 ab	109.09 d	2.10 bcd
3	Heidies brown bread	0.110 cd	0.069 abc	112.63 b	2.22 a
4	Gourmet brown bread	0.160 a	0.071 ab	106.99 f	2.20 a
5	Bunny's brown bread	0.14 ab	0.073 a	107.95 c	2.26 a
6	Sun brown bread	0.130 b	0.05 c	102.88 h	2.09 bc
7	Sona brown bread	0.089 c	0.063 c	110.01 c	2.23 a
8	Mary Gold brown bread	0.087 c	0.068 abc	105.07 g	1.98 dc
9	Wonder brown bread	0.11 cd	0.070 abc	100.87 i	2.00 dc
10	Shahab brown bread	0.088 e	0.059 abc	99.89 j	2.11 b
11	Broadway brown bread	0.094 dc	0.060 c	100.08 j	1.88 c

LSD 0.05 Thiamine - highly significant
Riboflavin - highly significant
Calcium - highly significant Iron- highly significant
Significant difference (P=0.05) as determined by DMR test

In a study conducted over 65,173 women it was concluded that a diet high in dietary fiber – especially fiber found in whole-grain cereals was protective against developing diabetes. Women who ate low fiber diets and more highly refined foods (white bread, white rice, cola beverages, etc.) were 2.5 times more likely to develop diabetes than women who ate a high fiber (25+ grams per day) diet (Liu *et al.* 1999).Moisture of the bread samples ranged from 28.0 – 34.0%. Available carbohydrate contents ranged from 53.16% for Wonder brown bread to 58.04% for Gourmet brown breads. In this way

carbohydrates present in brown bread samples contributed 212.64 to 232.16 kcal/100g food energy. Total food energy produced by brown bread samples varied from 271.0 calories for Shezan brown bread to 296 calories for Gourmet brown bread.

The mineral composition of the bread samples showed (Table-2) that Dawn brown breads and Bunny's brown breads had the high calcium 113.93 mg/100 g and 2.26 mg/100 g of Fe respectively. Maximum quantity of vitamin B1 was detected in Gourmet brown bread while B2 was in bunnys brown breads.

Significant difference (P=0.05) as determined by DMR test. Whole grains meal fight against obesity, many studies linked them to lower body weight and better fat distribution. A study showed that a dose response for every 1.4 ounces per day, people gained one pound less over several years (Dunkl 2004).

Table-3 shows the results of additive color detection . It was found that no added color was detected in 9 brands of breads while two brands showed the presence of chocolate brown color.

Table 3: Color estimation in brown breads

Samples	Colors	Remarks
Dawn brown bread	Nil	No added colour
Shezan brown bread	Nil	No added colour
Heidies brown bread	Nil	No added colour
Gourmet brown bread	Nil	No added colour
Bunny's brown bread	Nil	No added colour
Sun brown bread	Nil	No added colour
Sona brown bread	Nil	No added colour
Mary Gold brown bread	Nil	No added colour
Wonder brown bread		
Shahab brown bread	Little amount of chocolate	Little amount of chocolate brown
Broadway brown bread	Little amount of chocolate	Little amount of chocolate brown

Nutritive value

The bread contributed 26 - 33.0 Kcal/100g, 26.55 - 32.49Kcal/100g and 212.64 – 232.16Kcal/100g of the total food energy from protein, fat and available carbohydrate respectively. It was found that Gourmet brown breads were relatively good in protein 8.3% and vitamins as compared to the other bread types but they were relatively low in mineral contents (Table-2). The high crude fibre contents of Shezan and Dawn brown breads (Table-1) and their low caloric intake make them nutritionally important when planning for low calorie and high fibre diets.

CONCLUSIONS

From this limited study it was concluded that brown breads prepared by m/s Shezan International contained high fiber contents and low caloric values, which made them nutritionally important than the other locally available bread types. The addition of synthetic colors(chocolate brown) in two brands was detected. Although it is a permitted color according the pure food rules 1965, yet the addition of synthetic colors should be discouraged as they cause different hazardous effect on human health (Saadany and Nahrung 1991).

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Effect of maltogenic amylase on the shelf life of bread

Ijaz Ahmad, Naveed Ahmad, Tasneem Kausar and Muhammad Ashraf
Food & Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore

ABSTRACT

Significant differences were observed in moisture, fat, protein, NFE and farinographic characteristics. However ash and crude fiber of different commercial flours showed non-significant differences. The results showed that commercial flour contained moisture 9.95-11.58%, ash 0.52-0.68%, fat 0.94-1.51%, protein 10.32-11.58%, fiber 0.40-0.60% and nitrogen free extract (NFE) 74.62-77.74%. Teen-Sher, Sufi, Super and A-one exhibited higher protein contents than other commercial flours. The water absorption ranged from 55.7-59.7%, development time 1.4-2.0 minutes and stability 8.8-12.6 minutes. Farinograph quality number ranged from 18-30 which shows that these are weak flours. Storage intervals and treatments showed highly significant differences for all the internal and external characteristics of bread. However, interaction was found to be non-significant. Scores for all sensory characteristics of breads decreased gradually during storage for 144 hours in all the treatments. Maximum scores for crust color, evenness of bake, taste and texture was obtained by T18. However T5, T6, T1, T4 and T3 remained almost at the lower level in all the characteristics of bread. T18, T17, T16 and T12 got maximum scores and remained at the top for evenness of bake, and taste of bread. The results for texture of bread indicated that T18 got highest score followed by T16, T14, T12 and T11. The microbiological studies showed that T14 and T16 were more effective against mold growth. In the laboratory experiments T12 (calcium propionate 0.3%, calcium acetate 0.2% and maltogenic amylase 60ppm) gave better results. Trials conducted in Bunny's Ltd indicated that T16 (containing dextrose 6.0%, CP 3.0%, CA 2.0%, acetic acid (1:10) 0.275%, Bread improver B 1.375% and maltogenic amylase 90 ppm) and T18 (containing dextrose 2%, Bread improver B 1.375% and maltogenic amylase 90 ppm) were found to be the most suitable to enhance the shelf life of bread.
Keywords:

INTRODUCTION

Bread is perishable commodity having very short life and spoils within a short period. Unfortunately, the bread being marketed by some of bakery plants lacks in quality and has short shelf life. Hence a substantial loss is borne by the producers from unsold bread. Since, bread is an important part of our daily diet; therefore, ways and means should be explored to improve the quality and shelf life (Rehman and Ahmad 2003).

Bread is one of the most important staple food in the world and can be spoiled by many molds of which *Penicillium* species are most common. However, the dominant spoilage flora varies with the type of bread and the storage temperature (Legan 1993). Bacteria, mold and yeast play a key role in bread spoilage. Mold is heat sensitive and therefore destroyed during the normal baking process. Bread contains moisture and nutritive substances and provides a good nutrient medium. Even in clean and hygienic bakery and despite all efforts and care mold and rope still form. That's because mold spores, the 'seeds' of molds are present in air and they naturally settle on dough flour prior to baking. Generally, most of them are killed in the baking ovens. But some additional spores settle on bread during cooling, slicing and wrapping. They can also be reintroduced to the product by the equipments, employees or air. These spores are the source of subsequent mold growth. Therefore good sanitation practices are important to minimize the reintroduction of microbial organisms.

Dextrose, glycerol, invert sugar and high fructose corn syrup have high humectancy values. Watson (1985) described that sucrose esters, made from common table sugar are non-toxic, tasteless, odorless food additive having good emulsifying and dispersing abilities. When used in most foods they soften the crumb and improve volume and shelf life. The present study was undertaken to explore the effect of different chemical preservatives, additives, humectants and maltogenic amylase etc to extend the shelf life of bread. The findings of this research study will not only be useful to the industrialists but also to the consumers in terms of health and safety.

MATERIALS AND METHODS

Wheat flour, yeast, sugar, salt, cooking oil, preservatives and bread improver A etc. were purchased from the local market. Maltogenic amylase was imported from Denmark. Bread improver B was prepared in laboratory. The commercial flour samples of different brands were also collected from different baking plants and local market.

Flour was analyzed for moisture, ash, protein, fat, fiber and nitrogen free extract (NFE) according to their respective methods as given in AACC (2000). Farinographic characteristics of different commercial wheat flour were determined with Brabender Farinograph-E equipped with 300 g bowl capacity according to the instructions of AACC (2000).

Breads were prepared according to straight dough method and sponge & dough method as described in AACC (2000). Recipes are given in Table 1 and the treatments in Table 2. Sensory evaluation was carried out for external characteristics and internal characteristics at intervals of 3, 24, 48, 72, 96, 120, 144 hours after baking. Scoring was done according to 9-Point-Hedonic Scale.

RESULTS AND DISCUSSIONS

Chemical composition of commercial wheat flour

Significant differences were observed in moisture, fat, protein and NFE. However ash and crude fiber of different commercial flours showed non-significant differences. The results showed that commercial flour contained moisture 9.95-11.58%, ash 0.52-0.68%, fat 0.94-1.51%, protein 10.32-11.58%, fiber 0.40-0.60% and nitrogen free extract (NFE) 74.62-77.74% (Table 3). These results are comparable with those of Ahmed (2002).

Table 1 Recipes of bread

INGREDIENTS	Straight dough method	Sponge and dough method
Flour	100 g	100 g
Sugar	5 g	6 g
Dextrose	-	6 g
Active Dry Yeast	1 g	1 g
Oil	4g	2.5 g
Salt	1.75 g	2.0 g
Bread improver A	0.75 g	0.75 g
Bread improver B	-	1.375 g
Water	According to water absorption capacity	According to water absorption capacity

Highest moisture contents (11.58%) were found in Sufi flour followed by Crown (10.70%) flour and Top flour (10.30%). However the differences between the later two were non-significant. Highest fat contents were found in A-one (1.51%). All other flours tested in this study showed non-significant differences in fat contents. Highest protein contents were observed in Teen Sher (11.58%) followed by Sufi (11.50%) and Super (11.45%). However, the differences between these were non-significant. According to Halverson and Zeleny (1988) flours containing at least 11.0% protein is usually preferred for the production of yeast-leavened bread. These all the tested flours in the study fall approximately at the lower level.

Table 2 Treatments used in the preparation of bread

Treat-ments	Calcium propio-nate %	Calcium acetate %	Ascorbic acid %	Acetic acid % (1:10)	CMC %	Carra geenan gum %	Maltogenic Amylase
Straight dough method							
T_1	-	-	-	-	-	-	-
T_2	0.3	0.2	-	-	-	-	-
T_3	0.3	0.2	0.3	-	-	-	-
T_4	0.35	-	-	0.3	-	-	-
T_5	0.35	-	0.4	0.3	1	0.1	
T_6	0.35	-	0.5	0.3	0.5	0.1	-
T_7	0.4	-	-	0.275	-	-	-
T_8	0.4	0.2	-	0.275	-	-	-
Sponge and dough method							
T_9	0.4	-	-	0.275	-	-	-
T_{10}	0.4	-	-	0.275	-	-	60 ppm
T_{11}	0.4	0.2	-	0.275	-	-	60 ppm
T_{12}	0.3	0.2	-	0.275	-	-	60 ppm
Trials in Bunny's (1.66% fresh yeast)							
T_{13}	0.4	0.2	-	0.275	-	-	60 ppm
T_{14}	0.4	0.2	-	0.275	-	-	90 ppm
T_{15}	0.3	0.2	-	0.275	-	-	60 ppm
T_{16}	0.3	0.2	-	0.275	-	-	90 ppm
T_{17}	Bunny's recipe (Sugar 2%, Dextrose 2%)						60 ppm
T_{18}	Bunny's recipe (Sugar 2%, Dextrose 2%)						90 ppm

Note: In T13 & T14, 5.5% sugar and 5.5% dextrose was used.

Table 3 Proximate composition of different commercial wheat flours

Sr. No.	Flour	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	NFF (%)
1	Asia	10.01 c	0.58	0.95 b	10.32 c	0.40	77.74'a
2	Crown	10.70 b	0.52	0.94 b	10.58 c	0.45	76.81 abc
3	Super	10.00 c	0.62	1.00 b	11.45 a	0.54	76.39 bc
4	Supreme	10.02 c	0.56	1.00 b	10.33 c	0.50	77.59 ab
5	Sufi	11.58 a	0.68	1.02 b	11.50 a	0.60	74.62 c
6	Teen Sher	9.95 c	0.67	0.95 b	11.58 a	0.48	76.37 bc
7	Top	10.30 bc	0.63	1.01 b	10.40 c	0.48	77.18 ab
8	A-one	10.00 c	0.58	1.51 a	11.08 b	0.51	76.32 bc
LSD values		0.4973	-	0.3156	0.2910	-	1.150

Farinographic characteristics

Farinographic characteristics showed significant differences between different types of flours. Water absorption ranged from 55.7-59.7%, development time 1.4-2.0 minutes and stability 8.8-12.6 minutes (Table 4). These results are in accordance with earlier findings reported by Iqbal (2001).

Highest water absorption was observed in Crown flour followed by Top, Supreme and Teen Sher. However the differences between later three were non-significant. Teen Sher, Sufi, Asia and A-one showed higher development time. The differences between these were non-significant. Stability of Super was highest followed by Supreme, Teen Sher, A-one, Sufi, Asia, Top and Crown. However the differences between all these were significant. Thus the mixing requirements of these flours were different. Teen- Sher possessed greater farinograph quality number (30) than other commercial flours and exhibited non-significant differences with Asia, Supreme and Super which shows that these commercial wheat flours are weak flours.

Table 4 Farinographic characteristics of different commercial wheat flours

Sr. No.	Flour	Water absorption (%)	Development Time (Min)	Stability (Min)	Farinographic quality number
1	Asia	55.7 e	1.9 ab	9.7 f	28 a
2	Crown	59.7 a	1.4 d	8.8 h	18 b
3	Super	57.5 c	1.7 bc	12.6 a	26 a
4	Supreme	58.1 b	1.5 cd	12.0 b	24 a
5	Sufi	57.2 cd	1.9 ab	10 e	19 b
6	Teen Sher	57.6 bc	2.0a	11.8 c	30 a
7	Top	58.1 b	1.7 bc	9.2 g	18 b
8	A-One	56.8 d	1.8 abc	10.3 d	20 b
LSD values		0.4536	0.2376	0.1422	4.892

Sensory evaluation of bread

The bread was prepared from Teen Sher flour. T1 (control) to T12 were performed in laboratory. On the basis of laboratory experiments T11 and T12 were selected for further trials in Bunny's limited. Different trials were conducted in Bunny's Ltd.

Statistical analysis indicated that storage intervals and treatments showed highly significant differences for all the internal and external characteristics of bread. However, interaction was found to be non-significant. Scores for all sensory characteristics of bread decreased gradually during storage for 144 hours in all the treatments. Non-significant differences were observed between the storage of 3 hrs and 24 hrs. However, at the storage of 144 hrs all the treatments showed minimum scores for all the characteristics of bread (Table 5).

Maximum scores for color of crust, evenness of bake, taste and texture was obtained by T18. T5 and T6 got lower scores for all the sensory characteristics. This may be due to the reaction of gums with other preservatives. However T1, T4 and T3 also remained almost at the lower level in all the characteristics of bread.

Table 5 Effect of storage on different characteristics of breads

Treat-ment	Vol-ume	Crust color	Evenness of bake	Crumb color	Aroma	Taste	Texture
3 Hrs.	8	6.66 a	6.68 a	7.11 a	6.90 a	7.08 a	7.33 a
24 Hrs.	8	6.69 a	6.69 a	7.08 a	6.82 ab	6.94 a	7.19 a
48 Hrs	7	6.66 a	6.62 a	6.94 ab	6.55 b	6.50 b	6.69 b
72 Hrs.	7	6.53 a	6.15 b	6.83 b	6.15 c	6.14 c	6.37 c
96 Hrs.	7	6.30 b	6.11 c	6.33 c	5.72 d	5.8 c	5.97 d
120 Hrs.	7	6.02 c	5.94 d	6.19 c	5.14 e	5.00 d	5.40 e
144 Hrs.	7	5.75 d	5.66 e	5.74 d	4.55 f	4.15 e	4.86 f

The results for volume of bread indicated that T11, T16, T18 and T12 got higher mean scores than other treatments (Table 6).

Color of crust is very important parameter. Golden brown color of bread crust is liked by the consumers. T18, T17 and T16 got higher scores (7.0) for color of crust than other treatments (Table 6). T18, T17, T16 and T12 got maximum scores and remained at the top for evenness of bake, and taste of bread. Highest score for crumb color was exhibited by T12. T14 showed highest scores for aroma followed by T18, T12, T16 and T11 (Table 6). Degradation in aroma and taste may be due to staling of bread which transforms the rich aroma and flavor of the fresh crumb to a bland or off flavor (Setser 1996). In the laboratory experiments T12 (calcium propionate 0.3%, calcium acetate 0.2% and maltogenic amylase 60ppm) followed by T11 gave better results. This may be due to the addition of maltogenic amylase, bread improver B and suitable amount of preservative. These results are supported by the previous results of Gil *et al* (1999).To study the economical aspect and effect of using maltogenic amylase in commercial bread making, T17 and T18 were conducted in which Bunny's commercial recipe was used. The results indicated that dextrose may also be reduced for commercial production as observed in case of T17 & T18 (dextrose 2.0%). It was observed that 60ppm maltogenic amylase was sufficient for laboratory experiments (T12), while 90ppm maltogenic amylase gave good results in the industrial trials (T16 & T18). It may be due to

prevailing conditions in factory. T16 may be more economical because it contains less calcium propionate (0.3%).

Table 6 Effect of treatments on different characteristics of breads

Treatments	Volume	Crust Color	Evenness of bake	Crumb color	Aroma	Taste	Texture
T_1	6.21 c	5.78 b	6.28 ab	5.92 b	5.42 ab	4.78 d	5.57 cd
T_2	7.24 a	6.71 a	6.43 ab	6.78 a	5.57 ab	5.35 bcd	5.57 cd
T_3	6.71 abc	6.35 ab	6.01 b	6.85 a	5.85 ab	5.07 cd	5.71 bc
T_4	6.28 bc	6.28 ab	6.50 ab	6.42 ab	5.00 b	5.07 cd	5.5 cd
T_5	3.50 e	3.85 d	3.78 d	6.21 ab	4.85 b	4.97 cd	4.28 e
T_6	5.33 d	4.71 c	4.71 c	6.35 ab	4.92 b	4.81 d	4.71 de
T_7	7.00 abc	6.34 ab	6.50 ab	6.56 ab	6.07 a	6.07 abcd	6.21 abc
T_8	7.21 a	6.70 a	6.57 ab	6.64 ab	6.12 a	5.92 abcd	6.28 abc
T_9	7.14 a	6.64 a	6.50ab	6.49 ab	6.07 a	6.11 abcd	6.45 abc
T_{10}	7.14 a	6.57 a	6.58 ab	6.57 ab	6.43 a	6.07 abcd	6.64 ab
T_{11}	7.28 a	6.71 a	6.49 ab	6.71 ab	6.43 a	6.35 abc	6.92 a
T_{12}	7.27 a	6.92 a	6.71 ab	6.92 a	6.50 a	6.63 ab	7.00 a
T_{13}	6.85 abc	6.63 a	6.42 ab	6.57 ab	6.14 a	6.21 abcd	6.64 ab
T_{14}	7.00 ab	6.78 a	6.64 ab	6.85 a	6.57 a	6.57 ab	7.07 a
T_{15}	6.71 abc	6.71 a	6.64 ab	6.71 ab	6.28 a	6.28 abcd	6.85 a
T_{16}	7.28 a	7.00 a	6.85 a	6.85 a	6.50 a	6.71 ab	7.19 a
T_{17}	7.14 a	7.00 a	6.92 a	6.57 ab	6.24 a	6.92 a	6.85 a
T_{18}	7.28 a	7.00 a	6.92 a	6.85 a	6.57 a	7.07 a	7.21 a

Softness and shelf life of bread

Softness of bread is the most important factor from consumer point of view. Consumers press the bread with their fingers to check its softness to have an idea about bread freshness. If the bread is soft the consumer thinks that it is fresh otherwise the consumer rejects the bread. The results of texture of bread (Table 6) indicated that T11 & T12 (containing 60ppm maltogenic amylase) remained soft even at 144hrs. T16 and T18 retained enough softness up to 5 days (good scores = 7), so that it looks like fresh.

Table 7 Effect of treatment on microbiological analysis

Treatments	72 Hr.	96 Hr.	120 Hr.	144 Hr.
T_1	6.2×10^4	-	-	-
T_2	8.1×10^2	-	-	-
T_9	-	5.0×10^2	-	7.3×10^6
T_{14}	-	-	2×10^2	6.4×10^4
T_{16}	-	-	3×10^2	6.5×10^4

Softness of T18 was more than the respective normal bread of Bunny's limited. This confirms the role of maltogenic amylase, dextrose and bread improver B as emulsifier/ moisture retention and hence retardation of staling and increasing shelf life of bread.The microbiological studies of some selected treatments at different time intervals indicated that T1 and T2 showed yeast and mold growth 6.2×10^4 and 8.0×10^2, respectively after 72 hrs. T9 showed 5×10^2 after 96 hrs and 7.3×10^6 at 7th day. T14 showed 2×10^2 at 120 hrs (Table 7). T14 was proved more effective against mold growth followed by T16.

CONCLUSIONS

The bread prepared by the addition of dextrose 2.0-5.5%, CP 3.0%, CA 2.0%, acetic acid (1:10) 0.275%, Bread improver B 1.375% and maltogenic amylase 90 ppm was found to be most suitable to enhance/improve the softness and shelf life of bread. However, the dose of dextrose may be reduced and maltogenic amylase may be adjusted 60 to 90 ppm according to the recipe used.

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Microbial assessment of different fruit juices vended near roadside of Lahore city

Muhammad Nadeem, Qurat-ul-Ain Syed, Shahjahan Baig and Robina Nelofer
BFRC, PCSIR Laboratories Complex, Lahore-54600

ABSTRACT

Eighty samples of fresh squeezed juices of apples, carrots, mussambi, Kinnow, grapefruit, orange, pomegranate and mixed fruits, sold by street vendors at different localities of Lahore city were analyzed for their microbiological quality. Results showed that all the juice samples were contaminated with coliforms along with heavy bacterial load, yeast and mold count. Faecal coliforms and *E. coli* contamination were detected in apple, carrot, pomegranate and mixed fruit juice samples. *Staphylococcus aureus* (S. aureus) were only present in carrot juices. The nutritional values of juices were calculated on the basis of 100g of fresh fruits. Mixed fruit juice was found to be the best source of energy whereas maximum mineral contents were detected in carrot juice. It is concluded that fresh fruit juices should be encouraged on nutritional ground but steps must be taken to improve their microbial quality.

Keywords: Microbial quality, fruit juices, nutritional values.

INTRODUCTION

Fresh squeezed or pressed juices made from fruits and vegetables have high consumer preferences both in terms of taste and health effect through out the world. In Pakistan, especially in big cities, like Lahore people of all income and age groups consumed fresh squeezed juices around the year. These fruit juices are considered to be rich sources of vitamins and minerals but bearing in minds, their methods of washing and extraction.

On more of the social occasions, fresh fruit juices are the first choice among the beverages.However in current past, many juices have been shown to be potentional sources of bacterial pathogen, notably *E. coli* and salmonella (Ryu *et al.* 1998, Uljas *et al.* 1998, Zhuang *et al.* 1995). As juices are the only component closely examined by the consumers, it would be comparatively easier to make use of substandard fruits. Infection with molds could easily pass unnoticed and yet entry of the mycotoxins into the juices would represent a long-term risk that could not be ignored (Abrunhosa et al. 1991). The total colony account may exceed 1.0x 10^5 cfu/mL even for washed fruits (Splitttstoessor 1979, Harrigan 1998). Many of these micro- organisms are harmless but this confidence does not mean that *E. coli* may not be present at all (Bryan 1977). Fresh fruits and vegetable juices are an emerging cause of food borne illness (Parish 1997).In view of the threat posed by the bacterial pathogens in juices and the flourishing demand of such street vended juices, the present work was undertaken to assess the microbial quality of fresh juices as fresh pressed or squeezed juices, sold by street vendors.

MATERIALS AND METHODS

Eighty samples of different fruit juices (apples, carrots, mussambi, kinnow, orange juices, grape fruits, and mixed fruits) were collected in sterile container held at 4.0°C from different areas of Lahore city and were analyzed within two hours of collection.

Microbiological Analysis

AOAC (2002) methods of microbiological analysis were used for the microbial assessment of fresh fruits juices.

Total Plate Count

Standard plate count agar (Oxoid) was used to determine total bacterial count/mL of fresh fruit juices. 1 mL of each dilution was transferred to a sterilized Petri plate. Then 15mL of sterilized molten (40-45°C) standard plate count agar was poured in those plates. Medium was mixed well with sample by gently rotating the plate clockwise and anti clock wise. Then agar was allowed to settle down at room temperature and the colonies appear after 48 hours was counted.

Yeast and Mold Count

Potato dextrose agar (Oxoid) supplement with ug/mL chlortetracycline was added for yeast and mold count/mL. 1 mL of each dilution was taken and 15mL of potato dextrose agar was added in each Petri

plate. After mixing, these were allowed to settle and incubate at 25 °C for 5 days.

Total Coliforms

10 mL of Lauryl tryptose broth (Oxoid) was taken in test tubes with inverted Durham tubes and autoclaved. One mL of 1st three dilutions was added into three test tubes separately. These tubes were incubated at 37°C for 48 hrs for presumptive test. Tubes with gas production were used for confirmatory test.

Confirmatory Test

A loop full from positive presumptive tubes were transferred into brilliant green bile broth (Oxoid) having 10 mL volume with Durham tubes. These tubes were incubated 35°C for 48 hrs and the tubes with gas production were considered positive for coli forms. Total coli forms were calculated from MPN tables (AOAC 2002).

E. coli

EMB agar was used for *E. coli* detection. A loop full from positive tubes was streaked on EMB agar (Oxoid) plates and incubated at 35°C for 18-24 hours. Positive plates contained typical colonies with green metallic shine.

Faecal Coliforms

A loop full was added into the sterilized EC medium having Durham tubes from LT positive tubes. These tubes were incubated at 45.5°C for 48 hrs and examined for gas production.

Staphylococcus aureus

0.3 mL and 0.4 mL of each dilution were spread over Baired- Parker agar plate and these plates were incubated at 35°C for 48 hours. Black colonies surrounded by clear zone were added into 0.3 mL brain heart infusion broth (BHI) and placed at 35 °C for 18-24 hours. After that 0.5 mL reconstituted plasma with EDTA were added into BHI culture and incubated at 35°C and observed over 6 hours for positive coagulase test.

Nutritional Effect

Nutritional value and mineral content were estimated according to the methods described by Ronald and Ronald (1991).

RESULTS AND DISCUSSION

Fresh fruits juices are good source of minerals and vitamins so that they could be recommended ahead of drinks like coca cola and Pepsi cola. However, there are major risks of microbial contaminations during preparation because they are not subjected to any preservative treatment prior to being drunk which was shown by bacterial load in all the samples. 10 samples of each type of juices such as apples, carrots, mussambi, kinnow, orange, pomegranate, and mixed fruit juices were subjected for microbial examination. The results are shown in table-1 and means are given for each juice along with counts found.The data presented in Table-1 show heavy bacterial load in all types of juice samples.

Table 1: Microbial quality of fresh fruit juices vended near roadside of lahore city

Juices of Fruits	Total Bacterial cont / mL	Yeast & Mold count/ mL	Total Coliforms	Faecal Coliforms	*E. coli*	*Staph. aureus*
Apple	$3.1x10^4$ - $5.3x10^6$ $3.3x10^5$	$6.1x10^3$ - $3.2x10^4$ $1.2x10^4$	N.D - $1.3x10^5$ $8.2x10^2$	++	+	–
Carrot	$8.3x10^5$ - $2.2x10^8$ $2.6x10^5$	$5.0x10^2$ - $8.1x10^4$ $6.3x10^4$	$5.2x10^1$ - $8.7x10^3$ $6.6x10^5$	++	++	+
Mussambi	$7.9x10^4$ - $8.3x10^6$ $5.6x10^5$	$8.7x10^3$ - $2.4x10^4$ $4.7x10^4$	N.D - $3.8x10^5$ $2.3x10^5$	–	–	–
Kinnow	$9.1x10^4$ - $8.2x10^6$ $5.5x10^5$	$8.7x10^3$ - $5.4x10^4$ $4.7x10^4$	N.D - $1.4x10^3$ $1.5x10^2$	–	–	–
Grape Fruit	$9.4x10^4$ - $2.7x10^6$ $7.7x10^5$	$4.4x10^3$ - $8.1x10^4$ $5.7x10^4$	N.D - $2.5x10^3$ $1.8x10^2$	+	–	–
Orange juices	$8.7x10^4$ - $5.4x10^6$ $4.7x10^4$	$1.7x10^3$ - $4.5x10^4$ $2.6x10^4$	N.D - $3.8x10^4$ $5.3x10^3$	+	–	–
Pomegranate	$1.2x10^5$ - $3.4x10^6$ $4.8x10^5$	$4.4x10^5$ - $1.2x10^7$ $5.2x10^6$	$5.1x10^1$ - $1.4x10^4$ $9.7x10^3$	++	+	–
Mixed fruits	$1.8x10^6$ - $2.1x10^8$ $1.2x10^7$	$7.5x10^5$ - $1.2x10^7$ $6.7x10^6$	$8.2x10^1$ - $3.4x10^4$ $7.1x10^3$	++	+	–

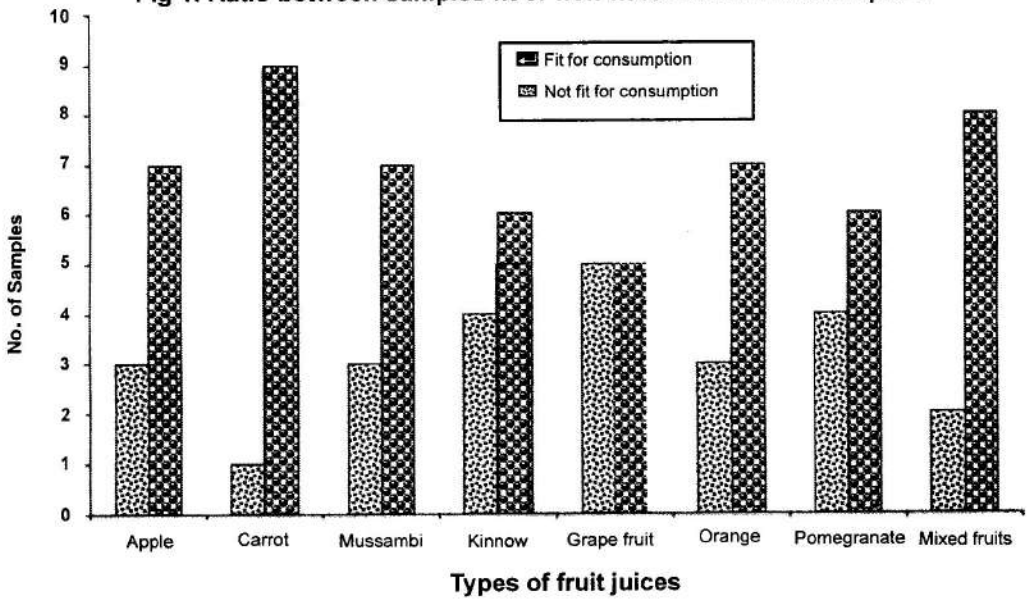
\+ : Detected in less than five samples
++ : Detected in More than Five samples
– : Not detected

Maximum total viable count (TVC) was found in carrot samples ($8.3x10^6$ -$2.2x10^8$) with mean value ($2.6x10^7$) followed by mixed fruit juices($1.8x10^6$ -$2.1x10^8$) with mean value $1.2x10^7$ (cfu/mL). Similar findings were reported by Al-Jedah and Robinson (2002). Carrots are transported from fields to central market and finally to retailers or street vendors who use them for juicing. Entry of microorganisms might be take place from point of field to point of retailers and finally due

to improper washing of carrots or with contaminated water. However, orange juice exhibited lower bacterial load ($3.7x\ 10^4$ -$5.4x10^6$ cfu/mL) with mean value $4.7x\ 10^4$ cfu/mL. Our findings in regard of orange juices are in accordance with the work reported by Babajide *et al.* (2002). The lesser bacterial load in orange juices might be due to removing of peel before extraction.

Yeast and mold count is significantly higher in mixed fruit juices ($8.2x10^2$ -$3.4x10^5$) with mean value $7.1x10^4$ as compared to the other fruit juices. It means that fruit juices carry a rich micoflora of yeast and molds. It is not surprising because the count in juices were often high which is above the Gulf standard as shown in table-4. However, it is revealed that juices of this type are consumed immediately after preparation and

Fig 1. Ratio between samples fit or non-fit for human consumption



Data summarized in table-1 indicates that maximum numbers of total Coliforms ($8.2x10^2$- $3.4x\ 10^5$) with mean value $7.1x10^4$ were found in mixed fruit juice samples followed by apple juice which have a mean value of $6.2x10^4$. Similar results were reported by Sandeep *et al.* (2003). The presence of Coliforms may also be due to the contamination of water or careless handling of fruit juices during the process of pressing.

Out of eight different types of fruit juices, five types show positive indication of faecal Coliforms as shown in Fig-1. Maximum number of samples was found to be contaminated in carrot juices. S. *aureus* was also observed in carrots juices which indicate the maximum contamination through handling. The possibility of contamination of carrot juices through improperly treated irrigation water can not be ruled out as investigated by Sandeep *et al.* (2003). that few yeast and mold are pathogenic to humans so that actual health risk should be minimized from this group. Nevertheless, the presence of yeast and molds in juices indicate that presence of handling fruits and extraction of juices leave a lot to be desired with respect to hygiene. Obgadu (1980) isolated toxin producing mold from fruit juices which indicate the undesirable health risk from juices.

As for minerals and energy are concerned, the carrot juices are useful source of dietary calcium and sodium (Table 3) while mixed fruit juices provide more energy to the body as compared to other juices (Table 2). It shows that fruit juices play vital role to provide minerals to our body in natural way.

Our results clearly indicate the poor hygienic quality of fruit juices and consumers are placed at a risk of contracting food borne infections. The practice of

consuming fresh fruit juices can not be stopped on nutritional ground nor did the street vendor prohibit such items from Pakistani sort community. Government agency must adopt measures to educate the vendors about the food safety and hygienic practice about fresh fruit juices.

Table 2: Potential nutritional value of fresh fruits based on the 100 gm of whole fruits

Juices of Fruits	Protein	Fat	Carbo-hydrates	Fiber	Energy (kcal/g)
Apple	0.39± 0.021	0.10± 0.010	11.2± 0.590	1.81± 0.142	44.10± 1.52
Carrot	0.58± 0.031	0.15± 0.012	6.8± 0.671	2.50± 0.135	29.19± 1.34
Mussambi	0.54± 0.022	0.11± 0.020	9.50± 0.533	2.45± 0.201	37.78± 2.03
Kinnow	0.65± 0.024	0.10± 0.015	11.38± 0.741	2.15±0 .152	45.27± 1.87
Grape Fruit	0.60± 0.021	0.08± 0.011	10.29± 0.622	1.98± 0.229	38.13± 2.09
Orange juices	0.72± 0.030	0.09± 0.003	10.80± 0.750	2.13± 0.215	41.23± 1.72
Pomegranate	0.18± 0.010	0.05± 0.001	11.80± 0.611	0.10± 0.032	45.45± 1.11
Mixed fruits	0.68± 0.023	0.13± 0.021	12.25± 0.824	1.55± 0.175	59.64± 2.35

Table 3: Potential minerals value of fresh fruits 100g of whole fruits

Juices of Fruits	Sodium (Na)	Calcium (Ca)	Iron (Fe)
Apple	2.0±0.16	4.0±0.28	0.3±0.01
Carrot	95±2.54	48.0±1.57	0.6±0.013
Mussambi	3.5±0.25	16.0±0.93	0.25±0.022
Kinnow	2.0±0.09	15±0.85	0.4±0.015
Grapefruit	2.5±0.15	17.0±0.59	0.3±0.017
Orange	3.0±0.25	41±1.07	0.32±0.020
Pomegranate	1.30±0.15	13.0±0.61	0.50±0.021

Table 4: The recommended microbial standards for any fruit juices sold in Gulf region, in all figures per mL juices are consumed.

	Total Colony Count	Yeast and Moulds	Coliforms
Maximum count anticipated	$5.0x10^3$	100	10
Maximum count permitted	$1.0x\ 10^4$	$1.0x10^3$	100

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Shelf stability of dhakki dates as influenced by water activity and headspace atmosphere

Shahzada A. Saleem[1] , Ahmad K. Baloch*, Malik Muhammad Hashim[1], Ambreen A. Saddozai[2], M. Niamatullah[3] and Waqar A. Baloch[4]

*Department of Food Science & Technology, Gomal University, Dera Ismail Khan, Pakistan.
1. Food Technology Section, Agriculture Research Institute, Dera Ismail Khan, Pakistan.
Email: sasdikpk@yahoo.com, 2. Food Microbiology Lab. NARC, Islamabad, Pakistan.
3. Gomal College of Veterinary Sciences, Gomal University, Dera Ismail Khan, Pakistan.
4. Department of Food and Environmental Chemistry, University of Glasgow, U.K.

ABSTRACT

Date palm (*Phoenix dactylifera* L.) is playing a vital role by providing food and shelter to millions. A prominent local cultivar "Dhakki" of Dera Ismail Khan is economically far more important for having jumbo size and weight with small stone, fine texture and delicious taste. However, being a late variety it is confronted with enormous environmental stresses. Stormy monsoon season coincidence with the period of dates ripening, unbalanced production/ consumption, and lack of preservation technology are few extremely disturbing factors causing quality deterioration and excessive wastage. In this purview sorption isotherm for Dhakki dates was constructed in the range of 0.12 to 0.97 a_w, and stability at 0.52 , 0.58 and 0.75 a_w under oxygen, air or nitrogen examined. Samples packed inside tinplate cans were stored for 4 months at elevated temperature of 40°C and quality evaluated monthly for darkening, pH, and titratable acidity, whereas slime appearance was examined twice daily. The sorption isotherm is sigmoid in shape, and water activity of 0.25 to 0.62 a_w represented for the monolayer, whereas the level of 0.61±0.01 a_w is regarded as the water activity of the freshly ripened Dhakki dates. The quality deterioration appeared as a function of both water activity and headspace atmosphere. Samples stored with water activities higher than 0.75 a_w deteriorated rapidly by slime formation, whereas those with lower levels displayed proportionately greater stability and with 0.52 a_w maintained characteristic color and flavor however gave a semi-dried look. The samples stored under the nitrogen afforded greatest stability. The rate of darkening, pH and titratable acidity was 2.2, 2.8 and 2.7 times higher respectively under oxygen than under the nitrogen. The impact of water activity and headspace atmosphere on quality parameter is statistically significant. In order to maintain freshness of the product with extended shelf life the Dhakki dates are to be stored under the inert atmosphere with a water activity close to its own level of 0.61±0.01 a_w.

Keywords: *Phoenix dactylifera* L, water activity, inert atmosphere, quality changes, shelf stability.

INTRODUCTION

A few plant species has developed into an agricultural crop so closely connected with human life, as has the date palm (*Phoenix dactylifera* L.). Certainly the date palm imparts close and everlasting association with mankind, and is legend for the Arabic world and for the Muslims in particular. It has been referred at 20 places in the Holy Quran, and the Prophet Muhammad (Peace Be Upon Him) stressed upon his followers to honor it "a blessed tree", and since then the date palm has become an integral part of Muslim culture. Date palm nourishes millions all over the world and contributes significantly towards their development and prosperity particularly to those living in the Arabian deserts. The dates are nutritious being high in carbohydrates, fiber, potassium, and certain vitamins and minerals, but low in fat, and virtually free from cholesterol and sodium. Being excellent source of energy the dates are taken as a staple diet. During 'Ramadan', which is annual fasting month for Muslims, the daily fast is broken after sunset with a few dates before taking sips of water. Pakistan is considered 4th largest dates producing country in the world, and the date is our important cash crop and a good source of foreign exchange earnings. The total cultivated area of all type of dates in Pakistan exceeds 78.1 thousand hectares with its estimated annual production over 630 thousand tonnes, which constitutes about 11 % of total world production (GOP 2002). Pakistan is exporting mostly dried dates worth Rs1.468 billion annually (GOP 2003). Cultivation of date palm in North West Frontier Province (N.W.F.) exceeds 1000 hectares with 6700 tonnes production out of which more than 50 % is furnished from Dera Ismail Khan area. Most of the plantations in Dera Ismail Khan are concentrated in Panyala, Paharpur, Chowdhwan and Dhakki, where summer is hot, a climate responsible for early ripening of the date fruits. The temperature during June - August normally ranges 38-48°C rising some times above 50°C with about 30-

cm rainfall. Among the local varieties "Dhakki" is the most promising cultivar with commercial importance. The date is quite popular for its extra large size (4-5 cm long and 2-3 cm thick) of small stone and heavy in weights (16-20 g/ fruit). It has fine texture, relish taste (Baloch 1999) and fetches high price in the market. However, concurrence of monsoon season with date ripening period, the crop receives heavy damages by rainstorm and insect bites. The losses are even greater in case of Dhakki date, which is a late maturing variety and very susceptible at mature/ ripened stage to hot humid climate. Moreover, during peak production period a large quantity of the fresh fruit is left over, and gluts the local market. Due to lack of appropriate processing and storage facilities the surplus produce is wasted.

A rapid darkening is also common in the dates on storage at the prevalent elevated summer temperature with high humidity, which causes much annoying situation for the date industry and calls for attention. Oxidation of phenolic compounds and involvement of sugar are the dominant factors causing darkening at elevated temperatures (Vandercook *et al* 1979). Mechanism for the browning in model systems (Hodge 1953) and in fruits and vegetables (McWeeny *et al* 1974; Wedzicha 1987) had been reviewed thoroughly. Packing under vacuum or inert gases (Rygg 1977 ; Mohsen *et al* 2003) or applying sulphite treatment (McWeeny *et al* 1974) had also been suggested for storage to prolong shelf life of high moisture dates and other moist foods. Previously we have reported that the Dhakki dates have about 0.62 water activity lying at the segment covering intermediate moisture levels (Saleem *et al* 1997). The information regarding the effect of storage atmosphere at elevated temperature as well as water activity on stability of dates in general and the Dhakki dates in particular is lacking. The objective of the present investigation is to explore the potential of inert atmosphere and optimize water activity close to freshly cured Dhakki dates in order to enhance storage stability at the elevated temperature of 40°C.

MATERIAL AND METHODS

Sample Preparation. Dhakki date at Khalaal stage having 200–250 mmHg.cm^{-2} hardness index (Baloch *et al* 2003) was procured from the local market. Well-developed fruits having good appearance were taken while unwanted discarded. To retain normal color and flavor of the dates during curing/ drying the fruits taken in a wire-mesh basket were dipped (1kg/L) for one minute in potassium metabisulfite (0.5 g/100 ml) solution at 70°C. The treated samples were allowed to drain, taken on to stainless steel trays with single layer loading of 6 kg/m^2, kept in a locally-made thermostatically controlled dehydrator equipped with hot air overflow system and then cured and dried at 40°C for 10 h until to about 24 % moisture contents. The dates were thoroughly mixed to ensure sample uniformity. Samples required for sorption studies were made into pulp after removing seeds, whereas whole cured fruits were used for further storage studies.

Sorption Isotherm and water activity evaluation

Pulp was macerated to obtain uniform mash, which was subjected to moisture equilibration with water activity in the range of 0.12 to 0.97 a_w at 40°C (Table 1). A 20-gram macerate was kept inside desiccators each containing saturated salt solution of required water activity. The sample was weighed twice daily until attainment of a constant weight. During equilibration for 5 days the solutions were maintained saturated by adding respective dry salt or distilled water as need arises. Equilibrium moisture content (EMC) of samples at each water activity was then determined using method of AOAC (1984). Sorption isotherm was constructed by plotting EMC against water activity using MSTATC package. Water activity of the date samples was then determined from the point of intersection with no change in weight of the sample and the sorption isotherm (Spiess and Wolf 1987).

Table 1. Saturated salt solutions of required water activity at 40°C.

S. No	Name of salt	Formula	A_W
1	Lithium chloride	$LiCl_2$	0.12
2	Potassium acetate	KCH_3COO	0.23
3	Magnesium chloride	$MgCl_2$	0.33
4	Potassium carbonate	K_2CO_3	0.44
5	Magnesium nitrate	$Mg(NO_3)_2$	0.52
6	Sodium bromide	NaBr	0.58
7	Sodium chloride	NaCl	0.75
8	Ammonium sulphate	$(NH_4)_2SO_4$	0.79
9	Potassium chloride	KCl	0.83
10	Potassium chromate	K_2CrO_4	0.88
11	Potassium nitrite	KNO_2	0.94
12	Potassium sulphate	K_2SO_4	0.97

Source: Troller and Christian (1978)

Evaluation for slime appearance

Cured date samples (100g) were maintained at water activity varying from 0.75-0.97 a_w for slime appearance

during the storage at 40°C by keeping inside glass desiccators each having saturated solution of different water activity. Slime formation was noted twice daily without opening the containers.

Biochemical studies

The samples were divided into three sets and water activity adjusted to 0.52, 0.58 or 0.75 a_w. Each set was subdivided into three lots for storage under controlled atmosphere of oxygen, air and nitrogen. About 200 g of equilibrated samples were sealed hermetically inside A1 (315 mL) size tin-plated cans fitted with two nozzles (valve-built-in) on cross sides for gas flushing. The cans were evacuated (125mm Hg) for one minute and the required gas was filled in. To retain the required water activity the flushing gas was passed through the desiccators having saturated solution of the required water activity. The process was repeated 4-times before soldering the nozzle outlets. The sealed samples were then incubated for 4 months in an oven at 40°C.

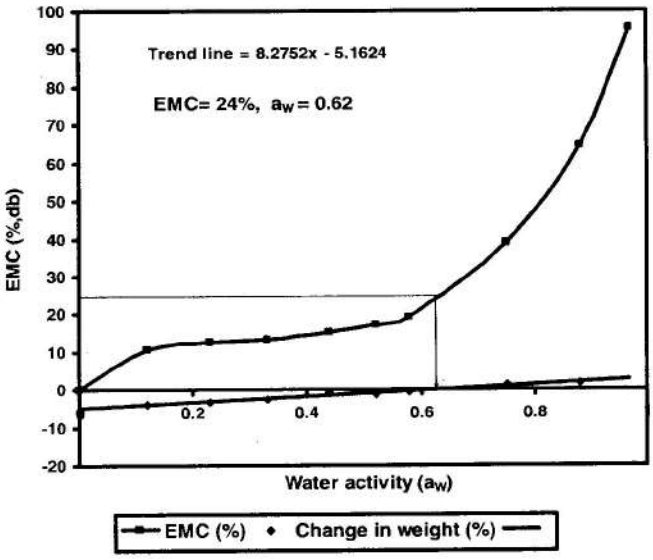
Samples were taken out from the oven periodically after every month and analyzed for darkening, pH and titratable acidity. The dates after removing the pits were cut into small pieces, and ground into a uniform mash. The mash was extracted with distilled water or dilute acetic acid (2 g/100 ml) for the measurement of pH and titratable acidity, and for darkening evaluation respectively. The pH was measured potentiometrically using digital pH-meter (Model 3010, Jenway England) equipped with temperature control probe. The titratable acidity (expressed as citric acid, mg/g) was assessed after titrating sample extract against known (4.0g/L) concentration of sodium hydroxide using pH-meter. The darkening was determined on the clarified extract by measuring absorbance at wavelength of 420 nm (Baloch *et al* 1973) using spectronic-20 spectrophotometer (Busch & Lamb USA). The experiment was conducted simultaneously and the data analyzed statistically by means of MSTAT-C version 2-10 software package applying completely randomized design (MSTAT-C 1987). The means are separated by LSD test using the same package. Slope of the plots from linear regression trend between measured parameters and time was taken as a rate for the quality deterioration and the effectiveness of a treatment assessed.

RESULTS AND DISCUSSION

Sorption Isotherm

The date samples stored at water activity of 0.58 a_w or below started loosing weight, whereas gain in weight was observed in samples kept under water activity of 0.75 a_w and above. The loss or gain in weight was rapid during initial equilibration periods, which leveled off after 5 days of equilibration. The equilibrium moisture content (EMC) increased from 10.6% to 95.4% with respective increases in water activity level from 0.12 to 0.97 a_w. A plot between EMC (%) and corresponding water activity values represents moisture sorption isotherms (Fig. 1). The figure depicts a typical sorption isotherm not segmented distinctly as frequently reported in theoretical representations. Heiss (1968) also reported a number of similar isotherms pertaining to fruits. The shape of the current isotherm indicates an overlap of moisture layers from one sorption region to the other. First segment of the isotherm extends to 0.25 a_w with relatively high rate of water uptake per unit change in water activity. The 2nd portion is larger in size approaching up to about 0.6 a_w and appears to be almost flat in shape. This portion most probably carries a moisture level for monolayer coverage. The last portion is enlarged one with highest slope for water uptake denoted for the region of vapor and capillary water. The isotherm depicts how the water activity interacts with food components and to its moisture, and thus helps in predicting stability of the dates during storage at various water activity levels.

Fig. 1. Sorption isotherm and water activity of Dhakki dates at 40°C.



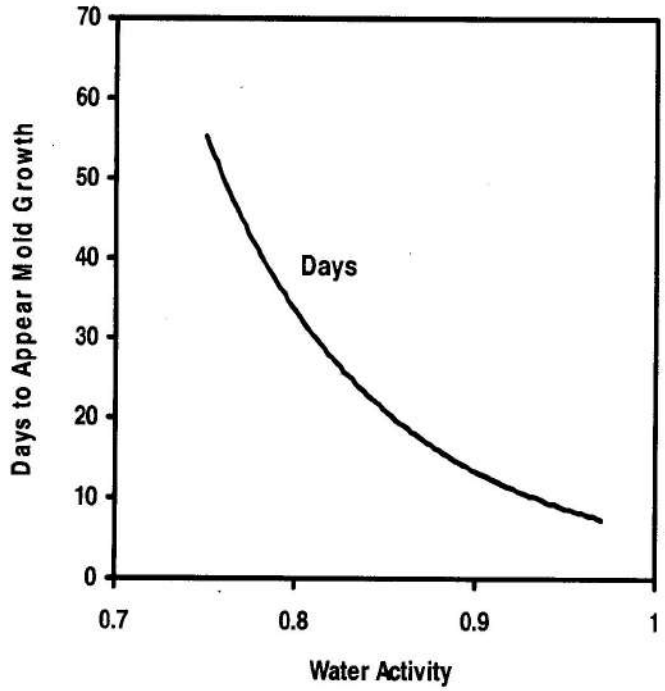
Water activity of the sample at zero weight change, calculated from the point of intersection from the plot between water activity and loss or gain in weight (%),

was found to occur at about 0.61-0.62 a_w, and supposed to be the water activity of the Dhakki dates. This water activity is within the reported range for dehydrated semi moist fruits (Davies *et al* 1976). The water activity of 0.61-0.62 a_w corresponded to 24-25% equilibrium moisture contents.

Studies on slime appearance

Slime appearance was observed during the storage in samples maintained at water activity levels beyond 0.75 a_w. The period of slime formation decreased rapidly as the level of water activity increased. In samples at water activity close to 1.0 a_w the mold growth became visible even prior to storage while the period of moisture equilibration. A plot between days prior to slime formation, and water activity represents mold free storage life of the dates (Fig. 2). The plot gives important information predicting mold free shelf life for the date fruits at various water activity levels during storage at the elevated temperature of 40°C. Safe shelf life of the date rapidly increased with the decrease in water activity. The results indicate that Dhakki dates can be kept much beyond 50 days without showing sign of slime formation provided it is stored at a water activity below 0.75 a_w.

Fig. 2. Limiting storage life at high levels of water activity



Biochemical studies

Taking preliminary studies as well as the sorption isotherm of the date into consideration three water activity levels of 0.52, 0.58 and 0.75 a_w were selected to further examine the effect of storage on darkening, pH changes and titratable acidity of the dates. The chosen limit of water activity covers the water activity of the dates (≈0.62 a_w), extends to the range of semi dried and moist foods stretching out to the sorption segment possibly intended for storage of freshly cured dates. Further, a temperature of 40°C was selected for storage studies so as to collect information at the elevated temperature. It is pertinent to note that the selected temperature lies within the range of most prevalent summer temperature corresponded with the high production season as well as rapid deterioration period of the freshly ripened dates.

Darkening

Irrespective of the environmental factors the darkening remained on the increase during storage at 40°C (Fig. 3). A maximum amount of darkening (0.089) was displayed by samples under oxygen at 0.75 a_w whereas a minimum (0.059) under the nitrogen at 0.52 a_w. Mean values (Table 2) for samples as regard to storage atmosphere are statistically significant ($P < 0.05$). The rate of darkening (12.1×10^{-3} /month) of samples stored with 0.75 a_w under the oxygen headspace was twice that of nitrogen and 1.4 times under the air (Fig. 4). It clearly indicated that oxygen accelerated while the nitrogen retarded the darkening as compared to air.

The samples under the nitrogen resisted the deterioration and looked normal in color and flavor at end of the storage. Whereas those under the oxygen appeared dark brown and smelled like burnt sugar, and gave absorbance close to 0.1 units - an indication to the exhaustion of shelf life (Baloch *et al* 1997; Baloch *et al* 2000). Since cured dates of tamar stage possesses sugar carbohydrates, amino acids and tannin polyphenolic compounds (Sawaya *et al* 1982), and moreover gives a continuous rise in the browning under any storage condition, testify the involvement of both oxidative and non-oxidative darkening at 40°C.

Similar findings appeared in the literature (Maier and Metzler 1965; Maier and Schiller 1960; 1961a, b). It is pointed out that the darkening was reduced by more than 30 % on storing the Dhakki dates under inert atmosphere at 40°C whereas a reduction of 20 % had been reported for Deglet Noor at 38°C (Maier and Schiller 1961a, b). The darkening was also influenced by water activity of the samples. The

mean values for water activity 0.52, 0.58 and 0.75 are statistically

Table 2. Mean values for darkening, pH and titratable acidity as affected by headspace atmosphere and water activity of Dhakki dates at 40°C for 4 months

Factors	Parameters	Darkening	pH	Titratable acidity
Headspace atmosphere	Oxygen	0.060 A	5.12C	52.67 A
	Nitrogen	0.049 C	5.79A	32.02 C
	Air	0.055 B	5.53B	39.80 B
Water activity (aw)	0.52	0.053 Y	5.58X	38.28 Z
	0.58	0.054 Y	5.52Y	40.36 Y
	0.75	0.065 X	5.34Z	45.85 X

Mean values bearing different letters (A–C), (X–Z) in each column for every factor differ significantly (LSD, $P \leq 0.05$).

significant ($P < 0.05$), whereas no significant effect appeared between 0.52 and 0.58 water activity under any headspace atmosphere (Table 2).

Adjusting the samples at the low water activity the stability increased under any atmosphere (Figs. 3, 4). The darkening rate was reduced by about 1.21 - 1.30 times on storing the samples at 0.52 instead of 0.75 a_w. The samples stored under the atmosphere of nitrogen at the lowest water activity (0.52) were found most stable. The finding is in accord with the reported observations (Mutlak and Mann 1984; Saleem *et al* 1997).

Fig. 3. Influence of oxygen, air and nitrogen as a headspace atmoshpere on darkening of Dhakki dates equilibrated at 0.52, 0.58 and 0.75 water activity levels during storage at 40°C.

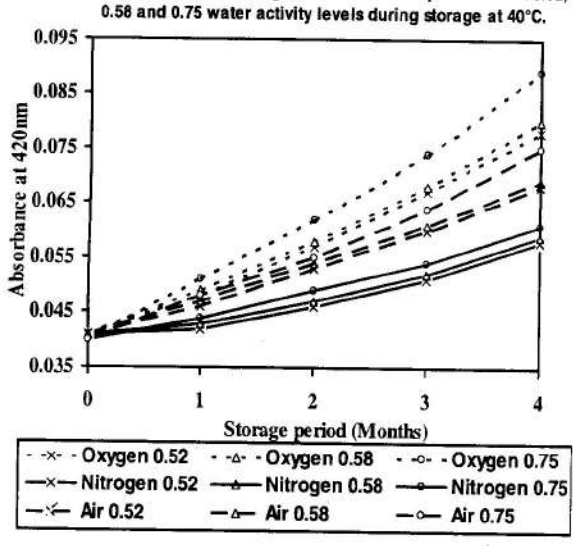
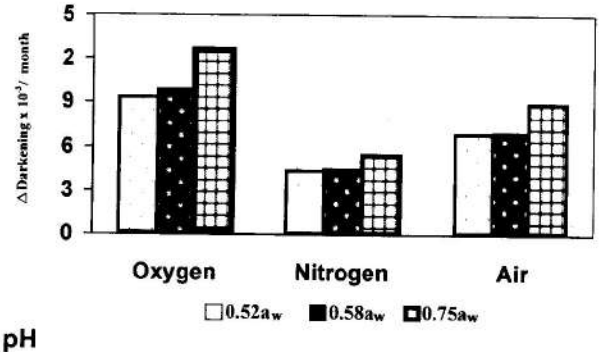


Fig.4 Influence of water activity and headspace atmosphere on the rate of change in darkening of Dhakki dates during storage



pH

A gradual decline in pH from 6.3 to 3.58 was seen during the storage, with a. rapid decline when the samples were stored under oxygen and over higher water activities (Fig. 5). A drop in the pH of 2.72, 1.6 and 0.92 units was found for samples under oxygen, air and nitrogen with 0.75 a_w respectively. A rate of fall (6.75×10^{-1} ΔpH/month, Δ stands for change) in pH corresponding to sample with 0.75 a_w under oxygen was reduced by 1.74 and 3.11 times as a result of change in the atmosphere to air and nitrogen respectively (Fig.6).

Similar observations had been reported in case of Deglet Noor variety (Maier and Schiller 1961a). Mean pH values as affected by controlled atmosphere are statistically significant at $P < 0.05$ (Table 2). It is once again found that storage under inert atmosphere is the most effective technique for controlling the deterioration. A continuous fall in pH during storage under atmospheres of oxygen, air or nitrogen demonstrates that both oxidative and non-oxidative mechanisms are responsible causing pH changes, similar to that found in case of the darkening reactions.

Water activity of the samples also played a vital role governing pH changes, and samples with reduced water activity displayed greater resistance against the deterioration. The mean pH values with respect to water activity are statistically significant ($P < 0.05$, Table 2). About 29 - 38 % reduction in the rate of pH change occurred on reducing water activity from 0.75 to 0.52 a_w. The manifestation that the samples with increased rate of darkening corresponded to increased rate of pH drop describes that the same process of quality deterioration are responsible for both phenomena (Baloch *et al* 1977, Rygg 1977).

Fig. 5. Influence of oxygen, air and nitrogen as a headspace atmoshpere on pH of Dhakki dates equilibrated at 0.52, 0.58 and 0.75 water activity levels during storage at 40°C.

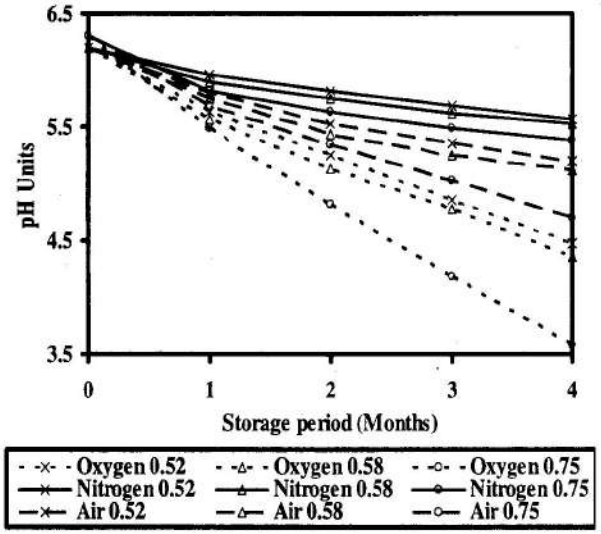
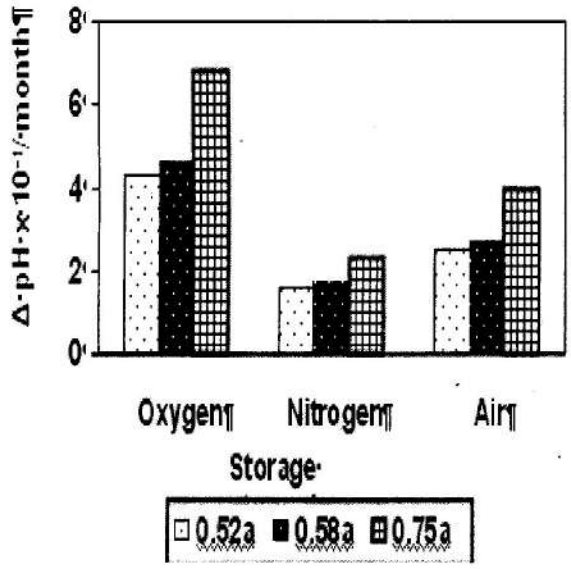


Fig. 6. Influence of water activity and headspace atmosphere on the rate of change in pH of Dhakki dates during storage at 40°C



Titratable acidity

A consistent rise in titratable acidity was observed for all the samples during the storage (Fig. 7). However, the rates were greatly influenced by storage atmosphere and water activity. Mean values of the determinant for both factors are statistically significant ($P < 0.05$, Table 2). Acidity of 22.15 x 10^{-2} - 22.37x10^{-2} mg/g prior to storage increased to 43.57 x 10^{-2} - 100.95 x 10^{-2} mg/g by keeping the equilibrated samples at 0.52 - 0.75 a_w for 4 month at 40°C (Fig 8). The rate of acid formation (19.14 mg/g month) for samples equilibrated with 0.75 a_w and stored under oxygen is about 1.76 and 2.86 times greater than for those under air or the nitrogen respectively, displaying highly significance difference ($P < 0.05$, Table 2). Moreover, it was found minimum of 5.43 (mg/g month) with 0.52 a_w and under nitrogen (Fig. 8). It is further noted that the samples yielding higher amount of darkening had produced greater amount of acidity and pH drop. It is therefore suggested that all such reactions have most likely a common reactive pool from where the determinants are emerging. Present findings are in line with those reported earlier (Saleem *et al* 1997; Saddozai *et al* 1998). Since water activity level of 0.62 a_w also touches to the segment of sorption isotherm containing capillary moisture range (Fig. 1), it can't give guarantee however, to the date fruit to remain unspoiled and safe over prolong storage at 40°C. Deterioration of dates may occur by the osmophilic yeast and xerophilic mold at water activity as low as 0.62 a_w, and even by chemical degradation (Brockmann 1973).

Fig. 7. Influence of oxygen, air and nitrogen as a headspace atmoshpere on titratable acidity of Dhakki dates equilibrated at 0.52, 0.58 and 0.75 water activity levels during storage at 40°C.

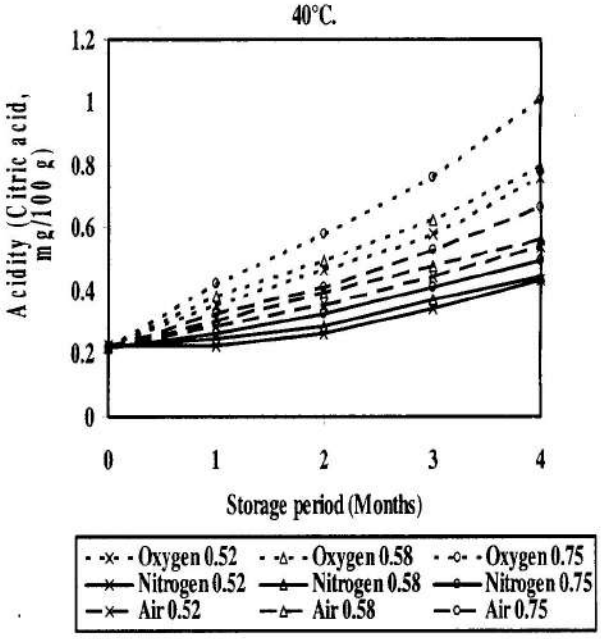
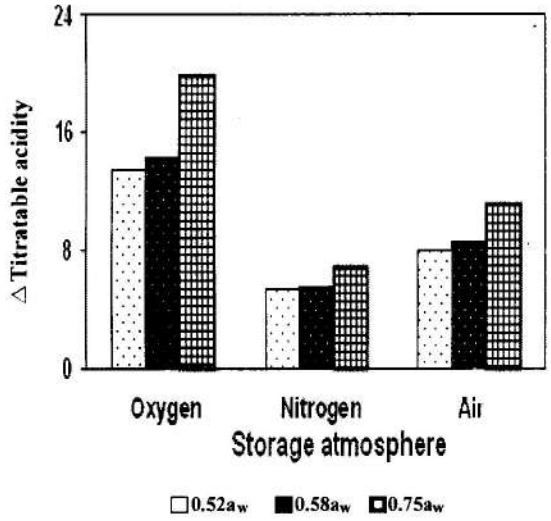


Fig. 8. Influence of water activity and headspace atmosphere on the rate of change in titratable acidity of Dhakki dates during storage at 40°C



Sorption isotherm of Dhakki dates is found to have sigmoid in shape similar to foods of high sugar contents. Water activity of Dhakki dates of 0.62 a_w occurs on a segment of the isotherm of intermediate moisture and semi-moist foods encouraging deteriorative changes of chemical nature. The darkening and other associated changes responsible for quality degradation of Dhakki dates are function of storage atmosphere and water activity. The investigated parameters slow down considerably on packing the dates under nitrogen and at 0.52 a_w, and the measures taken proved useful to combat against the deteriorative changes. Since the samples were stored at elevated temperature of 40°C and at water activity within the range of intermediate moisture limits, the degradative reactions eventuated at very rapid rate, and possibly interacted with each other bringing alteration in the deteriorating process sequence. The higher deterioration rates at the higher water activity (0.75 a_w) are attributed to the increased mobility of the constituents involved in the deteriorative process, which were further promoted by the oxygen.

Conclusions

The rate of deterioration in Dhakki dates becomes significantly low by storing the samples at a lower water activity level and under inert atmosphere of nitrogen. However the samples stored at water activities lower than 0.58 a_w suffer freshness and give hard texture. It is concluded that Dhakki dates preferably be stored under atmosphere free from oxygen and at relative humidity close to its own water activity level (0.60-0.61 a_w). Such pre-requisites will indeed provide sufficient stability and ensure for adequate shelf life of the Dhakki dates.

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Physico-chemical analysis of stored and fresh honeys

***Raheela Chaudhry, Salah-ud-Din and [a]Hina Arif**
*Corresponding Author: Food and Biotechnology Research Center,
PCSIR Labs. Complex, Lahore-54600. raheela.bpt@gmail.com
[a]Department of Chemistry, Government College, Lahore.

ABSTRACT

Honey is viscous and syrupy liquid has high nutritional and medicinal value and is extensively used in both Ayurvedic and Unani medicine. The composition of honey determines its value as nutritional and medicinal product and for that purpose physico-chemical analysis is carried out. In the present study different physical (pH, electrical conductivity, moisture contents, ash contents, refractive index, specific rotation) and chemical characteristics (nitrogen contents, hydroxy methyl furfural, free amino acids, total acidity, free acidity, lactone, fructose, glucose, fats and mineral elements) were determined in fresh and stored honey samples taken from Narran, Quetta and Rawalpindi. It was observed that the refractive index and specific gravity of the honey samples decreased with an increase in moisture content. All the samples were floral honey as they were levorotatory and moisture as well as ash contents of stored honey were less than the fresh ones. It contained traces of nitrogenous matter. Fresh honey contained small amounts of HMF as compared to the stored one while free amino acids of the stored one were found to be higher than that of fresh ones. Free acidity was same for all. Results of macro and microelements showed that K, Na, Ca and Mg were the macro-elements and Fe, Cu and Zn, microelements.

Key words: Honey, physico-chemical properties, metals.

INTRODUCTION

Honey is a natural, sweet, viscous liquid food produced in honey sacs of insect species of genus *Apis*, from nectar of flowers and to limited extent from juice of fruits and from honeydew. It was almost only source of sugar available to ancients and was valued for its medicinal and nutritional benefits (Crane 1976). It was an important commodity through out the ancient civilized world. It was favorite diet of the Holy Prophet (PBUH) as indicated by his saying:

"Honey is remedy for all illness of body and the Holy Quran is remedy for all illness of mind, Therefore, I recommend to you both remedies, the Holy Quran and Honey!"

Honey has high medicinal value. It helps to promote digestion and provides energy for muscle. It is used in treating ulcers, constipation, kidney problems, indigestion, bronchial infection, asthma, chronic disorders and sore throat. It is also used as a remedy for burns because of its anti-microbial activity. Appreciable increase in content of RBC and Hemoglobin is attributed to the presence of Fe, Cu, Mn and other elements in honey been taken. In spite of high sugar concentration, it can be given to diabetic patients. Honey dextran medium is used to preserve the cornea of the eye. Natural honey exhibits a very good performance as inhibitor for steel corrosion in high saline water, so also act as corrosion inhibitor (Abdullah 2000). The major components of honey are sugars (about 80%) and water (17-20%) and minor components include minerals, enzymes, lipids, amino acids, protein, organic acids etc. These minor elements determine its aroma, flavour and colour. The pleasant aroma and taste of this viscous liquid, ranging in colour from pale yellow to dark amber, also varies according to geographical and seasonal conditions (Rajalakshami 1999).

The high content of sugars, small amount of amino acids, lipids along with some vitamins and minerals imparts its high nutritional value. It contains about 75-80% fructose that gives substantial energy and makes it sweeter than glucose or sucrose (Latif and Manzoor-ul-Haq 1956a). Vitamin content in honey is also very low but includes water-soluble vitamins: thiamine, riboflavin, ascorbic acid, pyridoxine pantothenic acid, biotin folic acid and nicotinic acid. It was reported to contain amino acids such as lysine, arginine, proline, methionine, iso-leucine and leucine along with aspartic acid, glutamate, serine, glycine, histidine and alanine. Honey has been reported to exert beneficial influence on retention of calcium and hence covers requirements from infants to elders. It is rich in carbohydrates and therefore is a wholesome food. It consists of nearly equal portion of glucose and fructose with water and small quantities of

nitrogenous matter and acids and occasionally sucrose and mannitol (Jabbar *et al* 1996). Traces of alcohol are generally present. It also contains invertase and other enzymes derived from pollens. Due to certain specific characteristics like color, sweetness, viscosity, spread ability, miscibility, compatibility volume building and freezing point depression, honey is extensively used in bakery products, candies and confectionaries, cereal based car salad dressings, sauces, chocolates, fruit juices, puddings chilled desserts and microwave foods (Royden 1996).

The nutritional and medicinal importance of honey is directly related to its physico-chemical properties. The coloring matter present in honey includes chlorophyll, xanthphyll, anthocyanin, tannin and carotene. Darkening of honey either on heating or during storage is due to presence of amino acids in honey. Physical characteristics of honey (Bongdanov 1984; Jabbar *et al* 1996) include pH, moisture content, ash contents, viscosity, specific gravity, surface tension, rheological properties, refractive index, thermal conductivity, freezing point depression etc. The chemical characteristics include the determination of nitrogen, hydroxy-methyl furfural, free amino acids, free acidity, lactone, fructose, glucose, fats and mineral elements.

In the present study physico-chemical analysis of honey samples produced in different areas of Pakistan was done in order to characterize and compare Pakistani honey quality with international standards.

MATERIALS AND METHODS

Three honey samples were taken from Narran, Quetta and Rawalpindi. The sample taken from Quetta was stored for approximately 2 years while the other two were fresh samples. Different physical characteristics including pH, moisture contents, ash contents, refractive index, electrical conductivity, specific rotation and the chemical characteristics involving nitrogen, hydroxy-methyl furfural, free amino acids (proline), total acidity, free acidity, lactone, fructose, glucose, fats and mineral elements were determined.

Physical Characteristics

Refractive index was determined by using Abbey's refractometer. For the determination of Specific rotation, polarimeter was used and specific rotation was calculated using formula:

$$[\alpha]=100\alpha/L.C$$

where α is the angle of rotation, L is the length of polarimeter (in dm) and C is the concentration of honey solution. Moisture contents were determined by heating in Vacuum drying oven for overnight and maintaining pressure between 70-80 mm of Hg.

For the determination of ash content, honey samples were taken in pre-weighed crucible, heated till complete charring on a low flame and placed overnight in a muffle furnace at 550°C.

Electrical conductivity and pH was determined by using conductivity meter and pH meter, respectively.

Chemical Characteristics

Following methods were used for the chemical characteristics study of honey.

a) Nitrogen

Nitrogen in honey (Furnish and Tachell, 1989) was determined by digesting the sample in Kjeldhal's flask using 20mL conc H_2SO_4. Volume of the digested sample was made up and its 2mL were taken into Markham's distillation apparatus, 5mL of 40% NaOH was added to it and titration flask containing boric acid was placed under condenser. The contents were heated by passing steam through apparatus until volume becomes 4 times. Then the solution in the flask was titrated against 0.1N HCl and percentage nitrogen was calculated.

b) Hydroxy methyl furfural

For the determination of HMF, 15% $K_3[Fe(CN)_6]$ and 30% $Zn(CH_3COO)_2$ solution was added to the honey solution and filtered. 5ml of 0.3% $NaHSO_3$ solution was added to the filtrate and mixed well. Absorbance of the sample was noted against reference at 284nm and 336nm. HMF was calculated by using formula:

$$\text{HMF (\%)} = \frac{(A_{284}-A_{336}) \times 14.97 \times V/W}{100}$$

where V is the volume of the filtrate taken and W is the weight of the honey.

c) Free amino acid (Proline)

To 0.5mL honey solution, taken in three borosilicate tubes, 1ml each of formic acid and ninhydrin solution was added. Placed on a water bath for 15 minutes and after cooling 5mL isopropyl alcohol was added and absorbance was noted at 520nm. Proline in honey was determined by comparing the values with

calibration curve values, plotted with standard solution of proline.

d) Total acidity, free acidity and lactone

Honey solution was made in CO_2 free water. After inserting pH electrode in solution, 0.05N NaOH was added till pH became 8.5. 10 ml more NaOH was added and back titrated against standard HCl to pH 8.5. Blank reading was taken in the same way. Free acidity, total acidity and lactone were determined by following formula:

Free acidity (meq/kg) = $(V_1-V_B) \times 50 / W$
Lactone (meq/kg) = $(V_2-V_3) \times 50 / W$
Total acidity (meq/kg) = Free acidity + Lactone

where V_1, V2 and V_B are the volumes of NaOH used, V_3 is the volume of HCl used and W is the weight of sample.

e) Fructose and glucose

Fructose and glucose in honey samples were determined by TLC by using silica gel as an adsorbent. Mixture of ethyl acetate, isopropanol and water (4:2:1) was used as solvent. Sugars are located by spraying the film with a solution of locating mixture (anisaldehyde and conc. H_2SO_4 in ethanol) and drying chrome plate to 100ºC.

f) Fats

Fats were determined by cold extraction method using n-Hexane as solvent.

g) Mineral elemental study

For the determination of mineral elements (Na, K, Ca, Mg, Cu, Zn, Pb, Cd, Ni, Mn and Fe) acidic solution of ash was prepared with HCl and HNO_3 (9:1) and analyzed by Atomic Absorption Spectrophotometer using air-acetylene flame. Standard solutions of the elements were prepared by dissolving AR grade salts of the metals in 0.5N HNO_3 and diluting the stock solution to the desired range.

RESULTS AND DISCUSSION

The most common raw material for honey is nectar and other natural plant exudations. Nectar is a watery solution of sugars and originates in floral and extra-floral nectarines of plants. The composition of honey determines its value as nutritional and medicinal product and for that purpose physico-chemical analysis is carried out. Several properties like refractive index, density, viscosity, electrical conductivity, surface tension etc. are of great importance in honey industry as they influence its keeping quality, granulation and texture. Honey is basically composed of two important components i.e. moisture and sugars, along with various other minor components including minerals, HMF, amino acids, nitrogen etc.

Physical Characteristics

Refractive index

It is one of the important optical properties that provide an easy way of estimating the moisture content of honey, a value that determines its proneness to fermentations (Latif and Manzoor-ul-Haq 1956b). Fermentation of stored honey is uncommon. The refractive index of the honey samples decreased with increase in moisture contents at 20ºC from 1.5044 (Sample 1) to1.4840 (Sample 2). Refractive index of honey at 40ºC also decreased from 1.4998 to1.4794 (for Samples 1 and 2, respectively). Specific gravity decreases with the increase of moisture contents at 20ºC. In the honey samples its value decreased from 1.4457 to1.4794.

Specific Rotation

Honey has the property of rotating the plane of polarized light. This property depends largely on the sugars of honey, their types and relative proportion. Floral honeys are levorotatory. This is a consequence of normal preponderance in floral honey fructose, which has a negative specific rotation over glucose. The specific rotation of the samples was –15.00, -13.20 and -16.25.

Moisture and Ash

Moisture contents of the honey samples were approximately 14% and 16% (Table 1). It was also noted that the moisture contents of the stored honey were less than normal range. In the present study it was 12.81% for the stored sample. The ash contents of honey ranges form 0.24%-0.26% but ash contents of stored honey were less than normal range i.e. 0.14% only.

Electrical conductivity and pH

The electrical conductivity of honey determines its suitability for winter stores of bees. Krauze and Zalewshi (1991) reported the conductivity of different floral honey between 1.4×10^{-4}-1.75×10^{-4} mho/cm. In the present study the value (Table 1) ranged from 1.7 µohm/cm to 4.0 µohm/cm. pH of the samples taken from Narran, Quetta and Rawalpindi were 3.8, 4.0 and 3.6, respectively. This value is affected by amounts of various acids present, but mostly mineral

contents such as calcium, sodium, potassium and other constituent. Honey rich in ash generally showed higher pH values.

Table 1: Physical characteristics of honey samples

Physical Characteristics	Sample 1 (Narran)	Sample 2 (Quetta)	Sample 3 (Rawalpindi)
pH	3.8	4.0	3.6
Electrical conductivity (μohm/cm)	1.8	4.0	3.4
Moisture contents (%)	14.02	12.81	13.99
Ash contents (%)	0.2618	0.1469	0.2437
Refractive index (at 20ºC)	1.5044	1.4840	1.4931
(at 40ºC)	1.4998	1.4794	1.4830
Specific rotation	-15.00	-13.20	-16.25

Chemical Characteristics

a) Nitrogen

Honey usually contains traces of nitrogenous matter (Lothrop *et al* 1984) which is partially precipitated in colloidal forms and honey samples showed iso-electric point around pH 4.3. Nitrogen contents of samples taken from Narran, Quetta and Rawalpindi were 0.06%, 0.05% and 0.08%, respectively.

b) Hydroxy methyl furfural

It is produced by the decomposition of fructose in the presence of acid. Fresh honey contains small amounts of HMF as compared to the stored one. The calculated value of HMF (Table 2) for sample 1 and 3 was 0.06% and 0.07%, respectively, while for the stored sample (taken from Quetta) the value was higher i.e. 0.08%. Bechk *et al* (1998) also reported that HMF content of honey does not increase during storage.

c) Free amino acids (Proline)

Amino acids are the breakdown product of proteins, which, in normal honeys, also exist in minute quantities, contributed by bee rather than plants. Proline is the most important of free amino acids. It value was found to be 9mg/100kg for sample 1 and 7mg/100kg for sample 3. Free amino acids of the stored sample (taken from Quetta) were found to be higher than the normal range i.e. 11mg/100kg. It was proved that darkening of honey during storage is due to high amount of free amino acids that produced dark melaninoid coloring matter by reacting with fructose and glucose.

Table 2: Chemical characteristics of honey samples

Chemical Characteristics	Sample 1 (Narran)	Sample 2 (Quetta)	Sample 3 (Rawalpindi)
Nitrogen (%)	0.06	0.05	0.08
Hydroxy methyl furfural (%)	0.06	0.08	0.07
Free amino acids (proline)(%)	0.000009	0.000011	0.000007
Total acidity (meq/kg)	15	13	15
Free acidity (meq/kg)	10	10	10
Lactone (meq/kg)	5	3	5
Fructose glucose ratio	1.38	1.41	1.39
Fats	Nil	Nil	Nil

d) Total acidity, free acidity and lactone

The level of acidity of honey contributes to its stability towards microorganisms. The bees increase acidity of honey during ripening. The complexity of honey extends to the number of acids present. Gluconic acid is present in considerable excess over all other acids. It is produced by the action of enzyme on dextrose present in honey. Gluconic acid exists in solution in equilibrium with lactone or internal ester. Total acidity is sum of acidity and lactone and was found to be 15, 13 and 15 meq/kg for sample 1, 2 and 3, respectively, while the value of free acidity was 10 meq/kg for all the three.

e) Fructose and glucose

It was reported (Ivanov 1997) that the quantity of predominate sugars varies with honey type from 31.01 to 41.44% for fructose and from 23.26 to 30.28% for glucose i.e. fructose was the dominate monosaccharide. The main disaccharides were turanose and maltose while sucrose was present in lower quantities. In the present study thin layer chromatography was used for the determination of sugar contents. Glucose and fructose are located after spraying locating agent and their R_f value was determined. The fructose glucose ratio was about

1.40 for the three samples of honey while the amount of sucrose present was 3, 4 and 2% for sample 1, 2 and 3, respectively.

f) Fats

Fats were found absent in all the three samples of Pakistani honey.

g) Mineral elemental study

The scientific literature on honey ash falls into three categories i.e. the amount of total ash, amount of principle constituents and the identities of minor metallic constituents, which often appear in minute amounts. Macro and microelements (K, Na, Ca, Mg, Zn, Ni, Cd, Cu, Mn, Fe, Pb) were determined by using atomic absorption spectrophotometer. It was noted that potassium (579.5, 470 and 687.9 ppm for sample 1, 2 and 3, respectively) and magnesium (126.26, 113 and 86.71 ppm for sample 1, 2 and 3, respectively) contents were higher than other macro elements. It was reported by (Lopez-Garcia *et al* 1999) that calcium content of the honeydew honeys was smaller than that of floral honeys. Similarly the amount of iron was higher (6.27, 3.81 and 6.30 ppm for sample 1, 2 and 3, respectively) than other microelements.

Table 3: Mineral elemental analysis of honey samples

Mineral elements (ppm)	Sample 1 (Narran)	Sample2 (Quetta)	Sample3 (Rawalpindi)
K	579.50	470.00	687.90
Na	120.20	115.26	206.40
Ca	28.62	24.20	28.10
Mg	126.26	113.0	86.71
Zn	6.57	7.79	11.46
Ni	Nil	Nil	Nil
Cd	Nil	Nil	Nil
Cu	0.28	0.73	0.69
Mn	Nil	Nil	Nil
Fe	6.27	3.81	6.30
Pb	Nil	Nil	Nil

Results of macro and microelements (Table 3) showed that honey samples contains potassium, sodium, calcium and magnesium as macro-elements and iron, copper and zinc as microelements while manganese, nickel, cadmium and lead were absent in all the three samples. Iron is essential constituent of hemoglobin, which carries oxygen to our body tissues and copper promotes action of iron. Calcium is essential for bones and teeth. It was present in considerable amounts (28.62, 24.20 and 28.10 ppm for sample 1, 2 and 3, respectively).

CONCLUSIONS

The nutritional and medicinal importance of honey is directly related to its physico-chemical properties. Usually analysis of honey showed considerable differences according to their place of origin, but the three samples studied, have insignificant difference in values of almost all the physico-chemical characteristics. Fresh honey contained small amounts of HMF as compared to the stored one while free amino acids of the stored one were found to be higher than that of fresh ones. Results of macro and microelements showed that honey samples contains potassium, sodium, calcium and magnesium as macro-elements and iron, copper and zinc as microelements while manganese, nickel, cadmium and lead were absent in all the three samples.

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Detection of heavy metals in buffalo milk and dairy products

Saima Nazir, M. Khalid Saeed, Sakhawat Ali and Rauf Ahmad
Food and Biotechnology Research Centre,
PCSIR Laboratories Complex, Lahore-54600.

ABSTRACT

Heavy metal's toxicity is the result of their interactions with the enzymatic systems of the animal cells or some constituents of cells' membranes. The concentration of heavy metals iron, zinc, copper, manganese, cadmium and lead were determined in buffalo milk and two dairy products i.e. ice cream and processed cheese. Samples were initially treated to a wet digestion and afterward heavy metals were measured by atomic absorption spectrometry. The reliability of the approach was confirmed by analyzing data from literature. It has been declared that ingestion of heavy metals in large quantities may eventually lead to serious health problems.
Key words: Buffalo milk, dairy products, heavy metals, Pakistan

INTRODUCTION

The fact that trace elements (Fe, Zn, Cu, Mn, Cd and Pb) are present in trace amounts does not in any way diminish their importance as they perform remarkable physiological functions. Although feedstuffs are the primary source of these elements in milk, a certain quantity may originate from the water supply, from insecticides and other agrochemical residues, and from milk containers and processing equipments. Iron (Fe) is always present in milk ranging between 100-900 ug/L. Values much higher than these have also been reported but are probably of milks which have been contaminated with Fe from metal containers after milking (Lehninger *et al* 2000). Zinc (Zn), of all the trace elements found in milk, is consistently present in the largest proportion. An aver age value of Zn for normal cow milk is 3500-3900 ug/L. Zn is present in a number of enzymes such as carbonic anhydrase, alcohol and lactic acid dehydrogenase and various peptidases. Its toxicity has been reported on account of mineral supplementation of feed (Lopez *et al* 1991).

Copper (Cu) is a normal component of milk, present in amounts of 20-200 ug/L. Values higher than these have also been reported, which probably include an amount originating from processing equipments. High Cu in milk can come from Cu-rich feed, whereas low Cu in milk indicates animal feeding on pastures. Cu is essential for the formation of haemoglobin (Bermejo et al 1997 and Parikh 1992). While Mn in milk is reported to occur in very small amount of 0.08 mg/Kg (Carrion *et al* 1994), it is considered to be an essential trace element in human nutrition being it is an integral part of liver enzyme arginase. Ingestion of excessive quantities of Mn appeared to interfere with absorption of Fe, thus causing anemia. However, its absorption is significantly suppressed by (Cadmium) Cd as reported by Gruden and Mutausic (1989). Lead is also present in minute quantities in milk but it is known to be a metabolic poison and neurotoxin that binds to essential enzymes and cellular components and resulting in their inactivation (Cunningham and Saigo 1995).Its toxicity may lead to mental retardation and reduce intelligence (Schumann 1990). Cadmium is present in human body in significant quantities (26 ug/L) it binds to kidney SH-rich protein thus resulting in kidney cortex toxicity. Chronic toxicity of Cd resulted in mild liver damage and Itchi-Itchi disease (Sanchez *et al* 1996).

In many countries a lot of research has been done on the subject of trace elements in milk and dairy products. But in Pakistan there is not much awareness about their occurrence as well as their detection in food products. The main objective of present study was the detection of heavy metals (Fe, Zn, Cu, Mn, Pb, and Cd) in different samples of milk and dairy products such as ice cream and processed cheddar cheese and to compare the results with the international standard.

MATERIALS AND METHODS

In the current investigation, iron, zinc, copper, manganese, cadmium and lead contents from 30 samples of milk and milk products were determined. Samples of milk of local Buffalo and milk products were collected from different shops. The organic matter present in the sample was removed prior to analysis by digestion with mineral acids. All wares parts and sample vials were rinsed with 10% nitric acid and then with distilled deionized water before use.Each sample of milk and milk products (100 g) were dried at 100°C in an oven for 24 hours. Then placed in muffle furnace at 550°C for 8 hours for

charring. Ash was dissolved in 5mL of 6-N HCl and 5 mL of distilled water and warmed to ensure complete dissolution of ash. The ash solution was filtered and volume made to 50ml. All glassware used in the preparation of ash and ash solution was acid washed. Determination of heavy metals was done by aspirating the ash solutions in atomic absorption spectrometer (Hitachi Model 170-10) using air-acetylene flame following the procedure of Fricke *et al* (1997). Metal contents were calculated by comparison with the standard curves of respective metals. Specific hollow cathode lamps were used for each metal at their respective wavelengths: Fe (248.3), Zn (213.8), Cu (324.8), Mn (279.5), Pb (283.3) and Cd (228.8).

RESULTS AND DISCUSSION

The results of various metal ions in different milk and milk products were compared and evaluated with the maximum allowed limits as elaborated in Table 1

Table 1 Maximum permissible limits of metal ions in milk, ice cream and processed cheese adopted from Banu et al (1985)

Food Products	Fe (µg/100g)	Zn (µg/100g)	Cu (µg/100g)	Mn (µg/100g)	Pb (µg/100g)	Cd (µg/100g)
Milk	120.00	500.00	50.00	28.00	10.00	1.00
Ice-Cream	80.50	900.00	250.00	9.00	15.00	2.50
Processed Cheese	600.00	400.00	300.00	8.00	40.00	5.00

Table 2 Detection of heavy metals in milk

Sr. No	Fe (µg/100g)	Zn (µg/100g)	Cu (µg/100g)	Mn (µg/100g)	Pb (µg/100g)	Cd (µg/100g)
1	122.75	250.56	51.95	26.68	0.41	0.19
2	134.86	248.34	48.18	29.38	0.43	0.08
3	133.25	253.75	54.75	27.16	0.52	0.12
4	129.90	246.84	53.62	22.48	0.46	0.18
5	135.22	255.61	47.50	24.75	0.43	0.10
6	130.40	250.60	52.12	26.70	0.42	0.15
7	129.60	251.10	51.90	28.95	0.40	0.17
8	131.10	250.90	52.05	26.40	0.45	0.13
9	130.80	249.36	51.80	28.19	0.50	0.16
10	125.72	250.70	55.75	26.80	0.48	0.14
Average	130.36	250.78	51.96	26.75	0.45	0.15

The average values of Fe, Zn, Cu, Mn, Pb and Cd in milk are shown in Table 2, which are summarized as 130.36, 250.78, 51.96, 26.75, 0.45 and 0.15µg/100 mL of milk respectively. Ahmad and Sayed (1991) analyzed the Fe, Zn, Cu, Mn, Pb and Cd in fresh raw milk samples and found that Fe: 0.20, Zn: 0.50, Cu: 0.15, Mn: 0.29, Pb: 0.02 and Cd: 0.19 mg/Kg. The value of Fe and Mn were found to be lesser, while those of Zn, Cu, Pb and Cd were on the higher side as compared with findings of Ahmad and Sayed, 1990. However, it was found that average Fe and Cu values were high as compared with the maximum allowed limit of metal ions in milk. The average values of Zn, Mn, Pb and Cd were found well within the maximum allowed limit of heavy metals in milk. This may come from feeds eaten by the animals as natural constituents or from the containers and milk processing equipment as contaminants. Similar results were also reported by Carrion *et al* (1994). It was observed that in ice-cream the average value of Fe, Zn, Cu, Mn, Pb and Cd were 91.78, 680.50, 22.49, 8.13, 0.15, 0.10 µg/100 g respectively (Table 3). The values of metal ions were well with in the maximum allowed limit of heavy metals in ice cream.

In case of processed cheese, the average value of Fe, Zn, Cu, Mn, Pb and Cd were 651.65, 420.11, 29.13, 9.04, 0.23 and 0.61-µg/100 g respectively (Table 4). Carballo *et al* (1994) also analyzed the heavy metals in cheese and found that the mean contents of Fe 6.17, Zn: 37.86, Cu 1.03, Mn 0.098, Pb 0.0025 and Cd 0.0067 µg/g.

However the present investigation revealed that the mean values of Fe, Cu and Zn were lower whereas the mean values of Mn, Pb, Cd are slightly higher than those of Carballo *et al* (1994). Mn is considered an essential trace element in human nutrition since it is an integral part of the liver enzyme arginase, hexokinase, various decarboxylases and phosphoglucomutase. Ingestion of excessive quantities of Mn appears to interfere with absorption of Fe, thus causing anaemia, which is readily prevented by increasing the dietary Fe intake. Similarly, Cd is known to bind in significant quantities to a kidney SH-rich protein. In a study, India the milk was found to have significantly high levels of Pb and Cd than in the counterpart rural animals (Swarup *et al* 1997)

Table 3 Detection of heavy metals in ice cream

Sr. No	Fe (µg/100g)	Zn (µg/100g)	Cu (µg/100g)	Mn (µg/100g)	Pb (µg/100g)	Cd (µg/100g)
1	91.84	681.12	22.40	8.21	0.14	0.06
2	96.70	675.45	25.65	5.80	0.20	0.05
3	91.78	680.15	22.32	8.12	0.15	0.14
4	92.90	686.75	18.15	6.15	0.16	0.09
5	88.65	683.90	15.70	8.09	0.25	0.16
6	91.81	680.30	22.58	6.16	0.17	0.08
7	96.00	678.30	26.40	9.60	0.19	0.09
8	91.75	679.86	22.64	8.06	0.16	0.11
9	88.65	678.10	215.00	11.25	0.18	0.10
10	91.72	681.06	22.48	9.25	0.20	0.12
Average	**91.78**	**680.50**	**22.49**	**8.13**	**0.15**	**0.10**

Though the researchers did not detect any apparent toxicity symptoms, yet they have hinted at the risk of sub-clinical effects. It has been shown that Cd may not directly originate from milk but may come from glazes of packaging materials (Cabrera *et al* 1995). However, the value of Fe and Mn were found to be on the higher side when compared with the maximum allowed limit of heavy metals in processed cheddar cheese. Our research findings were comparable to those of Larson and Rasmussen (2003) and Zurera *et al* (1994).

Milk and milk products are an important part of daily food, because they provide all the essential nutrients necessary for the body growth. It is, therefore, desirable to compute and evaluate the level of trace elements in milk and milk products. The food quality control system in our country is far from adequate to meet the present challenges, which need to elaborate to make the effective system. This can only be achieved by awareness as well as adopting modern analytical technology (Saleem 2002).

Table 4 Detection of heavy metals in processed cheese

Sr. No.	Fe (µg/100g)	Zn (µg/100g)	Cu (µg/100g)	Mn (µg/100g)	Pb (µg/100g)	Cd (µg/100g)
1	645.30	420.26	28.92	8.99	0.24	0.57
2	648.90	416.70	30.70	7.45	0.17	0.66
3	656.75	419.96	29.30	9.05	0.23	0.58
4	653.65	412.80	25.75	11.55	0.22	0.65
5	651.95	424.10	26.80	6.75	0.29	0.59
6	650.70	420.32	29.40	9.11	0.21	0.63
7	652.45	419.86	28.84	5.80	0.19	0.62
8	653.65	420.15	27.40	8.95	0.25	0.64
9	652.12	425.65	29.21	13.65	0.24	0.57
10	650.58	421.30	35.0	9.08	0.26	0.59
Average	**651.65**	**420.11**	**29.13**	**9.04**	**0.23**	**0.61**

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Studies on the isolation and purification of carotenoids from fruits and vegetables

Ambreen Saddozai, Tabassum Hameed and Sadia Kousar
GQTL, National Agricultural Research Council, Chak Shehzad, Islamabad

ABSTRACT

Carotenoids were isolated and purified from such fruits as seabuckthorn, apricot, mango, peach, apple, loquat and vegetables including carrots, spinach, radish and tomato. These were processed and dehydrated at 60°C. Total carotenoids were extracted by solvent extraction method, percentage of carotenoids were determined spectrophotometerically at 452 nm against β-carotene. Results indicated that seabuckthorn had 5.3 mg/ 100g β-carotene content which was highest among all the fruits examined, whereas in vegetables the β-carotene content was high in carrots which ranged from 7.92-9.18 mg/100g.
Keyword: Carotenes, fruits, vegetables.

INTRODUCTION

The color of fruits and vegetables is very important from the point of view of ultimate quality of the product. The chief pigments of fruits and vegetables, which impart the color, are carotenoids, chlorophylls, anthoxanthin and anthocyanins. The name carotenoids is applied to all pigments chemically related to the carotenes. Carotenoids are plant pigments that are present in human diet as micro components of fruits and vegetables (Ranganne 1977). These are a group of aliphatic alicyclic, fat soluble, yellow to red compounds responsible for red, orange and yellow color of edible fruits and vegetables and widely distributed in nature (Bauernfeind 1981). Carotenoid pigments are important for human and animal nutrition because of conversion of some of them into vitamin A. Vitamin A is an essential micronutrient required for vision and variety of metabolic functions in the body. Its deficiency is a major cause of night blindness among preschool children (Graham 1988). In developing countries more than 80 % of the dietary vitamin A is supplied by carotenoids present in plant foods (Bhaskarachari *et al* 1995).

These act as antioxidants, can prevent the onset of cancer especially. lung cancer and reduce the risk of heart diseases (Shekelle *et al* 1981). β - carotene was the first synthetic carotenoid to be marketed as a food color which is added to many products like butter, popcorn, salad dressing, beverages, baked goods, dry mixes and soups (Gorden and Bauerfeind 1983). According to the statistical data provided by Federal Bureau of Statistics of Pakistan in 1990-91, Pakistan imported carotenes worth 22.44 million rupees in 1990.The main objective of the present study was to extract, purify and identify the carotene content of various locally available fruits and vegetables in order to find out their suitability for application in the industry.

MATERIALS AND METHODS

Collection of Samples

Different fruits (apricot, mango, persimmon, peach, grapefruit, loquat, citrus varities, seabuckthorn, and apple) and vegetables (carrots, spinach, radish, tomato) were procured from local market, processed and dehydrated at 60°C to constant moisture content. Moisture content was determined in dried samples according to AOAC (2005).

Preparation of Standard Curve

0.1 g β - carotene (Sigma C-0126) was dissolved in 20 mL petroleum ether and 3 mL. chloroform mixture, filtered and made various concentration of supernatant by taking 1, 2, 3, 4 and 5 mL of the filtrate in 100 mL volumetric flask and diluted to mark with petroleum ether. The concentration was 1.24, 2.49, 3.79, 4.98 and 6.23 mg/mL. Absorbency was measured at 452nm by using 1 mL chloroform and 20 mL petroleum ether as blank.

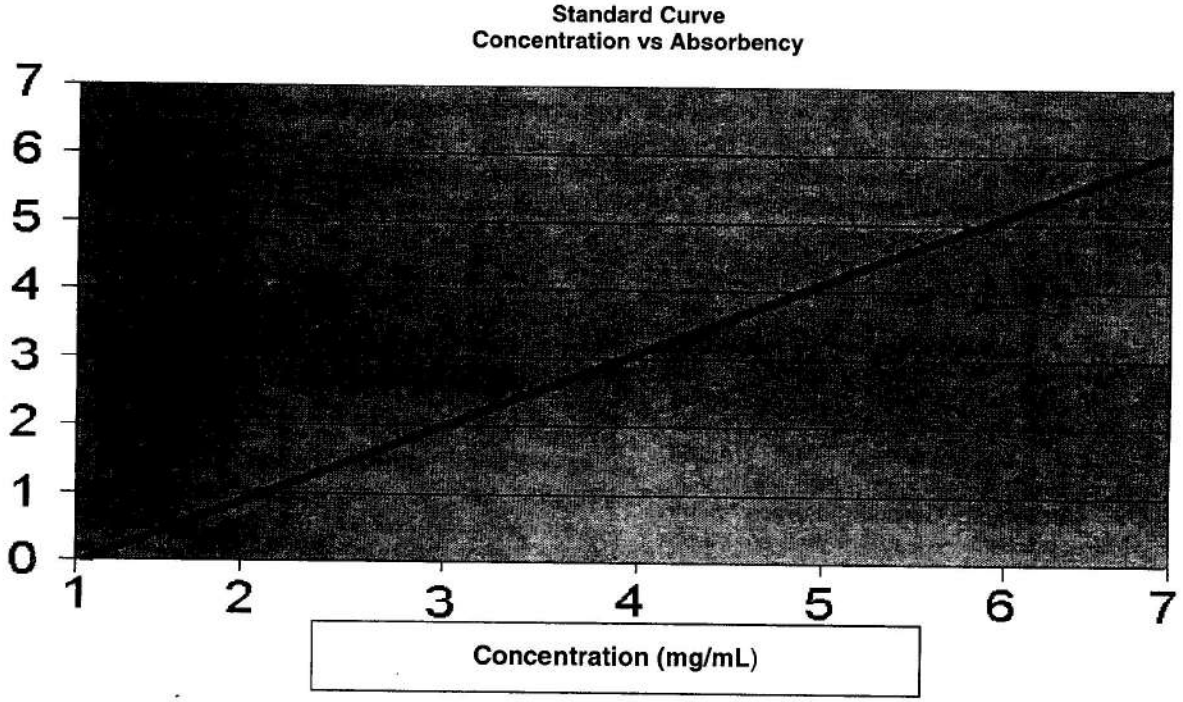
Extraction, Separation, Determination and Identification of Carotenes

Dried samples were ground and total carotenoids were extracted from the ground material with acetone-hexane mixture at the ratio of 3:7 by using solvent extraction technique and percentage of carotenoids were determined by using double beam spectrophotometer at 452 nm against β - carotene. The values were calculated from standard curve (Ranganne 1977).

Chromatographic column was prepared with 1:1 mixture of activated magnesia and diatomaceous earth (Supercel). One cm thick layer of anhydrous sodium sulphate was placed above the absorbent in

the column. 50 mL hexane was added in the column. After washing the column with hexane, 1 mL of carotene extract was added in the column. The top of the column was kept with a layer of solvent during entire operation.

0.52mg/100g in April and decreased in May. CV ranged from 2.04 to 5.89%. In methi, tomato, spinach leaves and radish carotene contents were 5.92 mg, 4.12 mg, 5.82 mg and 5.22 mg/100g respectively with CV of 0.61%, 0.33% 0.78% and 0.43%.



Carotenes passed rapidly through the column, whereas bands of xanthophylls, lycopene and chlorophyll were absorbed in the column. The elute was collected. Bands of lycopene, xanthophylls, and chlorophyll that were absorbed on the column were separated through solvent and collected (Ranganne 1977). After separating the different fractions from column chromatography, β-carotene was identified by thin-layer chromatography (Stahl 1965).

Statistical Analysis

The values were statistically analyzed for mean, standard deviation and coefficient of variation (Steel *et al* 1980).

RESULTS AND DISCUSSION

Total carotenoids content in vegetables were determined periodically during the season of each vegetable (Table 1). The mean value of carotene content in *Moringa oleirfera* leaves was 4.75mg/100g in February with CV of 0.25% which increased to 5.21mg/100g in March with CV of 0.60% and remained constant till May. Similarly, the maximum value of carotene content in calocasia was

In carrots, the results showed that value of carotene content increased with increase in time (Table-1).In October, carotene content in carrots was 7.91mg/100g which remained constant upto December, with CV of 0.12%.

There was an increase in carotene content upto the month of March in which the value was 8.84 mg/100g with CV of 3.08% which is the highest value in the present studies, March being the peak season of carrots. During this month the carrots are fully mature, sound and bright red in color. After the month of March the carotene content decreased gradually.

In the month of November the value decreased to 7.95 mg/100g with CV of 0.12%. The results obtained in these studies could be related to the findings of Bhaskarachari *et al* (1995), who reported 8.85 mg of pigment /100g of dry carrots.Different fruits have also been analyzed for the determination of total carotenoids content. The results have been summarized in Table 2. Carotene content in peach, apricot, apple, grape fruit, mango, persimmon and seabuckthorn were 2.5, 3.95, 0.05, 1.34, 3.97, 4.41 and 5.46 mg/100g respectively. Carotene content

was also studied in citrus peel. Total carotenoids in orange peel, Kinnow peel and sour orange peel were 1.44, 1.93 and 0.63 mg/100g respectively. The value of carotene content in Kinnow peel was high as compared to sour orange and orange peel. It was observed that among all the analyzed fruits carotene content was high in seabuckthorn fruit, while low in apple with CV of 2.67 to 2.56% respectively. Similar studies were also carried out by Bhaskarachari et al (1995). The present studies revealed that out of all fruits and vegetables carrots and seabuckthorn are rich in carotene content.

Table-1 Total carotenoids content in different vegetables

S.No	Sample	Month	Carotene content mg/100g		S.D	C.V
			Range	Average		
1	Carrots	October	7.90-7.93	7.927	0.015	0.19
		November	7.92-7.95	7.937	0.015	0.19
		December	7.97-7.99	7.980	0.010	0.12
		January	8.19-8.21	8.200	0.010	0.12
		February	8.83-8.85	8.840	0.010	0.11
		March	8.69-8.97	8.837	0.140	1.59
		April	8.43-8.44	8.423	0.020	0.24
		May	8.49-8.50	8.497	0.005	0.06
		June	8.00-8.03	8.013	0.015	0.19
		July	7.90-7.92	7.910	0.010	0.12
		August	7.91-7.94	7.927	0.015	0.19
		September	7.94-7.96	7.950	0.010	0.12
		October	7.89-7.93	7.913	0.020	0.26
		November	7.94-7.96	7.950	0.01	0.12
2	Moringa Oleirfera (leaves)	February	4.74-4.76	4.750	0.01	0.21
		March	5.02-5.08	5.053	0.030	0.6
		May	5.19-5.25	5.213	0.032	0.61
3	Colocasia Antiquorum	March	0.40-0.45	0.427	0.025	5.89
	(Arum)	April	0.50-0.54	0.523	0.020	3.98
		May	0.48-0.50	0.490	0.010	2.04
4	Moringa pods	June	0.55-0.58	0.563	0.015	2.71
		July	0.61-0.65	0.627	0.020	3.31
5	Spinach	April	5.80-5.85	5.827	0.025	0.43
6	Radish (red)	April	5.20-5.25	5.223	0.025	0.48
7	Methi	May	5.90-5.94	5.920	0.020	0.33
8	Tomato	May	4.10-4.15	4.123	0.025	0.61

CV= Coefficient Variance

TABLE-2 Total carotenoids content in different fruits

S.No	Sample	Month	Carotene content mg/100g		S.D	C.V
			Range	Average		
1	Apricot	May	0.81-0.86	0.833	0.025	3.02
		June	2.90-2.94	2.920	0.02	0.68
		August	3.90-3.95	3.927	0.025	0.64
2	Mango	August	3.95-4.00	3.977	0.025	0.63
		September	3.50-3.56	3.527	0.030	0.86
		October	2.89-2.92	2.903	0.015	0.52
3	Persimmon	October	4.40-4.43	4.417	0.012	0.28
4	Peach	November	2.49-2.53	2.507	0.020	1.55
5	Grape fruit	March	1.32-1.36	1.343	0.020	2.05
6	Loqat	June	0.002-0.005	0.003	0.001	1.38
7	Citrus varieties					
A	Orange Peel	March	1.42-1.46	1.440	0.020	1.37
B	Kino Peel	March	1.90-1.95	1.930	0.026	2.39
C	Sour Orange Peel	March	0.62-0.65	0.637	0.015	2.79
8	Seabuckthorn pulp	Oct- Nov	5.30-5.60	5.467	0.152	2.67
9	Apple	October	0.056-0.059	0.057	0.001	2.56

CV=Coefficient Variance

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Determination of yield and fruit characteristics of various Baltistan dried apricot cultivars

Muhammad Shahnawaz* and Tariq Umar Khan**
*Department of Food Technology, Karakurum International University Gilgit, Northern Areas.
**Pakistan Council of Scientific and Industrial Research, Skardu.

ABSTRACT

Twelve apricot varieties of Baltistan i.e. Halmand, (V1) Wahphochuli (V2), Lonakpochuli (V3), Sherakarpochuli (V4), Shakhanda (V5), Margulam (V6), Karpochuli (V7), Ambah (V8), Stachochuli (V9), Khochuli (V10), Brochuli (V11) and Bephochuli (V12) were evaluated for their characteristics as dried apricot. Two pretreatments namely sulfuring (1000 ppm) and sulphiting (3g/L water along with 0.5% citric acid for 5 minutes) were used. Fruits were dried by solar drying and semi-commercial dehydrator. Results revealed that there were non significant differences between V1 - V4 as showed similar color, texture, structure and taste. However, sufficient differences were observed in V5-V12 for various quality attributes particularly color/texture, taste and weight lost. The research work suggests that apricot samples V1-V4 are better varieties for having good characteristics after drying for commercialization. However, V5-V12 were correspondingly ranked for the same characteristics. It was also observed that drying whole-apricot gave better dried product than halved.
Keywords: Apricot varieties, sulfuring, sulphiting, sun-drying and dehydration.

INTRODUCTION

Apricot (*Prunus armeniaca L.*) is believed to have originated in the mountainous region of north central and north western China where it has been cultivated for 4000 years. Through the centuries, seeds were brought into Central Asia and then following Alexander the Great's Military expeditions, through into the Middle East. World production occurs generally in two broad bands between about 25^0 and 45^0 latitude and seems well adapted to soils of around pH 6 to 8. (Wills 1987).

As far as Northern areas of Pakistan for apricot production are concerned, they are situated all along the water courses where more than 90% of the population depends upon agriculture. However, fruit production is the major source of income. According to the UN-PK/FAO/2003 survey report, Northern areas produce about 170680 tons fruits per year; apricot alone contributes 107737 tons (UN-PK-FAO. 2003). It is reported that due to lack of know-how, 50-70% fruits goes wasted. It is further reported that in Northern areas particularly in Baltistan, apricot is used in routine diet of the people in an orthodox manner like apricot mixed with barley flour, cooked dried apricot with wheat flour in winter and kernel oil used to meet fat requirements, etc. However, latest methods are needed to introduce to utilize this natural wealth in good manner and also enable people to generate income.

Fruit pieces are widely used in food formulations and maintenance of natural color, flavor and satisfactory texture for consumer acceptance (Dall'Aglio et al 1986; Mastrocola et al 1995). Fruit ingredients should also show well defined functional properties in order to be compatible with the food system without affecting shelf life. The compatibility of the fruit with other components depends, basically, on the equilibrium of the respective water activity values (Maltini et al 1993).

Likewise, an increase in soluble solids uptake and further reduction of water activity, were achieved by a direct osmosis pretreatment before dehydration of fruits (Hawkes and Flink, 1978; Lerici et al 1985; 1988).

Objectives

The objective of this study was to determine most suitable varieties of Baltistan apricots for yield and quality attributes/characteristic on drying. The second aim was to facilitate apricot growers and dealers to select suitable apricot varieties available at Baltistan for commercialization.

MATERIALS AND METHODS

Materials

Twelve apricot varieties of Baltistan i.e. Halmand, (V1), Wahphochuli (V2), Lonakpochuli (V3), Sherakarpochuli (V4), Shakhanda (V5), Margulam (V6), Karpochuli (V7), Ambah (V8), Stachochuli (V9), Khochuli (V10), Brochuli (V11) and Bephochuli (V12) were purchased from local farmers of Skardu.

Determination of weight and TSS

5 Kg samples were weighed by a digital balance from each variety. Total soluble solids (TSS) were determined by using Atago Refrectometer at ambient temperature (22 ^{0}C).

Preparation of samples

All varieties were cleaned off from all impurities and sorted out for homogeneous shape and size. Fruits were then thoroughly washed and blanched (submerging whole apricot in boiling water for 1 minute to avoid browning and enzymatic activity). Water was drained from blanched samples through fanning. Fruits were kept in sulfuring chamber for 3-4 hrs for sulfur fumigation.

Use of chemicals

Powdered sulfur 1-2g and potassium metabisulphite 5g were used as preservatives prior to and during drying process. In addition, citric acid at the rate of 0.5% was also used in dipping solution to improve the functioning of $S0_2$ and preserve the color.

Determination of SO_2

To determine SO_2 in dried apricot, titration method was used and the final results were calculated by the following formula:

$$\text{ppm } SO_2 = \frac{32 \times 1000 \times \text{Normality of NaOH} \times \text{NaOH used}}{\text{Wt. of sample}}$$

Drying

Two types of driers i.e. solar dryer (average sun heat range 40-45^{0}C) and cabinet type electric dryer (temperature adjusted at 50^{0}C) were used.

Analytical methods

To determine moisture content in dried apricots a vacuum oven was used. For testing quality attributes, sensorial evaluation was practiced.

RESULTS AND DISCUSSION

Weighed apricot samples were cleaned off from all impurities and blanched in boiling water for 1 minute and then removed water from the blanched fruits through fanning. Pretreatment i.e. blanching, sulfuring and sulphiting to enhance certain features like color/texture and shelf-life were applied accordingly. Mahmutoglu *et al* (1996) reported that pretreatment effects the drying method combinations on the drying rates, quality and storage stability of apricot. Gowda *et al* (2000) also evaluated certain pretreatments like blanching and sulfuring for good quality raisin making. Gowda *et al* (2000) assess the combination of pretreatments consisting of lye-treatment + dipping oil+ sulfuring + shade drying was best for producing food quality raisins.

The pretreated samples were placed on the wooden trays without that they touch each other (left 1 cm between them). Trays were sterilized (given 12 hrs sulfur fumigation) before use to the process. Fruit loaded trays were shifted into sulfuring box and subjected to continuous smoke for 3 hrs. Before and after smoking, ventilation window/holes remained opened for few minutes and than closed accordingly. For sulfur fumigation, air tight plastic tent absolutely closed from every side was utilized, however; a sulfur burning generator outside the tent was established and facilitated the smoke entering the tent continuously up to 3 hrs. McLaren (1996) applied sulfur for brown rot control in central Otago to stone fruit. Sulfured samples (5 kg of each variety whole/halved) were shifted to a solar hybrid drier which uses solar air collector for preheating the drier. After 1-2 sunny days at an average temp of 40-45 ^{0}C (fluctuation in the drying temperature due to uneven levels of solar radiation was observed and this shortcoming was overcome by use of a rotary tray system).

Table I: Fresh wt, stone wt, consistency and TSS of selected apricot varieties.

Variety	Fresh wt. (No/Kg)	Stone wt. (g/Kg)	Consistency	TSS
Halmand V1	22	100	More Flashy	29
Wahphochuli V2	25	110	do	27
Lonakpochuli V3	24	110	do	26
Sherakarpochuli V4	23	112	Light Juicy	24
Shakhanda V5	22	100	Flashy	28
Margulam V6	18	120	Juicy	22
Karpochuli V7	23	115	Light Juicy	20
Ambah V8	20	118	Juicy	22
Stachochuli V9	30	110	Light Juicy	19
Khochuli V10	32	110	Light Flashy	18
Brochuli V11	35	120	do	16
Bephochuli V12	38	100	Juicy	25

Likewise, other samples (5Kg of each variety whole/halved) were kept into the electric drier at 50 ^{0}C for 18 and 14 hrs for whole and halved apricot respectively and these times have observed up to the final product. The semi-dried apricot were de-stoned

and again kept for more drying. Tsamparlis (1990) studied solar drying for real applications.

Table 2: The varieties name, natural color, maturity time and manual picking quality/status.

Variety	Natural color	Maturity time	Picking quality
Halmand V1	Bright red	End July-August	Easy
Wahphochuli V2	do	Do	do
Lonakpochuli V3	Light red	Early August-Mid September	Little difficult
Sherakarpochuli V4	White	Early July-Mid August	Easy
Shakhanda V5	Light red	Do	do
Margulam V6	Light brown	Do	do
Karpochuli V7	White	Do	do
Ambah V8	Light yellow	Do	do
Stachochuli V9	Red-Light brown	July-September	do
Khochuli V10	do	Do	do
Brochuli V11	Light yellow	Mid August-Mid October	Difficult
Bephochuli V12	Light red	July-August	Easy

Table 3: Percentage moisture content, weight lost dried weight and shelf life of dried apricot.

Variety	%moisture content	Wt. lost (%/g)	Dried wt. (%/g)	Fresh wt. (g)	Shelf life	Storage temp.
Halmand V1	18-20	70 (3500)	30 (1500)	5000	12 months	5-10 °C
Wahphochuli V2	18-20	71 (3550)	29 (1450)	do	do	do
Lonakpochuli V3	18-20	71 (3550)	29 (1450)	do	do	do
Sherkarpchuli V4	18-20	72 (3600)	28 (1400)	do	do	do
Shakhanda V5	18-20	70 (3500)	30 (1500)	do	do	do
Margulam V6	18-20	80 (4000)	20 (1000)	do	do	do
Karpochuli V7	18-20	75 (3750)	25 (1250)	do	do	do
Ambah V8	18-20	78 (3900)	22 (1100)	do	do	do
Stachochuli V9	18-20	71 (3550)	27 (1450)	do	do	do
Khochuli V10	18-20	71 (3550)	27 (1450)	do	do	do
Brochuli V11	18-20	80 (4000)	20 (100)	do	do	do
Bephochuli V12	18-20	71 (3550)	29 (1450)	do	do	do

Senhaji *et al* (1991) investigated the data on apricot drying kinetics and product quality. The next day, dried apricots were dipped in the solution of potassium metabiusulphite and citric acid at the rate of 3g and 0.5% per liter of water for 5 minutes respectively was maintained to control browning and bring additional shineness/brightness of the end product. Reports revealed that sulfuring and sulphiting for dried apricots are prominent methods which improve color and shelf-life of the dried products.

Table 4: Structure, texture, color and taste of the dried apricot

Variety	Structure	Texture	Color	Taste
Halmand V1	Best	Best	Best	Best
Wahphochuli V2	Better	Better	Better	Better
Lonakpochuli V3	Better	Better	Better	Better
Sherkarpchuli V4	Better	Better	Better	Better
Shakhanda V5	Good	Good	Good	Good
Margulam V6	Good	Good	Good	Good
Karpochuli V7	Fair	Fair	Fair	Fair
Ambah V8	Fair	Fair	Fair	Fair
Stachochuli V9	Poor-Fair	Poor-Fair	Poor-Fair	Poor-Fair
Khochuli V10	Poor-Fair	Poor-Fair	Poor-Fair	Poor-Fair
Brochuli V11	Poor	Poor	Poor	Poor
Bephochuli V12	Poor	Poor	Poor	Poor

The fruits were again shifted to the solar dryer for 3-4 hrs (depends upon the intensity of sunrays and dehydrator for half hr and average final moisture contents was maintained 18-20%. Dried fruit were cool down before packing and then packed in clean thick plastic bags (food grade). Stored the packed fruits in the dark cool place and checked their shelf life periodically up to one year (results shown in Table V). Mahmutoglu et al (1996) studied the effects of initial SO_2 concentration as well as the SO_2 source (dried SO_2 gas treatment vs. Dipping into sodium metabisulphite solution on drying rate and storage stability of dried apricot throughout a time of 8-12 months were investigated after making paired comparisons. Alizai *et al* (1997) studied the treatments with polyphenol oxidase (catechol oxidase inhibitors prior to drying by sun or dehydration gave brighter, shiny, flexible product than treatment with SO_2. However, sulfuring was more effective for maintaining the shelf life of fruits beyond 6 months.

Sensory evaluation

After one year storage, the sensory characteristics of all the dried apricot samples for various quality

attributes like texture, structure, color, taste, shelf life and general acceptability was carried out by using a technical rating chart filled by the experienced judges. Table VI indicated conclusive results that all the dried apricot samples as showed their response during storage. The score chart was used for final effects as good, better and best grade.

CONCLUSIONS

This research work narrates the variety characteristics of Baltistan apricot for drying and dehydration. According to the results, V1-V4 ranked best drying varieties among all. However, the other varieties V5-V12 were correspondingly ranked good for drying purposes. This achievement enables the dried apricot producers and entrepreneurs to priorities the apricot varieties/cultivars on the basis of their high quality characteristics for the best results and return.

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Submerged fermentation of acidic protease by *Rhizopus arrhizus* PTCC-1

Muhammad Nadeem*, Quratulain Syed, Shahjahan Baig and Fouzia Iqbal
Food & Biotechnology Research Center, PCSIR Laboratories Complex, Lahore-.
*Corresponding author (mnadeempk@yahoo.com)

ABSTRACT

Different agro-industrial by-products such as rice husk, rice straw, wheat bran, soybean meal, sunflower meal and cotton seed meal were screened for the production of extra-cellular protease by *Rhizopus arrhizus* PTCC-1 in submerged fermentation. Maximum enzyme synthesis was detected in soybean meal (16.23±1.11 PU/mL), followed by rice husk medium cultivated at temperature 37°C for 72 hrs. Effect of various pH values and agitation speed were studied to increase the yield of acidic protease. Maximum proteolytic level was obtained at pH 5 with agitation speed of 140 rpm.

Keywords: Acidic protease, soybean meal, submerged fermentation and *Rhizopus arrhizus* PTCC-1

INTRODUCTION

Proteases execute a large variety of functions in different industries. The main sources of these enzymes are animals (e.g. calf stomach) and plants (e.g. pineapple, fig, papaya). Due to irregular production associated with the plant sources and large numbers of ethical and moral issues related to animal sources, microbial sources occupy an important place in the production of all the three major types of proteases viz: acidic, neutral and alkaline protease (Rao *et al* 1998). Microorganisms, especially fungi, owing to their GRAS (Generally Regarded as Safe) nature, have now become popular, especially with respect to enzyme applications in the food industries (Pandey 1992).

Rhizopus sp. are important major moulds in fermentation. Several reports describe the efficient protease biosynthesis by fungi belonging to the genera *Aspergillus* (Fan-Ching and Lin 1998), *Penicillium* (Chrzanowska *et al,* 1993), *Rhizopus* (Farley and Akasari 1992), and *Humicola* (Aleksieva and Peeva 2000). Although bacterial proteases have long been used in the industry, the main drawback of their use is that they require cost-intensive filtration methodologies to obtain a microbe-free enzyme preparation. On the other hand, proteases of fungal origin offer an advantage, that is the mycelium can easily be removed by filtration (Phadatare *et al* 1993).

The present work was undertaken to produce acidic protease by employing different substrates in submerged fermentation. Various process parameters such as temperature, incubation period, pH and agitation speed were optimized to get the maximum yield of enzyme from *Rhizopus arrhizus* PTCC-1.

MATERIALS AND METHOD

Mould Culture

The fungus culture of *Rhizopus arrhizus* PTCC-1 was obtained from PTCC (Pakistan Type Culture Collection), Food and Biotechnology Research Center, PCSIR Laboratories Lahore. The culture was revived on Potato Dextrose Agar (PDA) at 37°C and was maintained on PDA (Oxoid) at 4°C.

Preparation of Spore Suspension

A spore suspension of $1x10^{6-8}$ was prepared by adding 10 mL sterile distilled water in 7 days old slant. The spores were scratched by sterile wire loop to break clumps to form homogenous spore suspension. Five mL of the spore suspension was used for inoculation.

Screening of Substrates

Different agro-industrial residues such as rice husk, rice straw, wheat bran, soybean meal, sunflower meal, cotton seed meal and rape seed cake were used for the selection of best substrate for acidic protease production.

The 2.0 % quantity of each substrate was used in growth medium consisting of yeast extract (0.5%), KH_2PO_4 (0.4%), NaCl (0.1%) and $MgSO_4$ (0.05%). The pH of the medium was adjusted at 5.0 with 1 N HCl/ NaOH before sterilization.

Screening of substrates was carried out in triplicates in 250 mL Erlenmeyer flasks, each with 50 mL medium. The flasks were agitated at 37°C for 72 hrs on a water bath shaker (Eyela Japan) operating at 140 rpm.

Optimization of process parameters

Different process parameters influencing the production of protease such as fermentation time (24, 48, 72, 96 and 120 hrs), fermentation temperature (16, 23, 30, 37 and 42^{0}C), pH of the growth medium (3, 4, 5, 6 and 7) and agitation speed (80, 100, 120, 140 and 160 rpm) were optimized for maximum production of proteolytic enzyme. All experiments of the process parameters were performed in triplicates.

Analytical Procedure

Proteases Assay

Proteases activity was assayed by the modified method of Anson *et al* (1938) using casein as a substrate. The reaction mixture containing 2 mL 1.0% casein in 0.5 M phosphate buffer (pH 5.0) and 1.0 mL suitably diluted enzyme was incubated at 40^{0}C for 30 minutes. The reaction was terminated by adding equal volume of 10.0% w/v of trichloroacetic acid and filtered through Whatman No. 1 filter paper. To 1 mL of the filtrate 5 mL 0.5 M Na_2CO_3 solution and 0.5 mL of three fold diluted Folin-Ciocalteau reagent were added and mixed thoroughly. The color developed after 30 min of incubation at 30^{0}C was measured at 660 nm. One unit of the proteolytic activity was defined as the amount of enzyme required to liberate 1µg tyrosine in 30 minutes at 40^{0}C.

Determination of Protein

Protein concentration was measured by the method of Lowery *et al* (1951) using bovine serum albumin as a standard.

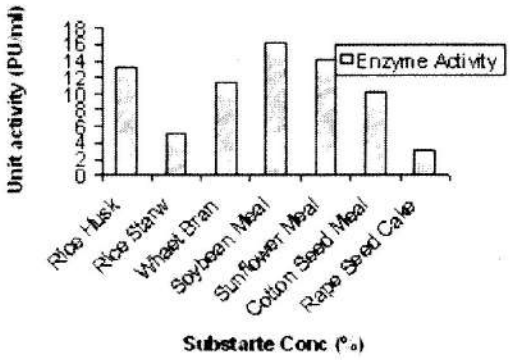
RESULTS AND DISCUSSION

Screening of substrates

Fermentation process is governed by a large numbers of physical, chemicals and biological factors. However, selection of the nutrient contents of both carbon and nitrogen sources have a great impact on enzyme production and play a vital role in the production processes.

The effect of different substrates on the production of acidic protease by *Rhizopus arrhizus* was investigated (Fig 1). Maximum yield of protease (16.23 ±1.11) was obtained from soybean meal based medium followed by sunflower meal (14.19 ±1.23) as a substrate. However, minimum enzyme activity (3.12 ±0.81) was observed in rape seed cake that might be due to the presence of some inhibitors in it. Cinthia and Rosana (2000) also found that soybean meal is the best substrate for the production of enzymes (160 U/ mL) followed by wheat bran (120 U/ mL). Ikram ul Haq *et al* (2004) also observed that soybean meal medium produced maximum enzyme activity 58 U/g.

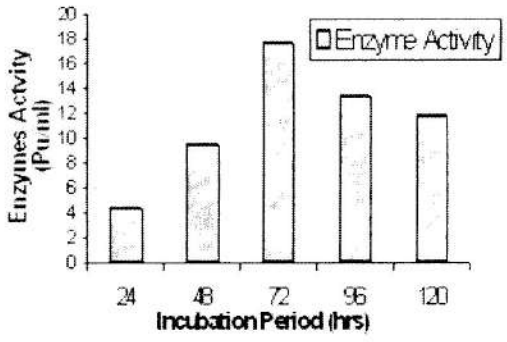
Figure 1. Screening of different carbon source for maximum acidic protease productin by *Rhizopus archizus* PTCC-1



Optimization of process parameters

Time course experiments (Figure 2) revealed that maximum enzyme production (17.56 PU/ mL) was obtained after 72 hrs fermentation period. Thereafter, enzyme activity decreased subsequently that could possibly be due to cessation of the production as enzymes are primarily metabolites and it also could be due to the inactivation of the enzymes. Sumantha *et al* (2005) reported that maximum enzyme production after 48 hrs fermentation period by *Aspergillus sp.* This might be due to the difference in the culture and mode of fermentation. Our findings are in line with Samarntarn *et al* (1999) who observed maximum enzyme activity after 72 hrs by genetically engineered *Aspergillus oryzae* U1521.

Figure 2. Effect of in incubation period on acidic protease production by Rhizopus arrhizus PTCC-1



Figures 3 and 4 depict the effect of temperature and pH on the protease production respectively. Maximum yield of protease was found at 37°C and pH 5.0. As the temperature increases beyond the optimized temperature, the protease production decreases. Ikram and Hamid (2004) found maximum enzyme activity at 30°C and pH 5.0 by *Rhizopus oligosporus* IHS_{13} in low cost medium. Other workers (Tremacolidi and Eleonora 2005) found maximum enzyme activity in a culture medium containing glucose and casein at 1.0 % (w/v) as a substrate at 25°C by *Aspergillus clavatus.*

Figure 3. Effect of temperature on acidic protease production by *Rhizopus arrhizus* PTCC-1

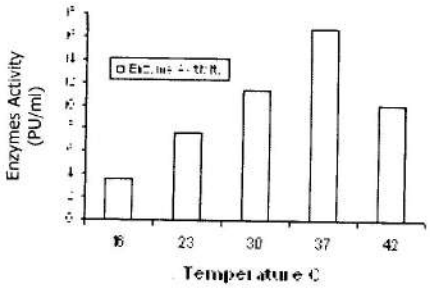
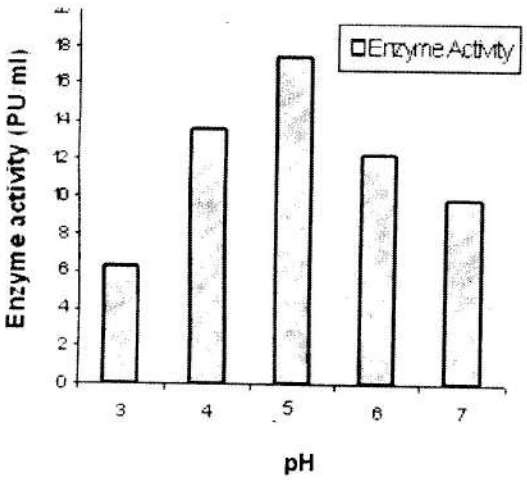
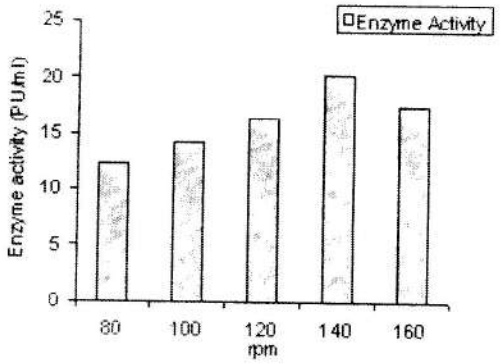


Figure 4. Effect of pH on the production of protease production by Rhizopus arrhizus PTCC-1



Proper aeration is the basic need for maximum growth of aerobic microorganisms. Agitation speed plays an important role in aeration and even distribution of oxygen throughout the growth medium. Effect of different agitation speed on protease production is shown in Figure 5. Maximum yield of acidic protease (20.12 PU/ mL) was recorded at 140 rpm shaking in Eyela, Japan. Dahot (1993) obtained maximum proteases at 220 rpm in 1.0% rice husk medium by *Penicillium expansum.* Cesar and Facundo (2003) studied the effect of agitation rate and aeration on the production of protease. They obtained maximum enzyme yield (5.28 units/mg) at 700 rpm/m and 0.5 v/v/m. All these findings indicate that hydrodynamic conditions affect the enzyme production in submerged fermentation.

Figure 5. Effect of agitation speed (rpm) on the production of acidic protease by *Rhizopus arrhizus* PTCC-1



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For further information please visit our booth H17 at FI Asia 2006 and learn more about ORAFTI's ground-breaking research programme, the most up-to-date application developments and the latest successful products from around the world.

[1] International Osteoporosis Foundation.
[2] Abrams, S.A., Griffin, I.J. Hawthorne, K.M., Liang, L., Gunn, S.K., Darlington, G., Ellis, K.J. (2005)
A combination of prebiotic short- and long-chain fructans enhances calcium absorption and bone mineralization in young adolescents. *American Journal of Clinical Nutrition*, 82, 471-476.

About ORAFTI Group

ORAFTI Group is a subsidiary of the Belgian agro-food group Raffinerie Tirlemontoise/ Tiense Suikerraffinaderij and is part of the ORAFTI/ PALATINIT Ingredients Group of Südzucker (Germany).

ORAFTI Group produces Beneo™ inulin and oligofructose, Raftifeed® for animal nutrition, as well as Raftisweet® (fructose syrups), from chicory roots. ORAFTI Group's head office is in Tienen, Belgium and the company operates in more than 75 countries worldwide, with production units in Oreye (Belgium), Chilean (Chile), Wijchen (Netherlands) and Wijgmaal (Belgium). The ORAFTI Group also includes REMY INDUSTRIES, world leading producer of rice starches (Remy®/Remyline®/Remygel®), rice flours (Remyflo®) and rice proteins (Remypro®).

ORAFTI Asia Pte. Ltd.
41 Science Park Road, #01-17
The Gemini Science Park II
Singapore 117610
Singapore
Tel: +65 6778 8300
Fax: +65 6778 2997
Email: afi.asia@orafti.com
www.orafti.com

For more information

For more business information:
Koen Van Praet
ORAFTI
Regional Manager Asia
Email: koen.van.praet@orafti.com

For more press information:
Mark Tho
ORAFTI
Marketing Coordinator Asia
Email: mark.tho@orafti.com