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ASSESSMENT OF QUALITY PARAMETERS IN PUNJAB WHEATS

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ABSTRACT

232 samples were procured in 1999-00 and 2000-01 from all over Punjab to determine wheat quality parameters – test weight, 1000 Kernel weight, protein contents, flour yield, ash percent, pearling index, foreign matter, fungal and insect damage. Quality of wheat was found good. The results were consistent both years. Wheat samples had high test weight, protein and flour yield. The kernels were medium in size. The foreign matter tended to be high. The values indicated that Punjab wheats meet international quality standards as applied in Australia and USA. There were, however, wide variation in samples so the ranges had wide margins. This indicated that varied quality needs of end-users can be met and that there is a further scope for quality improvement. This was supported by differences in values between years. The general pattern of data over both years was similar, yet the first year (1999-00) values tended to be high than the second year (2000-01) values. This was due to dry spell and shortage of canal water in the second year. The water stress resulted in lower average test weight, 1000 kernel weight and flour yield during 2000-01. The lower limits of ranges were pushed lower in the second year also. So improvement in crop agronomy and harvest technology can make Punjab wheat further competitive in world market.

INTRODUCTION

Pakistan has attained self sufficiency in wheat production (FAO, 2001) and is ready to enter export market where she will have to compete with long established wheat exporters. International market is dominated by few countries – USA, Canada, Argentina, Australia, W. Europe – who produce sizeable export surplus and together contribute over 90 % to wheat export channel annually (FAO, 2000). The crop production system in these countries is geared to export needs and they have well defined wheat quality grades and standards.

Pakistan has been a food deficit country for long. It has been averagely importing two million tons wheats each year during the past decade to meet its growing food needs (Government of Pakistan, 2001). Production was geared to local market which is neither quality conscious nor sufficiently diversified to demand exacting standards. Crop improvement programs were more bulk oriented than catering for quality needs of various end-users. The wheat varieties developed were general purpose and put to all uses – chapati, bread, confectionery, noodles and spaghetti etc. These end-uses have different quality requirements and, therefore, specific wheat varieties are developed and put into use to meet the requirements of these products in advanced countries (Williams, 1986; Pomeranz, 1987). Pakistani wheats which are developed mainly for their chapati quality may not fair well in wide world markets. However, Punjab wheats are grown over wide agroclimatic range and are expected to exhibit yield and quality differences (Khalifa, 1970; Al-Mashhadi et al, 1989; Chaudhry et al, 1995; Borghi et al, 1996).

It was decided to determine quality of Punjab wheats to know their competitiveness in international market. The study was initiated in Punjab where exportable surpluses are available.

MATERIALS AND METHODS

Two hundred and thirty two wheat samples were collected from the Food Department Stores all over Punjab from the purchased stock of two consecutive crops, 1999-00 and 2000-01.

Samples were drawn from August to September in 2001. The 1999-00 samples were, therefore, a year older than 2000-01 samples. Efforts were made to obtain samples from each district in Punjab. The Table 1 summarizes the number of samples obtained each year from various districts. Edible foreign matter consisting of grains of barley and oats; non edible foreign matter comprising of dirt, dust, stones, straw and weed seeds; insect damaged kernels and fungus affected/black tipped grains were hand picked and calculated as percent on weight basis. The samples were cleaned with 2 mm sieve to remove the shrunken/broken kernels for calculation of their proportion, again on weight basis. Protein contents (on dry basis Nx5.7), wet gluten, moisture, flour yield and ash percentage were determined as described by American Association of Cereal Chemists (AACC, 1983). 1000 kernel weight was taken on Sortirious electronic balance after counting cleaned samples on Numigral seed counter. Test weight (Kg/hl)) was determined with Schopper Chondrometer (Pomeranz, 1987). Pearling Index was calculated by pearling 20 g sample with Barley Pearler (Strong-Scott) for 60 seconds (Williams, 1986) to appraise hardness according to guideline given below:

Table 1. Distribution of samples drawn from different districts of Punjab.

No.	Locations	1999-00	2000-01	Total
1	Bahawalnagar	5	5	10
2	Bahawalpur	-	10	10
3	Mianwali	2	8	10
4	Lahore	4	6	10
5	Okara	4	6	10
6	Sheikhupura	-	10	10
7	Vehari	2	8	10
8	Kasur	6	4	10
9	Rajanpur	-	7	7
10	D.G. Khan	4	5	9
11	Sargodha	5	5	10
12	Sahiwal	5	5	10
13	Pakpattan	5	5	10
14	Gujrat	-	4	4
15	Khanewal	2	8	10
16	Sialkot	-	4	4
17	Jhang	5	5	10
18	Faisalabad	6	4	10
19	Bhakkar	4	6	10
20	Muzaffargarh	2	8	10
21	Lodhran	1	8	9
22.	Multan	4	6	10
23	T.T. Singh	5	5	10
24	Gujranwala	-	9	9
25	Rahim Yar Khan	5	5	10
	Total	76	156	232

Pearling Index	Wheat Hardness
30–40	Very hard
41–50	Hard
51–60	Medium
61–70	Soft
Over 70	Very soft

Mean, mode, ranges and standard deviation were worked out for each parameter using statistical "Minitab" computer software.

RESULTS AND DISCUSSION

The data are given in the Table 2 & 3. The results of the parameters measured were consistent over both years. The values were close and, therefore, bore confirmation for each other. Test weight, 1000 kernel weight, protein contents and flour yields were all high. Foreign matter and insect/fungal affected grains were over one percent each and were on slightly higher side (Table 2, 3). In 1999-00 samples, mean test weight, 1000 kernel weight and flour yield values were higher but those of wet gluten, pearling index and ash contents were lower than in 2000-01 samples. The mean test weight values were 77.1 and 76.5 Kg/hl for the years 1999-00 and 2000-01, respectively. The mode values in 1999-2000 were 76.2 and 78.2 Kg/hl which means more samples fell in these measurement groups. In 2000-01, the mode values were 76.2 and 76.5 Kg/hl showing a margin between samples in two years. The ranges showed narrow margins during first year than the second. The analysis of mean, mode and ranges, therefore, bespeak of deeper difference than visible through mean analysis alone. The 1000 kernel weight and flour yield almost followed the same pattern. The mean differences were not large. But range and

mode analysis of these traits showed narrow range and higher frequency of upper values in 1999-00 than in 2000-01. Apparently the year 1999-00 was a better year in expressing test weight, 1000 kernel weight and flour yield than the year 2000-01. This is consistent with the crop yields. The farmers harvested better yields in 1999-00 than in 2000-01. Since test weight and kernel size are component of yield, their increase in good harvest year is a well founded corollary.

Table 2. Quality analysis of Punjab Wheat* (1999-00)

Trait	Range	Mean	Mode	S.D
Test Weight (Kg/hl)	71.4 – 1.7	77.1	76.2, 78.2	2.1
1000 Kernel Weight (g)	33.0 –52.6	41.8	41.2, 47.6	3.8
Protein %	10.6 –14.1	13.0	12.1	0.7
Wet Gluten %	24.4 – 5.1	29.0	28.1	2.3
Moisture %	9.2 – 10.5	9.7	9.6 ,9.6, 9.7, 9.7, 9.9	0.3
Edible Foreign Matters %	0.1 – 4.5	1.2	0.1	1.1
Non-edible F. Matters %	0.1 – 3.3	0.5	0.0	0.7
Shrunken/Broken kernels %	0.0 – 4.6	1.1	0.2	1.1
Insect Damaged %	0.2 – 2.6	0.8	0.2	0.6
Fungus (Black Tipped Grains) %	0.0 – 1.9	0.4	0.8	0.6
Pearling Index %	15.0 –47.1	31.3	29.2, 33.1, 34.7, 35.2, 37.1	6.2
Flour yield %	64.3 –73.6	69.9	68.3, 69.2, 69.7, 69.8, 70.1, 70.3, 70.5, 72.2	1.9
Ash %	1.4 – 1.9	1.6	1.7	0.1

* Based on 76 samples

Table 3. Quality analysis of Punjab Wheat* (2000-01)

Trait	Range	Mean	Mode	S.D
Test Weight (Kg/hl)	69.3 –81.4	76.5	76.2, 76.5	2.0
1000 Kernel Weight (g)	26.4 – 56.2	41.0	38.5, 39.1, 39.3, 42.6	4.1
Protein %	10.7 –14.7	12.8	13.0	0.7
Wet Gluten %	24.8 –36.6	30.4	28.7, 30.4	2.2
Moisture %	9.1 –10.5	9.7	9.7	0.2
Edible Foreign Matters %	0.1 – 5.3	1.2	0.8	1.0
Non-edible F. Matters %	0.0 – 3.0	0.3	0.0	0.4
Shrunken/Broken kernels %	0.1 – 4.9	1.4	0.4, 0.4, 0.6	1.1
Insect Damaged %	0.1 – 1.1	0.3	0.1	0.3
Fungus (Black Tipped Grains) %	0.1 – 4.4	1.0	0.4	0.9
Pearling Index %	20.2 -48.6	35.5	40.9, 42.7	6.8
Flour yield %	63.4 –73.9	69.6	69.3	1.8
Ash %	1.4 – 1.9	1.7	1.7	0.1

* Based on 156 samples

Protein content is generally inversely related with the test weight and 1000 kernel weight. The mean protein value was slightly higher in 1999-00 when the grain weight and test weight were higher. This apparent anomaly was offset by an examination of the ranges and their mode values during the two years. The mode protein value was almost one percent higher during the second year – the year of falling test weight and 1000 kernel weight. The protein content range was higher also in second year (2000-01). Wet gluten contents which are the function of protein contents in the grain showed this relationship better. The mean, mode and range all had higher values in 2001-02 than in year 1999-00.

Black tipped grains were more in the second year crop (0.4 vs. 1.0%). This was due to rains during crop harvest in 2000-01. The fungi causing black-tip disease are known to be more active if rains occur during harvest (Rees et al, 1984). The mode moisture content values reveal rain effect better than the mean values in the two years comparison.

The year to year differences were due to rains and canal water availability. The year 2000-01 was drier than the year before. There was no rain during the crop season and canal water availability was lesser too. The drier conditions in 2000-01 resulted in lower kernel weight and test weight. One interesting expression of this water differential is visible in the ranges. The lower limits of test weight, 1000 kernel weight and flour yield ranges were higher in 1999-00 than in 20001-01. The higher limits were almost comparable. The lower limits of ranges are expression of resource-limited crop production and high limits those of resource-rich. The water deficit during 2000-01 pushed the marginal resource-poor-crops further down.

Foreign matter and broken kernels had almost similar ratio and ranges both years. Highest contents of any single non-wheat entity were barley and oats grains; 1.2% in both the years 1999-00 and 2000-01. The highest contents were 4.49 % but dominant sample class had very low mixture of these grains (0.1 %) during 1999-00. In 2000-01 the highest contents of edible foreign matter ranged upto 5.3 % with a mode value of 0.8 %. Non-edible contents were low; 0.5 % and 0.3 % in 1999-00 and 2000-01, respectively. The highest non-edible contents ranged to about 3 % in both years. Shrunken and broken kernels averaged 1.1 % and 1.4 % during both years in tandem. The mode values during these years were low though the highest ranges were over 4 percent (4.6 and 4.9 %, respectively). Insect damage was higher (0.8 %) in 2000-01 but low (0.3%) in 2001-02. It ranged from 0.2 to 2.6 % in 2000-01 with majority of samples having 0.2 % insect damage. The range in 2000-01 was from 0.1 to 1.1 % with mode value a mere 0.1 %. Apparently the insect damage was higher in 1999-00 crop. This was probably due to longer storage of 1999-00 crop where produce is liable to storage pest attack.

An examination of the data indicates that the quality of Punjab wheats is comparable to international standards of Australia and USA. The 1000 grain weight, test weight, flour yield and protein contents are high; approximately 41g, 77 Kg/hl, 70% and 13%, respectively. These wheats are hard and can meet the quality requirements of many varied end-uses. Insect damage, foreign matter and fungal damage tend to be on higher side. Careful agronomic practices – seed treatment, weed control, use of balanced fertilizers and improved harvest technology can further boost our quality and reduce objectionable foreign matter and fungal infestation. Wide range of quality in Punjab wheats and the boosting effect of rains and canal water availability on lower range limits in1999-00 are clear indications of quality enhancement possibilities through agronomic improvement. The varied production situations, however, need to be looked into. Punjab wheats are grown under wide agroclimatic conditions which influence yield and quality parameters (Khan and Saleem, 1981; Guzy et al, 1989; Chaudhry et al, 1995; Lil and Purchase, 1995; Borghi et al, 1996). So a survey of wheats on regional basis is in order to determine if regional quality differences exist. This would further enhance our understanding of wheat quality and, as a result, strengthen our marketing strategy in wide world market.

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MILLING AND COOKIE BAKING CHARACTERISTICS OF SPRING WHEAT VARIETIES OF PAKISTAN

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ABSTRACT

Milling and cookie baking characteristics of forty-four spring wheat varieties were studied. Flour yield and flour protein showed significant variation due to wheat varieties and crop years and varied from 65.78 to 74.40% and 9.63 to 13.14%, respectively. Significant differences existed in thickness, spread factor and crispness of cookies but non-significant differences were found in diameter, density, color, taste, surface characteristics and overall score of cookies between crop years. However, all the objective and subjective parameters of cookies differed significantly due to wheat varieties and interaction of wheat varieties with crop years. The diameter, spread factor and density of cookies ranged from 41.31 to 46.83mm, 5.56 to 8.04 and 0.421 to 0.657 g/cc, respectively. The wheat varieties Punjab 96, Faisalabad 83 and Bahawalpur 79 produced cookies of better quality as indicated by the greater spread factor and taste of cookies. The present investigation suggests that different milling and cookie baking characteristics are variable among wheat varieties and are also influenced by the growth conditions i.e. crop years.

INTRODUCTION

Pakistan has made an excellent progress in its wheat-producing sector by the development of high yielding, disease resistant and fertilizer responsive semi dwarf wheat varieties. The old tall wheat varieties previously grown in Pakistan possessed an excellent milling and baking properties but had become susceptible to diseases, insects/pests and had less fertilizer responsiveness. The wheat breeders in Pakistan were paying more attention to evolve new wheat varieties possessing an improved yield potential to meet the demand of food for rapid growing population.

Wheat is the staple diet of the people of Pakistan and mainly consumed in the form of chapaties. Some of the wheat is used in bakery products like bread, cookies, cakes and pastries. The composition and nutritive value of wheat is affected both by inheritance (class, variety) and environmental factors like climate, soil and cultural practices (Kent and Evers, 1994). Significant variations in milling, protein content and baking quality were observed due to the effects of genotype, environment and their interaction (Gaines *et al.* 1996; Bergman *et al.* 1998).

There are three factors that determine wheat type i.e. hardness, gluten strength and protein content. In general soft wheats with weak gluten and low protein are not suitable for bread making but suitable for cookies, pastries, crackers and flat breads while hard wheats with strong gluten and high protein content are preferred for leavened breads. This study was undertaken to find out variability in milling and cookie baking characteristics of different Pakistani wheat varieties. This information would help to prepare breeding program in developing new genotypes.

MATERIALS AND METHODS

Forty-four spring wheat varieties (*Triticum aestivum* L.) were planted during crop years 1995-96 and 1996-97 at Wheat Research Institute, Faisalabad. One kg sample of each variety was tempered to 15.5% moisture level. The tempered wheat grains were milled in Quadrumate Senior Mill and flour protein was determined according to AACC (1983).

Cookies were prepared from each flour sample according to AACC (1983). Biscuit cutter (diameter 36 mm) was used to cut the cookies from the rolled dough sheet. The cookies were weighed, their volume was recorded by using rapeseed displacement method to calculate density. The cookies were subjected to sensory evaluation by using hedonic scale (Haridas Rao and Shurpalekar, 1976). The statistical analysis was carried out according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Flour yield

The highest break flour yield was obtained in wheat variety Blue Silver followed by C 271 and Punjab 81 and the lowest break flour yield was found in Shahkar 95 during both the crop years. The break flour yield ranged from 15.60 to 28.30% and 16.30 to 30.31% during 1995-96 and 1996-97 crop years, respectively (Table 1). The wheat variety Dirk gave the highest yield of reduction flour while the lowest yield of reduction flour was obtained in Blue Silver during both the crop years. The variation in reduction flour was found from 41.85 to 56.68% when the years were pooled (Table 1).

Significant variation existed in flour yield (break + reduction flour = flour yield) due to difference in wheat varieties and crop years ($P \leq 0.01$). The grains produced during 1996-97 crop year gave higher flour yield than the grains produced during 1995-96 crop year. The combined effect of both the years showed variation in flour yield from 65.78 to 74.40% (Table 1). The wheat varieties Dirk, Bahawalpur 79, C 228, C 591 and LU 26 gave maximum flour yield as compared to other wheat varieties. Most of the wheat varieties were overlapping statistically with respect to flour yield. The present study is corroborated with the results reported by Butt *et al.* (1997) who reported variation in flour yield from 66.03 to 74.06% and 66.35 to 74.02% in 1993-94 and 1994-95 crop years, respectively. Paliwal and Singh (1985) reported a variation in flour yield from 55.48 to 72.56% for *aestivum* wheats of Uttar Pradesh.

Gaines *et al.* (1996) have also observed significant effects of genotype, environment and their interaction on milling of wheat. In the present study significant effect of wheat varieties and crop year on flour yield suggests that flour yield is not only influenced due to differences in wheat varieties but it is also affected by growing conditions especially crop years.

Flour protein

It is obvious that flour protein was significantly affected by the wheat varieties, crop years and interaction between wheat varieties and crop years. Statistically higher protein content was obtained in flour of wheat varieties grown during the crop years 1995-96 than the flour of wheats produced during 1996-97 crop year. The variation in crude protein content was found from 9.63 to 13.14% when the crop years were combined (Table 1). The flour of wheat variety Barani 83 contained the highest protein content followed by Arz, Punjab 96, Faisalabad 85, Pavon, Chakwal 86 and Bahawalpur 79. The differences in all these wheat varieties were found to be non-significant when the years were pooled.

Significant variation in protein content has been reported by various researchers (Huebner *et al.* 1995; Bergman *et al.* 1998) due to genotype, environments and their interactions. A wide variation in crude protein has also been found due to differences in wheat varieties, crop years and interaction between crop years and wheat varieties. This reflects that both environmental and genetic factors have influenced the protein content. It can be inferred from the results that the wheat varieties Barani 83, Arz, Punjab 96 and Faisalabad 85 possessing higher protein content get due attention by the wheat breeders.

Cookie baking quality

Significant differences exist in thickness and spread factor due to crop years but non-significant differences were found in diameter due to crop years. However, diameter, thickness and spread factor were influenced significantly by the wheat varieties and interaction between wheat varieties and crop years. The diameter of cookies ranged from 41.31 to 46.83 mm with the mean value of 43.95 mm when the data of years were combined (Table 2). The wheat variety Punjab 96 exhibited the highest cookie diameter followed by Faisalabad 83, Chenab 70, Shalimar 88, C 273 and Punjnad 88 on combining the crop years. The thickness of cookies ranged from 5.69 to 7.57 mm (Table 2).

The wheat varieties Punjab 96, Faisalabad 83 and Bahawalpur 79 showed higher spread factor as compared to other wheat varieties when the data of two years were combined. The spread factor of cookies ranged from 5.56 to 8.04 on combing the years (Table 2). Leelavathi and Haridas Rao (1993) have reported thickness 7.25 mm, diameter 50.02 mm, and spread ratio 6.99 for biscuits. Abboud *et al.* (1985) described that cookie diameter is a function of the rate of spreading and the setting point of the cookie dough. Rate of spreading was greater and expantion time longer for good quality (soft wheat) cookie doughs as compared to poor quality (hard wheat) cookie doughs. The present results also agree with the work already carried out on Pakistani wheat varieties by Anjum *et al.* (1993).

Density of cookies

The Density of cookies differed non-significantly by the crop years but differed significantly by the wheat varieties and interaction of wheat varieties with crop years. The results given in Table 2 showed that density of cookies was relatively higher in wheat varieties produced during 1996-97 crop year than the wheat varieties produced during 1995-96 crop year. The data further showed that cookies of wheat variety C 217 exhibited the highest value for density when the years were combined. The cookies of Faisalabad 83 and Punjab 96 got the lowest value for density in both the crop years. The density of cookies ranged from 0.421 to 0.657 g/cc when the data of two years were combined. Leelavathi and Haridas Rao (1993) have also reported 0.6168 g/cc density of biscuits.

Sensory evaluation of cookies

Crispness differed significantly by the crop years, while color, taste, surface characteristics and overall score of cookies were not affected significantly by the crop years. However, all the attributes of cookies assessed organoleptically differed significantly due to differences in wheat varieties and interaction of wheat varieties with crop years.

The wheat variety Punjab 96 got maximum score for cookie color and surface characteristics in both the years. The wheat variety Kohinoor 83 got maximum scores for crispness of cookies when the data of two years were combined. The maximum score for taste was assigned to the cookies of Faisalabad 83 followed by Punjab 96, Bahawalpur 79 and C 273 when the years were pooled.

It was observed that cookies produced from the wheat varieties produced during 1995-96 crop year got higher scores as compared to the cookies prepared from wheats produced during crop year 1996-97 (Table 2). The cookies of Punjab 96 got the highest scores for overall cookie quality characteristics on combing the data of two years. It was observed that the wheat varieties; Lyallpur 73, C 518, C 228 and C 217 produced cookies of poor quality. The wheat varieties Punjab 96, Faisalabad 83, Bahawalpur 79, C 273 and Blue Silver yielded cookies of better quality as compared to other wheat varieties.

The research work already carried out on Pakistani wheat varieties by Anjum *et al.* (1993) has shown that cookies of Faisalabad 83 were ranked the best and the varieties having soft grain texture and poor quality of gluten strength were better for cookie making quality. Faridi (1990) also stated that cookies made from soft and weaker gluten flours tended to have a more tender and desirable texture. The present study is also supported by the findings of Anjum *et al.* (1998) who stated that color, texture and overall acceptability of cookies differed significantly between wheat varieties.

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Table 1. Variability in milling and cookie characteristics of wheat varieties.

	Varieties	Break flour (%)	Reduction flour (%)	Flour yield (%)	Flour Protein (%)	Diameter (mm)	Thickness (mm)	Spread factor
1	C 518	23.33	48.68	72.01 abcdefgh	12.38 bcdef	41.89 qr	7.16 b	5.86 l
2	C 591	21.53	52.05	73.59 abc	11.20 lmn	42.75 op	7.18 b	5.97 kl
3	C 228	21.43	52.19	73.63 ab	11.81 fghijk	41.40 st	7.20 b	5.76 lm
4	C 217	21.15	50.37	71.52 bcdefghi	10.71 nop	41.96 qr	7.57 a	5.56 m
5	C 250	19.75	52.96	72.71 abcde	11.75 ghijkl	42.97 mnop	6.62 defg	6.51 ghi
6	C 271	27.63	44.91	72.54 abcde	10.68 nop	45.06 ef	6.62 defg	6.81 ef
7	C 273	17.87	51.60	69.98 efghijkl	11.30 klm	46.31 b	5.96 kl	7.77 b
8	Dirk	17.71	56.68	74.40 a	10.91 mno	42.17 q	6.57 defgh	6.42 hij
9	Mexi Pak	22.18	48.02	70.21 defghijk	10.25 pq	43.64 ij	6.53 defgh	6.69 fgh
10	Barani 70	20.97	48.06	69.04 ijklmn	11.53 ijkl	41.76 rs	6.70 cde	6.25 ij
11	Chenab 70	24.03	48.90	72.93 abcd	10.41 opq	46.46 b	6.47 defghi	7.24 cd
12	SA 42	19.84	47.53	67.37 lmno	9.63 r	42.86 nop	6.11 jkl	7.02 de
13	Blue Silver	29.30	41.85	71.16 bcdefghi	10.12 qr	45.06 ef	5.86 lm	7.69 b
14	Pari 73	22.91	47.66	70.57 defghij	11.98 defghij	43.44 ijkl	6.50 defgh	6.64 fgh
15	Lyallpur 73	24.15	45.51	69.67 fghijklm	11.87 efghijk	41.86 qr	7.11 b	5.91 l
16	Sandal 73	22.37	48.91	71.28 bcdefghi	12.46 bcde	44.54 g	6.55 defgh	6.80 ef
17	Pothohar	18.58	50.66	69.24 hijklmn	11.97 defghij	42.18 q	7.07 b	5.98 kl
18	Yecora	24.95	46.04	70.99 bcdefghi	12.01 defghi	44.87 efg	6.62 defg	6.78 ef
19	SA 75	21.57	49.84	71.41 bcdefghi	12.20 bcdefgh	41.31 t	6.93 bc	5.97 kl
20	Arz	21.91	47.74	69.65 fghijklm	12.75 ab	43.35 jklm	6.38 fghij	6.80 ef
21	LU 26	22.94	50.55	73.49 abc	11.64 hijkl	41.46 st	6.70 cde	6.19 jk
22	Punjab 76	18.64	50.49	69.13 ijklmn	12.17 bcdefgh	42.75 op	6.60 defg	6.47 hi
23	Pavon	19.97	50.41	70.39 defghijk	12.70 ab	43.56 ijk	6.22 ijk	7.01 de
24	WL 711	19.51	50.15	69.67 fghijklm	11.82 fghijk	43.18 klmn	6.37 fghij	6.78 ef
25	Chenab 79	20.61	47.57	68.18 jklmno	11.60 hijkl	43.77 i	6.74 cd	6.50 ghi
26	Bahawalpur 79	23.07	50.66	73.73 ab	12.65 abc	44.65 g	5.69 m	7.85 ab
27	Punjab 81	26.39	43.75	70.15 defghijkl	11.48 jkl	43.02 mno	6.65 def	6.48 hi
28	Pak 81	21.98	50.23	72.21 abcdefg	12.27 bcdefg	44.87 efg	6.19 ijk	7.25 cd
29	Barani 83	20.78	51.06	71.84 abcefghi	13.14 a	43.10 lmno	6.39 fghi	6.76 efg
30	Kohinoor 83	17.70	49.45	67.16 mno	12.52 bcd	45.47 d	6.30 hij	7.23 cd
31	Faisalabad 83	19.98	51.27	71.26 bcdefghi	12.44 bcde	46.58 ab	5.88 lm	7.93 ab
32	Faisalabad 85	23.28	47.54	70.82 cdefghij	12.70 ab	45.19 de	6.62 defg	6.84 ef

33	Punjab 85	21.45	48.95	70.40 defghijk	12.44 bcde	45.04 ef	6.94 bc	6.50 ghi
34	Satluj 86	18.41	49.31	67.73 klmno	12.05 defghij	44.84 efg	9.75 cd	6.65 fgh
35	Chakwal 86	22.18	48.38	70.57 defghij	12.68 abc	45.90 c	6.36 fghij	7.23 cd
36	Rawal 87	24.06	45.40	69.46 ghijklm	12.09 cdefghi	43.33 jklm	6.75 cd	6.42 hij
37	Punjnad 88	21.80	47.56	69.36 hijklm	12.40 bcdef	46.30 b	6.44 efghi	7.21 cd
38	Shalimar 88	22.38	47.66	70.04 efghijkl	11.89 efghij	46.40 b	6.36 ghij	7.32 c
39	Rohtas 90	18.22	49.87	68.10 jklmno	12.04 defghij	42.62 p	7.12 b	5.99 kl
40	Pasban 90	16.61	49.16	65.78 o	12.16 bcdefgh	44.17 h	6.68 cde	6.61 fgh
41	Inqulab 91	19.30	53.00	72.31 abcdef	12.18 bcdefgh	44.72 fg	6.94 bc	6.45 hi
42	Parwaz 94	20.21	47.91	68.12 jklmno	12.05 defghij	44.63 g	6.95 bc	6.43 hij
43	Shahkar 95	15.95	50.55	66.51 no	11.60 hijkl	45.56 cd	6.93 bc	6.58 fgh
44	Punjab 96	23.90	46.60	70.50 defghijk	12.73 ab	46.83 a	5.99 kl	8.04 a
	F-value	-	-	6.2975	19.6187	169.6231	22.6738	53.2954
	Mean	21.42	49.04	70.47		43.95	6.60	6.71

Mean values for varieties carrying same letters in a column are not significantly different (P>0.05)

Table 2. Variability in cookie characteristics among wheat varieties.

	Varieties	Density (g/cc)	Color	Crispness	Taste	Surface Characteristics	Total Cookie scores
1	C 518	0.656 a	6.88 ijkl	6.26 n	7.02 lmn	6.55 qrs	26.64 qr
2	C 591	0.652 a	6.42 lm	7.11 ijkl	7.20 ijklm	6.52 qrs	27.25 opq
3	C 228	0.655 a	6.00 m	7.32 fghijk	7.09 jklmn	5.68 t	26.02 rs
4	C 217	0.657 a	6.92 ijkl	6.50 mn	5.80 o	6.20 s	25.42 s
5	C 250	0.597 hi	7.25 fghijk	7.58 cdefghij	7.51 defghijkl	7.61 efghijkl	29.95 hij
6	C 271	0.508 st	7.75 bcdefg	7.70 bcdefghi	7.75 cdefghi	8.00 defg	31.20 d
7	C 273	0.453 y	7.75 bcdefg	8.33 ab	8.15 abc	8.58 abc	32.82 bc
8	Dirk	0.573 j	7.25 fghijk	7.60 cdefghij	7.90 abcdef	6.85 nopqr	29.60 ijk
9	Mexi Pak	0.555 l	7.25 fghijk	7.20 ghijkl	7.87 bcdefg	7.92 defgh	30.25 ghij
10	Barani 70	0.591 i	6.75 kl	7.75 bcdefghi	7.32 fghijkl	7.36 hijklmno	29.17 kl
11	Chenab 70	0.478 v	8.25 abc	8.04 abcde	7.79 bcdefghi	8.06 cdef	32.14 c
12	SA 42	0.539 m	7.25 fghijk	7.58 cdefghij	7.83 bcdefgh	7.70 defghij	30.37 efghi
13	Blue Silver	0.470 wx	8.25 abc	8.25 ab	8.04 abcd	8.25 bcd	32.79 bc
14	Pari 73	0.601 gh	6.90 ijkl	7.33 fghijk	7.21 hijklm	7.20 jklmnop	28.64 lm
15	Lyallpur 73	0.642 b	6.67 kl	6.65 lmn	6.90 lmn	6.62 pqrs	26.84 pq
16	Sandal 73	0.514 rs	7.77 bcdefg	8.06 abcd	7.75 cdefghi	7.62 efghijk	31.18 de
17	Pothohar	0.633 c	6.85 jkl	7.46 defghijk	6.57 n	6.83 nopqr	27.72 no
18	Yecora	0.595 hi	7.50 defghij	7.17 hijkl	7.03 lmn	7.07 klmnopq	28.77 lm

19	SA 75	0.634 c	6.92 ijkl	6.98 jklm	6.67 mn	6.92 mnopq	27.48 op
20	Arz	0.508 st	7.54 defghi	8.04 abcde	7.92 abcdef	7.70 defghij	31.28 d
21	LU 26	0.619 d	7.67 cdefg	6.87 klm	7.08 klmn	6.78 opqr	28.41 lmn
22	Punjab 76	0.530 no	7.83 bcdef	7.82 abcdefg	7.47 defghijkl	7.01 mnopq	30.21 ghij
23	Pavon	0.512 rs	7.75 bcdefg	7.75 bcdefghi	7.96 abcde	7.36 hijklmno	30.97 defg
24	WL 711	0.504 t	7.96 bcde	7.87 abcdef	7.38 efghijkl	7.91 defgh	31.13 def
25	Chenab 79	0.614 de	7.12 ghijk	7.40 efghijk	6.92 lmn	6.83 nopqr	28.27 mn
26	Bahawalpur 79	0.439 z	8.35 ab	8.29 ab	8.30 abc	8.15 cde	33.26 b
27	Punjab 81	0.517 qr	7.90 bcdef	7.79 abcdefgh	7.25 ghijklm	7.67 defghij	30.59 defgh
28	Pak 81	0.522 pq	7.46 efghij	7.96 abcdef	7.50 defghijkl	7.35 hijklmno	30.27 ghij
29	Barani 83	0.603 fg	7.62 cdefgh	7.21 ghijkl	7.05 lmn	6.84 nopqr	28.73 lm
30	Kohinoor 83	0.471 wx	8.00 bcde	8.40 a	7.77 cdefghi	8.07 cdef	32.23 c
31	Faisalabad 83	0.423 z	8.75 a	8.06 abcd	8.50 a	8.75 ab	34.06 a
32	Faisalabad 85	0.497 u	7.98 bcde	8.10 abcd	7.90 abcdef	7.41 ghijklmn	31.39 d
33	Punjab 85	0.520 pq	7.73 bcdefg	7.71 bcdefghi	7.70 defghijk	7.50 fghijklm	30.64 defgh
34	Satluj 86	0.566 k	7.75 bcdefg	7.02 jklm	7.71 cdefghij	7.07 klmnopq	29.52 jk
35	Chakwal 86	0.475 vw	8.10 bcde	8.29 ab	7.97 abcde	7.92 defgh	32.26 c
36	Rawal 87	0.535 mn	7.50 defghij	7.70 bcdefghi	7.29 fghijkl	7.81 defghi	30.30 fghij
37	Punjnad 88	0.475 vw	8.10 bcde	8.25 ab	8.04 abcd	7.82 defghi	32.21 c
38	Shalimar 88	0.469 x	8.25 abc	8.15 abc	7.80 bcdefghi	8.14 cde	32.34 c
39	Rohtas 90	0.615 d	7.00 hijkl	6.88 klm	7.00 lmn	6.32 rs	27.21 opq
40	Pasban 90	0.605 fg	6.65 kl	7.12 ijkl	7.46 defghijkl	7.16 jklmnop	28.56 lm
41	Inqulab 91	0.526 op	8.15 abcd	7.75 bcdefghi	7.47 defghijkl	7.02 lmnopq	30.38 efghi
42	Parwaz 94	0.524 p	7.96 bcde	7.98 abcde	7.40 efghijkl	6.97 mnopq	30.31 fghij
43	Shahkar 95	0.609 ef	7.71 bcdefg	6.63 lmn	6.90 lmn	7.22 ijklmno	28.47 lmn
44	Punjab 96	0.421 z	8.75 a	8.08 abcd	8.41 ab	8.92 a	34.33 a
	F-value	1164.9213	9.8396	8.5810	8.5012	15.3313	69.6694
	Mean	0.548	7.55	7.59	7.49	7.39	30.03

Mean values for varieties carrying same letters in a column are not significantly different (P>0.05)

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OPTIMIZATION OF SHELF LIFE OF BREAD WITH CHEMICAL PRESERVATIVES USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Among various bakery products, bread holds a distinct position with reference to its production and utilization. Major problem for a bread manufacturer is the spoilage of bread due to the growth of certain fungi and bacteria. Use of preservative in the bread is the way to minimize the spoilage. The present study was undertaken to determine the possibility of use of different acidulants and their salts in combination to boost shelf life of bread. Response surface models generated at 24, 48, 72 and 96 hours of storage of bread showed highly significant results. In all models, the coefficient of determination was more than 85% indicated the good fit of the models. Optimum levels of calcium propionate, calcium acetate and lactic acid were 0.2%- 0.5%, 0.2% and 0.2% respectively which extended the shelf life of bread upto 96 hr.

INTRODUCTION

Wheat is unique among all cereals due to the presence of gluten which imparts viscoelastic characteristics to bread. The most commonly grown species of wheat all over the world are *Triticum aestivum* (Common wheat), *Triticum compactum* (Club wheat), and *Triticum durum* (Durum wheat) (Branlard and Dardevat, 1985).

There is a possibility to shorten the processing time and improving the dough properties of flour by the addition of various additives. The production of good quality bread with shorter processing time, and longer shelf life requires the use of good quality preservatives. These preservatives affect processing time, volume, texture, taste, aroma, colour and shelf life of the bread.

Optimization of bread preservatives has vital importance for the production of bread of higher quality by modern processes. It may be achieved by applying response surface methodology, (RSM) which uses the quantitative data to determine and simultaneously solve multivariate equations that specify the optimum product for a specified set of factors through mathematical models (Giovanni, 1983).

The RSM techniques have been applied successfully in the determination of no time dough (Baker *et al.* 1988), optimization of Chinese wet noodle formulation (Shelke *et al.* 1990), production of enhanced nutritional value savoury chapaties (Rehman *et al.* 1996).

Keeping in view of these developments, the present investigation was carried out to extend the shelf life of bread and to optimize the levels of chemical preservatives by using RSM and to determine the responses of calcium propionate, calcium acetate and lactic acid which acted as independent variables at various storage intervals on microbiological load of bread.

MATERIALS AND METHODS

This research project was carried out in the Department of Food Technology University of Agriculture, Faisalabad during year 2000. The proximate analysis of flour was carried out using standard analysis methods (AACC, 2000) for moisture, crude protein, crude fat, crude fibre, total ash and nitrogen free extract.

The flour sample was run through Brabender Farirograph equipped with 50 g bowl capacity to determine the physical dough behavior of flour sample (AACC, 2000). The parameters such as water absorption, dough development time, dough stability, arrival time, departure time, resistance to dough, tolerance index and softening of the dough were derived from the farinogram (AACC, 2000).

The bread was prepared using recipe; flour 100g, water 60 ml, sugar 3g, salt 1g, oil 5g, yeast 1g, improver 1g, and preservatives according to the Table 1. The bread was prepared by using standard mixing and baking procedures (AACC, 2000).

The preservatives i.e. calcium propionate, calcium acetate and lactic acid were added to the dough according to following model which was generated with response surface methodology using randomized block design as shown in Table 1.

Table 1. Response surface model for level of Preservatives used to extend the shelf life of bread.

Treatment	Ca. Propionate %	Ca. Acetate %	Lactic acid %
T_1	0.4	0.2	0.2
T_2	0.3	0.3	0.3
T_3	0.2	0.2	0.4
T_4	0.4	0.4	0.4
T_5	0.3	0.3	0.3
T_6	0.2	0.4	0.2
T_7	0.2	0.2	0.2
T_8	0.4	0.4	0.2
T_9	0.3	0.3	0.3
T_{10}	0.4	0.2	0.4
T_{11}	0.3	0.3	0.3
T_{12}	0.2	0.4	0.4
T_{13}	0.3	0.3	0.1
T_{14}	0.1	0.3	0.3
T_{15}	0.3	0.3	0.3
T_{16}	0.3	0.1	0.3
T_{17}	0.3	0.3	0.3
T_{18}	0.3	0.3	0.5
T_{19}	0.5	0.3	0.3
T_{20}	0.3	0.5	0.3

Microbiological analysis of bread such as total count of microorganisms at 24, 48, 72, and 96 hrs intervals of storage was carried out (Frazier, 1967). The data of microbiological tests obtained for optimization were statistically analysed using response surface methodology (Gacula and Singh, 1984). Two dimensional contour plots were generated to determine the effect of independent variables on the selected responses (Minitab, 8.2).

RESULTS AND DISCUSSION

The composition of commercial flour was analysed using standard procedures, showed that the flour contained moisture 8.23%, ash 0.55%, crude protein 10.25%, crude fat 0.75%, crude fibre 0.68% and nitrogen free extract 79.54% (Table 2). The values obtained in this study are in close agreement with the findings that were reported by Ali (1980) and Tarrar (1999) who analyzed different wheat varieties and observed 12.5-14.4% moisture, 8.23-12.71% crude protein. 1.17-1.50% crude fat, 0.42-0.70% crude fibre and 0.42-0.66% ash.

Table 2. Proximate Composition of Flour

Characteristics	%age
Moisture	8.23
Crude protein	10.25
Ash	0.55
Crude fibre	0.68
Fat	0.75
Nitrogen free extract (NFE)	79.54

The farinographic characteristics of flour as determined by using Brabender Farinograph revealed that the flour has water absorption 56.4%, arrival time 2 min, peak time 3 min, departure time 8.5 min, dough stability 6.5 min, tolerance index 30 B.U and softening of dough 50 B.U. (Table 3). The farinogram of the flour is shown in Fig.1. The results are comparable with earlier findings of Mumtaz (1997). He used improvers in the dough and stated that the water absorption ranged from 58.8 to 61.4%, arrival times increased from 1 to 4.5 min, while dough development time accentuated from 3.5 to 5 min.

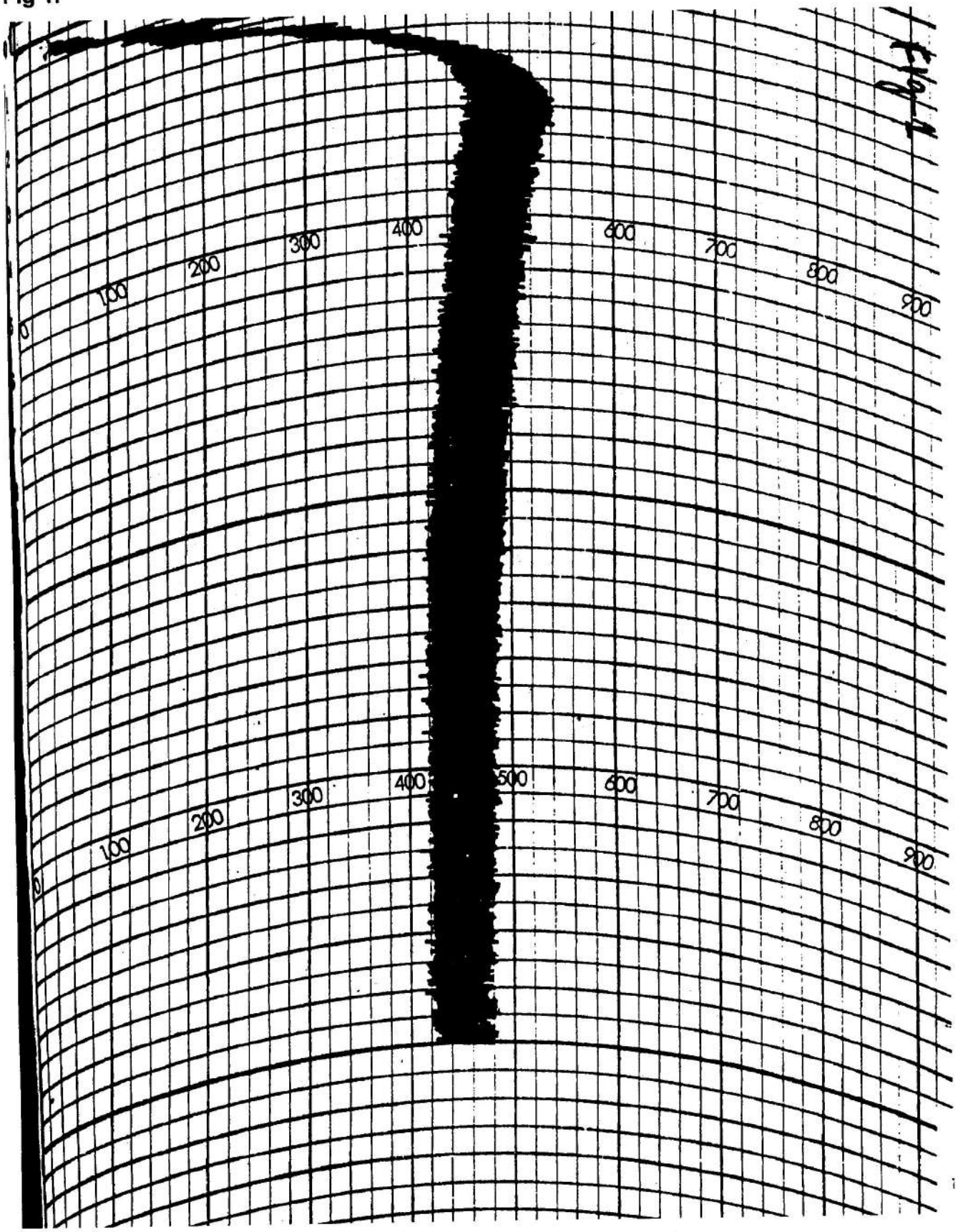
Table 3. Farinographic characteristics of flour

Water absorption (%)	56.4
Arrival time (Minutes)	2.0
Peak time (Minutes)	3.0
Departure time (Minutes)	8.5
Dough stability (Minutes)	6.5
Tolerance index BU	30
Softening of the dough BU	50

BU = Brabender units

Total microbial count observed at different storage intervals is presented in table 4. Minimum bacterial load was observed in case of T4 and T18 and maximum in case of T7 at all intervals of storage.

Fig 1:



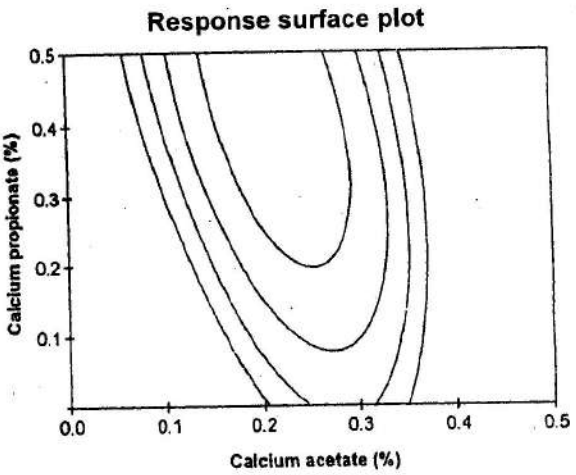


Fig 2: Contour plot for total microbial count after 96 hours of baking of bread as affected by Calcium propionate and Calcium acetate.

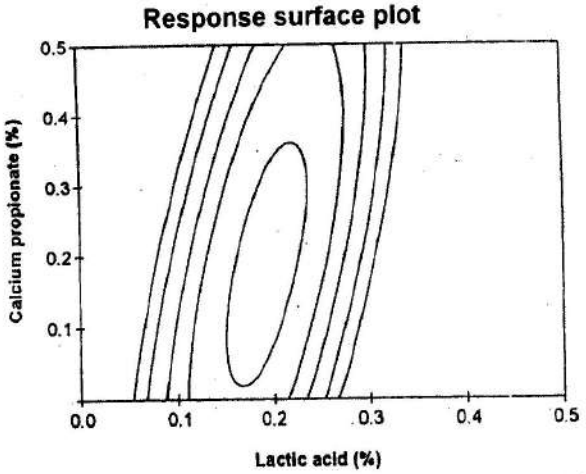


Fig 3: Contour plot for total microbial count after 96 hours of baking of bread as affected by Calcium propionate and Lactic acid.

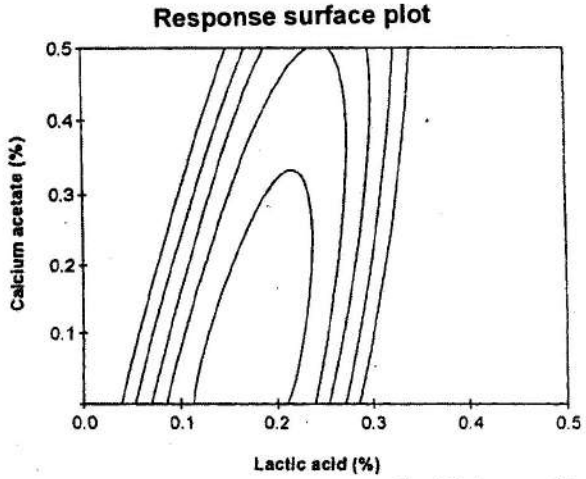


Fig 4: Contour plot for total microbial count after 96 hours of baking of bread as affected by Calcium Acetate and Lactic acid.

Table 4. Total microbial count observed at different storage intervals.

Treat-ment	Storage interval (hours)			
	24	48	72	96
T1	4×10^2	5×10^3	5×10^4	6×10^4
T2	3×10^2	2×10^3	4×10^4	5×10^4
T3	2×10^2	2×10^3	3×10^4	3×10^4
T4	0×10^2	1×10^3	1×10^4	2×10^4
T5	3×10^2	2×10^3	4×10^4	5×10^4
T6	6×10^2	6×10^3	7×10^4	9×10^4
T7	8×10^2	7×10^3	9×10^4	10×10^4
T8	3×10^2	3×10^3	4×10^4	5×10^4
T9	3×10^2	2×10^3	4×10^4	5×10^4
T10	1×10^2	2×10^3	2×10^4	2×10^4
T11	2×10^2	2×10^3	4×10^4	5×10^4
T12	2×10^2	3×10^3	3×10^4	3×10^4
T13	6×10^2	4×10^3	7×10^4	9×10^4
T14	5×10^2	5×10^3	6×10^4	8×10^4
T15	3×10^2	2×10^3	4×10^4	5×10^4
T16	4×10^2	4×10^3	5×10^4	6×10^4
T17	3×10^2	2×10^3	4×10^4	4×10^4
T18	0×10^2	1×10^3	1×10^4	2×10^4
T19	1×10^2	2×10^3	2×10^4	2×10^4
T20	2×10^2	2×10^3	3×10^4	3×10^4

The data was analyzed with response surface methodology. Response surface methodology was used to optimize the levels of calcium propionate, calcium acetate and lactic acid used in bread in order to extend the shelf life. Best fitting models were generated by regression procedure and used as predictors for the treatment factors and to estimate the properties of independent variables (preservatives) on microbial load. The signs of regression coefficient within each table reveals the direction of the effect of each independent variable, the square and interaction.

The R^2 values for the best fitting models for bacterial load of bread at various storage intervals were high (96.5%) at 24 (Table 5), (87.9%) 48 (Table 6), (96.5%) 72 (Table 7) and (94.6%) at 96 hr (Table 8) of storage. Satisfactory levels of R^2, CV and model significance indicated good fit of models with no significance lack of fit. The results are consistent with previous findings (Shelke *et al.*, 1990).

The contour plot for total microbial growth at 96 hr as affected by ca-propionate and ca-acetate is shown in figure 2. The region for optimized response as defined by calcium propionate was 0.5% and ca-acetate was 0.22%. Milatovic and Martinek (1971) used 0.3% calcium acetate and 0.2% ascorbic acid to improve the shelf life of bread through inhibiting the growth of rope producing organisms.

Table 5. Analysis of variance for response surface model at 24 hours for total microbial growth.

Source of variation	d.f.	S.S.	M.S.	F. value
Regression	11	76.20	6.927	20.15**
Error	8	2.75	0.344	
Total	19	78.95		

** = Highly significant

Response surface model at 24 hours.

Predictor	Coefficient	St. Dev.	t-ratio	Probability
Constant	2.830	0.2832	0.1001	0.759
Block 2	0.167	0.2785	1.6677	0.232
Block 1	-0.083	0.3078	-3.7084	0.090
Factor A	-1.130	0.1406	-0.1244	0.733
Factor B	-0.500	0.1427	-0.2854	0.607
Factor C	-1.750	0.1361	-0.0778	0.787
A x A	0.003	0.1097	36.5667	0.000
B x B	0.083	0.0897	1.0807	0.328
C x C	0.083	0.0991	1.1940	0.306
A x B	0.001	0.0373	37.3000	0.000
A x C	0.500	0.1875	0.3750	0.557
B x C	0.250	0.1871	0.7484	0.412

s = 0.5863 R^2 = 96.5%

(Factor A = Calcium propionate, Factor B = Calcium acetate, Factor C = Lactic acid)

The contour plot for total microbial growth at 96 hr as affected by ca-propionate and lactic acid is shown in figure 3. The region of optimized response as defined by ca-propionate was 0.2% and lactic acid was 0.2.

The contour plot for total microbial growth at 96 hr as affected by ca-acetate and lactic acid is in shown in figure 4. The region of optimized response as defined by ca-acetate was 0.05% and lactic acid was 0.2%. Chakre *et al.*, (1984) found that the doses of 15 g/kg ascorbic acid and 10 ml/kg acetic acid were more effective and could prolong the shelf life of bread upto one month.

It may be concluded that optimized levels of ca-propionate, ca-acetate and lactic acid were 0.2-0.5%, 0.2% and 0.2%, respectively which could control the spoilage of bread affectively upto 96 hrs.

Table 6. Analysis of variance for response surface model at 48 hours for total microbial growth.

Source of variation	d.f.	S.S.	M.S.	F. value
Regression	11	76.20	4.230	5.27*
Error	8	6.417	0.802	
Total	19	52.950		

** = Significant

Response surface model at 48 hours.

Predictor	Coefficient	St. Dev.	t-ratio	Probability
Constant	2.7083	0.4478	6.05	0.000
Block 2	-0.1667	0.5171	-0.32	0.755
Block 1	-0.8959	0.5007	-1.79	0.111
Factor A	-0.8125	0.2239	-3.63	0.007
Factor B	-0.4375	0.2239	-1.95	0.086
Factor C	-1.1875	0.2239	-5.30	0.000
A x A	0.4375	0.1828	2.39	0.044
B x B	0.3125	0.1828	1.71	0.126
C x C	0.1875	0.1828	1.03	0.335
A x B	-0.3750	0.3166	-1.18	0.270
A x C	0.3750	0.3166	1.18	0.270
B x C	0.3750	0.3166	1.18	0.270

s = 0.8956 R^2 = 87.9%

(Factor A = Calcium propionate, Factor B = Calcium acetate, Factor C = Lactic acid)

Table 7. Analysis of variance for response surface model at 72 hours for total microbial growth.

Source of variation	d.f.	S.S.	M.S.	F. value
Regression	11	76.200	6.927	20.15**
Error	8	2.750	0.344	
Total	19	78.950		

** = Highly significant

Response surface model at 72 hours.

Predictor	Coefficient	St. Dev.	t-ratio	Probability
Constant	3.8333	0.2932	13.08	0.000
Block 2	0.1667	0.3385	0.49	0.636
Block 1	-0.0833	0.3278	-0.25	0.806
Factor A	-1.1250	0.1466	-7.68	0.000
Factor B	-0.5000	0.1466	-3.41	0.009
Factor C	-1.7500	0.1466	-11.94	0.000
A x A	0.0833	0.1197	0.70	0.506
B x B	0.0833	0.1197	0.70	0.506
C x C	0.0833	0.1197	0.70	0.506
A x B	0.0000	0.2073	0.00	1.000
A x C	0.5000	0.2073	2.41	0.042
B x C	0.2500	0.2073	1.21	0.262

s = 0.5863 R^2 = 96.5%

(Factor A = Calcium propionate, Factor B = Calcium acetate, Factor C = Lactic acid)

Table 8. Analysis of variance for response surface model at 96 hours for total microbial growth.

Source of variation	d.f.	S.S.	M.S.	F. value
Regression	11	113.300	10.300	12.68**
Error	8	6.500		
Total	19	119.800		

** = Highly significant

Response surface model at 48 hours.

Predictor	Coefficient	St. Dev.	t-ratio	Probability
Constant	4.8333	0.4507	10.72	0.000
Block 2	-0.3333	0.5204	-0.64	0.540
Block 1	-0.0833	0.5039	-0.17	0.873
Factor A	-1.3750	0.2253	-6.10	0.000
Factor B	-0.5000	0.2253	-2.22	0.057
Factor C	-2.1250	0.2253	-9.43	0.000
A x A	0.0833	0.1840	0.45	0.663
B x B	-0.0417	0.1840	-0.23	0.827
C x C	0.2083	0.1840	1.13	0.290
A x B	0.0000	0.3187	0.00	1.000
A x C	0.7500	0.3187	2.35	0.046
B x C	0.2500	0.3187	0.78	0.455

s = 0.9014 R^2 = 94.6%

(Factor A = Calcium propionate, Factor B = Calcium acetate, Factor C = Lactic acid)

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ISOLATION OF L-LYSINE PRODUCING CORYNEBACTERIUM FROM LOCAL HABITATS

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ABSTRACT

Seventy strains of bacteria were isolated from soil, water and milk samples. These were screened for L-Lysine production. The isolate, designated as RN-12 was selected and identified as *Corynebacterium glutamicum*. It produced maximum amount of L-Lysine i.e., 2.784 mg/ml in fermentation medium.

INTRODUCTION

L-Lysine is an essential amino acid. It is often deficient in cereal proteins especially in proteins of wheat, rice and maize (March et al. 1950 and Stainer et al. 1986). These are the major ingredients of food and feed products. L-Lysine deficiency causes nausea and dizziness in humans and animals (Meister, 1965). Therefore, L-Lysine is supplemented in foodstuffs to increase their nutritive value. It is used in poultry feed as rapid growth promoter. L-Lysine is consumed approximately 80,000 tonnes / year in animal feeds (Kinoshita, 1987). In chemical industry, it is used to produce isocyanate resins (Brock et al. 1986).

The microbial processes for the production of amino acids are gaining importance. Using these processes, L-Lysine can be synthesized from simple and inexpensive raw materials such as glucose, acetate, molasses etc. The chemical and enzymatic methods are not suitable for the production of amino acids from simple materials (Soda et al. 1983). Only about 5% of the total L-Lysine production, is produced by enzymatic techniques whereas the rest is produced via fermentation (Hsiao et al. 1988).

Consumption of poultry products has increased rapidly in our country in the last few years. L-Lysine demand has also increased indirectly. In order to meet this demand, it is imported from foreign countries involving foreign exchange to the tune of million of rupees every year. So it is needed that L-Lysine is prepared locally. From an industrial point of view, it is necessary to improve bacterial strains, culture conditions and process for L-Lysine production.

In the present studies screening of amino acid producing bacterial isolates from local habitats was made. Repeated tests were exhibited on them to isolate L-Lysine producing strains. Morphological and cultural characteristics of maximum L-Lysine producing strain were also studied for its identification.

MATERIALS AND METHODS

Sample Collection and Isolation of Bacteria

Bacterial strains were isolated from soil samples of agricultural areas and canal bed, water from canal and river and raw milk. Sampling was carried out within Lahore metropolitan area. Soil samples were collected in sterilized plastic bags. Milk and water samples were collected in 500 ml sterilized plastic bottles.

Ten gram of each soil sample was added in 50 mL buffered peptone water at pH 7.0. One ml of milk and water samples were diluted to 10 mL with buffered peptone water. Further dilutions in saline water were made if required and 0.1 ml of each dilution was incubated in nutrient agar plates at 37°C. Colonies were picked up after 48 to 72 hours of incubation (Calam, 1980). Seventy colonies from different plates were selected and incubated on nutrient agar slants. Purity of the cultures was confirmed microscopically and by Gram's staining. Amino acid producing strains of *Corynebacterium* are all Gram-positive, therefore,

only Gram-positive bacteria were screened for amino acid production.

Culture Conditions of Bacterial Isolates for Amino Acid and L-Lysine Production

Fermentation was carried out in 250 ml Erlenmeyer flasks. The composition of inoculum medium was as follows (g/100ml): glucose 4.0, peptone 1.0, yeast extract 1.0, casamino acids 0.1, KH_2PO_4 0.04, K_2HPO_4 0.04, $MgSO_4$. $7H_2O$ 0.04, NaCl 0.1 and urea 0.2. The pH was adjusted at 7.0. The fermentation medium contained: glucose 17.5g, peptone 0.5g, yeast extract 0.5g, KH_2PO_4 0.05g, K_2HPO_4 0.1g, $MgSO_4$ 0.04g, $(NH_4)_2SO_4$ 2.5g, NaCl 0.2g, casamino acids 0.05g, $FeSO_4$ 0.2mg, $CuSO_4$ 0.2mg, thiamine-HCl 0.1mg and biotin 4µg in 100ml distilled water. The pH was adjusted at 7.0 with NH_4OH (Pham et al. 1993).

A loopful of seed was inoculated from isolated bacterial strains in 25 ml inoculum medium and incubated on a rotary shaker with 100 r.p.m. at 30°C for 24 hours. Then 3 ml of inoculum was added aseptically in 50 ml sterilized fermentation medium and fermentation was carried out on rotary shaker with 110 r.p.m. at 30°C for 72 to 96 hours. The fermented broths were centrifuged at 10,000 r.p.m. to separate cell mass. Supernatant of these broths were then tested for amino acid presence and broths of amino acid producing isolates were analyzed for L-Lysine. Maximum L-Lysine producing strain was chosen and its morphological, cultural and biochemical characteristics were studied according to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) and its genus was confirmed by using API Identification Kit (API Coryne. Ref. 20900).

Analytical Methods

The amino acids produced in fermented broths were determined by colorimetric method using Cd-ninhydrin reagent (Doi et al. 1981). L-Lysine was detected by Paper Chromatography (Nadeem et al. 1996).

Microbiological Media and Chemicals

All biochemicals and prepared media used for isolation and fermentation were of OXOID Ltd., Basingstoke, Hampshire, England. API Identification Kit for *Corynebacterium* (API Coryne. Ref. No. 20900) was used for confirmation of genus.

RESULTS AND DISCUSSION

A variety of microorganisms accumulate amino acids in the culture fluids. However, only few bacteria have sufficient productivity to warrant the commercial production of amino acids. Therefore, bacteria are most commonly used microorganisms for industrial production of amino acids. In the present studies bacterial strains were isolated from local habitats and screened for L-Lysine production. Only Gram-positive, non-motile coccal or rod shaped bacteria were studied for L-Lysine production. The commonly used bacteria for amino acid production such as *Corynebacterium glutamicum*_(Kinoshita, 1987), *Brevibacterium lactofermentum* (Nahn et al. 1976), *Brevibacterium thiogenitalis* (Kanzaki et al. 1967), *Microbacterium ammoniaphilum* (Miyai et al. 1964) and *Corynebacterium glutamicum* (Pham et al. 1993) are all Gram-positive, non-spore forming, non-motile, coccal and rod like and all requiring biotin for growth.

Seventy strains of bacteria were selected by microscopic studies and were grown in a medium used for L-Lysine production (Pham et al. 1993) containing sufficient quantities of biotin which was added to the medium to prevent the cells from producing L-glutamate (Soda et al. 1957), as wild strain of bacteria usually produces glutamic acid, valine and alanine (Nadeem et al. 1996). Fermentation broths were analyzed after 72 to 92 hours of fermentation for amino acid presence. Only 14 out of 70 bacterial strains produced some quantities of amino acids (Table-1) which is about 20% of the total bacterial isolates. Maximum number of amino acid producing bacteria were isolated from milk samples (Number was 4 i.e., 28% of total amino acid producing bacteria). Only 2 out of 12 (16.6%) isolated bacteria from soil of agricultural areas produced amino acids. Bacterial isolates 3 out of 17 (17.64%) produced amino acids from canal soil. Only 2 out of 9 bacterial isolates from canal water and 3 out of 15 bacterial isolates from river water produced amino acids equal to 20% and 23.52% respectively (Table-1).

Table 1. Isolation of bacteria from different sources.

No.	Source	Number of Samples	Number of Isolates	Number of Amino Acid producing cultures	Strain No.
1	Agriculture soil	10	12	2	1,2
2	Canal soil	10	17	3	3-5
3	Canal water	10	9	2	6,7
4	River water	10	15	3	8-10
5	Milk (raw)	10	17	4	11-14

These results showed that the presence of amino acid fermenting bacteria in raw milk was higher than water and was lowest in soil samples. There may be one reason for this difference is that milk is a nutritively rich medium for microbes. Furthermore, it is not contaminated with chemicals. On the other hand, water and soil are contaminated with chemicals such as pesticides, detergents and industrial wastes which are toxic for microorganisms and they also carried less quantities of nutrients. Soil is more affected with pesticides as these are directly sprayed on soil, therefore, it contained lowest number of beneficial bacteria. Pesticides and other chemicals are diluted in water, in this way their toxicity is reduced. Therefore, water carried higher number of amino acid producing bacteria than soil. Similar results were obtained by Ahmad et al. (1993) and Nadeem et al. (1996) from bacterial strains isolated from soil and water samples.

Only four bacteria out of 14 produced L-Lysine (Table-2). L-Lysine producing bacterial strains were RN-3, RN-6, RN-12 and RN-13 which produced 1.236, 2.048, 2.784 and 1.020 mg/ml L-Lysine respectively. These quantities are not sufficient enough for industrial scale production. The reason is that naturally occurring bacteria normally produce amino acids in very small quantities. There is always need to improve upon these microbes (Nadeem et al. 1996). Mutation by chemicals and recombinant DNA through genetic engineering are common methods used for increasing production of amino acids. Mutation by chemical mutagen NTG (N-methyl-N-nitro-N-nitrosoguanidine) of isolated strain will be included in future plan of studies. RN-12 was chosen due to its maximum L-Lysine producing ability in fermentation broth and its morphological, cultural and biochemical characteristics were studied to identify it.

Table-2. Production of L-Lysine by submerged fermentation*.

Strain No.	Presence of L-Lysine production	Concentration of L-Lysine produced (mg/ml)
RN-1	-	-
RN-2	-	-
RN-3	+	1.236
RN-4	-	-
RN-5	-	-
RN-6	+	2.048
RN-7	-	-
RN-8	-	-
RN-9	-	-
RN-10	-	-
RN-11	-	-
RN-12	+	2.784
RN-13	+	1.020
RN-14	-	-

* Shake flask experiments
(-) : Not detected
(+): Detected

Identification of Strain RN-12

Characteristics of strain RN-12 with respect to morphological, cultural and biochemical properties are described in Table-3. After examining these characteristics, it has been concluded that RN-12 belongs to class *Corynebacteriaceae* according to the taxonomic identification of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). Its genus was confirmed as

Corynebacterium by using API *Corynebacterium* Kit (API Coryne. Ref. No. 20900, Analytab. Products Inc.). Therefore, RN-12 was identified as *Corynebacterium glutamicum* as it is the well known species of *Corynebacterium* which produce amino acids and having the same characteristics as exhibited by RN-12 (Abe, 1972). Therefore, RN-12 was selected as L-Lysine producing bacteria and identified as *Corynebacterium glutamicum*.

Table 3. Morphological, cultural and biochemical characteristics of strain RN-12.

Morphological characteristics	
Nutrient agar plate at 37°C for 24 hours.	
Shape:	Rods, beaded and club shaped showing coccoid forms.
Motility:	Non-motile
Staining:	Gram- positive
Occurring:	Single or in pairs
Size:	(0.4 - 0.9) x (1.0 - 7.0) µm
Source:	Raw milk samples
Cultural characteristics	
Colony Forms:	Creamy white colonies of small size on nutrient agar. (Not rich growth)
	Yellow creamy colonies on the fermentation medium (Heavy, raised growth)
Fermentation reactions	
Monosaccharides:	Fermented with acid formation
Disaccharides:	Fermented with acid formation
Polysaccharides:	Not fermented
Blood agar:	No haemolysis
Growth factors:	
Oxygen:	Aerobic
Temperature:	Optimum 30°C (Range 28-32°C)
pH	Optimum 7.0 (Range 6.8-7.4)
Biochemical characteristics	
Toxin production:	No toxin produced
Pathogenecity:	Non-pathogenic

Selected strain RN-12 did not produce L-Lysine in quantities higher enough to be utilized at industrial scale. In further studies fermentation conditions will be optimized for L-Lysine production by RN-12. Carbon and nitrogen sources will be exchanged with cheaper raw materials. Mutation will be induced if necessary and it is expected to obtain L-Lysine production at industrial scale with less expenses and in large quantities.

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COMPARATIVE ANALYSIS OF QUALITY OF MILK COLLECTED FROM RAWALPINDI/ISLAMABAD REGION

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ABSTRACT

This study involves the comparison of the physico-chemical properties of milk collected from five different sources i.e, dairy farms, milk producers, processed milk, milk vendors and milk consumers. Fifteen samples each of five sources were analyzed for the compositional quality i.e. for fat, protein, lactose, total solids and ash contents. Results showed that the milk collected from dairy farms had the highest percentage of fat content, which ranged from 6.88 to 10.20%. Milk supplied to milk consumer through milk vendors was contaminated with up to 36% water. Milk supplied by the vendors showed significantly low fat and total solid contents (3.68 and 8.07%, respectively).

Milk samples of dairy farms, milk producers, and processed milk showed good quality, with average % fat 8.54, 5.55 and 3.50 respectively and % mean lactose contents 5.62, 5.33 and 4.54 respectively. Mean of protein (%) contents of these three samples were 2.95, 2.5 and 3.46 respectively while % ash contents are 0.66, 0.62 and 0.72 respectively. Milk samples of vendors and milk consumers showed % inferior quality.

INTRODUCTION

Milk is an ideal food for all age groups as well as for infirm people. Milk and milk products constitute a good source for proteins of animal origin. At the same time, it is highly perishable commodity and an excellent medium for the growth of microorganisms. Hygienically produced fresh milk is wholesome, nutritious and reasonably stable. Soon after leaving the udder it gets exposed to all types of detrimental changes i.e., changes in chemical composition, nutritive value, bacterial load, taste, flavour and appearance (Hanjra et al., 1989). Per capita availability of milk and milk products in Pakistan is highest in the Asia –Pacific Region. Yet under the prevalent conditions, the required care is not observed in handling of milk. The quality of such milk is thus often adversely affected. Milk from different sources contains the same kinds of constituents but in varying amounts. Within a given species, genetic factors and environmental conditions such as the climate and the stages of lactation influence the composition (Roadhouse., 1950). Cow's whole milk contains not less than 3.00 to 3.80 % fat (3.25% is more prevalent and 8.25%SNF (Solid non fat) (Anonymous. 1967).

Milk supplied to the consumers through milk vendors was found to be adultered with upto 43% of water, without significant difference in the fat and total solids contents of milk, supplied by milk producers, milk collectors and dairies (5.80 and 15.24%, 5.70 and 14.34%; 5.78 and 14.50% respectively. It was also found that the buffalo and cow milk of NARC dairy farms contained 6.5 and 16.6% and 3.8 and 12.8% fat and total solid respectively (Athar and Ali., 1986). It was found that mean fat contents is 3.9± 0.1, and protein is 3.25± 0.03 (Drake et al., 1997).

Composition of market and skim milks

Sr. No.	Composition	Market Milk	Skim Milk
1.	Water	87.40	90.40
2.	Fat	0.10	0.20
3.	Protein	3.50	3.60
4.	Lactose	4.90	1.50
5.	Ash	0.70	0.70
6.	SNF	8.00-8.50	8.15-8.50
7.	TS	11.50-12.30	8.25-12.00

(Louis., 1970).

In an other report mean percentage of protein was 3.32 (ranges from 2.98 to 4.22) and the mean percentage of fat was 3.93 (range 2.89 to 5.45; SD=0.35); While the correlation between milk and protein yield, milk and fat yield, and protein and fat yield 0.96, 0.87 and 0.91, respectively (Sargeant et al., 1997). A comparison between percentage composition of milk samples obtained from different sources indicated that from milk production to it's retailing, there was gradual deterioration in the quality of milk (Athar., 1991). The present study was conducted to compare physico – chemical properties of milk collected from dairy farm, milk producers, processed milk, milk vendors and milk consumers in mounth of summer (from May to Augest). Lot of work has been done to evaluate the quality of milks from different sources.

MATERIAL AND METHODS

The study was conducted at the Dairy Technology Research Laboratory (DTRL) of Animal Sciences institute at National Agricultural Research Centre (NARC), Islamabad.

Collection of samples:

Seventy-five milk samples were collected in 250ml-sterilized bottles from five different sources.

1. Dairy Farms (NARC and Military, Islamabad and Rawalpindi, respectively)
2. Milk producers (Rawalpindi and Islamabad)
3. Milk vendors (Gawala)
4. Consumers (Rawalpindi and Islamabad)
5. UHT processed milk (polyfilm pack from Halla Milk Center, NARC, Islamabad).

Milk samples collected from each source were brought to the Dairy Technology Research Laboratory, (DTRL, NARC) for analysis at fresh stage.

Chemical Analysis

Following tests were performed in chemical analysis,

The lactometer is used as an aid in detecting milk to which water might have been added as describe by Lampert (1965). Lactometer reading and Specific Gravity is determine by Eckles., et al method (1957). Ph was determined according to the method described by Anonymous (1990). Total titratable acidity was estimated by using Atherton and Newlander method (1977). Fat determination was carried out using method as described by Anonymous (1973). Total solid contents were measured by using method described by Ling (1957). Determination of Protein was done by using Kjeldahl method (Anonymous., 1990). Estimation of Lactose was done by method describe by Patel and Mistry (1997) and ash contents was determined by using Khalil and Manan method (1990).

RESULTS AND DISCUSSION

The data regarding various parameters are presented in tables and discussed as under.

Lactometer Reading (LR) and Specific Gravity

The average results obtained for LR and Specific gravity of different milk samples are shown in table 1. The standard milk showed the highest LR and Specific gravity, i.e. 28.80 and 1.028 respectively. LR of dairy farm's milk ranges between 23-31 and its specific gravity range is 1.022-1.033 while the LR and Specific gravity values of milk producers are 22-30 and 1.026-1.033. These two values are non-significant from each other ($P>0.05$). Whereas milk samples obtained from milk vendors and milk consumers showed significant difference to each other and were adultered on the basis of LR and Specific gravities.

pH and Total Titratable Acidity (TTA)

Each milk sample was tested for pH and TTA at fresh stage. The TTA is a simple acid base titration. This test allows a calculation of percentage acidity in milk. The values of pH and TTA in various milk samples are given in table 2.

The maximum value of pH (6.89) was of dairy farm milk while the maximum TTA (0.15%) was of sample of milk consumer. The pH value (6.67) of processed milk sample is almost similar to results studied by Gervilla et al., (1997), who found the pH value of 6.66 of milk sample. In this study the percentage acidity (0.115%) was the lowest in milk producer's milk sample in contrast the consumer's milk sample that showed highest percentage (0.15%) of acidity. Similarly, pH of milk vendors and dairy farm milk sample were

non-significant different from one another ($P<0.05$). Marked changes occurred in values of pH and acidity in UHT processed milk stored at different temperatures like 2°C and 7°C for 10 and 7 days respectively (Nakanishi et al., 1976). These findings support this study in respect to processed milk as compared to other milk types.

Table 1. Lactometer Readings and Specific gravities of different milk samples.

Source	LR (Range)	LR (Mean)	Sp. Gravity (Range)	Sp. Gravity (Mean)
Dairy Farms	23 - 31	26.87B	1.022 – 1.033	1.027C
Milk Producers	22 - 30	26AB	1.026 – 1.033	1.028B
Processed Milk	26 – 31	28.5A	1.029 – 1.033	1.033A
Milk Vendors	15 - 24	19.67C	1.081 – 1.022	1.02D
Milk Consumers	15-24	19.5C	1.018-1.028	1.028D

Table 2. Total Titratable Acidity (TTA) and pH of different milk samples

Source	TTA(%) (Range)	TTA (Mean)	pH (Range)	pH (Mean)
Dairy Farms	0.11-0.16	0.135	6.80-6.98	6.89A
Milk producers	0.10-0.13	0.115	6.35-6.90	6.63E
Processed Milk	0.13-0.15	0.14	6.49-6.85	6.67D
Milk Vendors	0.10-0.16	0.13	6.50-6.94	6.72B
Milk Consumers	0.11-0.19	0.15	6.44-6.95	6.695C

Fat, Total Solid and SNF

Determination of fat content is a satisfactory measure for estimation of quality of milk because as milk adultered with water its fat decreases. Table 3 shows the values and there respective range. Fat and total solid contents of the milk of dairy forms and processed milk were 8.54%, 17.93% and 5.55%, 14.12% respectively. Which adequately met with the standards of pure food laws (Kazmi., 1983). Whereas the milk supplied by milk vendors have3.68% and 10.32% fat and total solid contents, which is significantly lesser than other sources.

These results are in accordance with the findings of Shah (1975) and Ather and Ali (1986). Processed milk contains 3.50% and 11.57% and 8.57% SF, total solid and SNF respectively. Pure food laws support these results (Kazmi., 1983). The milk supplied by vendors contains 3.68% fat and 6.64% SNF and these values do not meet the requirements of pure food laws.

Total solids of milk other than fat are called as SNF or solid non-fat. The mean values of SNF with its range are given in the Table 4. The milk with high in fat would be high in SNF. However, the results in this study showed significant differences ($P<0.05$), among the milk of dairy farms (9.39%), milk producers (8.57%) and of processed milk (8.07%). In contrast the SNF values of milk vendors and milk consumers (6.64%and6.7%) showed insignificant difference. Results showed that milk collected from theses two sources is of inferior quality.

Table 3. Percentage Fat of different milk samples.

Source	% Fat (Average)	Range %
Dairy Farms	8.54A	6.88-10.20
Milk Producers	5.55B	4.60-6.50
Processed Milk	3.50D	3.50-3.50
Milk Vendors	3.68C	2.50-5.00
Milk Consumers	3.70C	3.10-5.10

Table 4. Percentage solid non-fat (SNF) of different milk samples

Source	SNF % (Range)	%SNF (Mean)
Dairy farms	8.40-10.38	9.39A
Milk producers	7.30-9.84	8.57B
Processed milk	7.80-8.34	8.07B
Milk vendors	5.88-7.40	6.64C
Milk consumers	5.30-8.10	6.7C

Protein Content

Fat, protein is most important and variant constituent of milk. In present study the percentage protein varies from 1.80% (vendors) to 3.59% (Consumer). These results are based on the estimation of total nitrogen in milk (Nx6.38). These results are shown in Table 5.

These results showed that the processed milk has good range (3.42-3.50) of protein and highest protein contents (3.46%). However the milk of dairy farms (2.96%) and processed milk (3.46%) are significantly different ($P<0.05$) from each other. Milk collected from milk vendors showed lowest protein contents (2.29%) which showed the non-significant difference ($P < 0.05$) from the protein contents of milk obtained from milk producers (2.5%). Louis and Pien (1970) support the result obtained from this study to some extent.

Table 5. Protein content (%) of milk collected from different sources.

Source	Range (%)	Protein(%) Mean
Dairy farms	2.50-3.42	2.96B
Milk producers	2.20-2.80	2.5D
Processed milk	3.42-3.50	3.46A
Milk vendors	1.80-2.79	2.29E
Milk consumers	1.86-3.58	2.72C

Milk Sources

Lactose Content

Lactose is the main carbohydrate of the milk. Lactose contents of different milk samples are shown in Table 6. Milk sample of consumer showed lowest lactose contents (3.21%) and dairy farms milk showed highest contents of lactose (5.62%). Milk obtained from the dairy farms (5.62%) and of milk producers were non significant by different form to each other while the milk obtained from milk vendors and milk consumers showed significant difference ($P<0.05$).

Table 6. Lactose content (%) of milk collected from different sources.

Source	Range (%)	Lactose (%) Mean
Dairy farms	4.62-6.60	5.62A
Milk producers	4.55-6.10	5.33A
Processed milk	4.20-4.82	4.54B
Milk vendors	2.60-5.62	4.13C
Milk consumers	1.60-478	3.21D

Ash Content

When water of milk is evaporated the whitish ash left. It contains the minerals substances. Values of ash of milk samples collected from different sources are given in Table 7. Values vary from 0.18% to 0.76%. These values partly match with the normal range of ash contents in milk obtained from various species (Louis, 1970).

Table 7. Ash content (%) of milk collected from different sources

Source	Ash (%) Range	Ash(%) Contents
Dairy farms	0.58-0.76	0.66AB
Milk producers	0.60-0.64	0.62B
Processed milk	0.68-0.77	0.72A
Milk vendors	0.18-0.52	0.37D
Milk consumers	0.36-0.55	0.46C

Milk Sources

The comparison of mean values by Duncan's Multiple Range Test (DMRT) showed that the ash contents of processed milk (0.72%) and that of dairy farms (0.66%) and of milk producers (0.62%) and milk consumers (0.46%) were

significantly different ($P<0.05$). While the lowest range of ash contents (0.37%) was found in the milk sample of vendors. However the milk collected from dairy farms, milk producers and processed were in the range found by Louis (1970).

CONCLUSION

Milk collected from dairy farms, milk producers and processed milk showed good quality. In contrast, milk collected from vendors and milk consumers are of inferior quality due to adulteration with contaminated water and adultrants. Good quality milk contains all the measured contents such as protein, Lactose, ash, fat, solid non-fat and total solid, pH, acidity in optimum amount while the milk sample of inferior quality shows the high acidity, low pH, low solid fat, low SNF, low lactose and low protein contents.

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PREPARATION AND CHARACTERIZATION OF CHOCOLATE FLAVORED SOYMILK DRINK

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ABSTRACT

Soymilk, the extract of soybean, is a good source of protein. In the present study chocolate flavored drink was prepared by using 1:12, 1:14 and 1:16 dry bean to water ratio with the addition of 0, 5 and 10 percent skim milk powder. The prepared drink was stored at 4-10°C for 1 month. The chemical and sensory analysis of all the treatments was carried out after 7 days interval. Analysis of variance revealed highly significant effect of storage and treatments on the over all quality attributes of chocolate flavored soymilk drink. It is was observed that T6 (having dilution of 1:14 dry bean to water ratio and 10% skim milk powder) showed better performance than the other treatments through-out the storage study. Addition of skim milk powder and flavor masked the characteristics undesirable beany flavor and astringency of soymilk.

INTRODUCTION

The steadily increasing costs of animal protein have compelled the food industry to focus attention on low cost vegetable sources of protein. Soybean has a greater potential for use in food products because of high quality protein contents (Smith and Circle, 1980). Soymilk and cow milk have approximately the same protein contents (3.5 – 4.0%) and show fairly similar A. A profile except that soybean milk is deficient in sulphur containing amino acids. Methionine supplementation raises its nutritive value to essentially the same level as that of cow milk. Soymilk also contains increased amounts of niacin and vitamin B (Leiner 1977).

Soymilk ordinarily produced does not have the bland flavor or smooth texture of cow milk. The enzyme lipoxygenase is mainly responsible for its characteristics beany flavor. Off flavor is hardly detectable in the raw whole soybean but it develops during breakdown of cell structure. Another flavor defect in soymilk is astringency.

The flavor of soy traditionally has been an obstacle to beverage makers. 60 percent, skimmed milk powder along with 1.62 percent cream and 3 percent sugar was previously used to overcome these problems (Shahid 2000). Intense flavors can mask the characteristics beany flavor (Anonymous 2001). Hence chocolate flavor with reduced amount of skimmed milk powder was used. Present study focused the preparation of pleasantly flavored nutritious drink with reduced cost and high consumer acceptance.

MATERIALS AND METHODS

Soybean was procured from Ayub Agriculture Research Institute, Faisalabad. Sugar, Cocoa powder, skimmed milk powder and chocolate flavor was directly purchased from local market. Soymilk was prepared from whole healthy soybeans. Husk of Soybeans was removed after soaking at 100°C in water (pH 8) for 1 hour. Dehulled soybeans were changed into slurry by the addition of little amount of water. Soymilk was prepared from the slurry with different dry bean to water ratios i.e. 1:12, 1:14 and 1:16 to improve the acceptability of product. Sugar, cocoa powder and flavor were blended at a level of 10, 1 and 0.2 percent respectively. Skimmed milk powder at a level of 0, 5 and 10 percent was used as shown in Table (1). Drink was subjected to pasteurization at temperature of 70°C for 15 minutes (Chien and Synder, 1983). Pasteurized drink was filled in 250

mL glass bottles, cooled and stored at 4-6°C for chemical and sensory evaluation for one month.

Table 1. Different treatments used in the study

Treatments	Dilution (Dry bean to water ratio)	Skim milk
T_0 (Control)	-	-
T_1	1:12	0%
T_2	1:12	5%
T_3	1:12	10%
T_4	1:14	0%
T_5	1:14	5%
T_6	1:14	10%
T_7	1:16	0%
T_8	1:16	5%
T_9	1:16	10%

Drinks were analyzed after 7 days intervals for total solids, protein, pH, acidity and ash contents according to the methods described in AOAC (1995).

For sensory evaluation, drinks were presented to a panel of 5 judges to evaluate color, flavor, taste and overall acceptability, using 9-point hedonic scale as described by Larmond (1987).

The data obtained was analyzed by analysis of variance techniques (Steel et al., 1996).

RESULTS AND DISCUSSION

The results of chemical analysis are shown in Table 2. Total solids, protein and ash contents increased significantly with increasing level of skim milk powder and decreasing dilution of soymilk. Statistical results regarding storage were non significant for all these attributes (Table 3). There were no significant differences in total solids, ash and protein contents. The present results are in corroborated with the previous findings of Kwoke and Nirangon (1995), Singh (1978) and Evans (1997). Statistical analysis of pH and acidity values were highly significant both for treatments and storage. A slight decrease in pH and slight increase in acidity was also reported by Luttrell *et al.* (1981) and Uboldi and Ferreira (1984) respectively. The decrease in pH was due to the fact that Hydrogen ion concentration increased during storage because of the formation of organic acids (Tanaka *et al.*, 1985).

Table 4 shows the effect of treatments on sensory characteristics of the drinks. The values for color, taste, flavor and overall acceptability increased with increasing level of skim milk powder as it improves smoothness in the drink (Shahid 2000). Drink having 10 percent skim milk powder got

Table 2. Chemical analysis of chocolate flavored soymilk drinks

Treatments	Total solids	pH	Ash	Acidity	Protein	Fat
T_0	16.11g	6.95b	0.58d	0.076e	3.46g	1.5a
T_1	13.52h	6.90c	0.18h	0.086d	2.59h	0.5f
T_2	17.05d	6.8f	0.508e	0.112b	4.26b	0.8c
T_3	21.05a	6.69g	0.88a	0.138a	5.95a	1.10b
T_4	13.18i	6.94b	0.16c	0.08e	2.3e	0.70d
T_5	16.92e	6.86e	0.47f	0.098e	3.93c	0.80c
T_6	20.68b	6.67h	0.86b	0.132a	5.68b	1.0b
T_7	12.83i	6.99a	0.14j	0.067f	2.04j	0.4g
T_8	16.58f	6.88d	0.45g	0.086d	3.71f	0.6e
T_9	20.33c	6.67h	0.84c	0.116b	5.46c	0.8c

highest score whereas among dilutions, maximum scores were obtained by 1:14 dry bean water ratio.

degradation as found by Chien and Synder (1983) affecting overall acceptability were recorded. But all the drinks remained acceptable throughout the

Table 3. Effect of storage on chemical characteristics of soymilk drinks

Days	Total Solids	pH	Ash	Acidity	Protein	Fat
0	16.850a	6.88a	0.508a	0.91c	3.835a	0.812a
7	16.849a	6.87a	0.508a	0.91c	3.83a	0.818a
14	16.85a	6.87a	0.508a	0.91c	3.84a	0.821a
21	16.849a	6.80b	0.508a	1.04b	3.835a	0.821a
30	16.85a	6.74c	0.507a	1.185a	3.84a	0.818a

Table 4. Sensory evaluation of chocolate flavored soymilk drinks

Tretments	Color	Taste	Flavor	Overall acceptability
T_0	6.91c	6.96b	6.96b	6.95b
T_1	4.92h	5.82h	5.36h	5.49g
T_2	5.89f	5.93f	5.96f	5.96f
T_3	6.5d	6.32e	6.33e	6.55c
T_4	5.1g	5.92g	5.96f	5.46h
T_5	6.99b	6.95b	6.96b	6.96b
T_6	7.98a	8.45a	7.96a	7.96a
T_7	4.72i	4.95i	5.54a	5.15i
T_8	5.92f	6.65d	6.56d	6.19e
T_9	6.3e	6.73c	6.67c	6.41d

Table 5. Effect of storage on organoleptic quality of soymilk drink

Days	Color	Taste	Flavor	Overall acceptability
0	6.20a	6.529a	6.48a	6.354a
7	6.20a	6.529a	6.48a	6.354a
14	6.08a	6.49b	6.48a	6.354a
21	5.94b	6.41c	6.40b	6.298b
30	5.94b	6.31d	6.34c	6.205c

The T_6 when compared with a commercial chocolate flavored dairy drink scored higher points when evaluated for color, taste, flavor and overall acceptability.

During storage a slight degradation in sensory quality was observed (Table 5). Color fading as reported by Kayani (1987), taste and flavor

storage period of 30 days under refrigeration temperature.

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STUDIES ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF SOME IMPORTANT INDIGENOUS AND IMPORTED APPLE CULTIVARS

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ABSTRACT

Nutritional quality of some indigenous and imported varieties of apples was investigated in this study. Physical characteristics i.e. shape, size, colour and taste of indigenous varieties of apples (Amri, Kandhari, Kulu, Mashadi, Golden Delicious) and imported apple variety (Red Delicious) were compared. Wide variations in most of the physical characteristics were found in these varieties of apples. The shape and size of these apple varieties varied from round oblique to round oblong and weight ranged from 125 – 166 gm per fruit piece. All these varieties had thin tough skin and sweet taste except Kulu. The colour of golden and red delicious varieties were dark brownish red while colour of other four varieties were almost pale greenish. Chemical analysis results revealed that protein (1.27%) and total sugars (12.70%) contents in imported variety were slightly higher than indigenous apple varieties. (0.67-0.96% protein, 8.58-12.20% total sugars). Ash contents were almost the same in indigenous and imported varieties of apples (1.60-1.80%). However, the contents of vitamin A (0.73 mg/100 gm) and C (10.1 mg/100gm) were lowest in case of imported variety. Variable amounts of dietary fibre components in indigenous and imported apple varieties were observed. However, amount of hemi-cellulose was found comparatively higher than cellulose in these varieties. Differences among the indigenous and imported varieties of apples were observed for mineral contents.

INTRODUCTION

Apple (*Pyrus malus)* is a delicious and nutritious fruit, which is widely grown and consumed in many areas of world including Pakistan. Total sugars, minerals and vitamins are the main nutrients of apple. Dietary fibre is another important component of various fruits (Guangya *et al.*, 1991 and Sachio, 1991). Dietary fibre mainly consists of cellulose, hemicellulose, lignin and pectin. Apple juice is also usually used by people of all ages for its high nutritional value and instant energy source. Several reports appear in literature regarding the nutritive value of fruits and vegetables (Salunkhe, *et al.*, 1974; Sehgal, 1975; Johnson *et al.*, 1984; Khan *et al.*, 1990; Zhijun *et al.*, 1998 and Rehman *et al.*, 1999). Apples are a rich source of soluble sugars, minerals and vitamins. (Castillo etal 1997). Apart from these nutrients, apples also contain sufficient quantity of soluble and insoluble dietary fiber components. (Gheyas et. al. 1997). However, little information is available in literature about the physico-chemical composition of apples particularly the locally grown cultivars.

The present work was undertaken to collect data about the chemical composition of indigenous and imported varieties of apples to compare the quality of locally grown apples with imported variety.

MATERIALS AND METHODS

Fresh apple fruit of different varieties (Amri, Kandhari, Kulu, Mashadi, Golden Delicious and Red Delicious) were purchased from the local market. The fruit was sorted, discarding the bruised and damaged ones and leaving only the wholesome to carry out this study. Physical

characteristics of the fruits were examined for shape, weight, skin, colour and taste.

The chemical composition (moisture, protein and ash contents) of different apple varieties was determined according to standard methods of A.O.A.C. (1990). Total sugars were estimated by dinitrosalicylic acid method as described by Miller (1959). Vitamin A was measured using the method of Valadone and Mummery (1959) after extracting it in petroleum ether and acetone mixture. Vitamin C was determined according to Bajaj and Gurdeep (1981) method on a spectrophotometer. Neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicellulose and lignin contents in the apple samples were determined according to the procedures described by Van-Soest *et al.* (1991). For the determination of minerals (Na, K, Mg, Ca, P, Fe, Zn, Ca, Mn), the ash was dissolved in 5 ml of 20% HCl and volume was made up to 50 ml. All minerals except Na, K and P were determined with the atomic absorption spectrophotometer (Model Hitachi 170-10), Na and K were determined with a flame photometer (Bechman Aline Flame). Phosphorus was determined spectrophotometrically using the procedure of

Table 1. Physical characteristics of different varieties of apples

Variety	Shape	Weight/piece (gm)*	Skin	Colour	Taste
Amri	Round conical oblique	125	Thin very tough	Pale greenish	Sweet
Kandhari	Roundish truncate	125	Thin and tough	Greenish to pale greenish	Sweet without any trace of acidity
Kulu	Round conical to oblong conical	166	Thin and tough	Pale greenish	Slightly acidic sweet
Mashadi	Round oblique	125	Thin and tough	Pale greenish	Sweet without any trace of acidity
Golden Delicious	Oblate	142	Thin and tough	Dark brown	Sweet
Red Delicious	Round oblong	142	Thin and tough	Dark brown	Sweet

*Average of eight pieces.

Table 2. Chemical composition of indigenous and imported apple varieties*

Variety	Moisture (%)	Protein (%)	Total Minerals (Ash) (%)	Total Sugars (%)	Vitamin C (mg/100g)	Vitamin A (mg/100 g)
Amri	80.01 ±1.27**	0.96 ±0.12	1.80 ±0.12	8.58 ±0.32	13.2 ±0.32	0.90 ±0.21
Kandhari	80.70 ±1.08	0.91 ±0.11	1.70 ±0.11	8.95 ±0.72	12.7 ±0.45	0.95 ±0.18
Kulu	82.80 ±1.66	0.73 ±0.10	1.60 ±0.13	12.20 ±0.77	13.0 ±0.41	0.88 ±0.17
Mashadi	78.77 ±1.44	0.67 ±0.13	1.70 ±0.11	9.85 ±0.38	11.7 ±0.55	0.79 ±0.13
Golden Delicious	81.00 ±1.35	1.05 ±0.11	1.65 ±0.10	9.81 ±0.65	10.5 ±0.49	0.82 ±0.20
Red Delicious	80.00 ±1.27	1.27 ±0.10	1.72 ±0.09	12.70 ±0.51	10.1 ±0.42	0.73 ±0.19

* : Average of triplicate determinations
**: Standard deviation values

Watanabe and Olsen (1965). Triplicate determinations were carried out and standard deviation was calculated following the method of Steel and Torrie (1980).

RESULTS AND DISCUSSION

Physical characteristics of indigenous and imported varieties of apples are given in Table-1. Amri, Kandhari, Kulu, Mashadi and Golden

Table 3. Insoluble polysaccharides in indigenous and imported apple varieties*

Variety	NDF (%)	ADF (%)	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)
Amri	8.65 ±0.65**	4.35 ± 0.15	3.50 ±0.05	4.30 ±0.13	0.85 ±0.21
Kandhari	7.74 ±0.41	3.79 ±0.11	3.09 ±0.08	3.95 ±0.11	0.70 ±0.11
Kulu	2.67 ±0.11	0.98 ±0.14	0.78 ±0.09	1.69 ±0.09	0.20 ±0.10
Mashadi	9.01 ±0.45	4.46 ±0.12	3.66 ±0.07	4.55 ±0.17	0.81 ±0.13
Golden Delicious	6.49 ±0.32	3.49 ±0.10	2.89 ±0.05	3.00 ±0.11	0.62 ±0.17
Red Delicious	4.31 ±0.21	3.80 ±0.09	1.75 ±0.05	1.91 ±0.11	0.65 ±0.15

* : Average of triplicate determinations
** : Standard deviation values
NDF : Neutral detergent fibre
ADF : Acid detergent fibre

Table 4. Macro and micro minerals in indigenous and imported apple varieties*

Variety	Macro Elements mg/100 g					Micro Elements mg/100 g			
	Na	K	Mg	Ca	P	Fe	Zn	Cu	Mn
Amri	26.50 ±0.02**	850.0 ±0.02	130.0 ±0.05	98.0 ±0.04	65.0 ±0.01	4.5 ±0.01	2.0 ±0.01	0.5 ±0.01	0.4 ±0.01
Kandhari	20.0 ±0.04	800.0 ±0.03	135.80 ±0.05	71.0 ±0.03	58.0 ±0.02	6.0 ±0.02	0.5 ±0.02	0.5 ±0.01	0.3 ±0.01
Kulu	18.50 ±0.05	905.0 ±0.02	140.0 ±0.03	125.0 ±0.02	72.0 ±0.04	1.8 ±0.01	0.2 ±0.02	0.3 ±0.01	0.1 ±0.01
Mashadi	10.50 ±0.03	1024.0 ±0.04	120.50 ±0.04	80.50 ±0.11	65.0 ±0.04	2.5 ±0.02	0.3 ±0.03	0.4 ±0.02	0.2 ±0.01
Golden Delicious	12.80 ±0.07	780.0 ±0.01	135.0 ±0.05	51.25 ±0.04	80.0 ±0.03	2.5 ±0.01	0.7 ±0.01	0.1 ±0.02	0.9 ±0.01
Red Delicious	8.90 ±0.06	1050.0 ±0.05	148.0 ±0.03	48.0 ±0.03	95.0 ±0.03	6.0 ±0.01	2.5 ±0.01	0.7 ±0.02	0.8 ±0.02

* : Average of triplicate determinations
** : Standard deviation values

Delicious are indigenous varieties whereas Red Delicious is an imported one. The shape and size of these apple varieties varied from round oblique to round oblong and weight of fruit per piece ranged from 125 – 166 gm/fruit. It was observed that, in general, the Golden Delicious and Red Delicious varieties had about 13.7% more weight than Amri and Kandhari varieties. On the other hand, Kulu and Mashadi varieties showed more weight (by about 17%) compared to Golden and Red Delicious varieties of apples. All these varieties had thin tough skin and sweet taste except Kulu which had thin skin and was slightly acidic in taste. The colour of Golden and Red Delicious varieties was observed to be dark brownish red while the colour of other four varieties was almost pale greenish. In general, wide variations in most of the physical characteristics were found in these varieties.

Table-2 shows the chemical composition of apples belonging to different indigenous and imported varieties. It may be observed (Table-2) in general, that the amount of the crude protein contents (N x 6.25) in the indigenous and imported apple varieties ranged from 0.67 to 1.27%. The protein content was higher in the imported variety (1.27%) compared to indigenous varieties. Minimum amount of protein (0.67%) was observed in case of Mashadi. However, ash content was almost the same in indigenous as well as imported varieties. These results also revealed that total sugars contents of Red Delicious (12.70%) were higher compared to local varieties (8.58 – 12.20%). Vitamin C and vitamin A contents were found to be the lowest in case of imported variety. However, there was not much difference in vitamin C and vitamin A contents of the local varieties. These observations agree well with the reported values in literature (Karklina and Melgalve, 1977, Marcelle, 1995).

The results of the analysis of insoluble polysaccharides of the indigenous and imported varieties of apples are shown in Table-3. Variable amounts of dietary fibre components in indigenous and imported varieties of apples were observed. It is apparent from this data that the amount of hemicellulose was comparatively higher than cellulose in these apple varieties. The contents of NDF and ADF in indigenous varieties varied from 2.67 – 9.0% and 0.98 – 4.46% respectively whereas 0.78 – 3.50% cellulose, 1.69 – 4.55% hemicellulose and 0.20 – 0.62% lignin contents were present in the local varieties. On the other hand, the amount of NDF, ADF, cellulose, hemicellulose and lignin in imported variety was 4.31, 3.80, 1.75, 1.91 and 0.65% respectively. It appeared from these results that minimum amount of NDF and other related polysaccharides were present in local variety i.e. Kulu. It is also evident from the data that the amount of hemicellulose was comparatively higher than cellulose in all the indigenous as well as imported varieties.

Results of macro mineral analysis show that K is the most abundant mineral element present in the indigenous and imported varieties (Table-4). Magnesium followed by calcium and phosphorus is the next highest in concentration in decreasing order. Sodium is in the lowest concentration in all the varieties. In comparison to imported variety, the local varieties other than Mashadi, are clearly deficient in K content and accordingly inferior in this regard. The lowest concentration of Na (8.90 mg/100 g) is recorded in the case of imported variety while it is highest (26.50 mg/100 g) in the local variety "Amri". The concentration of Mg and P is found to be the highest in the imported variety compared to local varieties of apples. Among the micro elements, Fe is present in higher concentration in all the varieties followed by Zn, Mn and Cu (Table-4). In comparison to local varieties, the imported variety contains distinctly high amount of Fe, Zn, Cu and Mn. The local variety 'Kulu' contains the least amount of all these micro elements. Maria (1969) reported minerals composition in different fruits particularly in various varieties of apples. The present results also agree with these findings.

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EFFECT OF DIFFERENT STABILIZERS ON BRIX, ACIDITY AND ASCORBIC ACID CONTENT OF TOMATO CONCENTRATE

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ABSTRACT

The objective of this study was to compare the effect of different stabilizers on ascorbic acid, acidity and ^{O}Brix of tomato concentrate during one-month storage at room temperature. A gradual increase in ^{O}Brix and acidity was observed. Maximum rise in ^{O}Brix was in the sample containing carboxymethyl cellulose (CMC) @ 0.2%, and least in the sample containing 0.1% guar gum. Maximum increase in acidity was observed in the sample containing CMC @ 0.2 % and least increase in the one containing guar gum @ 0.2 %. A decrease in ascorbic acid was observed, minimum decrease in sample containing CMC @ 0.2 %, while more decrease was observed in containing guar gum @ 0.2 %. It is concluded that tomato concentrate of good quality can be prepared by the addition of CMC @ 0.2 %.

INTRODUCTION

Tomato (*Lycopersicon esculentum L.*) was grown in Pakistan on the area of 19.3 (000) ha and 193.8 (000) tons of production is obtained during 1997-98. Apart from fresh consumption as salad, tomato is virtually part of every dish cooked at home or in the restaurants all over the country. Nutritionally it ranks quite high. Its caloric value is 20 calories per 100 g (Myres and Croll, 1921). It is a good source of vitamins A and C (Shah and Zafar, 1975). It also contains acids such as citric, malic , aminobutyric, cis-aconitic and formic acid along with a fair amount of histidine, lysine and certain minerals (Tressler and Joslyn, 1962.).

The major organic acids present in tomatoes are citric and malic. Citric acid is most abundant and accounts for upto 90 percent of acid content (Hammer and Maynard, 1945). Presently tomatoes are used in fresh state as well as in different processed products such as ketchup, pickles, sauces, soup, salad, purees, concentrates, pastes, etc. (Cruess, 1958). The principle reason for food concentration is to reduce weight and bulk (Norman 1973). Now-a-days, tomato concentrate is an important product in world food trade. Its use in the preparation of food has increased rapidly (Gould, 1974).

Synersis or separation of tomato paste occuring during storage for few days may be due to reaction of acids with starch and pectin, thus affecting the viscosity of the product .It may be possible to control this separation by the use of stabilizers. Among the different locally available stabilizers, carboxymethyl cellulose(CMC), guar gum and pectin were selected for this work. The objective of this research was to study the stability of tomato concentrate at room temperature using different concentrations of selected stabilizers.

MATERIALS AND METHODS

Tomatoes at maturity stage were procured from local vegetable market and used to prepare concentrate. The tomatoes were sorted to get fruit in uniform color. Trimming was done with a stainless steel knife to remove the unwanted portions of the fruit. After this the fruits were washed thoroughly in running water to remove the contaminants . The juice was extracted by " hot break " method. The fruit was crushed and immediately heated. The hot juice was passed through the pulper to separate seeds and skin. The juice was concentrated to 18 ^{O}Brix. After the preparation of concentrate to 20 degree Brix the three stabilizers i.e. carboxymethyl cellulose, pectin and guar gum were added individually at the rate of 0.1% and 0.2% respectively coupled

Table: 1. Effect of stabilizers on brix, acidity, and ascorbic acid of tomato concentrate.

		Brix				Acidity %				Ascorbic acid mg/100ml			
Treatments	Stabilizers	Day-0	Day-15	Day-30	Mean	Day-0	Day-15	Day-30	Mean	Day-0	Day-15	Day-30	Mean
T1	Pectin 0.1%	18.05	18.15	18.35	18.18	1.29	1.43	1.52	1.42^{d}	10.0	9.55	9.25	9.60^{b}
T2	Guar gum 0.1%	18.0	18.1	18.3	18.13^{d}	1.27	1.42	1.54	1.41^{d}	9.4	9.0	8.25	8.88^{c}
T3	CMC 0.1%	18.15	18.3	18.4	18.37^{b}	1.28	1.55	1.58	1.47^{c}	9.1	8.75	8.5	8.78^{c}
T4	Pectin 0.2%	18.20	18.25	18.4	18.28^{b}	1.27	1.57	1.73	1.52^{b}	10.5	10.0	9.5	10.0^{b}
T5	Guar gum 0.2%	18.15	18.25	18.3	18.23^{c}	1.34	1.39	1.45	1.39^{d}	9.25	7.83	7.63	8.26^{d}
T6	CMC 0.2%	18.4	18.55	18.6	18.52^{a}	1.37	1.63	1.74	1.58^{a}	11.5	11.25	10.75	11.17
T7	Control	18.05	18.15	18.2	18.13^{d}	1.25	1.32	1.37	1.32^{e}	10.4	9.25	8.25	9.3^{bc}

Figures showing common letters are non significant
DMR test = 0.05
NS = Non-significant

with control sample. The samples were analyzed just after processing and on fortnightly basis for a period of one month storage at room temperature.

Determination of ^{o}Brix: The degree Brix was measured by method No. 970.59 (AOAC,1990).

Titratable Acidity: Acidity of tomato concentrate was determined by method No. 943.03 (AOAC,1990).

Ascorbic acid: Ascorbic acid was determined with the help of a spectrophotometer at 540 nm as described by Ruck (1963).

Data Analysis: The methods of Steel and Torrie (1980) were followed for statistical analysis of the data.

RESULTS AND DISCUSSION

^{o}Brix: The data on effect of stabilizer on ^{0} Brix change in tomato concentrate is presented in Table-I. This table shows there was a gradual increase in ^{0} Brix with the passage of time. The possible reason for this increase may be the increase of soluble solids in concentrate with time. Maximum increase in ^{0} Brix was observed in the sample containing CMC @ 0.2% and least increase in the sample containing guar gum @ 0.1%.

Statistical analysis of the data revealed significant effect (< 0.05%) of different stabilizers used and storage period on ^{0} Brix in all samples of tomato concentrate (Table I). This is due to certain chemical reactions in which conversion of insoluble pro-pectin to soluble pectin occurred. These results are in line with the findings of Hummel and Okey (1950). It was further observed that during storage an increase in T.S.S. (Total soluble solids) occurred which may be due to acid hydrolysis of polysaccharides especially gums and pectin. Luh and Woodroof (1975) also observed similar results.

Acidity: The change in acidity of control and stabilized samples is presented in Table-I. It was observed that there was a gradual increase in acidity. Maximum increase was observed in the sample containing CMC @ 0.2 % and least increase in acidity in the sample containing guar gum @ 0.2 %.

Statistical analysis of the data as in Table I revealed significant difference (<0.05%) in different stabilizers and storage periods on acidity in all samples.

The rise in acidity may be explained by the fact that the concentration of weakly ionized acids and their salts increases during storage, resulting in increase in acidity. Similar views were expressed by Hummel and Okey (1950) and Ahmad (1997).

Ascorbic Acid. Data given in Table-I further reveals that there is a gradual decline in ascorbic acid content during storage period in all concentrations. This declining trend was more in sample containing guar gum @ 0.2 %. Minimum fall in ascorbic acid was observed in sample containing CMC @ 0.2 %. Statistical analysis of data shows significant effect of different stabilizers in tomato concentrate.

During storage of tomato concentrate losses in ascorbic acid are noted. These losses are due to aerobic destruction or oxidation reaction. Ascorbic acid can easily be destroyed by oxidation that takes place in the presence of molecular oxygen and is greatly accelerated by traces of metals. These results are in accordance to those observed by Naeem *et al.* (1999).

On the basis of these observations it is concluded that there was a significant difference among tested stabilizers on tomato concentrate. This study also suggests that among the examined stabilizers, CMC @ 0.2% can be used to produce a quality tomato concentrate.

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ELECTRON SPIN RESONANCE ANALYSIS OF GAMMA-IRRADIATED SPICES AND DRY FRUITS

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ABSTRACT

Electron spin resonance (ESR) spectroscopic method has been employed to know the irradiation history of four dry fruits and four spices. ESR signal based upon the free radicals produced upon irradiation. The dry fruit samples were irradiated to the gamma-ray doses of 1 and 1.5 kGy and the spice samples were irradiated to 5 and 10 kGy doses. The ESR spectra of dry fruits were recorded after one week of irradiation while that of spices after three weeks of irradiation. The spectra of all the irradiated and un-irradiated samples were compared in order to distinguish them. The results show that the intensity of base line spectra increases with the absorbed dose but no extra radiation specific feature appeared in the ESR spectra of irradiated samples which can discriminate the irradiated items from the un-irradiated control. The 'g' values and the peak width "ΔH_{PP}" have also been compared to evaluate the ESR spectra of all the irradiated and un-irradiated control samples.

INTODUCTION

Gamma-radiations within the recommended doses as prescribed by the food irradiation authorities make the food wholesome, enhancing its shelf life without affecting the food value and flavour (Maloney et al., 1992). To know about the irradiation history and correct labelling on the irradiated food, it is very important to develop an irradiation identification tool for the food-stuffs (Delincee, 1991).

During the irradiation process radicals are formed and some of them are trapped within the matrix of food. In a study conducted by Swallow 1988, it was found that a dose of 10 kGy produces about 5×10^{-3} moles of free radicals per kilogram of food material. A very valuable technique used for the detection of free radicals and can serve as detection method for irradiation treatment is an ESR spectroscopy. This method may also detect the radicals produced normally, for example, by the decomposition of food oils and during grinding in hard foods. This technique can serve effectively as an identification method, if the ESR spectrum of the naturally occurring radicals must differ from the spectrum of the radicals produced by irradiation. It is also very important that the radicals produced upon irradiation must also persist in the material for appreciable period during storage.

In some laboratory trials this technique has been employed only for foods having hard parts such as bones in meat and chicken and seeds in fruits (Delincee and Ehlermann, 1989; Gray and Stevenson, 1989; Rafi et al., 1988).

The increased use of irradiated foods in the recent years has encouraged the efforts to develop certain analytical methods for identification of irradiated foods as well as the radiation dose absorbed by the food items. We have used ESR method for evaluation of dry fruits and spices in the present study since this is relatively fast, simple, sensitive and appears to be closest to the practical implementation to detect changes produced during irradiation. In Pakistan dry fruits are produced in bulk, in addition to it more than 50,000 tons of various dry fruits are imported annually from Afghanistan. Dry fruits are a good source of income and foreign exchange in some countries like Afghanistan, Algeria, Argentina, Australia, Bulgaria, Greece, Iran, Iraq, Portugal, Turkey and Yugoslavia. A substantial amount of fruits, which is about one fourth of the total produced in the world is dried during the glut season by various techniques for storage and consumption. They are utilized at homes and specially during space, naval and other military operations as raw, shelled or after roasting in cream, bakery and confectionery goods. Some species of dry fruits have also been

used as medicines or a part of medicines since the time immemorial. The major part of dry fruits has been spoiled due to the lack of scientific storage method. Usually indigenous methods are used for their storage such as sun drying, salting, fumigation with ethylene oxide, freezing etc. Gamma irradiation is a modern, safe and very simple method used to enhance the shelf-life of the foods because no residue is left which may affect the food quality. Food irradiation can participate more to the social welfare of mankind by offering more benefits than those available from alternative procedures of preservation. It does not even increase the temperature of goods appreciably (1-4 °C at the maximum). Therefore, it can be applied even to the food materials in the frozen state without changing the consistency of the products (Wahid et al., 1989).

MATERIALS AND METHODS

The food samples investigated in the present study were purchased from local market of Peshawar, N.W.F.P, Pakistan. The samples include four types of spices (Cumin, Coriander, black pepper and red pepper) and four dry fruits (peanut, pinenut, almond and walnut). Prior to irradiation, all the samples were packed in polyethylene bags and subjected to irradiation at a temperature of (20 ± 2) °C using Co-60 gamma ray source at Nuclear Institute for Food and Agriculture (NIFA) Ternab, Peshawar. All the samples were irradiated at the dose rate of 2.6 kGy/hour, which was determined using Fricke dosimeter (Schested, 1970). The doses given were within the permissible limit as recommended by international agencies FAO/IAEA/WHO (Delincee, 1991).

The ESR spectra were recorded at room temperature using JEOL/JES-FEIXG ESR spectrometer at the Department of Chemistry, Quaid-i-Azam University, Islamabad. All the measurements were performed at the same conditions as given below:

Magnetic field = 3371 ± 250 Gauss

Modulation of magnetic field = 1.6 Gauss

Amplification = 3.2×10^{-2}

Sweep time/Scan rate =16 min/360 mm

Micro-wave power = 0.8 Mw

Micro-wave frequency = 9.44 G Hz

The limitation of this instrument is that it can only operate in a single scan mode. For each measurement, 200-300 mg sample was taken in the Pyrex ESR cuvette and the ESR spectrum of each irradiated and un-irradiated sample was recorded. As the position of the absorption varies with the field applied and with the instrument used at different operating conditions. Therefore, the absorption in terms of 'g' values has been calculated using the relationship as:

$$g = \frac{hu/g}{hu/g_1\beta - \Delta H_{pp}}$$

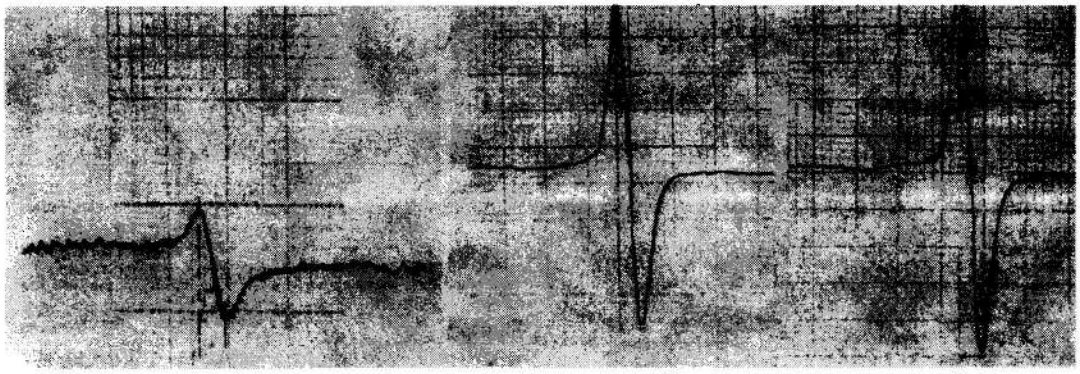
Where 'h' is Plank's constant has the value 6.62×10^{-27} erg sec., u is the frequency of the magnetic field, β is Bohr's magneton which has the value 9.2×10^{-21} erg G^{-1}, g_1 is the standard factor for Mn^{++} marker having numerical value 1.981 which is a calibration factor for ESR spectrometer and ΔH_{pp} is the peak width.

RESULTS AND DISCUSSION

It has been reported that in the ESR spectra of irradiated food material there should be same special new feature, which are not present in the un-irradiated control sample (Tabner and Tabner, 1993; Maloney et al., 1992). It has also been reported that some types of features are not necessarily present in all parts of the same food and in different foods. The ESR spectra examined for the skin, seeds and stalk of irradiated black grapes are not same.

The ESR spectra of dry fruits irradiated to the absorbed doses of 1 and 1.5 kGy and un-irradiated control sample was recorded. The ESR spectra shows that in the un-irradiated control sample the intensity (height of the ESR spectrum) is very small which is due to the radicals which are produced during grinding. In the irradiated samples the concentration of the free radicals has been increased during the irradiation process and hence the height of the ESR signal has also been enhanced. The ESR spectra of the dry fruits were recorded after one week of irradiation and the spices were recorded after 3 weeks of irradiation, which are shown in figures 1-8 respectively.

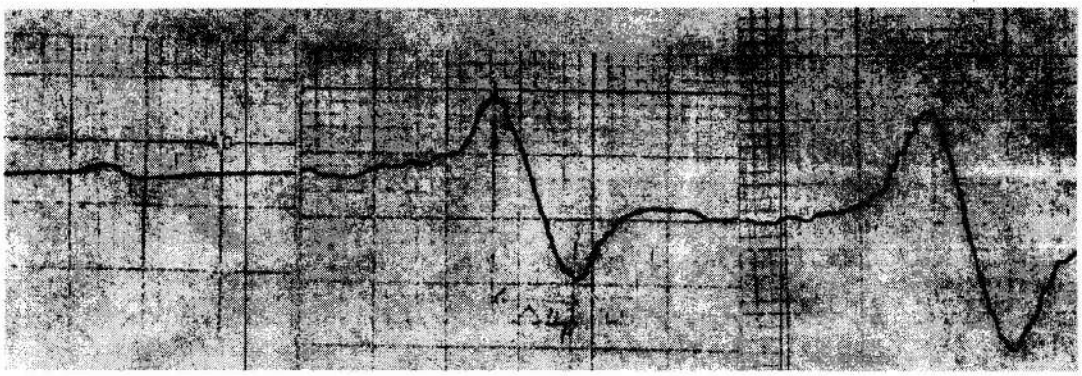
The ESR spectra shown in figures 1-4 were recorded for the pinenut, peanut, almond and walnut respectively. It was found that there was no special characteristic feature appeared in irradiated dry fruit sample which can differentiate the irradiated from un-irradiated sample. Although the population of free radicals produced during radiation is many fold high in the irradiated sample as compared to the un-irradiated where the radicals are generally produced during grinding. The presence of the radicals produced is



A B C

FIGURE 1: ESR SPECTRA OF PINE NUT

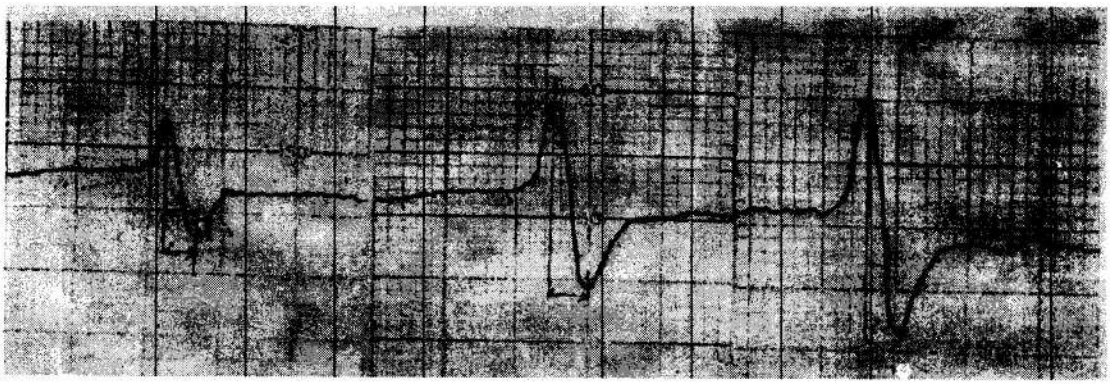
A) Un-irradiated control
B) Irradiated to a dose of 1.0 kGy
C) Irradiated to a dose of 1.5 kGy. The spectra were recorded ca. one week after irradiation.



A B C

FIGURE 2: ESR SPECTRA OF PEANUT

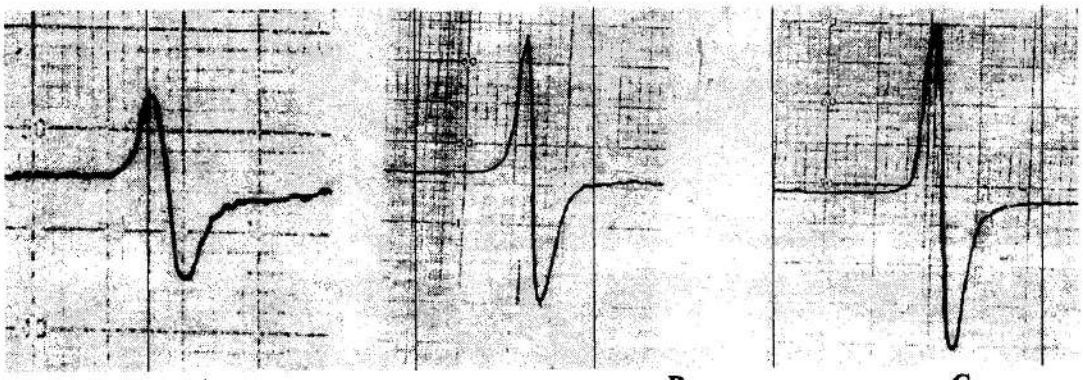
A) Un-irradiated control
B) Irradiated to a dose of 1.0 kGy
C) Irradiated to a dose of 1.5 kGy. The spectra were recorded ca. one week after irradiation.



A B C

FIGURE 3: ESR SPECTRA OF WALNUT

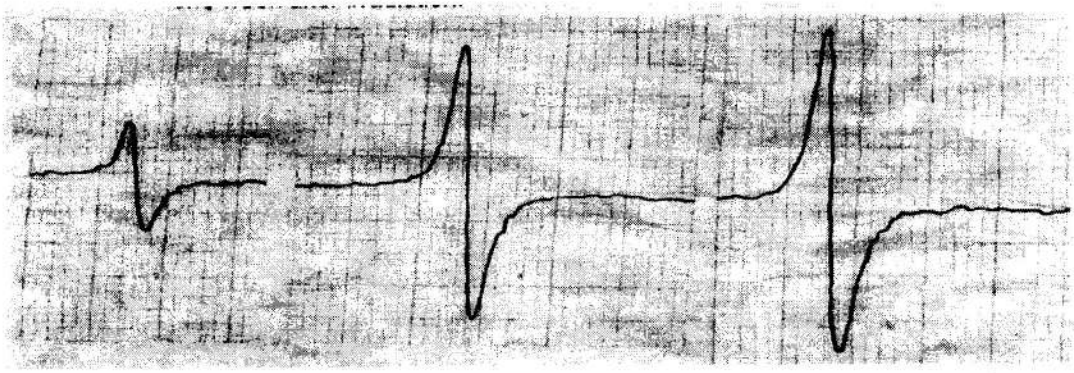
A) Un-irradiated control
B) Irradiated to a dose of 1.0 kGy
C) Irradiated to a dose of 1.5 kGy. The spectra were recorded ca. one week after irradiation.



A B C

FIGURE 4: **ESR SPECTRA OF ALMOND**

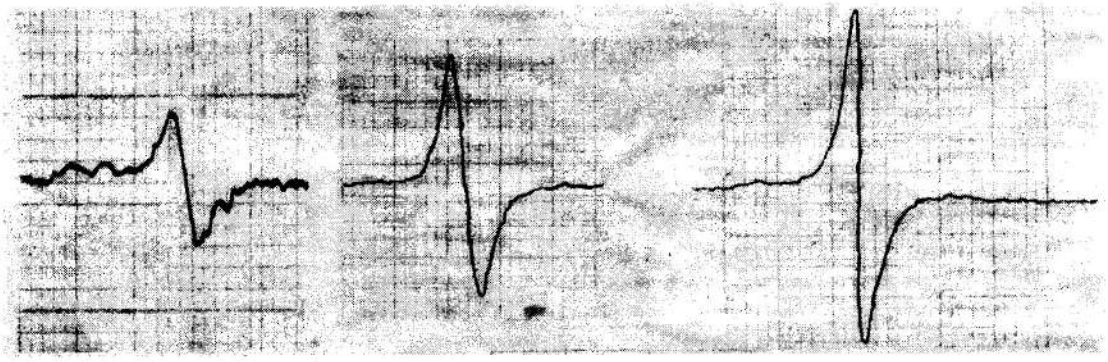
A) Un-irradiated control
B) Irradiated to a dose of 1.0 kGy
C) Irradiated to a dose of 1.5 kGy. The spectra were recorded ca. one week after irradiation.



A B C

FIGURE 5: **ESR SPECTRA OF BLACK PEPPER**

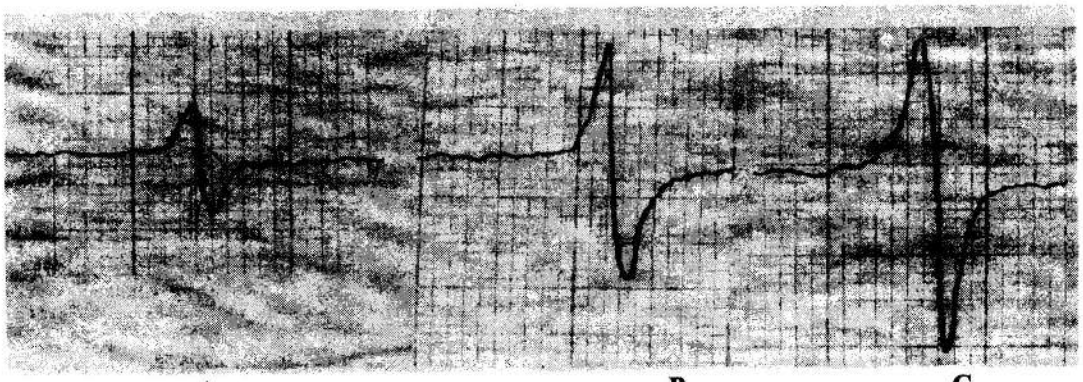
A) Un-irradiated control
B) Irradiated to a dose of 5.0 kGy
C) Irradiated to a dose of 10.0 kGy. The spectra were recorded ca. one week after irradiation.



A B C

FIGURE 6: **ESR SPECTRA OF RED PEPPER**

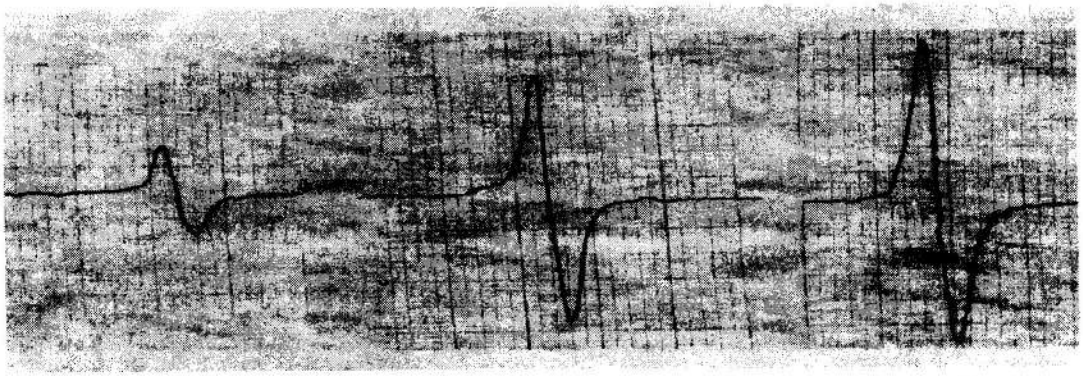
A) Un-irradiated control
B) Irradiated to a dose of 5.0 kGy
C) Irradiated to a dose of 10.0 kGy. The spectra were recorded ca. one week after irradiation.



A B C

FIGURE 7: **ESR SPECTRA OF CUMIN**

A) Un-irradiated control
B) Irradiated to a dose of 5.0 kGy
C) Irradiated to a dose of 10.0 kGy. The spectra were recorded ca. one week after irradiation.



A B C

FIGURE 8: **ESR SPECTRA OF CORIANDER POWDER**

A) Un-irradiated control
B) Irradiated to a dose of 5.0 kGy
C) Irradiated to a dose of 10.0 kGy. The spectra were recorded ca. one week after irradiation.

Table-1: ESR constants of irradiated and un-irradiated dry fruits and spices

Samples	Dose (kGy)	Intensity (Arbitrary units) cm	ΔH_{pp} (G)	g value
1. DRY FRUITS				
Pine nut	0 (control)	1.3	6.94	1.985
	1.0	11.2	8.328	1.986
	1.5	12.8	9.728	1.989
Pea nut	0 (control)	1.2	9.4	1.993
	1.0	3.3	19.43	1.993
	1.5	4.6	20.82	1.985
Walnut	0 (control)	2.3	5.968	1.985
	1.0	3.6	9.72	1.986
	1.5	4.7	8.33	1.986
Almond	0 (control)	2.3	8.33	1.986
	1.0	7.9	8.53	1.995
	1.5	10.0	9.40	1.998
2. SPICES				
Black Pepper	0 (control)	4.3	6.944	1.985
	5.0	10.9	8.944	1.985
	10.0	12.7	9.164	1.983
Red Pepper	0 (control)	1.5	8.33	1.992
	5.0	9.9	12.50	1.985
	10.0	13.2	13.25	1.988
Coriander	0 (control)	4.4	6.9	1.985
	5.0	12.0	5.5	1.984
	10.0	14.9	8.5	1.989
Cumin	0 (control)	3.0	4.16	1.983
	5.0	6.5	6.94	1.985
	10.0	8.8	7.92	1.985

confirmed by calculating the 'g' values and values of peak width "ΔH_{pp}" as shown in the table-1. The radicals are the reactive species which form some secondary stable and unstable products through the radiolytic reactions, some of them are trapped in the hard part of the food material which can be detected after several days or weeks or months or even years after irradiation depending upon the nature of food (Raffi and Angel, 1989). The signal intensity of the free radicals has also been studied using other techniques such as chemiluminescence which shows that intensity may also depends upon the particle size, storage time etc. (Bhatti, et al., 2002).

Four spices, black pepper, red pepper, cumin and coriander were irradiated to the absorbed doses of 5 and 10 kGy and their ESR spectra were recorded. The results obtained in this study are in consistent with the study carried out previously on the irradiated fruits (Raffi et al., 1988. Maloney et al.,1992). Only one signal, which represents the intensity of the radicals, was appeared in the irradiated specimen that was virtually identical to that observed in the spectra of un-irradiated control samples. Therefore, the results shown in the figures 4-8 were disappointing and this method cannot serve as potential diagnostic test in order to discriminate the irradiated and un-irradiated spices. All the spectra obtained did not contain radiation specific peak, although the intensity of the base line feature is enhanced with the radiation dose. These observations confirm that the additional signal observed in ESR spectra arise from unique radiolytic product. It has also pointed out that the irradiation of foods which have appreciable water contents and have no hard part in them cannot trap the radicals and an additional feature is appeared which is short-lived and cannot be detected in spectra recorded few days after irradiation (Raffi and Angel, 1989).

The ESR method is very promising for those foods having inherently tough or hard part such as cellulose in the plant based foods and bones in the animal products such as beef, meat, chicken

and sea foods (Gray and Stevenson, 1989). During irradiation, radicals are produced in the bone part of the food due to high contents of minerals and hydroxyapatite crystalinity which shows an additional radiation specific feature in the ESR spectra of the irradiated food that is totally absent in the un-irradiated control sample (Goodmann et al., 1989; Gray et al., 1990).

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We are very grateful to the Ex-Director Nuclear Institute for Food and Agriculture (NIFA) Peshawar, late Dr. Abdus Sattar (May the Almighty Allah rest his soul in peace) for providing the facility of Co-60 gamma-ray source. Special thanks for Dr. A.Y. Khan and Mr. Abid Latif, Quaid-i-Azam University Islamabad, for extending the ESR spectrometer facility.

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EFFECT OF ENVIRONMENTAL CONDITION ON THE JUICE CONTENT OF CARROT DURING STORAGE.

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ABSTRACT

Shelf life extension trial on T-29, a commonly cultivated carrot variety was conducted at Post Harvest Research Centre laboratories. Total soluble solids, juice percentage and weight loss were determined along with organoleptic evaluation. The results show that carrot could be stored at 0°C at 85-90% relative humidity for 28 days. It was found that the qualities of stored carrot were found much better organoleptically.

INTRODUCTION:

Carrot (Daucus carota) is one of the best-relished winter vegetable consumed as raw and in cooked form. It is cultivated through out the country and its annual production in Pakistan is 186 million tones, whereas in Punjab147 million tones of carrots are produced (Brash, 1996).

In processed form carrot is used as pickles, preserves and desserts. Carrot is very nutritive and rich in vitamin A (11000 I.U./100 g) and Beta Carotene (5). It also contains appreciable amount of thiamin and riboflavin besides calcium and phosphorus. Due to its specific composition, doctors recommend the use of carrot juice as preventive measure against blood and eyes diseases.

The harvesting time of carrot is December to February. During this month the fresh produce is abundantly available in the market and people consume it in the form of ready to serve fresh juice.

The storage stability of carrot depends mainly upon post harvest operations. Bruises should be avoided during its harvesting. The storage potential of mechanically harvested carrot lower up to 30 % as compared manual harvesting because maximum protection is given during harvesting while during mechanical harvesting it seems difficult due to fast action. Keeping in view the importance of this biennial vegetable, research studies were designed to determine the effect of storage conditions on the quantity and quality of juice.

MATERIAL AND METHODS:

Carrot (T-29 variety) was procured from vegetable areas of Ayub Agricultural Research Institute Faisalabad. After sorting and grading carrots were washed in running tap water. Carrots were stored in net bags @ 2-3 kilogram per bag and each treatment was replicated four times. Then the samples were placed at 0,2 and 4°C with relative humidity 85-90 % in different cold stores compartments. Control samples were placed at ambient condition (18-20°C) for comparison.

Total soluble solids, weight loss and juice percentage were determined by Bench type Refractometer, electric balance and juice extractor respectively and data was recorded after seven days intervals while organoleptic evaluation was conducted through a panel of five judges at the start and after each interval.

RESULTS AND DISCUSSION:

Total Soluble Solids

Total soluble solids (Table-I) of carrot at 0 day (control) were 8.5. Increase in TSS was noticed as 12.4 °B after the study period of 28 days. Similar trend in increase of TSS of carrot was observed in all treatments. In T1, increase in TSS was noticed as 14.5% after storage period of 28 days. Similar trend in increase in T2 and T3

was noticed as 28.7 and 41.6 % respectively after the storage period of 28 days. The increasing trend in TSS during all storage periods might be due to the conversion of insoluble solids to soluble solids of the carrot.

Juice Percentage

The results in Table-II showed a decrease in juice percentage of carrot during storage, which was noticed 33 % after 28 days in control sample. While the actual juice contents of fresh carrot were determined as 52% on weight bases. Similar trend of decrease in juice percent of carrot was noticed in T1, T2 and T3 as 14.8, 17.0 and 23.8% after 28 days of storage. Decrease in juice percentage of carrot during storage was due to moisture loss.

Weight Loss Percentage

In case of weight loss of carrot during storage, increasing trend was noticed after 28 days of storage. Maximum weight loss was observed in control sample (50 %) where as minimum weight loss was noticed in T1, which was stored at 0 °C for 28 days (Table-III).

The results of investigation of this study revealed that carrot could be stored at 0°C for 28 days without altering on the quality of the juice.

Organoleptic Evaluation

The 0 day results showed highly acceptability of all mentioned characters by scoring 8.2 points as overall acceptability because at the time carrots were fresh in all respects. But when the panel evaluated these at the end of trial then T1 scored highest marks in overall acceptability, which was 7.8 while control sample just scored only 3.5 showing the significant success of the trail (Table-IV).

Table I. Effect of Storage on Total Soluble Solids of Carrorts

	Storage period (in days)				
Treatments	**0**	**7**	**14**	**21**	**28**
T_0	8.5	9.0	10.7	11.9	12.4
T_1	8.3	8.6	8.8	9.2	9.5
T_2	8.7	9.1	9.6	104	11.2
T_3	8.4	9.2	9.9	108	11.9

Table II. Effect of Storage on Juice Percentage of Carrot

	Storage period (in days)				
Treatments	**0**	**7**	**14**	**21**	**28**
T_0	52.5	47.3	43.3	38.1	35.1
T_1	51.7	50.6	48.3	46.5	44.0
T_2	51.9	50.3	47.7	46.1	43.0
T_3	52.8	50.1	46.0	44.0	40.3

Table III. Effect of Storage on Weight Loss Percentage of Carrot

	Storage period (in days)				
Treatments	**0**	**7**	**14**	**21**	**28**
T_0	0	18.0	30.0	40.3	50.0
T_1	0	7.8	12.2	16.3	20.0
T_2	0	9.5	14.9	20.3	25.1
T_3	0	109	17.1	22.4	28.1

Table IV. Effect of Storage on Organoleptic Evaluation of Carrot Juice

	Storage period (in days)				
Treatments	**Days**	**Color**	**Taste**	**Flavor**	**Overall Acceptability**
	0	8.0	8.5	8.4	8.2
T_0	28	3.4	3.7	4.0	3.5
T_1	28	7.5	7.4	7.9	7.8
T_2	28	7.0	7.1	7.4	7.2
T_3	28	6.8	6.7	6.9	6.8

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EFFECT OF DIFFERENT STABILIZERS ON THE QUALITY OF READY-TO SERVE MANGO DRINK

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ABSTRACT

Ready-to-Serve mango drink was prepared by using different stabilizers viz., carboxymethyl cellulose (0.05-0.075), guar gum (0.05%, 0.075%), pectin (0.05%, 0.075%) while the remaining formula was kept constant. The beverage was evaluated at zero day and after 45 days. Beverage even after 45 days of storage showed no appreciable changes and remained fit for consumption. Sample containing CMC at 0.075% level i.e. (T_2) showed better results in all sensory quality attributes. This shows that the drink prepared by the use of stabilizers was of better quality than without addition of stabilizer.

INTRODUCTION

Mango (*Mangifera indica*) a rich source of vitamin A and C contains on an average 4800 IU of vitamin A and 40 mg vitamin C by weight of 100 gram edible portion (Malik et al.1994). Mango being one of the oldest tropical fruits, being cultivated since 4000 years, originating apparently in the Indo-Burma region, is most popular fruit among millions of people in the orient (Litz, 1997). It occupies, almost the same position in the tropics as the apple in Europe and North America (Singh 1971). Pakistan produces about 937.7 thousand tons of mangoes sharing about 5.86 % of the total world's production of mangoes (1998-99) (jang.com 2001). On the basis of its appealing taste and flavor it is considered as "King of fruits".

Mango fruit is consumed in large quantities during season in the subcontinent but its availability in fresh state is only for a short period of time. In order to prolong its consumption period, different food products are prepared at different stages of maturity, including unripe and ripe stage of maturity. Among all the products ready to serve mango drink is also being prepared by different processing concerns which is very popular not only in Pakistan but also in other parts of the world.

Cloudiness in mango drink is due to finely divided pulp and natural pigments. This defect gives undesirable visual separation of cloudy fruits not acceptable by the consumer. Therefore, the stabilizers are the most important ingredients in commercial juice manufacturing. Juice made without stabilizer lack mouth feel, quality and gives separation instability and cloudiness problem (Hicks, 1990). Three different stabilizers at two levels were used in this study to find out the best one to over come the problem of settling without effecting the quality of ready-to-serve product, and consumer's risk.

MATERIALS AND METHODS

Procurement and Packing of Samples

Fully matured "Desi" mangoes were purchased from local market of Faisalabad. The fruits were thoroughly washed with potable water for 5 minutes to remove dirt, dust and pesticide residues and to reduce microbial load. The mangoes were selected on the basis of uniformity for pulp extraction whereas, over ripe and damaged fruits were discarded. Mangoes were passed through a coarse mango pulper to separate the pulp from stones and skin. Water was added at the rate of 50% to the mangoes prior to passing through the coarse pulper. The pulp obtained was screened through fine pulper to get fine textured pulp which was stored for one month in plastic cans with Potassium metabisuphite (@ 700 ppm) at pH 4.0 adjusted with the help of citric acid. The pulp thus obtained was diluted with water so that it contained 12% fruit content. Total soluble solids (15 °Brix) were adjusted with sucrose at acceptable level (Dauthy, 1995). Following levels of stabilizers were used to stabilize ready-to-serve mango drink.

Table 1. Doses of stabilizers used during study period.

Treatment		Dose(%)
Control	(T_0)	0.00
Carboxymethyl cellulose	(T_1)	0.05
Carboxymethyl cellulose	(T_2)	0.075
Guar gum	(T_3)	0.05
Guar gum	(T_4)	0.075
Pectin	(T_5)	0.05
Pectin	(T_6)	0.075

Note: The drink was pasteurized at 95°C for 4 minutes and filled in 250 ml clean and sterilized bottles, then crowned and stored at room temperature.

Formula of Mango Drink

Mango pulp	12 %
Citric acid	1 %
Acidity	0.23 %
pH	3.56
Stabilizer	(0.075-0.05) %
Brix➡	13.5 %

Physico-chemical analysis

Chemical analysis for the following parameters were carried out at 0, 15, 30 and 45 days as under:

1. **Acidity:** Determined by method as described by Ruck (1963).
2. **pH:** pH was directly recorded by using pH meter (Model Beckman-43 pH meter).
3. **Total Soluble Solids (°Brix):** TSS were directly measured by using hand refractometer (Ruck, 1963).
4. **Ascorbic Acid:** Ascorbic Acid was determined by "Indophenol method" (Ruck, 1963).
5. **Total and Reducing Sugars:** Determined by using Lane and Eynon method (Ruck, 1963).
6. **Cloud Stability:** Cloud stability was measured in centimeters from the bottom to the top of the bottles. Results were expressed as percent cloud retention as described by Bhatti (1988).
7. **Sensory evaluation:** The ready-to-serve mango drink samples were evaluated organoleptically for colour, flavour, taste, mouth feel and over all acceptability at 0, 15, 30 days interval, and 45 days of storage according to methods as described by Larmond (1977).
8. **Statistical Analysis:** The data was analysed statistically according to the method described by Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

Acidity: Data shows that there was increase in acidity in all the samples during storage. Minimum acidity (0.20%) was found at 0 day and maximum (0.26%) at 45th days of storage. The increase in acidity might be due to the formation of acid by degradation of polysaccharides and oxidation of reducing sugars or by break down of Pectic substances. Data regarding the effect of different doses of CMC, pectin and guar gum on titratable acidity of ready-to-serve mango drink during storage is presented in Table 2.

pH: The data regarding the effect of different doses of CMC, pectin and guar gum on pH of ready-to-serve mango drink during storage is presented in Table 3. The statistical analysis shows that treatment had non-significant effect on pH of drinks, while storage period had highly significant effect on the pH value of the drink. A decreasing trend of pH in all the samples was found during storage period of 45 days. As the acidity increased there was a proportional decrease in pH value during storage of mango drink. Maximum pH was found in T_0 (3.50) on 0 day and minimum was in T_2 (3.14) on day 45.

Total Soluble Solids (°Brix): The data on total solids of all treatments during storage is presented in Table 4. The statistical analysis showed that treatments had non-significant effect on °Brix of drink. However, storage period had highly significant effect on the °Brix of the ready-to-serve mango drink. It means that stabilizers and their doses have no effect on total soluble solids of drink.

Ascorbic Acid: The data on ascorbic acid content in ready to serve mango drink during 45 days of storage at room temperature is given in Table 5. The statistical analysis showed that treatment had non-significant and storage had highly significant effect on ascorbic acid. The loss of ascorbic acid is probably due to detrimental effect of storage time, storage temperature, exposure to light and oxygen contents.

Total and Reducing Sugars: The data on the effect of different doses of CMC, pectin and guar gum on total sugars of mango drink during storage is presented in Table 6. Data on reducing sugars of ready-to-serve mango drink during 45 days of storage at room temperature is presented in Table 7. The statistical analysis showed that treatments had non-significant effect on total and reducing sugars. However, storage periods had highly significant effect on total and reducing sugars of ready to serve mango drink. The increase in total sugar might be due to conversion of polysaccharides. While the increase in reducing sugars might be due to interconversion of sugar to reducing sugars by acid, enzymes and temperature, is continued throughout the storage of mango drink.

Cloud Stability. Cloud stability was measured in centimeters from the bottom to the top of the bottles, and expressed in percent cloud stability. The statistical analysis showed that treatment and storage had highly significant effect on cloud stability of ready-to-serve mango drink. Result showed that CMC at both levels (0.05%, 0.075%) and pectin at 0.075 level was effective to check the cloud settling. Means of storage intervals show loss in cloud stability from 100 to 84.71% during 45 days of storage. Maximum cloud loss was observed in control sample. The data regarding cloud stability are given in Table 8.

Table 2. Effect of storage and treatments on percent acidity of ready-to-serve mango drink.

Treatment	Duration of Storage(days)			
	0	15	30	45
T_o	0.20	0.21	0.23	0.25
T_1	0.21	0.21	0.22	0.26
T_2	0.21	0.22	0.24	0.26
T_3	0.20	0.21	0.23	0.25
T_4	0.21	0.22	0.23	0.25
T_5	0.20	0.21	0.23	0.26
T_6	0.21	0.20	0.24	0.25

Table 3. Effect of storage and treatment on pH of ready-to-serve mango drink

Treatment	Duration of Storage(days)			
	0	15	30	45
T_o	3.50	3.43	3.25	3.16
T_1	3.52	3.43	3.25	3.15
T_2	3.53	3.42	3.24	3.14
T_3	3.52	3.43	3.23	3.16
T_4	3.51	3.43	3.24	3.15
T_5	3.51	3.42	3.25	3.15
T_6	3.51	3.44	3.23	3.16

Table 4. Effect of storage and treatment on total soluble solids (°Brix) of ready-to-serve mango drink.

Treatment	Duration of Storage(days)			
	0	15	30	45
T_o	15.00	15.23	15.50	15.85
T_1	15.00	15.30	15.47	15.90
T_2	15.10	15.30	15.43	15.90
T_3	15.00	15.24	15.51	15.91
T_4	15.10	15.23	15.46	15.98
T_5	15.00	15.21	15.43	15.97
T_6	15.00	15.20	15.47	16.00

Table 5. Effect of storage and treatment on ascorbic acid (mg/100) of ready-to-serve mango drink.

Treatment	Duration of Storage(days)			
	0	15	30	45
T_o	3.36	3.04	2.30	1.21
T_1	3.34	3.00	2.20	1.40
T_2	3.40	2.90	2.20	1.35
T_3	3.35	3.05	2.30	1.24
T_4	3.42	2.90	2.40	1.20
T_5	3.27	2.80	2.20	1.64
T_6	3.41	3.00	2.00	1.43

Table 6. Effect of Storage and treatments on total sugar (%) of ready-to-serve mango drink.

Treatment	Duration of Storage(days)			
	0	15	30	45
T_o	14.39	14.45	14.48	14.50
T_1	14.38	14.43	14.48	14.51
T_2	14.36	14.44	14.45	14.53
T_3	14.38	14.41	14.46	14.51
T_4	14.37	14.43	14.45	14.54
T_5	14.38	14.42	14.44	14.53
T_6	14.39	14.41	14.46	14.54

Table 7. Effect of Storage and treatments on reduc- ing sugars (%) of ready-to-serve drink.

Treatment	Duration of Storage(days)			
	0	15	30	45
T_o	3.36	4.50	5.11	6.40
T_1	3.19	4.65	5.13	6.41
T_2	3.39	4.67	5.12	6.39
T_3	3.29	4.61	5.20	6.43
T_4	3.32	4.55	5.14	6.41
T_5	3.40	4.67	5.00	6.44
T_6	3.68	4.32	5.14	6.42

Table 8. Effect of storage and treatments on cloud stability (%) of ready-to-serve mango drink.

Treatment	Duration of Storage(days)			
	0	15	30	45
T_o	100	69.5	60.8	56.4
T_1	100	100	100	100
T_2	100	100	100	100
T_3	100	91.3	82.6	73.4
T_4	100	82.6	78.2	70.2
T_5	100	100	95	93
T_6	100	100	100	100

Sensory Evaluation: The prepared drinks with different doses of stabilizers were evaluated organoleptically for colour, flavour, taste, mouth feel and over all acceptability. Each observation was made after 15 days of interval, by a panel of 5 trained judges. The statistical analysis showed that treatment effect and storage have highly significant effect on colour of the drink. The general trend from the data, appears that samples containing stabilizers were superior as compared to control sample. The judges gave maximum scores to T_2 (7.9) and minimum to T_o (7.5) on 0 day. The scores were decreased to 7.5 and 6.8 respectively after storage period of 45 days.

CONCLUSION: The treatment T_2 containing 0.075 % CMC was liked by the judges and the minimum separation was noted in the sample.

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12th ALL PAKISTAN FOOD SCIENCE CONFERENCE
HELD ON JANUARY 12, 2002
AT THE UNIVERSITY OF ARID AGRICULTURE, RAWALPINDI.

MINUTES OF THE CONFERENCE

In the Inaugural Session, Prof. Dr. Atta-ur-Rehman Federal Minister of Science and Technology was the chief guest. Prof. Dr. Khalid Mehmood Khan (S.I.), Vice Chancellor University of Arid Agriculture Rawalpindi, delivered the welcome address which was followed by the keynote address by Dr. Wazir Hussain Shah President PSFST and address by Dr. S.M Aboul Naga FAO Representative in Pakistan. Prof. Dr. Atta-ur-Rehman in his inaugural address, highlighted the polices for the uplift of science and technology in Pakistan. Two Technical Sessions were arranged, the 1st Technical Session was chaired by Prof. Dr. Riaz Hussain Qureshi Vice Chancellor University of Agriculture, Faisalabad. He was assisted by Dr. Muhammad Shafiq Chaudhry, Director Ramna Food Lahore. In this session seven technical papers were presented. Special Dr. Abdus Sattar memorial lecture was presented by Prof. Dr. Riaz Hussain Qureshi. In the second session Dr Shahzad Mufti, Chairman Pakistan Science Foundation and Dr. Muhammad Amjad, Chief Executive Vety Care Islamabad acted as Chairman and Co-Chairman respectively. In this session nine technical papers were presented on different aspects of food technology.

The Business Session was presided by Dr. Wazir Hussain Shah President PSFST. The activities of the Society were presented by the Secretary Prof. Dr. Faqir Muhammad Anjum, Chairman Department of Food Technology, University of Agriculture, Faisalabad. The most significant achievement was that PSFST had been accorded affiliation as an allied organisation number 28 of IFT (Institute of Food Technologists) USA. The activities of Lahore Chapter were presented by Hamid Ahmad, Chairman Lahore Chapter. Dr. Muhammad Shafiq Chaudhary proposed that since the tenure of Executive Council had expired on 31st December, and the meeting was being held on 12th January 2002 therefore, the house should condone the 12 days for the validity of Executive Council to regularize their function. The house unanimously approved the proposal. Dr. Javaid Aziz Awan announced the results of the elections of the various offices of the Executive Council for the years 2002-2003. Some offices remained vacant for technical reasons. The elections for the posts of Vice President (Technical

and Industry), Joint Secretary and Secretary Public Relation were held. The following office bearers for various offices of Executive Council were declared successful:

1. President	Dr. Wazir Hussain Shah, Head Biotechnology and Food Research Centre, PCSIR Laboratories, Ferozepur Road, Lahore.
2. Vice President (Industry)	Mr. Muhammad Haroon Director Standard Manufacturing Company Pvt Ltd 4- Dyal Singh Mansion, The Mall, Lahore.
3. Vice President (Academic)	Mr .Jan Muhammad Khan, Research Officer, Agricultural Research Institute, Tarnab, Peshawar.
4. Secretary	Dr. Faqir Muhammad Anjum, Professor and Chairman Department of Food Technology, University of Agriculture, Faisalabad.
5. Treasurer	Dr Salim ur Rehman, Associate Professor, Department of food Technology, University of Agriculture, Faisalabad.
6. Secretary (Public Relation)	Mr. Tahir Zahoor, Lecturer, Department of Food Technology, University of Agriculture, Faisalabad.
7. Joint Secretary	Mr. Mustajab Ahmad Production Manager Vita Pakistan Ltd, Plot 20-D, Peshawar Road, Rawalpindi.

President Dr. Wazir Hussain Shah thanked the members for reposing confidence in his team and ensured that the new Executive Council will do their best for the betterment of the Society. Finally the business session was concluded with a vote of thanks.