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Extraction of starch from taro (*Colocasia esculenta*) by freeze-thaw method

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ABSTRACT

Taro (*Colocasia esculenta*) contains a large amount of starch, which has got certain preferences over other starches. Due to mucilage content, the extraction of starch from taro is cumbersome. The present study is an attempt to explore a simplified method for starch extraction from taro. Freeze-thaw method, a novel and simple technique of starch extraction has been used for the first time for starch extraction. Functional properties of extracted taro starch was compared with the properties of ammonia extracted starch. The hydration capacity, moisture absorption, freeze-thaw stability as well as swelling power for freeze-thaw extracted starch (FTS) were found generally higher than that of ammonia extracted starch (AMS). The solubility of FTS was lower than that of the AMS. The gelatinization pattern showed that FTS begins to swell at a slightly high temperature than AMS. The FTS has a high peak temperature and high peak viscosity in contrast with AMS. Similarly, the transition temperatures (onset, peak and completion temperatures) of FTS were higher than those of AMS. Taro starch extracted by freeze-thaw method could be exploited for product development in food industries as it showed significant improvements in certain functional properties.

Keywords: Starch, taro, extraction, freeze-thaw, functional properties

INTRODUCTION

Edible aroids are starchy staples in communities of humid tropical and subtropical regions of the world (Onweume, 1978; Gallant *et al.*, 1982). They are herbaceous plants belonging to the Aracease family, and consist of five genera of which *Colocasia esculenta* is the most important food crop. This specie is commonly known as Taro, kolocasia, Dasheen, Addoe, Old cocoyam or Arvi. Taro contains a large amount of carbohydrates, besides having protein, fat and some minerals in appreciable quantities.

It is a low-cost, high-starch food source (Vincent and Yamaguchi, 1995). Previous studies reveal that taro starch has preference over other starches in some aspects e.g. they have clarity at high solid concentration, form hard coating layer, have high swelling power and peak viscosity (Lauzon *et al.* 1995; Perez *et al.* 1998; Adebayo and Itiola, 1998). They form high gel strength and small granule size, which make them suitable for specific applications (Coursey 1967; Griffin and Wang 1983). In addition, taro has been used in baby foods, taro chips, taro bread (Moy and Nip 1983) and taro sorbet (Hong and Nip 1990). Small granular starch has been demonstrated to be a good filler for biodegradable plastic films (Lim *et al.* 1992) and also has been reported to provide a better mouth feel as a lipid substitute (Daniel and Whistler 1990). Taro starch is easily digested and non-allergenic.

The isolation of starch from fresh tubers of taro is a difficult task due to the mucilaginous material as it makes a sticky paste-like emulsion. The settling of starch thus takes a long time during which microbial growth starts and hence both quantity and quality are reduced (Moorthy 1991). Due to this extraction of starch from taro has never been given full attention. Moorthy (1991) used ammonia solution to prevent microbial growth for the extraction of starch but it disrupted starch granules to some extent and thus yield as well as quality decreased. Hence an easy and convenient method is desirable for extraction of Taro starch to produce good quality starch and better yield. An attempt has been made to use freeze-thaw method for the extraction of taro staroh and its functional properties were also studied.

MATERIALS & METHODS

Taro (*Colocasia esculenta*) was purchased from local market of Karachi. All chemicals were of analytical grade from Merck and Sigma.

Taro starch was extracted by two different methods. The method developed by Moorthy (1991) and a newly developed method i.e. freeze-thaw method.

Table 1. Comparison of functional properties of freeze thaw extraction starch (FTS) and ammonia extracted starch (AMS)

Functional properties	Freeze-thaw extracted starch (FTS)	Ammonia extracted starch (AMS)
Hydration Capacity (g water/g starch)	1.44 ± 0.02[a]	1.31 ± 0.02
Moisture Absorption (%)	12.0 ± 0.22	8.22 ± 0.15
Freeze-thaw Stability(%)	52.2 ± 0.3	48.6 ± 0.3
Swelling Power (ml./g)	3.5 ± 0.3	2.7 ± 0.4

[a]Mean + SD (n = 3)
All values are expressed on dry basis.

Table 2. Comparison of viscosities between freeze thaw extraction starch (FTS) and ammonia extracted starch (AMS) of 5% Solution

Viscosity of 5% starch solution	Freeze-thaw extracted starch (FTS)	Ammonia extracted starch (AMS)
V_P (cp)	3025	2980
$V_{95}°_C$ (cp)	2425	2350
V_H (cp)	1800	1740
V_C (cp)	2285	2210

V_P = Peak Viscosity
$V_{95}°_C$ = Viscosity at 95°C
V_H = Viscosity after holding at 95°C for 30 min.
V_C = Viscosity after cooling to 50°C temperature.

Table 3. Comparison of gelatinization temperature of freeze thaw extraction starch (FTS) and ammonia extracted starch (AMS)

Gelatinization temperature range	Freeze-thaw extracted starch (FTS)	Ammonia extracted starch (AMS)
T_O (°C)	68.0 ± 0.5	67.2 ± 0.4
T_P (°C)	79.8 ± 0.3	77.6 ± 0.3
T_C (°C)	93.6 ± 0.4	92.2 ± 0.3

T_O =Onset temperature
T_P = Peak temperature
T_C = Completion temperature
[a]Means ± SD (n= 3)

Extraction by Freeze-thaw method

Peeled Taro was ground in a grinder and blended in distilled water in a ratio of 1:2 and mixed well with a mechanical mixer for 1 hr. The slurry was filtered through an 80mesh sieve followed by 170 mesh sieve. The residue on the sieve was removed and filtrate was kept in a freezer at −10°C, for 5days. The frozen extract was thawed at room temperature for 4 hours. The separated water was decanted. Residue was carefully dried in the pettri dishes at 45°C and then pulverized.

Extraction by Ammonia

So far the best reported method is by Moorthy (1991). In this method starch is extracted by using 0.03M ammonia solution.

Table 4. Relationship between time vs viscosity of freeze thaw extraction starch (FTS) and ammonia extracted starch (AMS) of 5% Solution

TIME (min.)	FREEZE-THAW EXTRACTED STARCH VISCOSITY (Cp)	AMMONIA EXTRACTED STARCH VISCOSITY (Cp)
25	80	60
30	2680	2370
32.5	V_P = 3035	V_P = 2980
35	2900	2760
40	2650	2510
43.5	V_{95}= 2425	V_{95} = 2350
45	2415	2250
50	2285	2160
55	2150	2090
60	2100	1960
65	1985	1880
70	1910	1775
73.5	V_H = 1800	V_H = 1740
75	1890	1730
80	1925	1770
85	2040	1865
90	2125	1925
95	2175	2085
100	2260	2130
103.5	V_C = 2285	V_C = 2210

V_P = Peak Viscosity
$V_{95}°_C$ = Viscosity at 95°C
V_H = Viscosity after holding at 95°C for 30 min.
V_C = Viscosity after cooling to 50°C temperature

Moisture Absorption

Moisture absorption was determined by the method of Nyqvist (1998) with some modifications.

The starch powder was dried at 60°C or until the moisture level was< 1%. The hygrostate was prepared using a saturated Na_2SO_4 solution in wells of glass dessicator. A sample of 2g was placed in a watch glass and kept in hygrostate. Percent moisture absorbed was determined from the weight gained after 240hrs. Result used for calculation were means of triplicate measurements.

Hydration Capacity

Hydration capacity or water absorption was determined by the method of Komblum & Stoopak (1973).

Swelling Power

Swelling power was determined by using the method of Cheng *et al.* (1995).

Solubility

The solubility was determined by using 0.4%(w/w) starch dispersions according to the method of Leach *et al.* (1959). The amount of soluble material in the supernatant was estimated from its volume and concentration.

Freeze-Thaw Stability

A modified method based on Schoch (1968) and Narkrugsa (1992) was used to determine the freeze-thaw stability.

Starch sample of 15g was mixed with 300ml distilled water in a beaker at 95°C, with mechanical mixer for

20 minutes. The mixture was poured into a plastic cup and frozen at –10°C, for 7 days. The frozen mixture was then thawed in a water bath at 30°C, for 4 hrs. From this 100ml of the mixture was centrifuged at 8000rpm for 30 minutes. The amount of separated water from the mixture after centrifugation was measured. Results used for calculation were means of triplicate measurements.

Viscosity Behavior

There are different types of viscometers used for this purpose, in which Barbender viscoamylograph is the best. We used a new technique in which Haake Visco Tester 6L was used, and a set up was developed to get viscosity behavior similar to Brabender viscoamylograph.

A jacketed stainless steel container of 500ml capacity with an outlet and inlet was used with glycerin as heating medium. In a total volume, 400ml starch solution of 5% w/v was filled in the container and was placed on a magnetic stirrer. The mixture was heated at a rate of 2°C/min. with constant stirring. Viscometer was placed in such a manner that the spindle of viscometer was dipped in the mixture and viscosity was measured from 30°C to 95°C. The solution was kept at 95 °C for 30 minutes and then cooled back to 50°C at the same rate of 2° C/min.

Different parameters were measured during this cycle, i.e. onset temperature; T_o, peak temperature; T_P, completion temperature; T_C, peak viscosity; V_P, viscosity at 95°C;V_{95}, viscosity after holding at 95°C for 30 minutes; V_H, viscosity after cooling to 50°C;V_C, pasting temperature (at which viscosity started to increase), and maximum viscosity.

RESULTS AND DISCUSSION

The studies showed that settling of extracted starch from Taro (*Colocasia esculenta*) was poor. It took a long time resulting in microbial growth. The best extracted method reported so for is using ammonia solution, but it was observed that, although ammonia solution improve the settling of Taro starch and prevents the microbial activity but the starch obtained had brownish color, which could not be removed even by repeated washing. Ammonia also disrupted the starch granules to some extent, as its functional properties were affected.

The hydration capacity value for ammonia extracted starch (AMS) is generally lower than that of freeze-thaw extracted starch (FTS). The absorbed water is undoubtedly both absorbed by the granules and on their surfaces (Medcalf & Gillies, 1965). The lower values of AMS indicate that the ammonia breaks some starch granules and thus the tendency to absorb water by the granules decreases.

Similarly, results of moisture absorption show that FTS is much more hygroscopic than that of the AMS. It shows that much more surface area is available to absorb the moisture in case of FTS, which is superiority of FTS over AMS.

When starch gels are subjected to freeze-thaw cycling, water used in the preparation of the gel is separated due to the tendency of starch molecules to re-associate, thus forming insoluble aggregates. The gels are characterized as weepy, grainy or spongy. The stability of starch to freeze-thaw cycling enhances its suitability for use in the food products. The starches that are most stable to freeze-thaw cycling are also most stable to refrigerated storage (White et al, 1989). The freeze-thaw stability of a starch gel is evaluated by the amount (%) of water released (syneresis) when starch chain retrograde (reassociate) during the freeze-thaw cycle. The results show that FTS has high freeze-thaw stability than that of AMS. This is probably due to the greater degree of re-association between starch chains in FTS.

Solubility of starch is observed to be a function of temperature (60 to 90°C). At temperature below their gelatinization temperature starches are less soluble. Solubility of starch greatly increases at higher temperature (80 and 90°C). Solubility characterization of starch reportedly depends upon the degree of substitution and the degree of polymerization. The results show that the AMS is slightly more soluble than the FTS. It is probably due to weakening of starch granules during chemical treatment, which causes the ease in solubilization of AMS.

The swelling power of starch was found to be a function of temperature and it followed a pattern similar to that of solubility characteristics. Prior to gelatinization there is only a slight increase in swelling capacity of starches. Once the gelatinization process set in, however, swelling increases rapidly with increasing temperature. The swelling power rises drastically at gelatinization temperature. When the crystal region in the starch granule began to melt, it enhances the swelling power. As the temperature of an aqueous suspension of starch granules is raised above the gelatinization range, hydrogen bonds between polymers continue to be disrupted and water migrates into the interior of the molecule. The results showed that the swelling power of FTS is higher than that of the AMS and it is due to weakening of the hydrogen bonds by the ammonia to some extent.

Chart 1. Swelling Power of FTS & AMS

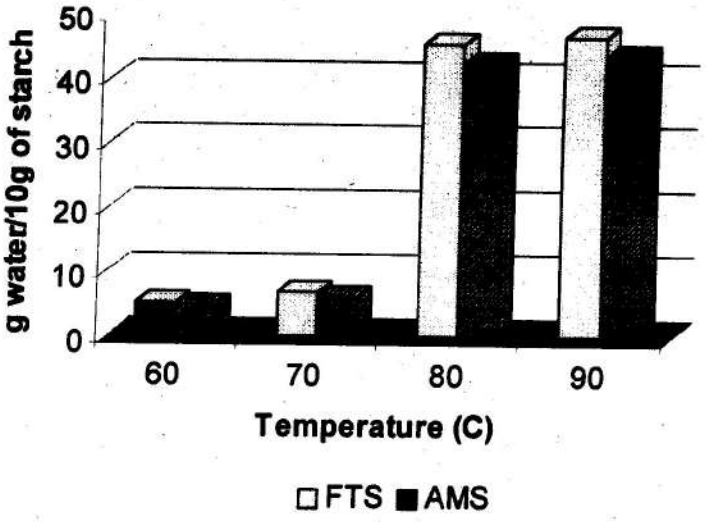
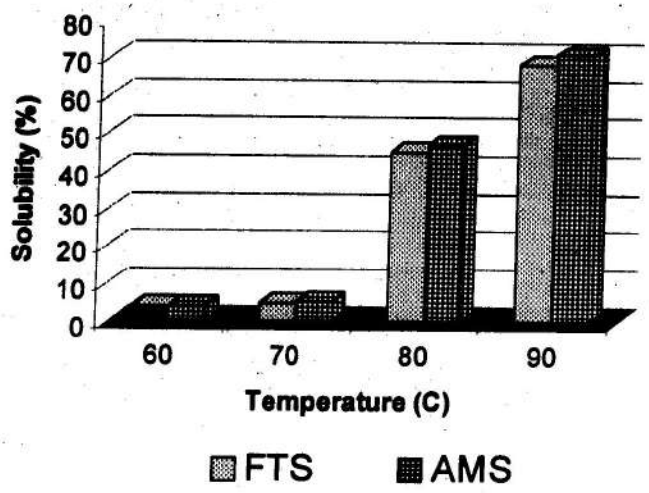


Chart 2. Solubility of FTS & AMS



Gelatinization curves of both extracted starch samples had the same general shape. Important points were recorded during the heating and cooling cycle including pasting temperature, onset temperature, peak temperature, viscosity at 95 °C, viscosity after holding at 95 °C for 30 min., and viscosity after cooling to 50 °C. The result shows that considerable viscosity stability was observed throughout the heating-cooling cycle. This is evidence of restricted swelling and solubilization and of resistance to mechanical disintegration. When the pasted starch was cooled, setback (retrogradation) was observed. Viscosity can be considered as a measure of the strength of starch granules that are intact, while the starch which has undergone chemical and microbiological damage loses viscosity (Moorthy, 1991). The data indicates that FTS begins to swell at a slightly high temperature than the AMS, this observation may be related to the

Chart 3: Solubility Of FTS & AMS

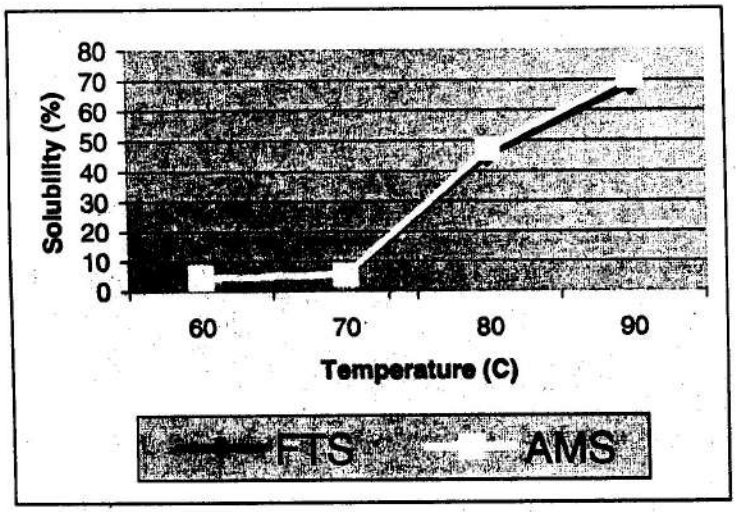
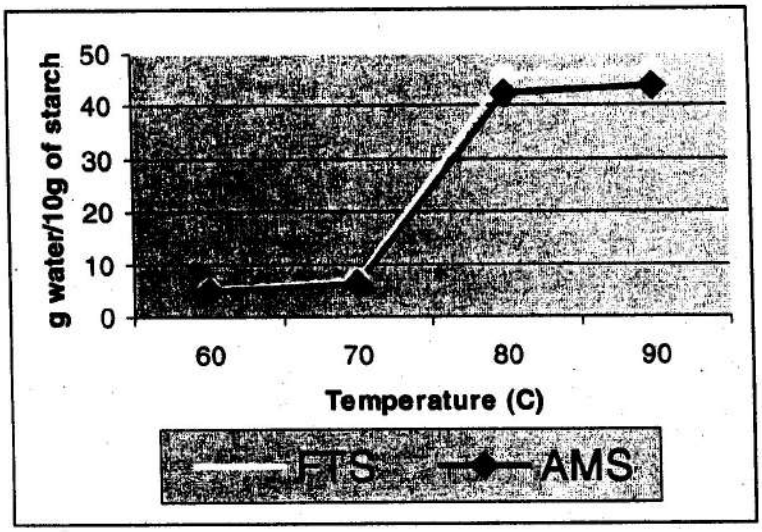


Chart 4: Swelling Power of FTS & AMS

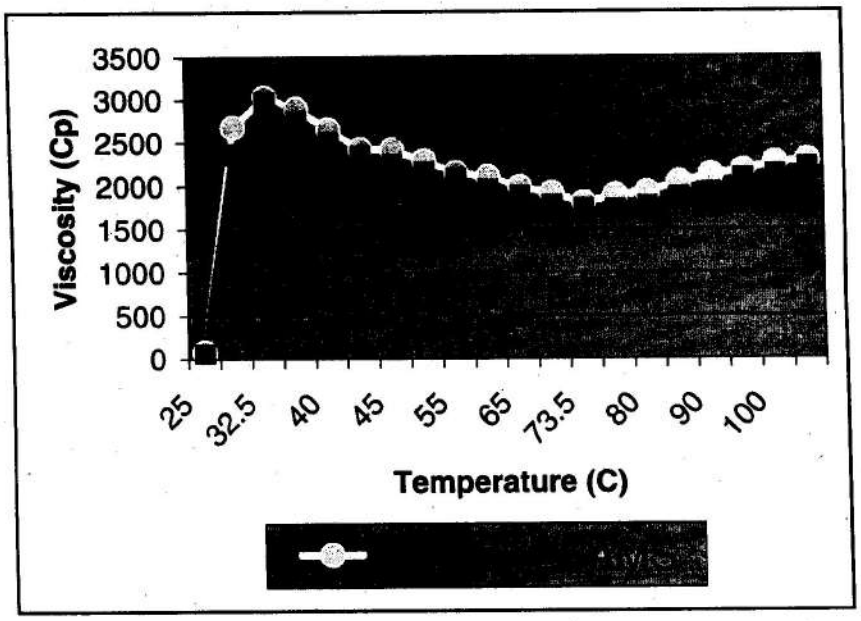


suggested more-compact granule structure of the FTS starch. Similarly, the FTS has the high peak temperature and the high peak viscosity measurement, in contrast with AMS. The relatively lower viscosity breakdown can be exploited in food uses, where a short non-cohesive texture is required. Data on the range of gelatinization temperature indicates that the change in gelatinization temperature range is a function of chemical treatment. Chemical treatment disrupts the structure of starch granules, rendering them more susceptible to hydration and swelling and eventually complete disintegration takes place at a lower temperature (Whilte *et al.*, 1989). The transition temperature (T_o; onset, T_p; peak and T_c; completion) of FTS were higher than those of AMS. It suggests that the crystalline association within its granules is of a higher order of magnitude than in AMS, in which ammonia may disrupt the crystallinity of the starch granules to some extent.

CONCLUSION

Previous study reveals that Taro *(Colocasia esculenta)* starch has several advantages over other starches as discussed earlier. However, extraction of

Chart 5. Relationship between Time vs Viscosity of FTS & AMS of 5% Solution



Taro starch is cumbersome. Extraction by ammonia disrupts granule and thus the yield as well as quality decreases. Therefore freeze-thaw method had definite advantages in increasing yield and also quality of Taro starch.

Better understanding of the functional and sensory properties of Taro starch may therefore lead to the new applications for the food industries. Taro raw and modified starches have tremendous potential as functional ingredients in food industry and their versatile application make them better suited as functional food additives. Further studies regarding its applications are in progress.

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Effect of chemical additives on the shelf life of wrapped bread

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ABSTRACT

Chemical additives (calcium propionate, sodium propionate) were added in flour during mixing at a level of 0.15%. Sodium propionate and calcium acetate were incorporated in the spray mixture, which was sprayed during different stages of bread production. Proximate composition and rheological characteristics of flour were determined before bread preparation. The prepared bread was stored at optimum temperature and evaluated microbiologically of intervals of 3, 24, 48, 72 and 96 hours. As regards sensory evaluation of bread, T_5 (coating of pan+ spray on dough + slicer + packaging material) got maximum score followed by T_7 (spray on packaging) for external and internal characteristics. Treatment T_5 proved most effective treatment against control of fungus and bacteria (total count: $2.3x10^2$ cfu/gram of bread after 96 hours) followed by T_7 ($3.4X10^2$ CFU/gram) and T_6 ($3.5x10^2$ cfu /gram after 96 hours). It is therefore concluded that bread treated by sodium acetate and calcium acetate mixture during panning, proofing, slicing and packaging has great potential against microorganisms and retains good sensory characteristics up to 96 hours storage

Keywords: Rheological characteristics, microorganism, bread, chemical additives, sensory evaluation

INTRODUCTION

Baking industry is an important food industry of the world (Secosmka 1979). Bread occupies a unique position in baking industry both for production and utilization as compared to other bakery products. The main ingredients of bread are flour, water, salt, sugar, shortening and yeast, etc. All these are supportive to growth of microorganisms and multiplication at different stages of bread production, processing and packaging. The growth of certain micro-organisms i.e. yeast is desirable in the leavened bread but the prevalence of some others i.e. mould and rope is troublesome for bakers. A significant number of breads are spoiled during storage as a result of microbial growth, which is a great loss to manufacturer.

The chief types of microbial spoilage in baked bread are usually moldiness and ropiness, termed "mould" and "rope". Chief moulds involved in the spoilage are *Rhizopus nigricans, Aspergillus niger, Penicillium expansum* and *P. stolonifer.* Moulding often begins within a loaf of sliced bread, where more moisture is available than at the surface, especially in the crease (Liaqat 1988).

Since, bread is an important part of our daily diet, therefore, ways and means should be explored to increase its shelf life. The shelf life of bread can be increased through improving the hygienic conditions of mixing and baking halls and ensuring sterilized environment and utensils.

Several chemical additives (antimicrobial agents) have also been employed to increase the shelf life of bread. Propionic acid and propionates (calcium and sodium), acetic acid and acetates (calcium) are organic antimicrobial agents which are freely soluble in water and highly active in the range of 5-6 pH of any food (pH of bread is 5.6). Moreover, these anti microbial chemicals also act as pH controlling agents, texturizers, antioxidants, firming agents and flavor enhancers.

It is, hence, necessary to standardize the quality and quantity of preservatives alongwith maintenance of hygienic conditions in the bakery to increase the shelf life of bread. This study was conducted to increase the shelf life of bread by using antifungal and antibacterial chemicals at various stages of bread preparation such as mixing, slicing and packaging.

MATERIALS & METHODS

Raw materials collection

The commercial wheat flour samples & chemicals were procured from local market & scientific stores.

Proximate analysis

The flour samples were analyzed for moisture, crude protein, crude fat, crude fiber and ash contents according to the procedure as given in AACC (1983).

Farinographic studies

Flour samples were run through Brabender Farinograph to assess the rheological properties of dough according to AACC (1983).

Bread production

During bread production chemical additives (calcium propionate, sodium propionate) were added in flour during mixing at the level of 0.15%. Sodium propionate and calcium acetate were incorporated in to spray mixture at the level of 33%. Above solution was sprayed/coated according to the plan as given in table 1.

Flour was mixed in Hobart mixer up to optimum dough development.

Table 1. Plan for treatments

Treatments	Application
T1	Without spray
T2	Coating of pan
T3	Coating of pan+spray on dough
T4	Coating of pan+spray on dough+slicer
T5	Coating of pan+spray on dough+slicer+packaging material
T6	Spray on slicer
T7	Spray on packaging material

Fermentation, dividing and moulding

Dough was allowed to rest for 2 hours at 29°C. Dough was divided in to pieces of 100 grams, molded in to loaves by hand and placed in to the pre-greased pans.

Proofing

The dough pieces were kept in a proofing chamber for 50 minutes.

Baking

Loaves were baked in a gas oven at 255°C for 20 minutes.

Cooling, slicing and packaging

The breads were cooled to room temperature and sliced with the help of slicer. The cooled and sliced breads were packed manually in polyethylene bags.

Microbiological analysis

Counting of bacteria was made on nutrient medium by plate count method (Harrigan and McCance (1976). Counting of moulds were made by pour plate method on Sabouraud agar medium (Beneke 1962).

Sensory evaluation

Sensory evaluation of bread was carried out by a panel of judges for external and internal characteristics.

Statistical analysis

The data obtained for each parameter was subjected to statistical analysis as described by Steel & Torrie (1980).

RESULTS AND DISCUSSIONS

CHEMICAL COMPOSITION

The results regarding chemical analysis of flour samples are given in table (2). The results are in close agreement to the findings as reported by Ali (1980) who analyzed different wheat varieties.

Table 2. Chemical composition of wheat flours

Flour samples	Moisture %	Grude protein%	FAT %	Crude fiber%	Ash %	NFE %
A	11.50	12.20	1.35	0.47	0.48	74.00
B	12.68	10.75	1.10	0.52	0.59	74.36
C	13.60	9.78	1.52	0.69	0.62	73.79

Table 3. Farinographic characteristics of wheat flours

Flour samples	Water absorption	Arrival time	Peak time	Departure time	Dough stability	Tolerant Index	Softening of dough
A	68.00	2	4	7	5	50 B.U	80 B.U
B	66.40	2	2.6	10	8	40 B.U	90 B.U
C	64.60	1.4	1.75	11.75	10.35	40 B.U	95 B.U

FARINOGRAPHIC STUDIES

The results of Farinographic study are presented in table (3). The samples had 64.6-68 mL water

absorption, 1.4-2 minutes arrival time, 7-11.75 minutes departure time, 40-50 B.U. tolerant index and 80-95 B.U. softening of dough.

Microbiological analysis

The results regarding colony count of bacteria are shown in table (4). Maximum numbers of bacterial colonies (2.7x102 colonies/gram of bread at 96 hours storage) were observed in T1 (without spray). It is exhibited from table (4) that treatment T5 (coating of pain + spray on dough + slicer + packaging material) was proved to be the most effective against bacterial spoilage.

Table 4. Total bacterial count (CFU/gram) at different storage intervals

Treatments	3 hours interval	24 hours interval	48 hours interval	72 hours interval	96 hours interval
T1	6×10^1	9×10^1	1.6×10^2	2.1×10^2	2.7×10^2
T2	5×10^1	8×10^1	1.6×10^2	2.1×10^2	2.5×10^2
T3	-	8×10^1	1.4×10^2	1.9×10^2	2.2×10^2
T4	-	6×10^1	1.2×10^2	1.7×10^2	2.0×10^2
T5	-	-	-	5×10^1	8×10^1
T6	-	5×10^1	8×10^1	1×10^2	1.5×10^2
T7	-	-	6×10^1	9×10^1	1.2×10^2

Table 5. Fungus colony count (CFU/gram) at different storage intervals

Treat-ments	3 hours interval	24 hours interval	48 hours interval	72 hours interval	96 hours interval
T1	1.8×10^2	2.1×10^2	2.4×10^2	2.7×10^2	2.9×10^2
T2	5×10^1	1.2×10^2	1.6×10^2	2.3×10^2	2.6×10^2
T3	-	8×10^1	1.4×10^2	1.8×10^2	2.4×10^2
T4	-	9×10^1	1.3×10^2	2×10^2	2.7×10^2
T5	-	-	-	1×10^2	1.5×10^2
T6	-	-	9×10^1	1.6×10^2	2×10^2
T7	-	7×10^1	1.2×10^2	1.8×10^2	2.2×10^2

The data on fungus count is reported in table(5). Maximum number of colonies were observed in T1 (without spray). In treatment T5, first colony appeared after 48 hours storage. Colony count was 1.5x10 2 CFU (colony formed unit/gram of bread) at 96 hours of storage. It indicated that the main source of contamination in bread are packaging material and slicer. It was also supported by Al-Mohizae et al.(1987), who found that microbial load of the air and relative humidity inside the package play a major role in bread spoilage.

Sensory evaluation

The effect of treatments on external characteristics i.e. volume, color of crust, symmetry of form, evenness of bake, character of crust, and internal characteristics i.e. grin, color of crumb, aroma, taste and texture of bread are shown in table(6).Maximum score (6.98) was obtained by T5 (coating of pan + spray on dough + slicer + packaging material) followed by T7 (spray on packaging material), having a mean score (6.93). The volume symmetry of form character of crust and grain of bread were affected significantly by treatments, while color of crust, aroma, texture, taste and color of crumb were not affected by treatments and results were non significant.

The effect of storage time on external &internal characteristics of bread are presented in table (7). Maximum mean score (7.35) was obtained at 3 hours storage interval. While minimum score (3.40) was observed at 96 hours storage interval. Color of crust, color of crumb, aroma, taste and texture were affected significantly by storage time. The volume, symmetry of form, character of crust and grain of bread were not affected by storage time and results were non significant.

Conclusion

In short, the preservatives have a positive influence on the shelf life and sensory characteristics of bread, whereas, storage caused a decrease in aroma, taste, texture and color of crust and crumb. Among the treatments, treatment T5 (coating of pan+ spray on dough + slicer + packaging material) showed no sign of micro flora up to 48 hours of storage and was comparatively better with respect to overall acceptability. Hence, the study reveals that the bread treated by spray mixture during panning, proofing, slicing and packaging has great potential against fungus and bacteria.

Table 6. Effect of treatments on external & internal characteristics of bread

Treatments	Volume	Symmetry of form	Character of crust	Color of crust	Evenness of bake	Grain	Aroma	Texture	Taste	Color of crumb	Mean score
T1	6.68	3.10	2.88	6.34	2.24	9.74	5.66	10.42	14.24	6.60	6.79
T2	6.60	3.18	3.12	6.18	2.14	10.72	5.80	11.80	14.12	6.76	6.48
T3	6.44	2.96	2.82	6.34	2.22	10.84	5.94	11.36	14.02	6.90	6.98
T4	6.16	3.38	3.00	6.46	2.18	10.00	5.68	11.18	14.04	6.98	6.90
T5	5.46	3.40	3.00	6.32	2.28	9.70	5.88	11.38	14.32	7.02	6.87
T6	6.02	3.18	2.90	6.56	2.24	10.40	5.78	11.30	14.24	6.76	6.93
T7	6.06	3.34	2.76	6.18	2.32	10.32	5.56	11.60	13.96	6.78	6.88
Mean+S.D	6.20± 0.41	3.22± 0.16	2.92± 0.12	6.34± 0.13	2.23± 0.05	10.24± 0.45	5.75± 0.13	11.29± 0.43	14.13± 0.13	6.82± 0.14	

Table 7. Effect storage time on external & internal characteristics of bread

Storage interval	Volu-me	Symm-etry of form	Char-acter of crust	Color of crust	Even-ness of bake	Grain	Aroma	Texture	Taste	Color of crumb	Mean score+- S.D
24Hours	6.35	3.38	2.92	6.70	2.28	10.47	6.00	11.73	14.69	7.00	7.15±4.0-3
3 Hours	6.48	3.61	3.10	6.84	2.31	10.50	6.32	12.14	15.09	7.15	7.35±4.1
48Hours	6.14	3.22	2.88	6.42	2.27	10.32	5.87	11.43	14.26	6.83	6.96±3.93
72Hours	6.14	3.08	2.85	6.02	2.17	10.02	5.47	10.80	13.99	6.64	6.71±3.82
96Hours	5.85	2.94	2.85	5.70	2.11	8.25	5.11	10.47	12.66	6.15	6.20±3.40
Mean+- S.D	6.19± 0.23	3.24± 0.26	2.92± 0.10	6.33± 0.47	2.22± 0.08	9.91± 0.94	5.75± 0.47	11.31± 0.67	14.13± 0.92	6.75± 0.38	

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Comparative storage studies on wheat flour prepared by different processing methods II. Effect on phytic acid content in flour and bread

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ABSTRACT

Three months study was conducted on the comparative storage behavior of wheat flour prepared by roller flour mill (FM) and chakki (Cki) milling. Flour was prepared by the two methods from the same lot of wheat, packed in polypropylene woven bags and stored at ambient temperature and relative humidity at Peshawar in the months of May to July. The flour samples were analyzed for phytic acid content in the beginning and at monthly intervals. Leavened and unleavened flat breads were prepared at each storage interval, and among other parameters, were analyzed for phytic acid content. The FM flour contained 0.32% phytic acid and Cki flour 0.55%. In both cases the phytic acid contents did not change for 2 months, but increased slightly in the third month, possibly due to feeding of insect on the endosperm part of the flour. Baking without leavening resulted in slight reduction of phytic acid content, whereas leavening resulted in significant reduction of phytic acid in the resultant bread. This effect of leavening was consistent at all storage intervals as well as in both types of flours. Results of both types of flour were comparable and no specific differences in the storage behavior could be noted with respect to changes in the phytic acid content

Keywords: Wheat flour, processing, storage, phytic acid

INTRODUCTION

Pakistan has a bread eating culture despite production of substantial quantities of rice. Different variants of flat bread (leavened, unleavened, nan, chapatie) are consumed in different parts of the country which are prepared from high extraction flour called 'atta'. The wheat is milled in roller flour mills or chakies (stone grinders or pin mill grinders). About 80% wheat is consumed in the form of chapaties. It is reported that the per capita consumption of wheat in Pakistan is 124 Kg per year or 340 gram per day (Khan 2001). Roller flour mills or modern milling industry grinds approximately 45% of the total wheat consumed in Pakistan and small scale grinders (chakkies) grind the remaining 55%. (Khan 2001).

In its natural state, wheat is a good source of vitamin B1, B2, B6, niacin as well as iron and zinc besides being a cheap source of process and calories. But since most of these nutrients are concentrated in the outer layer of the wheat grain, hence a significant portion is lost during the roller-milling process. For more refined wheat flour, the loss of vitamins and minerals is greater as in modern milling sector (Kent 1983).

Associated with the bran of cereal grains is a substance, phytic acid (inositole hexaphosphoric acid) which can form insoluble compounds with calcium iron and Mg More than 90% of the total phytic acid in wheat is localized in the aleurone layer (Pringle 1952) as aleurone grains, which consist almost entirely of phytin, the potassium-magnesium salt of phytic acid (Steven 1971). Phytic acid forms complexes with divalent and trivalent metallic ions such as Zn^{+2},Ca^{2},Cu^{2},Fe^{+2},Fe^{+3}, especially those which are not absorbed in gastrointestinal tract and thereby lowers the bio-availability of essential elements leading to deficiency diseases and also reduces nutritive value of cereals (Smith and Circle 1978). Brown flour and whole meal flour contain bran and aleurone, and therefore, phytic acid tends to immobilize the calcium and iron present in the flour itself and in other ingredients of diet.

Although studies aiming at establishing a direct cause and effect relationship have not yet been conducted, yet it is believed that high phytate diets and lower intake of iron rich food have contributed to the very high prevalence of Iron Deficiency Anaemia (IDA) in Pakistan, severely affecting large sections of the population, particularly young children, pregnant and nursing mothers and female adolescents (GOP, 1985-87).

The purpose of present research was to compare the storage stability and phytic acid contents of wheat flour produced by grinding in roller flour mill and chakki. Effect of baking unleavened and leavened chapatis of the two types of flours on the phytic acid contents was also studied.

MATERIALS AND METHODS

The present research was carried out at Nuclear Institute for Food and Agriculture (NIFA), Peshawar. The flour samples (85% extraction) were obtained from Dastagir flour mills Peshawar. Wheat from the same lot was milled by Chakki (traditional) milling machine (100% extraction rate) and were packed in small (1kg) polypropylene bags. The samples were stored at ambient temperature which ranged from 33.34 °C to 37.73 °C and relative humidity ranged from 40.14% to 64.58% during 3 month's storage period. Samples were analyzed for phytic acid (Haug and Lantzsch, 1983) at 0 days and at monthly intervals thereafter. Leavened (natural) and unleavened chapattis were prepared by traditional method at each storage interval and analyzed for phytic acid content.

Analysis of variance of the data was carried out (2 X 4 factorial RCBD), using MSTAT-C computer package. Means were separated using Duncon's New Multiple Range Test (DNMRT).

RESULTS AND DISCUSSIONS

Phytic acid in flour samples

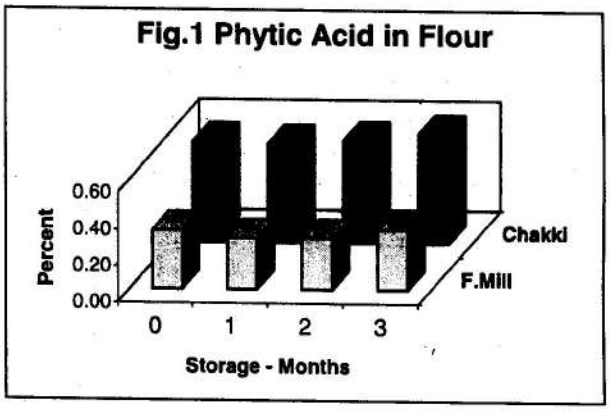
Two types of flours, prepared by roller Flour Milling (FM) and Chakki were used in these studies. Since the extraction rate, bran content and the proximate composition of both the flour types were different, therefore it was expected that the nutrient availability, storage behavior, acceptability and other parameters of nutritional significance would also be different in both types of Atta. (Khan, 2001).

Roller milled and chakki flours prepared from the same wheat lot were studied for their total phytic acid contents. The average phytic acid contents of the chakki flour were 0.56% as compared to 0.30% in the FM flour (Table 1). Effect of storage and milling method on phytic acid content are shown in Fig 1. The phytic acid contents were 0.32% in FM flour and remained almost unchanged during the entire storage period. Phytic acid contents of the chakki flour also did not change significantly for 2 months (0.55 to 0.56%) but increased significantly in the third month.

Table 1. Effect of flour type and storage on the phytic acid content (%) of wheat flour

	Storage – Months				
Flour Type	0 Month	1 Month	2 Months	3 Months	Mean
F. Mill	0.32 a ±0.04	0.28 a ±0.10	0.28 a ±0.02	0.33 a ±0.01	0.30 b
Chakki	0.55 a ±0.24	0.54 a ±0.03	0.56 a ±0.02	0.60 b ±0.01	0.56 a
Mean	0.43 ab	0.41 b	0.42 b	0.46 a	

Values are means of three replications ± standard deviation.
Valuesin a row or column followed by different letters are significantly ($p<0.05$) different from each other.



Increase in the phytic acid contents was not an increase in the amount of phytates *per se*, rather the concentration of phytic acid increased due to drying out of the samples as well as insect infestation, which was observed in present case during the last month of the storage (Tariq 2003). Since the insects feed upon the endosperm part of the flour, the concentration of the bran portion increased, and consequently resulted in an overall increase in the concentration of phytic acid.

Differences in storage behaviour of the two types of flours, in terms of PA contents, can be explained on the bases of composition of the two flours. Due to the removal of bran portion in the roller milling process the bran contents and hence the concentration of PA is reduced in the flour. That logically reduced the impact of insect infestation on the PA contents.

Phytic acid is present in the range of 1-5% of many cereals, legumes and oil seed (Reddy et al. 1982). The phytic content in the wheat flour varies according to the extraction rates. Batten (1994) compared the concentration of minerals and phytic acid in 47 different wheat samples and observed that Australian

wheat contained only 59-77% of the phytic acid as compared to the white and red wheat grown overseas. Becker and Lorenz (1978) found that the amount of phytic acid varied from 0.40 to 2.0% in legumes, from 0.5 to 1.89% in cereals and from 2 to 5.20% in oil seed.

Phytic acid in chapati samples

Table 2 shows the phytic acid content of the leavened (L) and unleavened control (C) chappaties prepared from freshly milled as well as stored flours of both types. The date revealed that the differences in phytic acid contents of the two types of flour were reflected also in the baked products. Chapatties from Chakki flour contained higher (P<0.05) concentration of phytic acid than those prepared from roller milled flour. Effect of leavening (natural fermentation for two hour) was significant (P<0.05) on the phytic acid contents of both the flours. As observed in the case of flours, the phytic acid contents increased in the chapaties prepared in the later stages of storage. This effect of storage, however, was more pronounce in the case of Chakki flour chapatties as compared to roller milled flour chapaties.

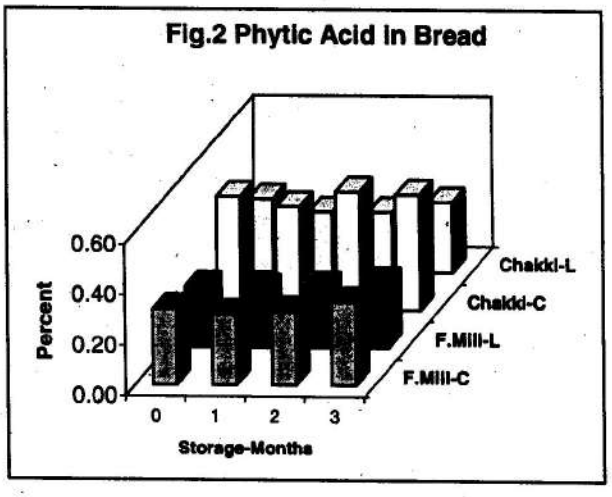
Studies on legumes, cereals and oilseeds have shown that phytic acid is generally stable under ordinary processing conditions (Thompson 1990). Pure phytic acid in aqueous solution at pH 6.0 was lost (about 50%) after 1-hour autoclaving (De Boland et al. 1975), but in biological systems e.g. in cereals and oilseeds less than 10% loss was observed with autoclaving for 0.5-2.0 hours (Leese 1966). Boiling of legumes also resulted in a significant breakdown of their phytic acid content (Kumar et al. 1978 and Reddy 1987). However, Ologhobo and Fetuga (1984) could not record a significant reduction in phytic acid of soybean due to cooking, autoclaving and soaking. Nevertheless, Microwave heating of soybean caused a 23% phytic acid reduction after 9 minutes and 46% after 15 minutes (Hafiz et al. 1989). Poonam and Sahil (1993) reported reduction in phytic acid content in the processed weaning food, after mixing locally available cereals (wheat, barley and pulses). Mameesh and Tomar (1993) observed that fermentation of soybean fortified wheat flour resulted in complete removal of phytic acid.

Table 2. Effect of flour type and storage on the phytic acid contents (%) of bread

	Storage-Months				
Flour Type	**0-Month**	**1-Months**	**2-Months**	**3-Months**	**Mean**
F.Mill-C*	0.30 ±0.02	0.28 ±0.03	0.29 ±0.01	0.33 ±0.01	**0.30 b**
F.Mill-L**	0.24 ±0.30	0.23 ±0.01	0.24 ±0.01	0.26 ±0.01	**0.24 d**
Chakki-C	0.45 ±0.03	0.41 ±0.01	0.47 ±0.01	0.46 ±0.01	**0.44 a**
Chakki-L	0.29 ±0.09	0.24 ±0.01	0.24 ±0.01	0.28 ±0.01	**0.26 c**
Mean	**0.32 b**	**0.29 d**	**0.31 c**	**0.33 a**	

C = Unleavened control, ** = Leavened
Values are means of three replications +- standard deviation
Valuesin a row or column followed by different letters are significantly (p<0.05) different from each other.
L-Leavened
C-Control

Fig.2 Phytic Acid in Bread



Advantages of the acidic environment and the lengthy fermentation of sourdough bread include the breakdown of phytates -increasing mineral bioavailability, increased digestibility, and decreased rate of spoilage. The binding of phytic acid with minerals is pH dependent and the complexes formed with different cations have varying solubilities. Phytic acid also binds protein molecules (Schwenke et al 1986 and Moth et al. 1987). Protein-phytic acid complex is insoluble but may become soluble when the pH decreases below 3 (Cheryan 1980).

Phytic acid is hydrolyzed to phosphoric acid and inositol by the enzyme phytase, present in the seed. Maximum phytase activity occurs at 55°C. Probably 60% of the phytic acid in flour is hydrolyzed during bread making (Kent 1983). Brune et al. (1992) reported that effective fermentation would increase bioavailability of iron in whole-wheat flour bread.

In conclusion it can be said that the amount of phytic acid was higher in Chakki flour as compared to roller flour milled atta. Leavening resulted in substantial reduction in the phytic acid contents of the flour. Although the sensory properties (Tariq 2003) of the flat bread prepared from the flour remained within the acceptable limit (>5), it can be concluded that a reasonable storage period for the wheat flour, under the given climatic conditions and the existing packing system, should not more than 45 days.

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Suitability of Pakistani wheat varieties for chapatie preparation

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ABSTRACT

The suitability of Pakistani wheat varieties grown during two crop years i.e. 1995-96 and 1996-97 for chapatie preparation was evaluated. The sensory characteristics texture, breakability, flavour, taste and overall chapatie scores were significantly affected by crop years. Interaction of varieties and years was found to be significant in all parameters except feel to touch, color and overall chapatie scores. The sensory parameters of chapatie differed significantly among the wheat varieties. Pak-81, Rohtas-90 and Pasban-90 got highest overall scores. Twenty-five wheat varieties fall statistically in the same group with respect to overall chapatie score. Rohtas 90 and Pasban 90 obtained highest overall score, which may be attributable to pelshenke value or the protein content of respective variety.

Keywords: Wheat, Pakistan, chapaties, flavor, taste

INTRODUCTION

Wheat is the economical and principal source of energy and protein for the inhabitants of Pakistan. It is one of the leading cereal grains in Pakistan, which has made substantial progress towards enhancement of grain yield per unit area in wheat by the introduction of new cultivars and development of a new package of production technology.

Wheat is classified on the basis of color, growth habit and grain texture. The utilization of wheat into different products varies from country to country and even region to region. However, in Pakistan chapatie bread is the principal use of wheat. The quality criterion for assessment of wheat is generally based on different physical, chemical, biochemical and rheological characteristics, but the real ordeal is the baking test of the particular wheat.

In the present study chapaties were prepared with flour of forty four wheat cultivars released in Punjab province up till 1996-1997 crop year. Chapatie were subjected to sensory evaluation to sort out the best quality produced. The consumption of wheat varieties for 1995-1996 and 1996-1997 was made for the parameter.

MATERIALS AND METHODS

Collection of wheat samples

The wheat samples were collected from Wheat Research Institute, Faisalabad. These samples comprised all old tall and new semi dwarf wheat varieties grown at Faisalabad during the crop years 1995-96 and 1996-97 by applying similar fertilizer doses of N-P_2O_5- K_2O at the rate of 60-90-60 Kg ha^{-1}, respectively. All the wheat varieties released in the Punjab Province of Paksitan since 1933 upto the year of study i.e. 1995-96 were selected in order to assess their quality parameters. The wheat samples were properly cleaned and stored for further use.

Milling

The wheat grain samples of each variety were tempered to a moisture level at 15.5 percent. The water required for tempering of grains was computed according to the expression outlined in AACC (2000). The samples were allowed to stand for 24 hours at room temperature in order to equilibrate the moisture content in the grains before milling. The tempered grains were milled through Quadrumate Senior Mill. The procedure for milling of wheat grain was followed as described in AACC (2000).

Whole wheat meal flour

The whole wheat meal flour was prepared by grinding wheat grains of each variety through Udy Cyclone Sample Mill.

Chapatie baking

The chapaties were prepared from whole wheat flour of each wheat variety according to the method developed by Rao *et al.* (1986). The dough was prepared by adding 65-70 percent water in 200 g flour and mixing was carried out for 3 minutes in a mixer (National Mfg. Co., Lincoln, Nebr.). The dough was allowed to rest for one hour at room temperature. A dough piece weighing 80 g was rounded and turned into chapaties by using specially designed platform with the help of rolling pin. The chapaties were of

uniform thickness. Baking of chapaties was done on a thermostatistically controlled hot plate at a temperature of 210 °C for 1.5 minutes.

Sensory evaluation of chapaties was performed according to the method described by Piggot (1988) for color, flavor, taste and texture using Hedonic scale by a panel of trained judges as per evaluation performed given below:

RESULTS AND DISCUSSION

Texture

The results for texture of chapaties prepared from different wheat varieties have been shown in Table-1. The analysis of variance showed that crop years, wheat varieties and interaction of crop years x wheat varieties were found to have significant difference with respect to texture of chapaties. The results indicated that during the crop year 1995-96 the wheat varieties C-591, Faisalabad-85, C-273 and Lyallpur-73got significantly the highest scores to the texture of chapaties followed by C-228. The lowest scores were given to chapaties of Barani-70 and Faisalabad-83 followed by Sandal, Chenab-79 and Rawal-87 when grown during the crop year 1995-96.

The wheat varieties SA-42 followed by Bahawalpur-79, Kohinoor-83 and Shahkar-95 were significantly at the top with respect to texture of chapaties. The lowest value for the texture was given to chapaties of Barani-70 and Sandal when produced during the crop year 1996-97.

The texture of chapaties prepared from flour of wheat varieties Lyallpur-73 followed by C-217, C-273, SA-42 and Faisalabad-85 got the highest scores while Barani-70 and Sandal got the lowest scores when the crop years were pooled. Texture variation is also an indication of under or over fermentation and may also be affected by sheeting (Pomeranz, 1988). The results for good texture score may be attributed to high protein content. The results are in line with earlier findings of various research workers (Butt 1997, Ahmad 2001) who found similar results while testing Pakistani wheats.

Feel To Touch

The results regarding feel to touch of chapaties prepared from different wheat varieties are presented in Table-1. It is indicated from the statistical analysis that effect of crop years and interaction of crop years x wheat varieties was found to be non-significant for this sensory parameter. However, the wheat verities were found to be highly significant different with one another for feel to touch characteristics of chapaties.

The feel to touch of chapaties of the wheat varieties C-228 was the highest scoring whereas C-273, Dirk, Chenab-70, Blue Silver, Arz, Pavon, Faisalabad-85, Chakwal-86, Rohtas-90, Shahkar-95 and Punjab-96 at the second level after C-228.. The lowest scores for feel to touch were given to the wheat varieties Punjnad-88 followed by second lower varieties Mexi Pak and SA-42 when grown during the crop year 1995-96. During second crop year 1996-97, the wheat varieties C-228, C-273, Blue Silver, Pari-73, Pothohar, Pavon, Faisalabad-83, Faisalabad-85, Pasban-90 and Inqulab-91 got significantly higher scores for feel to touch of chapaties. The lowest scores for feel to touch was given to chapaties of SA-42, Punjnad-88, C-518 and Barani-83 when grown during 1996-97.

When the results of two years were pooled the maximum score was assigned to chapaties of wheat variety C228 followed by C-273, Blue Silver, Pavon, and Faisalabad-85. The lowest score for this sensory parameter was assigned to chapaties of wheat varieties Punjnad-88 and SA-42.

The results obtained in this study showed that the effect of crop years on feel to touch character of chapaties was found to be non-significant while the effect of wheat varieties was observed to be highly significant. These findings are partly in concordance with the results of Ahmad (2001) who found that this sensory character was affected by wheat varieties; crop years and interaction of wheat varieties x crop years. The wheat varieties getting highest scores were of more soft and acceptable to the judges for good mouth feel.

Foldability

The results for foldability of chapaties prepared from different wheat varieties are presented in Table-1. The statistical results revealed that scores for foldability did not differ significantly between the crop year. However, the effect of wheat varieties and interaction of crop year with wheat varieties was found to be highly significant on this sensory parameter. It is obvious from the data that the wheat varieties Arz followed by Dirk got significantly higher scores for foldability, however, the wheat varieties WL-711 was lowest one, whereas, C-518, C-271, Pak 81, Barani-83 and Punjnad-88 got the second lowest scores but non-significant with each other for foldability of chapatie during crop year 1995-96. The highest scores for foldability was achieved by the chapaties prepared from wheat varieties SA-75 followed by C-217 and Rohtas-90 when grown during crop year 1996-97 whereas, significantly lowest scores were assigned to chapaties of C-518, C-591 and Dirk when grown during same crop year.

Table-1. Texture, feel to touch and foldability of chapaties prepared from different wheat varieties

S.No. Variety	Texture			Feel to Touch			Foldability		
	1995-96	1996-97	Mean	1995-96	1996-97	Mean	1995-96	1996-97	Mean
1. C-518	5.83 e-h	5.83 hij	5.83 lmn	6.50	6.50	6.50 ghi	4.83 gh	4.83 gh	4.83 i
2. C-591	7.67 a	6.83 c-h	7.25 a-d	8.50	8.33	8.42 abc	5.83 c-h	4.83 gh	5.33 e-i
3. C-228	7.33 ab	7.17 a-f	7.25 a-d	8.83	8.67	8.75 a	6.33 a-f	5.83 c-h	6.08 b-g
4. C-217	7.17 abc	7.67 a-d	7.42 ab	8.00	8.00	8.00 a-f	7.00 a-d	7.33 ab	7.17 a
5. C-250	6.33 b-f	6.00 g-j	6.17 h-n	7.83	7.83	7.83 a-f	6.00 b-h	5.83 c-h	5.92 c-h
6. C-271	5.83 e-h	6.00 g-j	5.92 k-n	7.33	7.33	7.33 d-g	4.83 gh	5.83 c-h	5.33 e-i
7. C-273	7.50 a	7.33 a-f	7.42 ab	8.67	8.67	8.67 ab	6.33 a-f	6.67 b-e	6.50 a-d
8. Dirk	6.67 a-f	7.33 a-f	7.00 a-g	8.67	8.33	8.50 abc	7.33 ab	4.83 gh	6.08 b-g
9. Mexi Pak	5.83 e-h	6.67 d-i	6.25 g-m	6.33	6.67	6.50 ghi	5.33 fgh	5.50 d-h	5.42 e-i
10. Chenab-70	6.17 c-g	6.33 f-i	6.25 g-m	8.67	7.00	7.83 a-f	5.83 c-h	5.00 fgh	5.42 e-i
11. Barani-70	5.00 h	5.00 j	5.00 o	8.33	8.17	8.25 a-d	5.83 c-h	5.83 c-h	5.83 c-i
12. SA-42	6.67 a-f	8.17 a	7.42 ab	6.33	6.33	6.33 hi	5.33 fgh	4.67 h	5.00 hi
13. Blue Silver	6.00 d-h	7.17 a-f	6.58 c-l	8.67	8.67	8.67 ab	5.33 fgh	5.33 e-h	5.33 e-i
14. Lyallpur-73	7.50 a	7.50 a-e	7.50 a	8.00	8.17	8.08 a-e	6.17 a-g	6.83 bcd	6.50 a-d
15. Pari-73	6.83 a-e	6.83 c-h	6.83 a-i	8.17	8.67	8.42 abc	6.83 a-e	6.33 b-f	6.58 a-d
16. Sandal	5.17 gh	5.67 ij	5.42 no	8.33	8.17	8.25 a-d	6.83 a-e	5.83 c-h	6.33 a-e
17. Pothohar	5.67 fgh	7.00 b-g	6.33 f-m	7.67	8.67	8.17 a-e	5.67 d-h	6.50 b-e	6.08 b-g
18. Yecora	6.83 a-e	7.33 a-f	7.08 a-f	8.00	8.17	8.08 a-e	5.33 fgh	6.33 b-f	5.83 c-i
19. Arz	5.83 e-h	7.33 a-f	6.58 c-l	8.67	8.33	8.50 abc	7.50 a	7.00 abc	7.25 a
20. SA-75	7.00 a-d	6.67 d-i	6.83 a-i	6.67	6.67	6.67 ghi	6.33 a-f	8.17 a	7.25 a
21. Punjab-76	6.83 a-e	7.67 a-d	7.25 a-d	8.17	8.17	8.17 a-e	7.00 a-d	6.33 b-f	6.67 abc
22. LU-26	6.67 a-f	7.50 a-e	7.08 a-f	8.17	8.33	8.25 a-d	7.17 abc	6.17 b-g	6.67 abc
23. Pavon	6.33 b-f	7.50 a-e	6.92 a-h	8.67	8.67	8.67 ab	5.50 e-h	5.00 fgh	5.25 f-i
24. WL-711	6.17 c-g	7.17 a-f	6.67 b-k	8.50	8.17	8.33 abc	4.67 h	5.33 e-h	5.00 hi
25. Chenab-79	5.17 gh	6.83 c-h	6.00 j-n	7.83	7.83	7.83 a-f	5.67 d-h	5.67 c-h	5.67 c-i
26. Bahawalpur-79	6.67 a-f	8.00 ab	7.33 abc	8.17	8.17	8.17 a-e	7.00 a-d	6.33 b-f	6.67 abc
27. Punjab-81	6.00 d-h	6.83 c-h	6.42 e-m	6.50	6.67	6.58 ghi	5.67 d-h	5.50 d-h	5.58 d-i
28. Pak-81	6.00 d-h	7.00 b-g	6.50 d-m	8.17	8.17	8.17 a-e	4.83 gh	6.33 b-f	5.58 d-i
29. Barani-83	6.83 a-e	7.50 a-e	7.17 a-e	6.67	6.50	6.58 ghi	4.83 gh	5.50 d-h	5.17 ghi
30. Kohinoor-83	6.67 a-f	8.00 ab	7.33 abc	8.50	8.33	8.42 abc	6.33 a-f	6.83 bcd	6.58 a-d
31. Faisalabad-83	5.00 h	6.50 e-i	5.75 mn	8.33	8.67	8.50 abc	6.67 a-f	6.67 b-e	6.67 abc
32. Faisalabad-85	7.67 a	7.17 a-f	7.42 ab	8.67	8.67	8.67 ab	6.50 a-f	6.67 b-e	6.58 a-d
33. Punjab-85	5.67 fgh	6.83 c-h	6.25 g-m	7.50	7.67	7.58 c-f	5.33 fgh	5.33 eh	5.33 e-i
34. Chakwal-86	6.33 b-f	7.17 a-f	6.75 a-j	8.67	8.33	8.50 abc	5.67 d-h	6.83 bcd	6.25 a-f
35. Satluj-86	6.67 a-f	7.67 a-d	7.17 a-e	8.17	8.17	8.17 a-e	6.67 a-f	6.67 b-e	6.67 abc
36. Rawal-87	5.17 gh	6.33 f-i	5.75 mn	6.50	7.83	7.17 fgh	7.00 a-d	5.50 d-h	6.25 a-f
37. Punjnad-88	5.67 fgh	6.00 g-j	5.83 lmn	5.83	6.33	6.08 i	4.83 gh	6.67 b-e	5.75 c-i
38. Shalimar-88	5.67 fgh	6.50 e-i	6.08 i-n	7.83	6.67	7.25 efg	5.33 fgh	6.00 b-h	5.67 c-i
39. Rohtas-90	7.00 a-d	7.00 b-g	7.00 a-g	8.67	7.67	8.17 a-e	6.67 a-f	7.33 ab	7.00 ab
40. Pasban-90	7.00 a-d	6.83 c-h	6.92 a-h	8.33	8.67	8.50 abc	5.50 e-h	6.33 b-f	5.92 c-h
41. Inqulab-91	6.83 a-e	7.83 abc	7.33 abc	8.33	8.67	8.50 abc	6.67 a-f	6.50 b-e	6.58 a-d
42. Parwaz-94	6.67 a-f	7.00 b-g	6.83 a-i	7.83	7.67	7.75 b-f	6.50 a-f	6.17 b-g	6.33 a-e
43. Shahkar-95	6.67 a-f	8.00 ab	7.33 abc	8.67	8.33	8.50 abc	6.33 a-f	6.33 b-f	6.33 a-e
44. Punjab-96	7.00 a-d	7.67 a-d	7.33 abc	8.67	8.33	8.50 abc	6.17 a-g	6.83 bcd	6.50 a-d
Mean	6.39b	7.01a	6.70	7.94	7.91	7.93	6.03	6.09	6.06

Table-2. Texture, feel to touch and foldability of chapaties prepared from different wheat varieties

S.No. Variety	Breakability			Flavor and Taste			Color			Overall chapatie		
	1995-96	1995-96	1996-97	1995-96	1996-97	1995-96	1996-97	1996-97	Mean	1995-96	1996-97	Mean
1. C-518	5.83 f-i	7.17 bcde	6.33 fgh	7.17 bcde	6.33 fgh	7.17 bcde	6.33 fgh	4.83 gh	4.83 i	7.33	5.00	6.17 a-f
2. C-591	6.67 c-h	7.00 cdef	7.83 abc	7.00 cdef	7.83 abc	7.00 cdef	7.83 abc	4.83 gh	5.33 e-i	6.67	6.17	6.42 a-d
3. C-228	7.33 b-e	6.17 f	6.33 fgh	6.17 f	6.33 fgh	6.17 f	6.33 fgh	5.83 c-h	6.08 b-g	6.50	5.67	6.08 a-f
4. C-217	7.83 bcd	7.00 cdef	6.17 gh	7.00 cdef	6.17 gh	7.00 cdef	6.17 gh	7.33 ab	7.17 a	6.67	5.83	6.25 a-f
5. C-250	6.67 c-h	7.83 abc	7.00 cdefg	7.83 abc	7.00 cdefg	7.83 abc	7.00 cdefg	5.83 c-h	5.92 c-h	6.00	5.33	5.67 c-f
6. C-271	6.83 c-h	7.00 cdef	6.17 gh	7.00 cdef	6.17 gh	7.00 cdef	6.17 gh	5.83 c-h	5.33 e-i	5.67	5.17	5.42 f
7. C-273	6.33 e-i	7.67 abcd	7.67 abcd	7.67 abcd	7.67 abcd	7.67 abcd	7.67 abcd	6.67 b-e	6.50 a-d	6.83	6.00	6.42 a-d
8. Dirk	7.00 c-g	7.67 abcd	6.67 defg	7.67 abcd	6.67 defg	7.67 abcd	6.67 defg	4.83 gh	6.08 b-g	6.33	5.83	6.08 a-f
9. Mexi Pak	5.17 i	8.17 ab	7.83 abc	8.17 ab	7.83 abc	8.17 ab	7.83 abc	5.50 d-h	5.42 e-i	5.83	5.33	5.58 def
10. Chenab-70	5.67 ghi	7.83 abc	7.67 abcd	7.83 abc	7.67 abcd	7.83 abc	7.67 abcd	5.00 fgh	5.42 e-i	6.17	5.33	5.75 c-f
11. Barani-70	5.83 f-i	6.33 ef	5.67 h	6.33 ef	5.67 h	6.33 ef	5.67 h	5.83 c-h	5.83 c-i	5.67	5.17	5.42 f
12. SA-42	5.17 i	7.00 cdef	6.67 defg	7.00 cdef	6.67 defg	7.00 cdef	6.67 defg	4.67 h	5.00 hi	5.83	5.50	5.67 c-f
13. Blue Silver	6.33 e-i	6.33 ef	6.33 fgh	6.33 ef	6.33 fgh	6.33 ef	6.33 fgh	5.33 e-h	5.33 e-i	5.83	5.67	5.75 c-f
14. Lyallpur-73	7.17 c-f	8.17 ab	7.33 abcde	8.17 ab	7.33 abcde	8.17 ab	7.33 abcde	6.83 bcd	6.50 a-d	6.67	6.00	6.33 a-e
15. Pari-73	6.00 e-i	8.33 a	7.17 bcdef	8.33 a	7.17 bcdef	8.33 a	7.17 bcdef	6.33 b-f	6.58 a-d	6.33	6.00	6.17 a-f
16. Sandal	6.00 e-i	7.17 bcde	7.17 bcdef	7.17 bcde	7.17 bcdef	7.17 bcde	7.17 bcdef	5.83 c-h	6.33 a-e	6.00	5.33	5.67 c-f
17. Pothohar	7.00 c-g	8.00 ab	7.33 abcde	8.00 ab	7.33 abcde	8.00 ab	7.33 abcde	6.50 b-e	6.08 b-g	6.17	5.67	5.92 a-f
18. Yecora	6.17 e-i	8.33 a	8.00 abc	8.33 a	8.00 abc	8.33 a	8.00 abc	6.33 b-f	5.83 c-i	6.33	6.00	6.17 a-f
19. Arz	8.50 ab	7.83 abc	7.83 abc	7.83 abc	7.83 abc	7.83 abc	7.83 abc	7.00 abc	7.25 a	6.17	5.50	5.83 b-f
20. SA-75	9.17 a	8.50 a	7.17 bcdef	8.50 a	7.17 bcdef	8.50 a	7.17 bcdef	8.17 a	7.25 a	7.17	5.83	6.50 abc
21. Punjab-76	7.83 bcd	7.50 abcd	8.00 abc	7.50 abcd	8.00 abc	7.50 abcd	8.00 abc	6.33 b-f	6.67 abc	6.83	6.50	6.67 ab
22. LU-26	7.33 b-e	7.00 cdef	7.83 abc	7.00 cdef	7.83 abc	7.00 cdef	7.83 abc	6.17 b-g	6.67 abc	6.17	5.50	5.83 b-f
23. Pavon	6.67 c-h	7.67 abcd	8.00 abc	7.67 abcd	8.00 abc	7.67 abcd	8.00 abc	5.00 fgh	5.25 f-i	6.33	5.50	5.92 a-f
24. WL-711	6.33 e-i	6.67 def	6.50 efgh	6.67 def	6.50 efgh	6.67 def	6.50 efgh	5.33 e-h	5.00 hi	5.67	6.00	5.83 b-f
25. Chenab-79	5.50 hi	7.67 abcd	7.33 abcde	7.67 abcd	7.33 abcde	7.67 abcd	7.33 abcde	5.67 c-h	5.67 c-i	5.67	5.17	5.42 f
26. Bahawalpur-79	6.50 d-i	8.17 ab	8.17 ab	8.17 ab	8.17 ab	8.17 ab	8.17 ab	6.33 b-f	6.67 abc	6.33	5.67	6.00 a-f
27. Punjab-81	6.17 e-i	6.83 cdef	7.83 abc	6.83 cdef	7.83 abc	6.83 cdef	7.83 abc	5.50 d-h	5.58 d-i	5.83	6.00	5.92 a-f
28. Pak-81	6.50 d-i	6.83 cdef	6.67 defg	6.83 cdef	6.67 defg	6.83 cdef	6.67 defg	6.33 b-f	5.58 d-i	7.00	6.50	6.75 a
29. Barani-83	6.00 e-i	8.17 ab	7.67 abcd	8.17 ab	7.67 abcd	8.17 ab	7.67 abcd	5.50 d-h	5.17 ghi	6.17	5.83	6.00 a-f
30. Kohinoor-83	6.33 e-i	7.17 bcde	7.00 cdefg	7.17 bcde	7.00 cdefg	7.17 bcde	7.00 cdefg	6.83 bcd	6.58 a-d	6.33	5.50	5.92 a-f
31. Faisalabad-83	6.33 e-i	8.50 a	8.00 abc	8.50 a	8.00 abc	8.50 a	8.00 abc	6.67 b-e	6.67 abc	5.83	5.17	5.50 ef
32. Faisalabad-85	7.33 b-e	8.50 a	7.67 abcd	8.50 a	7.67 abcd	8.50 a	7.67 abcd	6.67 b-e	6.58 a-d	7.00	5.33	6.17 a-f
33. Punjab-85	6.50 d-i	7.67 abcd	7.67 abcd	7.67 abcd	7.67 abcd	7.67 abcd	7.67 abcd	5.33 eh	5.33 e-i	5.67	6.17	5.92 a-f
34. Chakwal-86	7.17 c-f	8.50 a	7.33 abcde	8.50 a	7.33 abcde	8.50 a	7.33 abcde	6.83 bcd	6.25 a-f	6.67	5.50	6.08 a-f
35. Satluj-86	6.50 d-i	8.17 ab	7.83 abc	8.17 ab	7.83 abc	8.17 ab	7.83 abc	6.67 b-e	6.67 abc	6.17	6.00	6.08 a-f
36. Rawal-87	6.67 c-h	8.50 a	7.83 abc	8.50 a	7.83 abc	8.50 a	7.83 abc	5.50 d-h	6.25 a-f	6.00	6.00	6.00 a-f
37. Punjnad-88	6.33 e-i	8.33 a	7.50 abcd	8.33 a	7.50 abcd	8.33 a	7.50 abcd	6.67 b-e	5.75 c-i	5.33	5.83	5.58 def
38. Shalimar-88	5.67 ghi	8.33 a	8.00 abc	8.33 a	8.00 abc	8.33 a	8.00 abc	6.00 b-h	5.67 c-i	5.83	5.83	5.83 b-f
39. Rohtas-90	8.00 abc	8.33 a	8.33 a	8.33 a	8.33 a	8.33 a	8.33 a	7.33 ab	7.00 ab	7.00	6.50	6.75 a
40. Pasban-90	6.33 e-i	8.50 a	7.83 abc	8.50 a	7.83 abc	8.50 a	7.83 abc	6.33 b-f	5.92 c-h	7.17	6.33	6.75 a
41. Inqulab-91	7.17 c-f	7.50 abcd	7.83 abc	7.50 abcd	7.83 abc	7.50 abcd	7.83 abc	6.50 b-e	6.58 a-d	6.50	6.17	6.33 a-e
42. Parwaz-94	6.83 c-h	8.17 ab	8.17 ab	8.17 ab	8.17 ab	8.17 ab	8.17 ab	6.17 b-g	6.33 a-e	6.50	6.33	6.42 a-d
43. Shahkar-95	7.17 c-f	8.33 a	7.33 abcde	8.33 a	7.33 abcde	8.33 a	7.33 abcde	6.33 b-f	6.33 a-e	6.67	6.33	6.50 abc
44. Punjab-96	6.83 c-h	8.50 a	8.17 ab	8.50 a	8.17 ab	8.50 a	8.17 ab	6.83 bcd	6.50 a-d	6.50	6.17	6.33 a-e
Mean	6.65a	6.03b	6.34	7.71 a	7.38 b	7.55	7.83	7.84	7.84	6.30a	5.78b	6.04

Table 3. Mean squares for sensory evaluation of chapaties prepared from different wheat varieties

Source	d.f.	Mean Square						
		Texture	Feel to Touch	Foldability	Breakability	Flavor and Taste	Color	Overall Chapatie
Year	1	25.160**	0.077^{NS}	0.274^{NS}	25.160*	6.842**	0.004^{NS}	18.296*
Error	4	0.201	1.188	0.272	1.294	0.757	0.845	2.337
Varieties	43	2.396**	3.330**	2.498**	2.738**	2.290**	2.318**	0.815**
Year x Varieties	43	0.596**	0.342^{NS}	1.097**	0.962**	0.448**	0.372^{NS}	0.384^{NS}
Error	172	0.318	0.439	0.516	0.499	0.257	0.479	0.367

** = Highly significant (P≤ 0.01)
NS = Non-significant

The sensory scores for foldability ranged from 4.83 to 7.25 when data averaged across the two crop years. The maximum scores were obtained by the chapaties of Arz and SA-75 followed by C-217 when the data of two crop years were pooled ; however, these wheat varieties exhibited non significant differences among one another on combining the data of two crop years. The lowest scores for foldability were given to chapaties of C-518 followed by second lowest SA-42 and WL-711 on combining the two years.

The results obtained in this study regarding foldability in chapatie are in consistent with those of Ahmad (2001) who observed maximum scores for foldability in SA-75. The research work done by Butt *et al.* (1997b) also indicated that sensory parameter of chapatie are significantly affected by the wheat varieties and crop years × wheat varieties. The changing behaviour of varieties may be attributed to variation in protein content and quality as stated by Murty and Austin, 1963.

Breakability

The results for breakability of chapaties prepared from different wheat varieties are presented in Table-2. The statistical analysis indicated that effect of crop years was found to be significant while effect of wheat varieties and interaction of crop years × wheat varieties interaction was found to be highly significant for breakability of chapaties.

It is evident from the data that during the crop year 1995-96 the variety SA-75 got significantly the highest scores for breakability followed by Arz. The chapaties of Mexi pak and SA-42 got significantly the lowest scores when the wheat varieties were grown during the crop year 1995-96.

Significantly the highest scores for breakability as given to the chapaties of SA-75 and Faisalabad 85 followed by C-228, C-217 and Lyallpur-73 when the wheat varieties were produced during the crop year 1996-97 whereas, lowest scores for breakability were assigned to the chapaties of Barani-70, Mexi pak and Shalimar-88 during the same crop year.

The scores for breakability ranged from 4.92 to 8.17 scores when average of two crop years was taken. The highest scores for breakability were achieved by the chapaties of wheat SA-75 followed by C-217 attaining scores 7.42 whereas, Mexi Pak and Barani-70 showed 4.92 the lowest and 5.08 the second lowest scoring varieties respectively when data across the crop years were averaged. The results showed that the chapaties of crop produced during the year 1995-96 exhibited significantly better scores for breakability than the chapaties of wheats produced during 1996-97 crop years.

The study carried out by Butt *et al.* (1997) and Ahmad (2001) indicated that this sensory character of chapaties significantly affected by wheat varieties, crop years and the interaction of wheat varieties x crop years. Their study concluded that SA-75 got the highest scores regarding breakability while Barani-70 was found to be the poor in quality for chapatie making with respect to breakability. Farvili *et al.* (1997) concluded that optimum tanoor bread quality was produced from moderate protein flour (11.2%). Variation in quality characteristics based on sensory evaluation regarding breakability among the wheat

varieties may be due to differences in wheat varieties, their protein content and quality as it was also reported by Yamazaki and Lamb (1961), Murphy and Austin (1963) and Ahmad (2001). The results regarding this sensory attribute are fairly in concordance with the results reported by the workers as stated above especially for spring wheats grown in Pakistan during different crop years.

Flavor and Taste

The results for flavor and taste of chapaties prepared from different wheat varieties are given in Table-2. It is clear from the statistical results that flavor and taste of chapaties were significantly affected due to wheat varieties, crop years and interaction of crop years x wheat varieties. The result concludes that the wheat varieties SA-75, Faisalabad-83, Faisalabad-85, Chakwal-86, Rawal-87, Pasban-90 and Punjab-96 obtained significantly the highest scores (8.50) for flavor and taste of chapaties when grown during 1995-96. Significantly the lowest, scores for flavor and taste of chapaties were given to the wheat varieties C-228, followed by Barani-70 and Blue Silver grown during the first crop year.

The wheat varieties Rohtas-90 followed by Bahawalpur-79, Parwaz-94 and Punjab-96 were assigned significantly higher scores and the lowest scores were given to chapaties of Barani-70 followed by C-217 and C-271 when grown during 1996-97 crop year.

The interaction of wheat varieties x crop years also showed highly significant effect on this sensory parameter. When the scores for the two years were pooled the maximum scores were obtained by the wheat varieties Rohtas-90, Punjab-96 followed by Faisalabad-83 and ranked at the top showing non-significant difference with each other with respect to flavor and taste of chapaties on pooling the data across the crop years. However, the lowest scores were given to flavor and taste of chapaties prepared from Barani-70 followed by and C-228 on the combination of data of two crop years.

With reference to previous research work done on Pakistani spring wheats, the differences for this parameter in the present results may be attributed to the change in environmental factors. However, the results of present studies are in partial agreement with the findings of previous workers. Though, there are many factors such as preparation of atta, dough, diastatic activity, water absorption, damage starch etc. which influence the quality of chapatie but in the present study the differences are mainly attributed to the variation in genetic makeup of the wheat varieties and crop years as other conditions were identical for all chapaties.

Color of Chapatie

The results for color of chapatie prepared from different wheat varieties are shown in Table-2. The statistical analysis indicated that effect of crop years and interaction of crop years × wheat varieties did not show significant effect on color of chapaties but the effect of varieties was highly significant.

It is evident from the data that during the first crop year wheat varieties C-591 and C-273 got the highest scores for color of chapaties followed by Mexi Pak, Punjab-76, Rohtas-90 and Pasban-90. The lowest ranking with respect to color was assigned to chapaties of Punjnad-88 followed by Arz when grown during the crop year 1995-96.

The highest scores for color of chapaties were assigned to wheat varieties Barani-70 and Pak-81 followed by Punjab-76, Rohtas-90 and Shahkar-95 when grains produced during the crop year 1996-97. However, the wheat varieties Punjnad-88 and SA-75 showed the lowest score for color of chapaties.

When the scores for the two years were pooled, maximum scores were assigned to color of chapaties of wheat varieties Punjab-76 and Rohtas-90 followed by C-591, C-273, Pak-81 and Pasban-90 with non-significant difference with each other. The wheat varieties Punjnad-88 and Arz were ranked at the bottom when the data across the years were evaluated.

It is evident from the results that Punjab-76 and Rohtas-90 were excellent with respect to color of chapatie. However, in the present study all the chapaties received identical time for baking, therefore, this factor is ruled out. The results may be related to the study of Pomeranz (1988) who showed that burnt patches usually indicate over fermentation or high alpha amylase activity and grey color indicates poor flour quality. Although the variation in color of chapatie may be related to the choice of consumer, panel of judges who like or dislike the color of the product. However, the color of chapatie is in conformity to the results obtained by Ahmad (2001) who found differences in this sensory parameter due to variation in genotypes and crop years.

Overall Scores of Chapatie

The overall scores for chapaties prepared from different wheat varieties are presented in the Table-2. The statistical analysis revealed that crop years and wheat varieties showed a significant and highly significant effect respectively on overall scores of the chapaties. However, the interaction of crop years × wheat varieties did not show significant effect on this parameter.

Significantly the highest scores were attained by the wheat varieties C-518 followed by SA-75 and Pasban-90 when grown during the crop year 1995-96. The lowest overall scores were assigned to chapatie prepared from Punjnad-88 grown during 1995-96. During second crop year 1996-97 the wheat varieties Punjab-76, Pak-81 and Rohtas-90 got significantly higher overall scores followed by Pasban-90, Parwaz-94 and Shahkar-95. The lowest overall scores were assigned to C-518 followed by Barani-70, Chenab-79 and Faisalabad-83 when grown during crop year 1996-97. The overall score of chapaties ranged from 5.42 to 6.75 scores when average across the crop years was taken. The maximum overall score for chapaties was the highest in Pak-81, Rohtas-90 and Pasban-90 when data of the two years were pooled. The lowest overall scores were assigned to chapaties of C-271, Barani-70 and Chenab-79 on averaging data across the crop years.

Chapatie making quality is mainly dependent on protein. Qarooni *et al.* (1988) have observed that flour; with protein content from 10-12% have been found to be more suitable for Arabic bread production. The acceptability for overall chapatie scores was identical with the studies of Butt (1997).

The variation might also be due to the process conditions for preparation of chapatie such as dough consistency, thickness, size, shape of the dough, sheet and baking conditions which vary widely in different regions as well as laboratories Austin and Ram, (1971), Shurpalekar and Prabhavathi (1976).

In the present study although the process conditions were identical for the preparation of the chapaties but quality of chapaties varied significantly. Thus significant effect of crop year and wheat varieties suggested that chapatie quality depends upon the genetic make up of the wheat varieties as well as growing conditions prevailed during the crop year. In Pakistan 70% wheat is consumed for the preparation of chapaties, therefore, the wheat varieties producing good quality chapaties should be used by the breeders in their breeding programs. This information is also useful for the farmers to grow suitable wheat cultivars having better chapatie quality and the consumers to get wheat of good chapatie making quality.

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Studies on rheological and chemical properties of fortified whole wheat flour

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ABSTRACT

This study was designed to determine the effect of fortification on the rheological as well as chemical properties of whole wheat flour. Four different fortificants, Zinc sulphate, Zinc oxide, NaFeEDTA and Elemental iron were used as a potential vehicle for fortification. Furthermore impact of various sources of iron and zinc in different dose levels was also estimated. Results of this showed that these effects had a significant impact on the rheology and chemical composition of whole-wheat flour.

Keywords: Wheat flour, fortification, zinc, iron, physical tests, chemical tests

INTRODUCTION

The prevalence of anemia is estimated to be about 30% in the world population or about 1.3 billion people. It is further estimated that about half of these people, 600-700 million, suffer from iron deficiency anemia making iron by far the most wide spread nutrient deficiency worldwide (Demaeyer et al 1985).

Iron deficiency is the most common nutritional disorder in developing countries and common cause of nutritional anemia in young children and women of reproductive age. It is estimated that 2,150 million people are iron deficient and 1200 million of them are anemia (Anon 1988).

Zinc deficiency among women, infants and children might be as widespread globally as iron deficiency because the dietary sources of the most bioavailable Fe is animal meats and are also the most important sources of bioavailable Zn in people's diets (Gibson, 1994).Average intake of wheat flour per person per day is 318 g in Pakistan (OMN11996).

The significance of the wheat flour fortification in Pakistan to eliminate micronutrients deficiency cannot be overlooked in the light of the data available indicating its nutritional and economic importance. Multitude of technical areas needs to be investigated before implementing a fortification plan and to make it a success. In this very context it is imperative that biological and chemical properties of the fortified flour are to be studied to find out the effect of such fortification programs on the quality of the vehicle and the final product.

The objectives of the present study will be

1. To estimate the extent of changes in the Chemical properties like moisture, ash, protein and fat and rheological properties like water absorption, dough stability, dough development time and amylases activity of the flour as a result of fortification.
2. to find out the best combination of zinc, iron and folic acid as far as the chemical composition and rheological properties of the whole wheat flour are concerned.

MATERIALS AND METHODS

Procedures

Vehicle

Wheat variety Inqulab-91 was received from NIAB, for flour production. Whole-wheat flour (100% Extraction) was used for this study.

Fortificants

Elemental iron, Na Fe-EDTA, Zinc oxide and Zinc sulphate were used as fortificants for the sources of iron and zinc respectively.

Table 1. Chart of treatments designed for the study

Fortificant	T_1	T_2	T_3	T_4	T_5
Elemental Iron	Nil	Nil	40ppm	60ppm	Nil
NaFeEDTA	40ppm	60ppm	Nil	Nil	Nil
ZnO	Nil	20ppm	Nil	40ppm	Nil
$ZnSO_4$	20ppm	Nil	30ppm	Nil	Nil
Folic Acid	1.5ppm	1.5ppm	1.5ppm	1.5ppm	Nil

Dose level

Following levels of fortificants were used in the study:

Level of Fortificants:

1 - Elemental iron	60 mg/kg & 40 mg/kg
2- NaFeEDTA	60mg/kg & 40 mg/kg
3- Zinc oxide	20mg/kg & 30 mg/kg
4- Zinc sulphate	20mg/kg & 30 mg/kg

Combinations/Treatments

Keeping in view the toxicity and RDA % of zinc and iron, following combinations as treatments were used in this study.

Storage time

The fortified flour was will be stored for a period of 8 weeks and the samples will be analyzed after every four weeks i.e.O, 30, & 60 days.

Analytical tests

Sample of fortified whole-wheat flours will be analyzed for the following

1- Chemical Analysis
2- Rheological properties

Chemical analysis

Moisture %age, Ash %age. Crude protein %age (for gluten estimation) and were determined by prescribed standard methods of AACC, (2000).

Physical analysis

Physical Characteristics like water absorption, arrival time, dough development time, dough stability, resistance to the dough and departure time were studied by conducting the farinographic analysis of the treatments and amylase activity of treatments was studied by conducting the amylographic analysis by using Brabender Farinograph and Brabender Amylograph analytical equipment respectively,

Statistical analysis

The data obtained from the said research wad analyzed and assessed by following the methods described by Steel and others.

RESULTS AND DISCUSSIONS

Table number (1) indicates that a gradual increase occurred in moisture contents of flour and it has positive co-relation with storage interval but fortification level and type of fortificant has no relation with the moisture contents.

No co-relation could be found between fortification (either type or dose level) and protein contents but a very low level of interaction between storage interval and protein %age can be noted. It is evident from table (2) that protein % age decreases with the storage time. Ash %age changed with the change in level and type of fortificant but the change was not significant enough to be considered.

Table 2. Table for chemical analysis of treatments at 3 different storage intervals

	T-1	T-2	T-3	T-4	T-0
Moisture					
0 Days	6.37	6.41	6.55	6.54	6.98
30 Days	6.91	7.40	7.46	7.20	7.30
60 Days	7.55	7.90	7.90	7.70	8.20
Protein					
0 Days	12.19	12.74	12.55	12.69	12.21
30 Days	12.10	12.20	12.25	12.27	11.90
60 Days	11.92	11.90	11.90	11.75	11.69
Ash	T-1				
0 Days	1.72	1.72	1.76	1.77	1.63
30 Days	1.74	1.75	1.78	1.79	1.62
60 Days	1.74	1.74	1.76	1.78	1.60

The moisture content of wheat flour is influenced by the milling techniques as well as storage conditions (Kirk & Sawyer, 1991). The absolute moisture content in grain and flour is less important then relative humidity with which the food is hygroscopic equilibrium. Depending on the prevailing RH, the stored produce either release moisture or absorb moisture from the surroundings. The results of the moisture are not comparable with those found by; Butt et al. (1997) and Qamar (2002) who reported moisture content in different wheat fractions varies from 7.8 to 14.8 % the reason behind this fact is that the Wheat grains was not subjected to conditioning before milling and this resulted in low moisture % age or it might be the result of high temperatures and low humidity during the days of June and July in Faisalabad.

Table 3 shows the farinographic parameters of different treatments has not been significantly influenced by the level and type of fortificants but those were significantly affected by the storage intervals of 0, 30 and 60 days. As the moisture %age of fortified flour Table (1) showed gradual increase with the advancing storage intervals the farinographic characteristics comprehensively represent this change in moisture %age increase.

Water Absorption: Treatment 3 and 4 showed maximum water absorption.

Arrival Time: Treatment 4 shows the least arrival time (2.27) and treatment 3 and 5 has highest arrival time of (2.50).

Dough development time: Treatment 3 showed minimum time taken for dough development.

Dough stability: Treatment 3 represents highest dough stability.

Table 3. Table for farinographic characteristics of Treat

	T-1	T-2	T-3	T-4	T-0
Water Absorption					
0 Days	67.40	67.60	68.20	68.60	66.60
30 Days	66.75	66.60	66.60	67.10	66.40
60 Days	66.60	66.50	66.60	66.30	65.90
Arrival					
0 Days	2.50	2.60	2.50	2.70	2.50
30 Days	2.00	2.00	2.00	1.85	2.50
60 Days	2.36	2.50	3.00	2.25	2.50
Dough Developmen t Time	**T-1**	**T-2**	**T-3**	**T-4**	**T-0**
0 Days	6.50	5.50	5.50	6.50	5.50
30 Days	7.50	7.75	7.00	7.20	7.10
60 Days	6.25	6.50	5.75	7.50	7.10
	T-1				
Dough Stability	**T-1**	**T-2**	**T-3**	**T-4**	**T-0**
0 Days	12.50	13.10	13.50	13.80	13.00
30 Days	13.75	13.50	13.90	13.50	13.50
60 Days	14.90	14.10	14.00	13.75	13.20
Resistance to Dough	**T-1**	**T-2**	**T-3**	**T-4**	**T-0**
0 Days	490.00	480.00	490.00	510.00	480.00
30 Days	530.00	550.00	530.00	560.00	560.00
60 Days	565.00	560.00	560.00	515.00	550.00
Departure Time	**T-1**	**T-2**	**T-3**	**T-4**	**T-0**
0 Days	15.00	16.00	16.00	16.50	15.50
30 Days	15.75	15.95	15.00	15.95	16.00
60 Days	17.75	15.75	16.5	16.75	15.75

Resistance to dough: Treatment 3 showed minimum resistance to dough.

Departure Time: Treatment 3 and 4 showed maximum departure time.

Table 4. Table of mean analysis of amylase activity of different treatments at different storage intervals

	T-1	T-2	T-3	T-4	T-0
Amylograp h Reading					
0 Days	340	140	260	180	340
30 Days	260	230	175	325	325
60 Days	225	180	165	165	325

Table (4) shows that amylase activity was affected by presence of metals such as zinc and iron and it was positively related to the level of fortificant added and it was also seen that addition of ferrous sulphate decreased the amylase activity.

Following inferences were concluded from the research.

1. Fortification of wheat flour by the above mentioned fortificants did not affect the chemical properties of wheat flour as well as moisture, ash and protein %age are concerned.
2. Fortification under prescribed levels does not have any drastic effect on rhelogy of fortified wheat flour.
3. Treatment number 3 having $FeSO_4$ (40ppm), $ZnSO_4$(30ppm) and folic acid (1.5ppm) will be the most suitable fortificants combination keeping in view their stability, nascence, availability and economical aspects

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Preparation and evaluation of low calorific biscuits from composite flours containing wheat bran

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ABSTRACT

Biscuits were prepared from composite flours containing untreated and alkaline hydrogen peroxide (AHP) treated wheat bran. Composite flours were prepared by supplementing wheat flour with treated and untreated wheat bran @ of 0, 5, 10, 15 & 20 %. The moisture, ash, fiber, fat and protein content of the composite flours containing untreated wheat bran varied from 13.26 to 13.01, 0.54 to 1.87, 0.33 to 2.47, 1.91 to 2.50 and 11.0 to 12.47% respectively. While the moisture, ash, fiber, fat and protein content of the composite flours for AHP treated wheat bran varied from13.26 to 12.99, 0.54 to 1.90, 0.33 to 3.07, 1.91 to 2.25 and 11.02 to 12.23 %respectively. The calorific value of the biscuits decreased significantly by supplementation of wheat bran. The gross energy values of the biscuits decreased from 5049 to 4200 and 4149 for the 20 % addition of untreated and AHP treated wheat bran respectively. All the bran treatments were found acceptable by the panel of judges for their sensory characteristics. However the biscuits prepared from the AHP treated bran obtained higher score on hedonic scale than the biscuits prepared from composite flours containing untreated wheat bran.

Key words: Composite flour, AHP treated wheat bran, chemical composition, sensory characteristics and low calorific biscuits.

INTRODUCTION

Dietary fiber is residue of plant food resistant to hydrolysis by human alimentary enzymes. Dietary fiber is composed of cellulose, hemi cellulose and lignin. Dietary fiber plays an important role in human health. Dietary fiber in human diet helps in proper functioning of digestive system and other related health concerns (Trowell, 1976).

Wheat grain's outer layer is mainly composed of fiber and fulfills the dietary fiber needs of the masses. The refined wheat flour lacks fiber contents, associated with wheat bran, which is removed during milling. Wheat bran is a concentrated form of insoluble fiber (Andreson *et al.* 1987). According to Bread and Flour regulation 1984 at least 0.6% fiber content must be included in flour for consumption (Kent and Evers 1994). The fiber content has no calorific value. Dietary fiber also plays an important role to check the constipation (Kahcon *et al.* 2000)

Dietary fiber has emerged as leading factor in the prevention and treatment of chronic diseases. High fiber intake is associated with lower serum cadastral concentration, lower risk of heart disease, reduced blood pressure, enhanced weight control, better glycemic control and improved gastrointestinal function (Andreson *et al.* 1987).

The lack of fiber in the human diet possess problem like obesity (Baturin 1994). High fiber diets decrease the time that indigestible material remained in intestine, that decrease the risk of cancer (Lee *et al.*1997). High fiber diets can be used to prevent constipation and lower the risk of tumor and cancer development (Kahcon 2001). So the fiber addition into food products from the health point of view is very important. Bakery products introduced commercially are high in number than others and contribute highest amount of fiber in human diet.

Alkaline hydrogen peroxide treated delignified cellulose can be used to replace a portion of flour in various baked products without affecting their final quality. Compared to other fibers, alkaline hydrogen peroxide (AHP) treated lignocelluloses improves baked volume and water holding capacity of bread and cakes and increased along tensile strength as compared to other fiber sources without introducing gritty texture or off-flavor (Jasberg *et al.* 1989).

Present study has been designed to incorporate untreated and treated wheat bran in order to access the appropriate type of bran and its optimum percentage in flour, which may not affect quality but enhance the overall status of the fiber in the bakery products

MATERIALS AND METHODS

Procurement of raw material

Wheat variety Iqbal- 2002 was procured from Wheat Research section, Ayub Agriculture Research Institute, Faisalabad.

Milling

Wheat was tempered at 14% moisture by following the procedure of AACC (2000). Tempered wheat was milled in Quadrumat senior Mill. Wheat flour and bran were obtained separately.

Wheat bran preparation

Wheat bran was divided into two parts. One portion was supplemented (without any treatment) at different levels in wheat flour and second portion was treated with Alkaline-hydrogen peroxide.

Wheat bran treatment with alkaline hydrogen peroxide

Wheat bran was treated with alkaline hydrogen peroxide according to the procedure described by Gould.(1984).

Preparation of composite flour

Composite flours were prepared by supplementing wheat flour with treated and untreated wheat bran at the rate as given in Table 1

Proximate analysis of composite wheat flour

Composite wheat flour was analyzed for crude fat, crude fiber, crude protein, ash, moisture content and NFE according to the methods described in AACC (2000).

Preparation of biscuits

Biscuits were prepared from composite flour with some modification in the method described in AACC (2000).

Physical tests of biscuits

Biscuits prepared from composite flour were tested for width, thickness and spread factor according to method as described inn AACC (2000).

Sensory evaluation

To assess the quality and acceptability, the biscuits were presented to a panel of judges and the sensory evaluation will be carried out for color, taste and flavor characteristics according to methods as described by Land and Shepherd (1988).

Gross energy of biscuits

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

Statistical analysis

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

RESULTS AND DISCUSSION

The wheat flour supplemented with treated and untreated wheat bran for the preparation of cookies was analyzed for chemical composition. The results regarding the chemical composition of flour are given in Table 2. It is obvious from data that bran addition significantly affects the proximate composition of wheat flour. The moisture content and nitrogen free extract that are mainly carbohydrates decreases while the protein, fat, fiber and ash contents increases with bran addition. Moisture, protein, fat, fiber, ash and nitrogen free extracts varied significantly between 13.27 to 12.99, 11.01 to 12.47, 1.91 to 2.50, 0.33 to 3.06, 0.54 to 1.87 and 72.58 to 68.58 respectively. It is clear from results that chemical composition of wheat flour varied greatly for treated bran that the untreated bran. Fiber content of the wheat flour increased significantly by the addition of treated and untreated bran. However this increase was higher for AHP treated bran than untreated. The results regarding the chemical composition of wheat flour confirm the finding of Davis *et al* (1981), Kirk and Sawyer (1999), Kent- tones and Amus (1967), Butt (1997) and Kulp *et al* (1980).

Biscuits prepared were subjected to physical evaluation such as width, thickness and spread factor. The significant effect of bran addition was founded as indicated by data shown in Table 3. The physical parameters are greatly affected by untreated bran than the treated bran. Results values for width varied from 56.57 to 63.08mm, thickness varied from 270.0 to 241.4mm and spread factor was in the range of 47.73 to 38.26. The difference in physical parameter for treated bran up to 15 % supplementation is non significant which show that it does not affect the physical parameter. The reason is that AHP treated bran have lignocelluloses network in which half of lignin removes, this increase the ability of bran to

absorb more water. That is why the physical parameter is not so much affected. The results are comparable with Gould. (1984) and Sievert *et al* (1990).

Gross energy of the biscuits was also determined to know the calorific value of the product. The results regarding the gross energy of biscuits are given in Table 4. It is obvious from that gross energy value significantly decreased. It is also clear from the data that decrease in energy value for AHP treated bran was higher than in the untreated bran. The gross energy varies between 5049 to 4149. These results are in accordance with Gould. (1984) who reported that AHP treatment reduces calorific density of material.

One of the most important aspects of product development is its acceptance by the consumer. Thus biscuits prepared from bran supplemented flour were evaluated for sensory characteristics by a panel of judges. The color of biscuits was affected by bran significantly. AHP treated bran has higher impact on color than untreated bran. The reason is that AHP change the color of bran and ultimately of the product. The score value for color ranges between 7.67 to 5.33 as shown in Table 5. Similarly taste and flavour of the product were also significantly affected by bran. Panel of judges for AHP treated bran biscuits liked taste of biscuits. Te score given to different treatment vary between 8.33 to 6.00. Flavor was affected by the bran supplement higher score were obtained by the AHP treated bran biscuit. The score values vary between 5.33 to 7.67. Regarding the overall acceptability the score given by panel of judges vary between 6.00 to 8.33. So it can be said on the basis of over all acceptability score that all the treatment was acceptable. However AHP treated bran have higher score value than untreated bran.

Table 1. Combination of treatments used in study

Treatment	Wheat flour %	Untreated Bran %	Treated Bran %
T_0	100	0	0
T_1	95	5	-
T_2	90	10	-
T_3	85	15	-
T_4	80	20	-
T_5	95	-	5
T_6	90	-	10
T_7	85	-	15
T_8	80	-	20

Table 3. Mean for Physical evaluation of Biscuits

Treatment	Width (mm) D	Thickness (mm) T	Spread factor (D/T)
T_0 (Whole wheat Flour)	56.57	270.0	47.73
T_1 (5% Untreated bran)	57.44	267.5	46.50
T_2 (10% Untreated bran)	58.38	260.2	44.57
T_3 (15% Untreated bran)	64.21	256.1	39.90
T_4 (20% Untreated bran)	63.08	241.4	38.26
T_5 (5% AHP treated bran)	56.57	260.5	47.30
T_6 (10% AHP treated bran)	55.56	260.4	46.867
T_7 (15% AHP treated bran)	56.39	256.4	45.47
T_8 (20% AHP treated bran)	59.90	252.7	42.17

Table 4: Mean Values of gross energy of bran biscuits

Treatments	Energy values
T_0 (Whole wheat Flour)	5049a
T_1 (5% Untreated bran)	4900b
T_2 (10% Untreated bran)	4632d
T_3 (15% Untreated bran)	4530f
T_4 (20% Untreated bran)	4200h
T_5 (5% AHP treated bran)	4837c
T_6 (10% AHP treated bran)	4581e
T_7 (15% AHP treated bran)	4487g
T_8 (20% AHP treated bran)	4149i

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Table 2. Mean for proximate composition of composite flour

Treatments	Proximate composition					
	Moisture	Protein	Fat	Fiber	Ash	NFE
T_0 (Whole wheat Flour)	13.27a	11.01f	1.91f	0.33e	0.54e	72.58a
T_1 (5% Untreated bran)	13.26ab	11.39de	2.09e	0.87de	0.88d	71.64b
T_2 (10% Untreated bran)	13.22abc	11.56cd	2.10de	1.41cd	1.23c	71.07bc
T_3 (15% Untreated bran)	13.11bcd	11.95b	2.35b	1.94bc	1.54b	70.06d
T_4 (20% Untreated bran)	13.02d	12.47a	2.50a	2.47b	1.87a	68.98e
T_5 (5% AHP treated bran)	13.13bcd	11.23ef	1.95f	1.00d	0.89d	71.82ab
T_6 (10% AHP treated bran)	13.07cd	11.47be	2.05e	1.72c	1.26c	70.65cd
T_7 (15% AHP treated bran)	13.01d	11.78bc	2.15d	2.41b	1.57b	70.60cd
T_8 (20% AHP treated bran)	12.99d	12.23a	2.25c	3.06a	1.90a	68.58e

Table 5. Mean for the sensory evaluation of bran supplemented biscuits

TREATMENT	Color	TASTE	FLAVOR	OVERALL ACCEPTABILITY
T_0 (Whole wheat Flour)	5.33CD	6.00CD	7.00AB	7.00B
T_1 (5% Untreated bran)	6.33BC	6,33BCD	6.33ABC	7.33AB
T_2 (10% Untreated bran)	6.33BC	6.67BC	6.57ABC	7.33AB
T_3 (15% Untreated bran)	7.33AB	7.00BC	7.44AB	7.33AB
T_4 (20% Untreated bran)	5.33CD	5.33D	5.33C	6.00C
T_5 (5% AHP treated bran)	6.33BC	6.67BC	6.67ABC	7.33AB
T_6 (10% AHP treated bran)	6.33BC	7.00BC	7.33AB	7.33AB
T_7 (15% AHP treated bran)	7.67A	8.33A	7.67A	8.33A
T_8 (20% AHP treated bran)	4.67D	7.33AB	6.00BC	7.33AB

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Preparation and quality evaluation of vinegar prepared by *Acetobacter aceti*

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ABSTRACT

In present study vinegar was prepared from alcohol by using *Acetobacter aceti*. For this purpose culture was used at the rate of 30%, 40%, 50% and 60% with a viable count of 10^6 cfu/mL. The vinegar thus prepared was analyzed for different attributes like flavour, taste, overall acceptability, pH, acidity, total soluble solids, volatile acids and nonvolatile acids after 20 days of fermentation in orbital incubator at 30±2°C. On the basis of these results it was concluded that culture dosage significantly affected the physico-chemical and sensory quality of the vinegar.
Key words: *Acetobacter*, Vinegar, Vinegar analysis

INTRODUCTION

Vinegar is the product of a mixed fermentation of yeast followed by acetic fermentation. Acetic acid produced by the fermentation of alcohol (ethanol) gives the characteristic flavor and aroma to vinegar. It can be made from almost any fermentable carbohydrate source, for example fruits, vegetables, syrups and wine. The basic requirement for vinegar production is a raw material that can be subjected to alcoholic fermentation. Apples, pears, grapes, honey, syrups, cereals, hydrolyzed starches and wine are all ideal substrates for the production of vinegar. The raw material used as substrate should be well matured, clean and in good healthy conditions (Tortora *et al.*1995)

Strains of acetic acid forming bacteria (*Acetobacter*) and oxygen to enable the oxidation of alcohol are necessary in an alcoholic substrate. However, this process is very slow and vinegars produced by this method tend to be of inferior quality. Controlled fermentation conditions produce a more acceptable product by using a wide range of raw materials for vinegar (Battcock and Ali, 1998).

In Pakistan, although synthetic vinegar is mostly consumed yet fermented vinegar is also available. The culture used for the production of fermented vinegar is mostly contaminated with other unnecessary micro-organisms at industrial level. Hence it is the need of the time to develop pure vinegar cultures to improve the quality of fermented vinegar so that the use of synthetic vinegar may be avoided as it is prohibited in most European and overseas countries 9Rehman and Reed, 1983).

Furthermore the Food and Agriculture Organization of the United Nations (FAO) has established that vinegar is a liquid allowed for human consumption of two consecutive fermentations, first an alcoholic fermentation that transforms the sugar into ethanol and then acetic fermentation that converts ethanol to acetic acid, that is the main product of vinegar (Parrondo et al. 2003). Keeping in view the study was conducted for isolation of vinegar culture which subsequently used for vinegar production.

MATERIALS AND METHODS

Culture collection

Vinegar culture *Acetobactor aceti* after isolation, identification and characterization was produced in Food Microbiology and Biotechnology Laboratory, Institute of Food Science and Technology, University of Agriculture, Faisalabad by following the criteria suggested by Holt *et al.* (1994).

Vinegar preparation

Raw material

Alcohol: Alcohol was obtained from Mitchell's Fruit Farms Ltd. Okara.

Vinegar culture: A pure culture of *Acetobactor aceti* isolated with a viable count of 10^6 cfu/ml (colony forming unit/ mL) was used at different levels as given below. Viability was measured by the methods recommended by Awan and Rahman 2002 and Cappuccino and Sherman 1996.

Different levels of culture used for preparation of vinegar

Treatments	Culture percentage	Alcohol (%age)
T1	30	70
T2	40	60
T3	50	50
T4	60	40

Vinegar making

Acetobactor aceti was added in to alcohol (sterilized by filtration) in 250 ml conical flask and fermentation was carried out in an orbital shaker at 30°± 2°C with 250 rpm (Parrondo *et al.* 2003) for 20 days. Samples were examined for aroma and acidity on daily basis until specific aroma and acidity for vinegar preparation was achieved.

Pasteurization

When the required criterion of acidity and aroma was achieved, vinegar samples were pasteurized in water bath at temperature of 60°C for 90 seconds in sterilized bottles to check any further fermentation as suggested by Riaz and Ahmad 1996.

Cooling

Vinegar samples were cooled down to 25°C for evaluation.

Vinegar evaluation

Quality tests for vinegar

pH, acidity, soluble solids (Brix), total acids, non volatile acids and volatile acids were determined to evaluate the quality of vinegar according to the procedures explained in AOAC (1990).

Sensory evaluation of vinegar

In order to check the overall acceptability of vinegar, the samples were subjected for sensory evaluation. Vinegar was evaluated for its color, flavor, taste and overall acceptability by a panel of trained judges using the 9-point Hedonic scale as suggested by Larmond (1977).

Statistical analysis

Data obtained was statistically analyzed to see the effect of different doses of culture on the quality of vinegar by the method as recommended by Steel *et al.*, 1997.

RESULTS AND DISCUSSION

Physico-chemical analysis of vinegar

The results obtained are discussed as under.

pH

The results for pH given in Table 1 indicate that pH of vinegar produced was significantly effected by the culture dosage as the highest pH value was observed in case of T_1 (30 percent) with the value 2.60, whereas this figure was reduced to lowest figure 2.42 when T_4 was analyzed. It is obvious from the results that the pH value gradually decreased from T_1- T_4 (2.60, 2.50, 2.49 and 2.42) as dose was 30 percent, 40 percent, 50 percent and 60 percent respectively. Furthermore as analysis of variance given in Table 2 indicates that there was a significant effect of treatments on the pH of the end product however, the differences in pH of T_2 and T_3 were non-significant. The significant results obtained are similar to the recommendations of Rehm and Reed 1983 who recommended that vinegar has a pH range of 2.35 to 2.45.

Acidity (total acids)

The results for acidity as recorded are expressed in Table 1. It was found that the treatment T_4 gave significantly the highest mean value (5.58 percent) for acidity whereas the lowest mean value (2.60 percent) was seen in the treatment T_1. The mean acidity values measured for T_3 and T_4 were 3.96 percent and 4.51 percent respectively. Moreover statistical results given in Table 2 indicate that the effect of different culture percentages on the quality of vinegar was highly significant. Although T_4 was found to be the highest acidity value but the range for this parameter in the present study was 2.6-5.58 percent. The upper values of acidity in T_3 and T_4 fell within the range as concluded by Rehm and Reed 1983 who reported that usually vinegar has 4-7 percent acidity. T_2 (3.9 percent) was also nearest to the lowest acidity value quoted by them.

Total soluble solids

Results obtained for total soluble solids indicated (Table 1) that significantly the highest total soluble solids were found in T_1 with a mean value of 12.16 percent. Similarly the lowest total soluble contents were calculated in T_4 (10.16 percent). Non-significant in results for total soluble solids in T_2 and T_1as well as among T_2 and T_3 but significant difference was obtained in T_1 and T_4 Furthermore results for analysis of variance given in Table 2 exhibited that the effect of different percentages of culture was highly significant

on total soluble solids of the end product. It is due to the reasons that more the number of bacteria more will be the utilization of sugars and ultimately there will be reduction in total soluble solids in the end product.

Nonvolatile acids

Table 1 showed that significantly the highest nonvolatile acids were determined in T_4 with mean value 0.38 percent and significantly the lowest percentage of nonvolatile acids was measured in T_1, T_2 and T_3 each having a non-significant mean value of 0.35 percent. However, the effect of culture percentage was clearly found to be highly significant on the concentration of nonvolatile acids in vinegar as shown in Table 2.

Sensory evaluation of vinegar

The results regarding sensory evaluation are presented in Table 3 along with statistical data in Table 4.

Color

Color plays an important role in the visual evaluation and aesthetic appeal of a food product. Different culture percentages had different effect on the color of the vinegar. Significantly the highest scores were given to the color of the vinegar prepared by T_4 (8.20) while treatment T_1 obtained lowest score (6.70). It was further observed from the Table 3 that treatment T_4 was highly significantly different from the rest of the treatments whereas treatments T_1 (6.70), T_2 (6.90) and T_3 (7.00) were non-significantly with each other. Analysis of variance showed that there was a highly significant effect of culture percentage on color of the vinegar as indicated in Table 4.

Table 1. Effect of culture dosage on chemical composition of vinegar

Culture (%)	pH	Acidity (% acetic acid)	Soluble solids (%age)	Nonvolatile acids (%age)	Volatile acids (%age)
T_1 (30)	2.60a	2.60d	12.16a	0.35b	2.25d
T_2 (40)	2.50b	3.96c	11.66ab	0.35b	3.61c
T_3 (50)	2.49b	4.51b	11.16b	0.35b	4.16b
T_4 (60)	2.42c	5.58d	10.16c	0.38a	5.65a

Results are given as means of three observations
Values with same letters are non-significant with one another

Table 2: Analysis of variance for chemical composition of vinegar

Sources	DF	MS				
		pH	Acidity (% acetic acid)	Soluble solids (%age)	Nonvolatile acids (%age)	Volatile acids (%age)
Treatment	3	0.015933**	4.6239**	2.187**	0.000608**	5.9430**
Error	8	0.000233	0.0106	0.208	0.000133	0.0118
Total	11					

**Highly significant ($p > 0.01$)

Volatile acids

Results given in Table 1 indicate that all the treatments T_1, T_2, T_3 and T_4 were significantly different from each other. The treatment T_4 was significantly at the top with a mean value of 5.65 percent followed by T_4 while treatment T_1 was significantly at the bottom with volatile acid contents of 2.25 percent. The mean values for volatile acid contents in T_2 and T_3 were 3.61 percent and 4.16 percent respectively. Analysis of variance given in Table 2 revealed that the effect of culture percentage was highly significant on the volatile acid contents on vinegar.

Table 3. Effect of culture dosage on sensory attributes of vinegar

Culture %age	Color	Flavour	Taste	Overall acceptability
T_1 (30)	6.70b	5.70c	5.80c	5.80c
T_2 (40)	6.90b	6.20c	6.50c	6.80b
T_3 (50)	7.00b	6.80b	6.90b	6.90b
T_4 (60)	8.20a	7.70a	8.00a	8.20a

Results are given as means of scores given by four judges
Values with same letters are non-significant with one another

Table 4. Analysis of variance for sensory attributes of vinegar

SOV	dF	MS			
		Taste	Flavour	Taste	Overall acceptability
Treat-ment	3	2.3000**	3.700**	4.233**	4.8458**
Error	16	0.0500	0.200	0.156	0.0688
Total	19				

** Highly significant (p >0.01)

Flavor

Flavor also plays a key role in the sensory evaluation of a food product. The results expressed in the Table 3 showed that significantly the highest scores for flavour were given to the vinegar prepared from T_4 (7.70) followed by T_3 (6.80) and significantly the lowest scores were given to the vinegar of T_1 (5.70) with second lowest figure T_2 (6.20) which were non-significant with each other. Typical flavour of the vinegar is due to *Acetbacter aceti*, which produce acetic acid and more the number of bacteria fermentation becomes faster, more acetic acid is produced and hence best results for flavour are achieved. More acetic acid formation occurred with higher doses of vinegar culture and hence maximum flavour score was obtained.

Taste

For taste of vinegar, it was used in chicken soup and then soup prepared by using vinegar obtained from different treatments was subjected for evaluation. It was found from the Table 3 that vinegar prepared from treatment T_4 was significantly at the top with mean scores of 8.20 followed by T_3 with mean scores of 6.90 and it was highly accepted by the judges. Significantly the lowest scores were given to vinegar of T_1 and T_2 with mean values of 5.80 and 6.50 respectively and these treatments were non-significant with other. ANOVA results shown in Table 4 indicate that the taste of the vinegar was highly significantly affected due to different concentrations of the cultures used for acetic acid fermentation.

Overall acceptability

Overall acceptability is one of the important and basic features for the acceptance or rejection of a food product. It is clear from the Table 3 that vinegar prepared from treatment T_4 with average score 8.20 was highly accepted by the judges and treatment T_1 with mean scores of 5.80 was found significantly much poor. Further more treatment T_2 (6.80) and treatment T_3 (6.90) were non-significantly different from each other. Results for analysis of variance are expressed in Table 4 indicated that there was highly significant effect of treatments on overall acceptability of the vinegar prepared from different treatments.

Results are similar to the recommendations of Rehm and Reed 1983 who stated that the acetic acid is the typical constituent of vinegar and that more the number of viable count (culture percentage) more will be acetic acid production. If there is more production of acetic acid then ultimately sensory attributes and chemical composition will be affected.

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Studies on the seed oils of citrus cultivars, Shamber grapefruit and Minneola tangelo

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ABSTRACT

The seed oils of citrus cultivars Shamber grapefruit and Minneola tangelo were analysed for their physico-chemical properties. The yield of oils on dry seed basis were 25.01% and 40.8% respectively. The GLC analysis of methyl esters derived from the seed oils of Shamber grapefruit and Minneola tangelo revealed the presence of lauric (0.9%, 0.74%), myristic (2.1%, 4.38%), palmitic (54.65%, 35.38%), stearic (5.2%, 0.33%), oleic (24.37%, 39.47%), linoleic (4.67%, 14.63%) and linolenic acid (5.96%, 3.97%).
Key words: Shamber grapefruit, minneola tangelo, fatty acid

INTRODUCTION

The taxonomic classification of citrus species has been the subject of considerable controversy (Hodgson *et al.*, 1967) but within the past two decades chemo-systematic studies on secondary plant constituents has become an important tool in defining and differentiating the complex genus.

Shamber grapefruit is a variety of *Citrus paradisi* the commonly known grapefruit. They are largely globose with a slightly bitter acid pulp like other members of this genus. Minneola tangelo belongs to group that includes which consist of hybrids of tangerine and grapefruit. (Ali 1987) and Seminole, Orlando, Minneola, San Jacinto, Sunshine and Shampson. There are attractive because of high colour and smooth rind.

Although the production of citrus seed oils is of minor importance yet it is a non-conventional source of oils in some citrus growing countries where citrus fruit is processed in large quantities for juices and jams. The citrus seed oils can be used for edible as well as soap and cosmetic preparations. Extensive studies (Kefford and Chandler 1970) have been carried out on the fatty acid composition of the seed oils of various citrus fruits. Many seed oils of indigenous varieties have also been analysed (Saleem *et al.* 1977; Sattar *et al.* 1987) but the fatty acid composition of seed oils of Shamber grapefruit and Minneola tangelo have not been reported earlier. The present research work thus describes the fatty acid composition of these seed oils in continuation of a research programme to explore the indigenous and non-conventional source of vegetable oils (Saleem *et al.* 1977, Sattar *et al.* 1987).

MATERIALS AND METHODS

Selection of seeds

Fresh, mature and virus-free fruits of Shamber grapefruit and Minneola tangelo varieties were collected from Horticulture Research Institute, Sahiwal. The fruits were cut in halves and seeds were handpicked and transferred to petri-dishes. The seeds were then washed with distilled water and dried in the shade for further study and analysis.

Extraction of oil

The seeds of the two varieties were ground to a fine powder and extracted with distilled hexane a Soxhlet apparatus for 12 hours (AACC 2000). The hexane extracts were dried over anhydrous sodium sulphate and after removal of solvent gave pale yellow oils. The percentage yield of the oils of both varieties was noted as 25.01% and 40.08% respectively.

Seed oil analysis

The physical and chemical properties Table 1 of the oils were determined according to standard procedure (AOCS, 1998 and AOAC, 1990).

Table 1. Physico-Chemical Properties of Shamber grapefruit and Minneola tangelo seed oils

Physicochemical properties of seed oils	Shamber grapefruit Seed Oil	Minneola tangelo Seed Oil
1. Yield (%)	25.01	40.08
2. Colour	Yellow	Yellow
3. Refractive index at 30°C	1.4645	1.4637
4. Specific gravity	0.8879	0.8906
5. Acid value	0.7941	0.8085
6. Saponification value	191	187
7. Free fatty acid	0.413	0.419

Preparation of fatty acid methyl esters

Weighed amounts of oils (1.0 g) were transferred to a Teflon test tube. Methanolic potassium hydroxide (0.5 N, 10 ml) was then added to the oil samples. The mixture was refluxed until the globules of oil got into solution (90 minutes). Sulphuric acid (2 N) was then added to the cooled mixture to liberate the fatty acids. Esterification of the liberated fatty acids was carried out in the presence of catalytic amount of methanol BF3 reagent (10 ML) and boiled for about 20 minutes. The esterified mixture was cooled and extracted with hexane. Separated hexane layers were washed with water and dried over anhydrous sodium sulphate (Waheed et al. 2001).

RESULTS AND DISCUSSION

The seeds of Shamber grapefruit and Minneola tangelo were found to be a valuable, non-conventional source of fixed oils. In order to evaluate the oils with respect to their suitability both for industrial and edible purposes, the oils were subjected to physico-chemical characterization and determination of chemical composition. The percentage yield of oil from dry seeds of shamber grapefruit and Minneola tangelo were 25.01% and 40.08% respectively. The physico-chemical constants and chemical composition of the oils are reported in Table 1, 2, 3. .

Table 2. Percentage Fatty Acids Composition of Shamber grapefruit and Minneola tangelo Seed Oils

Fatty acids	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Shamber grapefruit	0.91	2.1	54.65	0.53	5.2	24.37	4.67	5.96
Minneola tangelo	0.74	4.38	35.38	-	0.33	39.47	14.63	3.97

Table 3. Saturated and Unsaturated fatty Acids composition of Shamber grape fruit and Minneola tangelo Seed Oils

	Saturated Fatty Acid (%)	Unsaturated Fatty Acid (%)
Shamber grapefruit	62.86	35.53
Minneola tangelo	40.83	58.07

Determination of fatty acids methyl esters

The fatty acids composition of the oils was determined by gas liquid chromatography (GC-14A, Shimadzu) using a column (1.5 m x 4 mm i.d.) packed with celite coated with 10% DEGS. The GLC operating conditions were; column temperature 200°C, FID temperature 250°C, Injector temperature 220°C and carrier gas nitrogen with flow rate of 40 ml/min. The determined percentage fatty acid composition is given in Table 2.

Saponification values of the oils indicated that the mean molecular weight of the combined fatty acids is normal showing the presence of predominantly C_{16} and C_{18} fatty acids. The physico-chemical characteristics of the oils from the two varieties (as listed in Table 1) are also normal. Because of this property the extracted oils can be used for edible purposes.

The chemical compositions of the oils were determined by GLC. Apart from the solvent peak, chromatograms of methyl esters of the species indicated the presence of lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids respectively in varying proportions. The presence of these acids were further confirmed by running standard methyl fatty esters mixtures under the same set of conditions and comparing the chromatograms. According to table-3 the Shamber grapefruit seed oil contained higher saturated fatty acids (62.86%) as compared to Minneola tangelo seed oil (40.83%), which had more a unsaturated fatty acids (58.07%). Shamber grapefruit oil contained palmitic acid (54.65%) and oleic acid (24.37%) as major fatty acids while lauric acid (0.91%) and palmitoleic acid (0.53%) were the minor fatty acids. In Minneola tangelo seed oil palmitic acid (35.38%) Oleic acid (39.47%) and linoleic acid (14.63%) were as the major fatty acid while lauric acid (0.74%) and stearic acid (0.33%) were present as minor fatty acids. It is interesting that the fatty acid profile of the oils from the two cultivars resemble each other and is close to that of Palm Oil (Lentner 1981). It is concluded from our investigations that these oils can find application both for edible and industrial purposes.

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Storage effects on physico- chemical and sensory characteristics of dried apricot diet jam.

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ABSTRACT

Sorbitol with cyclamate and aspartame were used as artificial sweeteners in dried apricot diet jam to reduce calorific value. Six different treatments were prepared and one of them was control to compare the response of apricot diet jam to different sweeteners. All the samples were analyzed for physico-chemical and sensory characteristics. Total soluble solids (TSS) increased gradually in all treatments during storage period. The mean for TSS was 68.95 at 0 day which rose to 69.60 at 60 days. There was a gradual increase in acidity and decrease in pH in all treatment. Acidity ranged from 0.65 to 0.70 at 0 and 60 days storage specifying a significant effect of treatment and storage. There was no effect of treatment and storage on ash content of dried apricot diet jam. The maximum reducing sugars content in diet jam was 33.51% in T0 and minimum was observed (2.43) in T_5 after 60 days of storage. All treatments remained acceptable during the entire storage period for two months. Treatments T_4 and T_5 were ranked best by all judges for their overall acceptability. To conclude dried apricot diet jam can be manufactured commercially in combinations as for T_4 and T_5.

Key words: Diet apricot jam, health benefits, storage impact, quality

INTRODUCTION

Recent research has indicated that excessive use of sugar results in obesity, increased risk of coronary heart diseases and other potentially lethal conditions (Coleman 1995). The main objective of using alternative sweeteners is to reduce the energy intake and thus to avoid the risk of heart disease. The demand of low calorie products without sacrificing sweet taste is increasing rapidly (Hyvonen and Torma 1983). Apricot is an important fruit of temperate regions with a distinct pleasant aroma and is used for preparing many products including jams and nectar. In Pakistan apricot is preserved mainly by the conventional method like sun drying without any chemical treatment. The dried fruit is available in the market round the year. Generally fruit jams contain higher concentration of sugar that limits their use to a greater extent for diabetics, obese and calorie conscious people. Nutritionists and many consumers do not consider sugar to be a satisfactory food, so there has been a growing desire to utilize artificial sweeteners replacing sucrose for nutritional and health point of view (Salminen and Hallikainen 1989). Sugar alcohols like sorbitol are mostly used in diabetic jams because of their slow absorption in the blood stream and thus help to avoid blood glucose level (Richard and Primack 1986) Evaluation of dried apricot diet jam prepared by incorporating a suitable combination of sorbitol, cyclamate and aspartame replacing sucrose and glucose syrups on an equivalent solid basis remains the main focus of the study and this further includes setting acceptable levels of artificial sweeteners and impact of storage on physico chemical and organoleptic characteristics of the product.

MATERIALS AND METHODS

The dried good quality apricots and artificial sweeteners sorbitol, cyclamate and aspartame were purchased from local market. Apricots after being washed thoroughly and cut into small pieces were soaked in 40% water for 24 hours for pulp extraction. After adding potassium metabisulphite @788 ppm and adjusting final pH 4 .0 with citric acid, pulp was preserved in glass containers. Jam was prepared by using sorbitol, cyclamate and aspartame replacing sucrose and glucose syrups in the following treatment combinations (%).

Preparation of jam

The pulp, sorbitol, cyclamate and aspartame were cooked for 10 minutes. Pectin was dissolved in sorbitol using high speed mixer and was added to the different lots near the end point 64- 66° Brix. After boiling, sodium benzoate, citric acid, color and flavor

Treatment	Pulp	Sugar%	Sorbitol	Cyclamate	Aspartame	Pectin	C.A	S.B
To	39.48	59.21	00.00	00.00	00.00	0.55	0.63	0.1
T_1	29.13	00.00	70.00	00.00	00.00	0.45	0.48	0.1
T_2	30.17	00.00	68.79	0.038	0.0056	0.45	0.48	0.1
T_3	31.29	00.00	67.59	0.078	0.012	0.45	0.48	0.1
T_4	32.49	00.00	66.29	0.120	0.018	0.45	0.48	0.1
T_5	33.79	00.00	64.89	0.170	0.025	0.45	0.48	0.1

S.B :Sodium Benzoate C.A : Citric Acid

were added. The cooking was ended when the final temperature reached between 104-108°C. After cooking, the jam was filled in pre sterilized wide mouthed glass-jars. The jars were allowed to cool further while still open and molten wax was applied at the top of each jar. Then the jars were closed with lids. The jars were labeled and stored for 60 days at room temperature (25-35°C) for subsequent analysis.

ANALYSIS OF JAM

Physico- chemical characteristics.

The diet jam samples prepared from in different treatment combination were analyzed after 0, 15, 30, 45 and 60 days of storage intervals to observe the changes in total soluble solids, pH, titratable acidity, ash content and reducing sugars.

1. Total soluble solids. TSS were directly measured by using Abbe's Refractometer according to the method of Ruck (1963) and expressed as degree Brix.

2. pH. pH was directly recorded by pH meter (Modal pH N81, Tacussel Electronque, France) according to the method given by Ruck (1963). pH meter was calibrated for every sample with known buffers of pH 4.00 and pH 7.00 use.

3. Titratable acidity. It was determined by the method given by Ruck (1963) using formula:

$$\% \text{ Titratable acidity} = \frac{1}{10} \times \frac{\text{Equiv. wt of acid X normality of NaOH X titer}}{\text{Weight of sample}}$$

4. Ash. Ash was determined by the method described by Ruck(1963).

$$\text{Ash\%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

5. Reducing sugars were determined according to the Lane and Eynon methods as described by Ruck (1963).

Sensory evaluation

The samples of diet jam were evaluated for sensory attributes such as colour, flavour, taste, and texture and overall acceptability after each storage interval by the scoring method as described by Land and Shepherd (1988).

Statistical analysis

The analysis of data was conducted using CRD to find out level of significance between treatment and storage intervals by using the procedure as described by Steel *et al.* (1997).

RESULTS AND DISCUSSION

Physico-chemical characteristics of dried apricot diet jam.

Mean values for TSS given in Table.1 revealed a highly significant effect of treatments on total soluble solids of jam .However T_1, T_2 and T_4 displayed non significant difference with one another but these values differed significantly from T_3 and T_0. It is evident from results in Table.2 that the total soluble solids of all treatments showed an increasing trend with increase in storage days while the interaction between storage time and treatments was found to be non significant. This increase in TSS may be attributed to the formation of water-soluble pectin from protopectin during storage, as reported by Bindra *et al.* (1974) and Zaheer (1986)

Mean values for treatments indicated in Table 1 clearly manifested a significant difference for pH. Nevertheless T_1 & T_2 and T_3 &T_4 were non significantly different from each other but significantly differed from rest of the samples. Statistical results have shown highly significant effects of storage intervals, while interaction between storage and treatments (T x S) was found to be non-significant on the pH of diet jam.

Table 1. Comparison of means for physico- chemical characteristics as influenced by treatments

Characteristics	T_0	T_1	T_2	T_3	T_4	T_5
TSS	69.12 b	69.74 a	69.92 a	68.46 c	69.86 a	68.60 c
pH	3. 30 d	3. 33 b	3. 33 b	3. 34 a	3. 34 a	3. 32 c
Acidity	0. 69 a	0. 67 b	0. 67 b	0. 67 b	0. 66 c	0. 66 c
Ash	0.480 a	0.475 a	0.475 a	O.475a	0.475 a	0.474 c
R*. sugar	33.51 a	3.07 b	2.66 d	2.79 c	2.66 d	2.43 e

R*=reducing Means sharing the same letter are non significantly (P ≤0.01)

Table 2. Comparison of means for physico- chemical characteristics as influenced by storage

Characteristics	S_1	S_2	S_3	S_4	S_5
TSS.	68.95 c	69.15 c	69.28abc	69.43 ab	69.60 a
PH	3. 35 a	3. 34 b	3. 32 c	3. 31 d	3. 29 e
Acidity.	0. 65 e	0. 66 d	0. 67 c	0. 68 b	0. 70 a
Ash	0.476 a	0.476 a	0.476 a	0.476 a	0.476 a
R* .sugar	7.13 e	7.46 d	7.84 c	8.23 b	8.61 a

R*=reducing Means sharing the same letter are non significantly (P ≤0.01)

Table 3. Comparison of means for sensory characteristics as influenced by treatment

Characteristic	T_0	T_1	T_2	T_3	T_4	T_5
Colour	7.20d	7. 35c	7.05e	6.96f	7.45b	7.77a
Flavour	6.43d	7.03c	7.10c	7.25b	7.45a	7.51a
Taste	6.00f	7.11d	7.01e	7.32b	7.21c	7.55a
Texture	6.l0f	6.28e	7.01d	7.14c	7.23b	7.51a
Acceptability	6.13e	7.16d	7.27c	7.31c	7.37b	7.46a

Means sharing the same letter are non significantly (P ≤0.01)

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

Characteristics	S_1	S_2	S_3	S_4	S_5
Colour	7.33a	7.50a	7.09a	7.25a	7.29a
Flavour	7.16a	7.14a	7.13a	7.13a	7.l0a
Taste	7.02a	7.03a	7.04a	7. 04a	7.04a
Texture	6.87a	6.88a	6.88a	6.88a	6.89a
Acceptability	7.l0a	7.09a	7.13a	7.14a	7.12a

Means sharing the same letter are non significantly (P ≤0.01)

The increase in pH among treatments may be attributed to the production of acid during a storage period of 60 days as indicated from the values of acidity in Table. 2 where an increase in acidity for the whole storage period was noted . Similar results were reported by Lindroth, (1980). Statistical analysis of data showed that there was highly significant effect of treatments and storage time on the percent acidity of jam. Increase in acidity might be due to the formation of acidic compounds by degradation or oxidation of carbonyl compounds present in jam as reported by Lodhi (1989). No effect of treatments except in T_5 and

storage period was observed on ash percentage of jam (Table 1&2) The results obtained for ash were in accordance with finding of Winton (1935) who reported that there were no effects of treatments and storage on ash content of jam. No relationship for the change in ash contents compared with all other samples could be established.

Mean values given in Table I and 2 for the jam samples showed a highly significant effect of storage time, treatments and treatments storage interaction on reducing sugars. It is obvious from results that reducing sugars increased with an increase in storage days. The extent of reduction of sugars decreased as the levels of aspartame and cyclamate increased. The maximum reducing sugars content in diet jam was recorded as 33.5 1% in To and minimum reducing sugars percentage was observed 2.43 in T_5. The significant increase in reducing sugars might be due to the prolonged storage time and high storage temperature with subsequent increase in catalytic oxidation and hydrolysis of sugars resulting in ultimate increase in acidity and decrease in pH. The similar results were given by Pandit (1991) and Saleemi (1999).

Sensory characteristics of dried apricot diet jam

Organoleptic evaluation is an important criterion to determine the acceptability of food products. Panel of experts conducted the evaluation of jam. The jam prepared from different sweeteners was evaluated organoleptically for colour, taste, flavour, texture and overall acceptability. The statistical data (Table 3 and 4) showed highly significant effect of treatments on the colour and non-significant effect of storage. Similar results for colour score were obtained by Pandit (1991) and Saleemi (1999). Treatment T_5 was ranked best for flavour by getting maximum scores i.e. 7.51 while T_0 got minimum scores 6.43. However, effect of storage was non significant while interactive effect of treatment and storage period was found to be highly significant for flavour. It was observed that difference in flavour could be due to difference in formulation of treatments. (Pasha *et al.* 1994). Similar findings were observed by Park *et al.* (1975), Lodhi (1989) and Saleemi (1999) who reported that differences in flavour is due to the differences in formation of treatments.

It is evident from Table.3 that T_0 remained at bottom for taste scores by securing mean score of 6.00 where as treatment T_5 was ranked to be at the top by securing 7.55 score. Statistical results manifested highly significant effect of treatments but non-significant effect of storage on the taste of apricot diet jam. The overall results showed that the treatments T_3, T_4 and T_5 were better in taste than other treatments. However, the difference in taste was significant among all treatments. The difference among the treatments for taste is attributed to different proportion of sorbitol, cyclamate and aspartame, used in formulation for diet jam. The results were in agreement with the finding of Zaheer, (1986), Lodhi (1989) and Saleemi (1999). It is obvious from the data in Tables 3 & 4 for the texture of diet apricot jam during storage that all the treatments were found to be acceptable during 60 days of storage. Like other organoleptic characteristics treatment T_5 got the highest scores for texture by securing maximum scores of 7.51 while T_0 and T_1 got minimum score of 6.10 and 6.28 respectively. A highly significant effect of treatments but non-significant effect of storage was observed for texture of apricot diet jam samples. The score recorded for acceptability showed negligible changes in various parameters like colour, flavour, taste and texture during 60 days of storage. Statistical data regarding overall acceptability revealed significant effect of treatments but non significant effect of storage and interaction between treatment and storage (Table 3 and 4). The data indicated maximum score for overall acceptability to be 7.46 recorded for T_5. However, the difference in overall acceptability of diet jam samples were not significant among T_2 and T_3.The similar findings were observed by Bindra (1974).

To conclude, treatments T_4 and T_5 had shown the best results for physico chemical and sensory characteristics of the dried apricot diet jam and could be manufactured successfully on commercial scale with a view point to reduce the increased health risks associated with higher intakes of sugar through the product like jam.

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Effect of different concentrations of caustic and soda solutions on the efficiency of evaporator scale removal

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ABSTRACT

Different concentrations of sodium hydroxide (15, 20, 25, 30, 35%) and sodium carbonate (5, 10, 15, 20, 25%) solutions were used to remove scales from cane juice evaporator tubes. The scale samples were collected from five bodies of a quintuple evaporation system. The scales were examined on compositional basis to find out the percentage of calcium, magnesium and sulphate. The effectiveness of different concentrations of both chemicals had significant effect on scale removal. The caustic and soda solutions of 35% and 15% strength gave the best results however complete (100 %>) scale removal was not achieved by any chemical.
Keywords: Sodium hydroxide, sodium carbonate, cane juice scale, evaporation

INTRODUCTION

Evaporation is an important processing operation in the sugar industry during production of refined sugar from cane juice. During this process, the juice is concentrated up to the required level *i.e.* 65° Brix. Generally the higher the syrup Brix, the more scale is produced. The cane juice vapors produced during this operation due to the multiple effect evaporators are supplied to primary heaters. Therefore the evaporators are of prime importance because the whole steam economy is based on their performance.

The cane juice contains a significant amount of impurities, the most important being the mineral salts. The solubility of these minerals decreases as the juice become concentrated. On the other hand some chemical reactions also take place such as caramelization and decomposition of monosaccharide which results in the formation of organic acids and these in turn react with calcium ions resulting in the formation and precipitation of organic calcium salts.

The suspended and colloidal matter present in cane juice defective clarification or filtration and improper operational factors result in the deposition of impurities on the heating surface of evaporators, forming a hard scale. The suspended and colloidal matter act as nuclei for the crystallization of various species, it may attach itself to the tube wall and encourage the deposition of scales. It is believed that juice velocities in the tube and pH are important. This accumulation of scale hinders heat transfer and process becomes defective and uneconomical (De Beer and Moult 1998).

Scale composition is influenced by process adopted for clarification, because sulphitation will result in more calcium sulphate and carbonation will give more calcium oxalate. On the other hand defecation will result in more calcium phosphate in the scales. Another important factor is proper juice flow as low or high juice flow rates increase the scale deposition. By controlling the operational factors, scale formation can easily be prevented. This is possibly the most important factor in cane sugar factory. Low and/or erratic juice flow rates results in severe fouling. In the worst cases, dry out occurs and tubes are blocked. Correct juice flow rates reduce fouling and allow a longer period of operation before cleaning is required Brunei *et a1.* (1981) and Jenkins (1979). In order to solve this problem and to remove the scale occasional stoppage for cleaning becomes necessary. It is found that caustic (sodium hydroxide) and soda (sodium carbonate) are helpful to remove the scale from evaporator's bodies without having any toxicity and are also economical to use in sugar factory. Therefore, the mandate of the present project was to determine the effective concentrations of caustic and soda for the removal of scales from evaporator's tubes.

MATERIALS AND METHODS

The scale samples were collected from Hussain Sugar Mills Ltd., Jaranwala, District Faisalabad during the crushing season 1999-2000. Five samples were collected. One from each body of the quintuple evaporation system. After washing and drying samples were analyzed on compositional basis to find

out the percentage of Calcium (Ca), Magnesium (Mg) and sulphate (SO_4) in each sample by following standard procedures of AOAC (1995). The caustic and soda solutions of 15, 20, 25, 30, 35 and 5. 10, 15, 20 and 25% were prepared, respectively and scale removal was carried out in these different concentrations of both chemicals as described by Hugot (1972). Finally the data collected for each parameter were subjected to statistical evaluation by following the techniques of Steel *et al.* (1996).

RESULTS AND DISCUSSION

The data composition of scale as calcium, magnesium and sulphate is depicted in Table 1. The maximum amount of calcium (31.40 %) magnesium (2.35 %) and sulphate (21.25 %) were obtained from the samples of evaporators B_1, B_3 and B_5, Where as the minimum amounts of calcium (13.45 %) magnesium (0.26 %) and sulphate (8.50 %) were obtained from samples B_4 and B_1. It was concluded that calcium content decreased from first to fourth body while it increases in the fifth body. Similarly, sulphate content gradually increased from first to last body. From the present findings it is evident that sulphate content of scales increased from first to last body i.e. (8.50 to 21.25%) which is in close agreement with the results reported by Gorizaley and Vega (1986) and Walthew and Turner (1995).

Magnesium content of scale varied from 0.26 to 2.35% and there was no symmetrical increase or decrease from first to last body. Similar results were also reported by Brunet *et al.* (1981) and Devillers *et al.* (1975b). The present findings about calcium and magnesium percentages also resemble with those reported by Nandagopal and Ramamurthy (1976a).

The chemical tests were carried out to find out the effectiveness of different concentrations of caustic and soda solutions for the removal of scale from evaporator tubes. The data regarding effectiveness of different concentrations of caustic solutions for removal of scale is given in Table 2. It is revealed from the data that 35% caustic solution gave the best result for the fifth body whereas same concentration showed the poorest results for the first body. All the concentrations except 20% had no significant difference, which was found to be less effective in almost all the bodies.

The data regarding effectiveness of different concentrations of soda solutions for scale removal is depicted in Table 2. It is revealed from the data that 15% soda solution dissolved maximum scales i.e. 0.247 g/0.5g in the fifth body, where as the 20% solution in the second body gave the poorest results. In the fifth body all the treatments showed the best results as compared to other bodies. It was also observed that 100% scale was never dissolved in case of any chemical (i.e. caustic and soda) used.

From present work 20-30 % NaOH was found most effective for 2nd, 4th and 5th body which is in comparison with earlier findings of different research workers as discussed below.

Patterson (1988) also suggested that concentrated caustic solution (up to 30%) should be used for descaling evaporator's tubes. Staub and Paturau (1993) used concentrated solutions of NaOH (30°Be) for evaporator scale removal. He further reported that efficient results can be achieved by using caustic soda in combination with soda ash. The concentration of soda ash ranged from 8-15 %. The findings are in close agreement with those reported by Leal *et al.* (1988) they descaled the low purity sweet water evaporators by using soda ash and caustic soda solutions. The concentration of sodium carbonate solutions was 5-15 %.

The present observations also partially confirm with that of Anonymous (1980), who used NaOH solution up to 25 % under atmospheric pressure (3-4 psi). The results are quite comparable with earlier findings of Ivin (1985), who got 90 % scale removal by using 20 % NaOH solutions.

Table 1. Chemical composition of evaporator scales

Evaporator body	Calcium %	Magnesium %	Sulphate %
Evaporator body 1	31.40	1.53	8.50
Evaporator body 2	25.19	1.29	9.58
Evaporator body 3	21.19	2.35	13.72
Evaporator body 4	13.45	0.26	16.90
Evaporator body 5	20.14	1.27	21.25

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Table 2. Effect of different concentrations of caustic and soda on scale removal (in g/0.5g).

Evaporator body	Caustic					Soda				
	T_1	T_2	T_3	T_4	T_5	T_1	T_2	T_3	T_4	T_5
1	0.188	0.043	0.045	0.084	0.036	0.037	0.036	0.053	0.080	0.016
2	0.077	0.135	0.175	0.223	0.146	0.092	0.078	0.041	0.012	0.066
3	0.178	0.082	0.065	0.051	0.104	0.075	0.078	0.045	0.059	0.046
4	0.149	0.127	0.158	0.174	0.157	0.118	0.162	0.133	0.031	0.093
5	0.177	0.277	0.290	0.237	0.321	0.196	0.234	0.247	0.194	0.205

For caustic **For soda**

T_1	=	15% caustic solution	T_1	=	15% caustic solution
T_2	=	20% caustic solution	T_2	=	20% caustic solution
T_3	=	25% caustic solution	T_3	=	25% caustic solution
T_4	=	30% caustic solution	T_4	=	30% caustic solution
T_5	=	35% caustic solution	T_5	=	35% caustic solution

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Storage studies on the stability of cooking oils

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ABSTRACT

Refined oils of sunflower, soybean, canola and hydrogenated vegetable ghee (Kisan), and locally prepared non-refined Desi ghee were selected to observe the effect of temperature on storage stability. The temperatures of 15°C, 40°C and 60°C, refractive index, free fatty acid, iodine value, saponification value and peroxide value assessed the oil stability. The changes in the quality parameters correlated to the storage temperature. A proportionate increase in free fatty acid value and peroxide value was observed in all the oil samples. Similarly, a considerable decrease in iodine values with simultaneous increase in saponification values was observed in all oil samples during storage at all temperatures. Lowest iodine value of 37.56 was observed in case of Desi ghee samples. Increase in refractive index was also noticed with increase in temperature and highest refractive index of 1.525 was observed in Kisan vegetable ghee. All the samples were found to have E_a values in the range of 13.0 to 71.7 kJ/ mole including those of higher amount range for fatty acid values (37.4 to 54.6 kJ/ mole) and peroxide values (37.5 to 68.2 kJ/ mole), and of lower amount range for Iodine values (13.9 to 29.4 kJ/ mole) and saponification values (13.0 to 45.9 kJ/ mole). The overall activation energy having minimum amount of range found in the Kisan vegetable ghee and Soybean oil whereas of a range with maximum amount associated for the Canola, sunflower and Desi ghee samples. The range of Q_{10} values varied with in the normal limit from 1.2 to 2.0. The hydrogenated Kisan ghee and soybean oil appeared relatively more heat stable products.

Key words: Cooking oils, stability, temperature, rate of deterioration, activation energy

INTRODUCTION

Oils and fats play an important role in the economy of any country. For providing energy, fats and oils occupy a higher position than any other energy supplying food. They in fact represent the most concentrated form providing 9 Calories/g fat. So vital is the contribution of fats and oils to the life processes of human beings which cannot synthesize certain of the unsaturated fatty acids like linoleic acid, and show symptoms of deficiency diseases and die prematurely if their food does not contain these essential fatty acids. However, these being polyunsaturated in nature are modified readily and are badly affected by environmental factors like oxygen and temperature.

Clark and Serbia (1991) reported that the free radicals formed by fatty acids react with oxygen to generate peroxides which enter into a multitude of reactions, producing numerous products such as aldehydes, ketones, acids, esters and polymerized fat. These compounds make the food unpalatable and even harmful. Rajesh and others (1992) studied the thermal stability of tertiary butyl hydroquinone (TBHQ) in ghee and observed the efficacy and stability of the diphenolic antioxidant, during continuous heating at 100, 150, 200C. The addition of TBHQ at conc. of 5, 10, and 20 mg /100 g resulted in retardation of oxidative deterioration of ghee heated at 100 and 150°C as assessed by the peroxide value. Ahmad and others (1996) used cooking oils to observe the effect of deep fat frying of potato-fillets for four consecutive days @ 3-4 minutes per day. Quality of frying oils was determined by the peroxide value (POV), anisidine value (AV), iodine value (IV), free fatty acid value (FFA) and color (O.D at 420 nm). The highest average POV (22.94 meq/kg), FFA (0.40) and IV (114.10 g/ 100g) were observed in sunflower oil while AV (109.8) in corn oil. The maximum change in color (discoloration) was observed in corn oil. All quality parameters increased during frying, except the IV, which showed a decreasing trend in each case. Huyghebaert (1998) conducted an experiment to follow the effect of heating on the chemical composition of selected oils and fats (crude soybean oil, refined and partially hydrogenated soybean oil, refined lard and refined beef tallow). He found that the change in free fatty acids was rather limited, saponification value was negatively correlated with high temperature. During heating there was a continuous conversion of the nonpolar fraction into the

polar fraction. The stability was inversely related to the number of double bonds.

The present research work was carried out with the objective to find out the effect of temperature (at which the fatty food is normally held in houses during summer) on the stability of various brands of fats and oils, which are commonly used for cooking purposes.

MATERIAL AND METHODS

Sealed pack of refined, bleached and deodorized (RBD) cooking oils was purchased from the local market. The sample includes Kisan vegetable ghee, Super Habib soybean oil, Sufi sunflower oil, Seasons canola oil and Desi ghee. According to the labels affixed on the packs, these samples were manufactured and packed in the year 2000. While Desi ghee (butterfat of cow) was bought from the countryside. The samples were brought to the laboratory of Food Technology and study conducted in the month of May 2000. Each of the sample was taken in long neck conical flask, fitted with condenser and allowed to heat on water bath at incubation temperatures of 40ºC and 60ºC for a period of 15 days. The samples were also placed at 15°C in the cold storage. After the storage the packs were left in a refrigerator under tightly packed conditions until required for further study purposes. The stability of the samples was studied by estimation of quality parameters including refractive index (RI), free fatty acid value (AV), saponification value (SV), iodine value (IV) and peroxide value (POV). Rate of deterioration, activation energy (Ea) and temperature quotient Q_{10} (40°-50°C) were also determined for each of the said quality parameter. The analysis was conducted in duplicate and the mean value is recorded.

Refractive index and acid value of oil samples were recorded in accordance with the method described by Triebold and Aurand (1963). Saponification and peroxide values were determined by the method of A.O.A.C (1984). Iodine value was estimated as described by Pearson (1970).

Rate of deterioration. Rate of deterioration per day for each parameter was determined by subtracting the fresh values of a given parameter from its final reading at a particular temperature.

Rate of deterioration/ day =
(Initial pre-storage reading – final post-storage reading) / incubation time.

Activation energy (Ea). The energy of activation for each determinant was estimated by Arrhenius plot (Baloch and others 1977) after plotting logarithmic rate of deterioration/day against 1/T, where T is absolute temperature (Fig. 6). The activation energy is calculated from the following equation:

Slope = $-E_a / 2.303\ R$

where R is a Gas constant = 1.987 cal/mol

From the Figure 6, given as a specimen, the slope can be determined, and hence from the same graph the apparent energy of activation (E_a) and the temperature quotient Q_{10} can be calculated for all of the quality parameters.

RESULTS AND DISCUSSION

Physico-chemical constants of oil samples were studied at different storage temperatures. Changes in quality parameters were recorded after exposing the oil samples to the specific temperature for a selected period of time. Moreover rate of deterioration, E_a, Q_{10} values in temperature range of 40°- 50°C were recorded for each of the above parameters. The results are tabulated and graphically presented at appropriate places.

Refractive Index

The refractive indices of 1.4767, 1.4787, 1.4737, 1.4450, and 1.5210 were recorded in freshly opened samples of Sufi sunflower oil, Super Habib soybean oil, Seasons canola oil, Desi ghee and Kisan hydrogenated vegetable ghee respectively. The data indicate that the hydrogenated ghee had highest RI value as compared to all other oil samples. All the samples showed an increase in the RI on storage and the rate increased proportionately with storage temperatures. Maximum increase was found at 60°C followed by 40°C. A slight increase was also observed on keeping the samples at 15°C (Fig. 1). These results are in agreement with the those reported by Triebold and Aurand (1963) and Pearson (1970).

Free fatty acid value

Free fatty acid value of 0.32, 0.02, 0.71, 3.20, and 0.33 were recorded in freshly opened samples of soybean (Super Habib), canola (Seasons), sunflower (Sufi), Desi ghee and Kisan ghee respectively. Free fatty acid contents were highest in Desi ghee followed by sunflower, Kisan ghee and soybean. Minimum amount of fatty acid values was found in the canola oil samples (Fig. 2). A considerable high amount of the free fatty acid values of Desi ghee samples indicate that the samples were not properly stored and turned deteriorated. In the rural areas normally the butter is

left for the collection over a week period or so before its conversion to Desi ghee. Most probably the butter was deteriorated considerably which eventually marred quality of the Desi ghee. All these samples after incubation showed an increase in free fatty acid values, which corresponded well with the incubation temperatures. Highest increase was found at 60°C followed by 40°C. A negligible increase was also observed at 15°C. Similar findings have also been reported by Stevenson and others (1984) and Huyghebaert (1998).

Peroxide Value

The initial POV of 0.33 was the lowest in case of Kisan ghee as compared to soybean (Super Habib), canola (Seasons), sunflower (Sufi), Desi ghee which had 4.01, 3.22, 6.02 and 8.21 respectively (Fig. 3). On evaluating the results after storage it is noted that there was corresponding relationship between POV and temperature, and highest increase was observed at 60°C but very little changes occurred at 15°C. These findings collaborate with the work of Steven and others (1994) who had also reported similar results.

Saponification Value

Freshly opened samples of soybean (Super Habib), canola (Seasons), sunflower (Sufi), Desi ghee and Kisan ghee gave initial SV of 185.41, 187.88, 188.34, 218.41and 190.78 respectively (Fig. 4). Although all the samples showed an increase in SV after incubation at higher temperatures but the extent of the increase did not correspond with the rise in the temperature. Huyghebaert (1998) reported similar observations in this parameter of cooking oils.

Iodine Value

Fresh sample of soybean (Super Habib), sunflower (Sufi), canola (Seasons), Kisan ghee and Desi ghee displayed the iodine value of 137.66, 134.56, 108.91, 98.43 and 37.56 respectively (Fig. 5). It appears that soybean, sunflower and canola samples had higher amount of unsaturated fatty acids whereas hydrogenated and Desi ghee samples had considerably lower amount of these acids. On storage, the incubation temperature considerably exerted a negative effect and the values were reduced after 15 days of storage with maximum reduction at 60°C. However, the decreases were not at 15°C as it was at the elevated temperatures. These results are in accordance with Ahmad and others (1996).

Activation Energy and Q_{10} values

The data regarding activation energy and Q_{10} are recorded in Table 1. The E_a varied from 13.0 to 71.7 kJ/ mole for all of the quality parameters studied. The overall minimum levels of activation energy varying from 13.9 to 35.1 kj/ mole and 13.0 to 45.9 kj/ mole were estimated for Iodine and Saponification values respectively. Whereas values of much higher level were found for the other quality parameters. Iodine values appeared to proceed with out being much affected by the temperature variation, but PO, Free fatty acid and RI values appeared to be highly temperature dependent. As regards the activation energies of the oil samples the Kisan vegetable ghee and Soybean oil possessing overall lower activation energies are likely much less affected by the elevation of temperature in the studied range. However, the Canola, Sunflower and Desi ghee samples are likely to depend on the temperature rises. The Canola, Sunflower and Desi ghee samples may not show much deterioration at relatively lower temperatures but these are very likely to deteriorate at much rapid rates by increasing the temperatures. The findings indicate that all of the cooking oil samples require fairly high amount of the energy to allow and to continue the deterioration process, and remain stable so long as they are placed at lower temperatures. The fact that all of the samples had comparatively lower activation energies with regard to IV and SV maintains that the samples are likely to be deteriorated initially by those factors which affect the measurement of iodine and saponification values. Similarly, Q_{10} values varied from 1.2 to 2.0 (Table 1). The results indicate that the rate of quality deterioration becomes 1.2 to 2.0 time fast with 10° rise in the temperature between 40° to 50°C. Similar findings have also been put forwards by Baloch and others (1977), and Grivas and others (2002).

CONCLUSIONS

From the above study, all the samples showed significant increase in their FFA and Peroxide values at elevated temperatures. Minute increase was also observed at 15°C. Desi ghee showed rapid increase in its free fatty acid and peroxide values while the refined and hydrogenated fats did not show such a trend in these parameters. There was increase in refractive index of all cooking oils. Whereas a decreasing trend is given out for the Iodine values in all oils samples and the trend was prominent at higher temperatures. The SV showed also a slight increasing trend in this study for all the samples. However this increase was not as significant. The hydrogenated vegetable ghee afforded higher stability whereas the Desi ghee samples responded ease to deterioration.

Table 1. Activation energy (KJ/Mol) and Q_{10} values of the oil samples during storage.

Sample	Refractive index		Free fatty acid value		Peroxide value		Saponification value		Iodine value	
	E_a	Q_{10}	E_a	Q_{10}	E_a	Q_{10}	E_a	Q_{10}	E_a	Q_{10}
Desi ghee	28.3	1.4	54.6	1.9	58.2	2.0	45.9	1.8	29.4	1.5
Soybean oil (S.Habib)	27.5	1.2	49.3	1.7	63.6	2.1	13.0	1.2	13.9	1.2
Sunflower oil (Sufi)	54.0	2.0	51.1	2.0	68.2	2.0	27.2	1.5	35.1	1.6
Canola oil (Seasons)	71.7	1.6	47.3	1.8	65.3	1.3	26.8	1.6	27.2	1.4
Kisan vegetable ghee	20.8	1.3	37.4	1.5	37.5	1.6	15.4	1.2	27.9	1.4

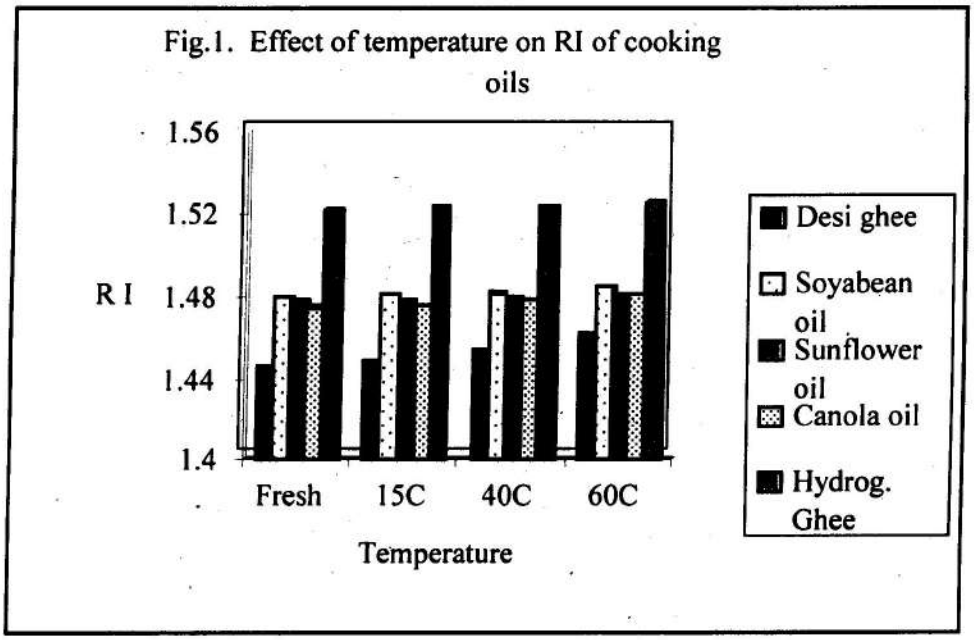


Fig. 2. Effect of temperature on free fatty acid values of cooking oils

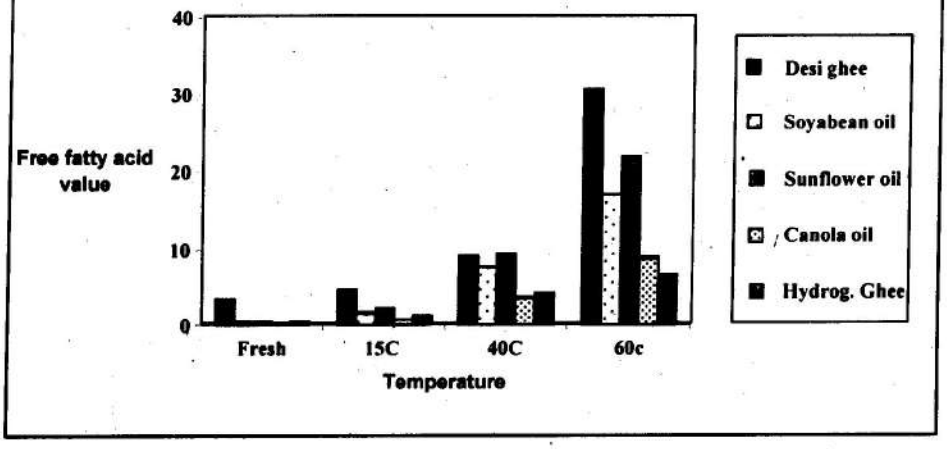


Fig. 3. Effect of temperature on peroxide value of cooking oils

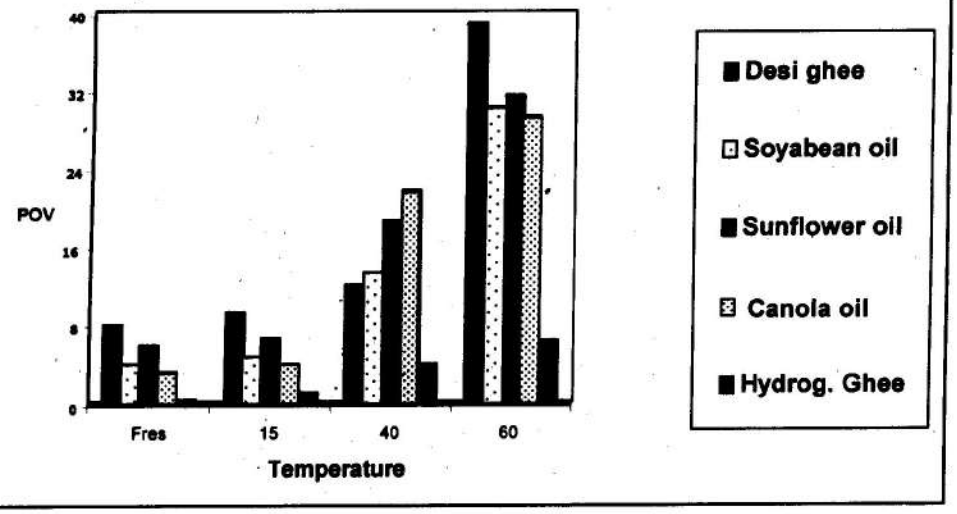


Fig. 4. Effect of temperature on saponification value of cooking oils

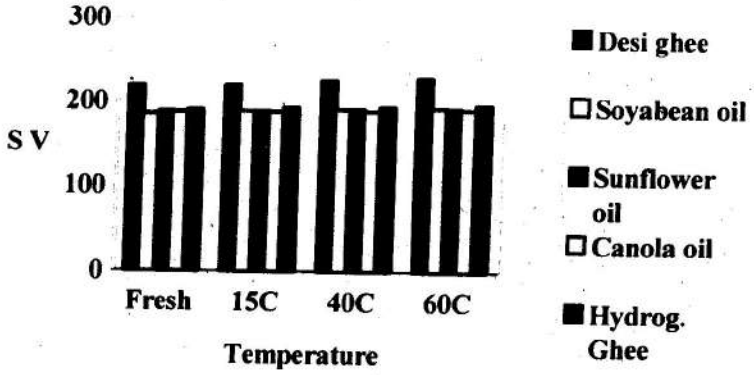
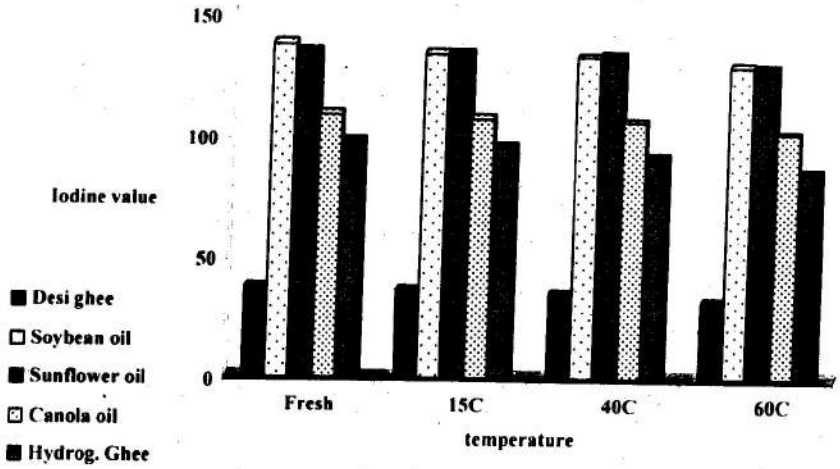
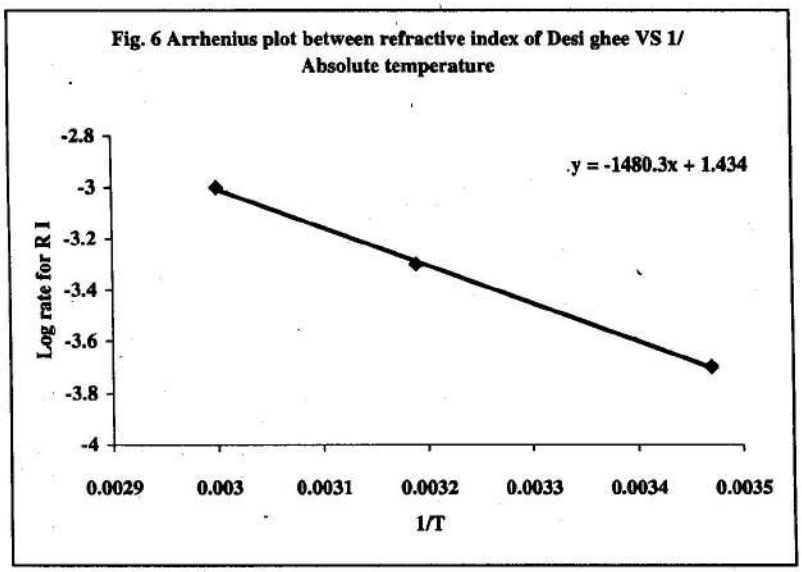


Fig. 5. Effect of temperature on iodine value of cooking oils





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Biosynthesis of fungal phytase from defatted rice polish

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ABSTRACT

Static cell culture was conducted for the biosynthesis of fungal phytase by *Aspergillus niger*-23 using defatted rice polish (DRP) as substrate. The parameters investigated during the studies were effect of agitation, different nitrogen sources, age and size of spore inoculum for the optimum yield of fungal enzyme. It was found that maximum yield of extra-cellular phytase was 96 hours old inoculum having a concentration of 40 mg/5g substrate. The present research concluded that the static culture is better, when compared with shake flask studies. Among nitrogen sources, ammonium nitrate was found to be best nitrogen source for the maximum yield (84μ/mL) of fungal enzyme after 72h of inoculation at 30°C.
Key words: Phytase, *Aspergillus niger*-23, Static cell culture

INTRODUCTION

Feed ingredients of plant origin contain a number of components that cannot be digested by mono-gastric species because of the lack or insufficiency of endogenous enzyme secretions. In addition to being unavailable to the animal, these components also lower the utilization of other dietary nutrients, leading to depressed performance. Examples of such anti-nutritive components include pentosans in wheat, β-glycans in barely and phytic acid. In recent years, with the development of enzyme products targeting specific substrates, the use of feed enzymes to ameliorate the effect of these anti-nutritive factors has received increased attention (Ravindran *et al.* 1999, Fredrikson *et al.* 2002,;l-Batal and del-Karem 2001).

The value of microbial phytase is to release phytate bound phosphorous and improve phosphorous bioavailability of plant ingredients for poultry and to reduce the phosphorous levels in effluent from intensive animal units (Ravindran *et al.* 2001, Lane *et al.* 2002).

Phytate, in its native state, is also complexed with various cations, proteins, lipids and starch. The significance of phytate on the utilization of nutrients other than phosphorous by poultry, however, has received little attention until recently. By releasing these phytate-bound nutrients and improving their utilization, dietary supplementation with microbial phytase would be expected to have protein/amino acid and energy effects in mono-gastric animals (Gill 1999. Musari *et al.* 2002; Popanich *et al.* 2003). Different experimentation with particularly broiler chicks have shown that phosphorous of Ca-phytate is utilized only ten percent as compared to disodium–phosphate. Only ruminants can efficiently utilize phytate-phosphorous, due to rumen microbial enzymes namely phytase which hydrolyze the plant phytates to inositol and inorganic phosphorous which are in turn, hundred percent utilized. Thus hydrolyzing the plant phytate prior to mono-gastric consumption would increase the availability of inositol and inorganic phosphorous in the diet (Khan *et al.* 2003; Halander *et al.* 1996) demonstrated that addition of phytase to poultry feed not only increases the bio-availability and absorption of phosphorous but also digestibility of dry matter, protein and fiber.

Thus many attempts have been made in order to biosynthesize the microbial phytase (EC. 31.3.8) by solid state fermentation for the hydrolysis of dietary phytates to improve the feed quality (Gargova & Sariyska 2003).

Against this background, the present investigation was initiated for the production of extra-cellular phytase by *Aspergillus niger*-23 as well as to maximize its yield by manipulating and optimizing the nutritional and cultural parameters of static cell culture.

MATERIALS AND METHODS

Organism

Aspergillus niger-23 obtained from NIBGE, Faisalabad, was grown and maintained on PDA slants at 30°C for 5-7 days in order to get maximum sporulation and kept in refrigerator at 5°C and sub-cultured monthly.

Spore Inoculum

Spore inoculum was prepared by suspending sterilized 0.01% MOT solution in PDA slants of *Aspergillus niger*-23. Spores over the slants were wetted and supernatant suspension was decanted off aseptically. Age and size of spore inoculum was investigated from 5-10 days and 100-500 mg of biomass/5g of DRP.

Fermentation Medium and Conditions

DRP was purchased from the local market of Lahore and was used as substrate throughout the present investigation. 100g of DRP was placed in1L cotton plugged conical flask with 40% moisture contents. Each flask was sterilized at 121°C for 15-20 minutes. After cooling to room temperature, spore inoculated with *Aspergillus niger* and incubated at 30°C for 120 hours for the synthesis of extra-cellular phytase.

Shake flask/ Agitation

For comparison, a set of flasks were placed on a rotatory shaker at 150 rpm for 120h in order to study the effect of agitation on the production of *Aspergillus niger*-23 phytase at 30°C.

Nitrogen Sources

Different nitrogen sources such as beef extract, yeast extract, urea, sodium nitrate, ammonium nitrate, ammonium sulfate were supplemented at the rate of 1% w/w with DRP in order to study the effect of different nitrogen sources on the yield of *Aspergillus niger*-23 phytase.

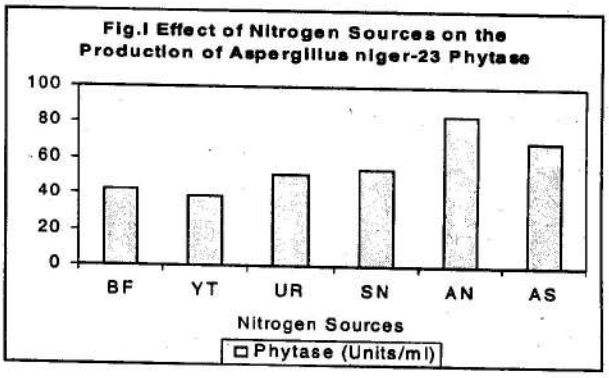
Enzyme Extraction and Activity

200ml of 2% $CaCl_2$ solution was added to each fermented flask for the extraction of extra-cellular phytase. Mash was passed through muslim cloth to remove the rice bran followed by centrifugation at 5000 rpm for 10 minutes. The supernatant was used for the phytase assay. Phytase activity was assayed by following the release of phosphorous from phytate in the form of orthophosphate. The liberated inorganic phosphate was determined by the method of Heinonen & Lathi (1981). One unit of phytate was defined as the amount of enzyme required to liberate 1uM of inorganic phosphate per minute under the assay conditions.

RESULTS AND DISCUSSION

Effect of Nitrogen Source on Phytase

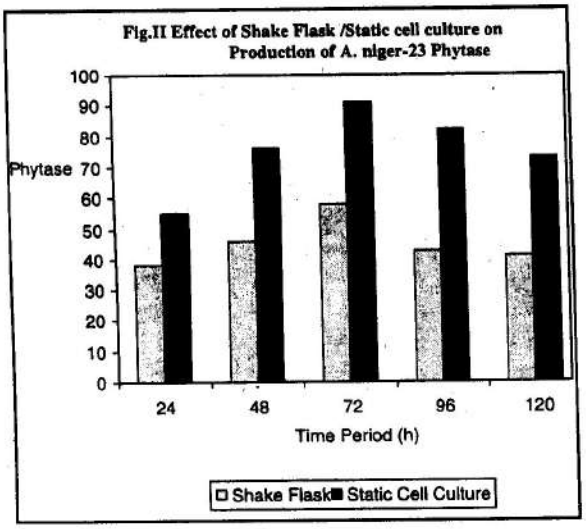
Various organic complex materials were evaluated as supplement for the biosynthesis of *Aspergillus niger*-23 phytase in 100g DRP at static culture and the results are summarized in Fig. I. These organic matters were added at the rate of 1% w/w in DRP in 1L conical flask. It was found that the synthesis of phytase was reached maximum (84 u/ml) with ammonium nitrate after 72 hours of static culture at 30°C. Other nitrogen sources have a much lesser effect on the production of fungal enzyme, probably due to an increase in pH which appeared after 72 hours or these nitrogen sources did not meet the basic demand of mycelium for the protein synthesis. Our findings are in complete association with that of Davison *et al.* (2001). Confirm please is it Davison or Davidson? These researchers declared ammonium nitrate as the best nitrogen source, when compared with other organic materials such as beef extract, yeast extract, urea, ammonium sulfate and sodium nitrate etc on the production of *Aspergillus niger* phytase from cassava dregs through solid state fermentation. They obtained a maximum yield of the extracellular fungal enzyme of 6.73 u/g of dry mass and the enzyme extract was found to be stable at feed processing temperature that is 75°C for 30 minutes. Similarly, Purva and Baneriee (2002) found maximum yield of *Aspergillu niger var teighm* phytase from rotton wood logs in shake flask culture. They concluded that the specific activity of the extracellular phytase was comparatively high with ammonium nitrate as a sole source of nitrogen in the culture media.



Effect of Agitation

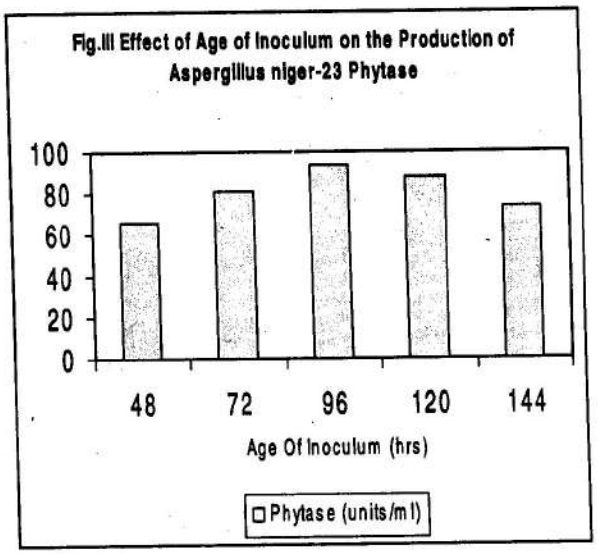
In order to study the effect of agitation for the production of *Aspergillus niger*-23 phytase, one set of sterilized 1L conical flasks of DRP with 40% moisture contents were placed on a rotary shaker at 150 rpm in order to compare the productivity of extra-cellular fungal enzyme with static culture. The results are shown in Fig. II. It was concluded that the static culture yielded much higher amount of fungal phytase enzyme when compared with the agitated culture at 30°C for 120 hours. Our findings are in association

with that of Papagianni *et al* (2001) Please confirm the Spellings! Is he/she Popagianni or "o" replaces 'a" who reported the effect of agitation and medium viscosity on the production of phytase by *Aspergillus niger* in submerged as well as on solid state fermentation. Their results showed that the specific growth rate and phytase production rate were found to be high at 150 rpm after 72 hours of fungal inoculation. However, upon inoculation of solid state fermentation with *Aspergillu niger*, increased productivities of the Phytase enzyme were obtained. Therefore, they concluded that the static culture led to higher amounts of extra-cellular phytase, when the results were compared to those obtained from agitated culture.

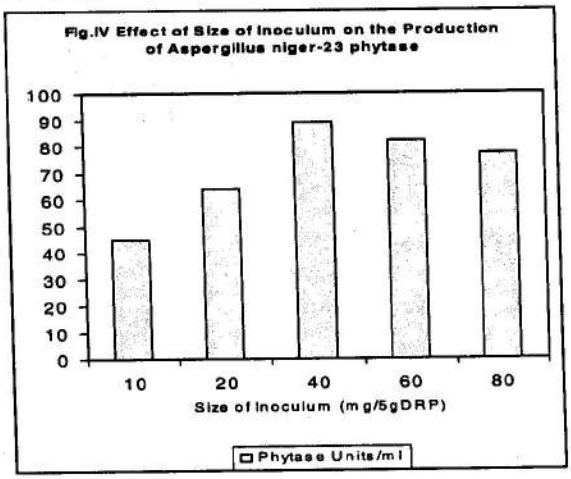


Effect of Age and Size of Inoculum

The effect of size and age of *Aspergillus niger*-23 spore inoculum was studied for the production of extra-cellular fungal phytase. *Aspergillu niger*-23 was grown on PDA slants at 30°C for a period of 48 to 144 hours for maximum fungal sporulation and the size of spore inoculum varied from 10-80 mg/5g of substrate in order to get maximum yield of enzyme. The results are shown in Fig. III and IV. It was found that the productivity of the enzyme reached maximum with 96 hours old spore inoculum i.e 93 u/ml and its best concentration at 40 mg biomass per 5g of substrate (89 u/ml). Our findings are in partial agreement with those of Kirshan and Nokes (2001). They used *Aspergillus niger* for the production of phytase on wheat bran and soy meal through solid state fermentation. The researchers used 7-14 days old fungal slants with liquid inoculum size of 60-480 mg biomass per 5g of substrate and conducted the static culture for a period of 10 days. Their results showed that phytase yield was not much affected by the size of spore inoculum that is over 60mg/5g substrate after 48 hours of fermentation. However, they obtained maximum yield of 94 u/g of substrate at 192 hours of solid state static culture fermentation. This variation in the results of age and size of spore inoculun in order to achieve maximum yield of extra-celluler phytase might be due to the difference in the substrate used during the present investigations.



Hence, it can be concluded from the above mentioned result findings that the production of *Aspergillus niger*-23 enzyme (Phytase) was found maximum by using seven days old fungal inoculum having a concentration of 45mg/5g of defatted rice polish with fortified ammonium nitrate at static cell culture after a period of 72h of incubation at 30°C.



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Dietary magnesium and its effect on human health status

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ABSTRACT

Different varieties of food materials before and after cooking, blood serum samples of normal persons (20-60 yrs. and above) and patients with cardiovascular diseases and with high blood pressure were analyzed for magnesium content by atomic absorption spectrophotometer. Magnesium concentration was found to be higher in fresh foods (range: 15-1592 mg/ Kg) as compared to the cooked. Serum magnesium level was found lower in the patients with cardiovascular diseases (0.17- 1.57 mg/ dL) and with high blood pressure (0.25- 1.59 mg/ dL) as compared to healthy controls (1.50-1.87 mg/dL).
Keywords: Cardiovascular disease, serum, blood pressure, magnesium

INTRODUCTION

Magnesium is a prosthetic ion in enzymes that hydrolyzes and transfers phosphate groups. Hence it is essential for energy requiring biological functions such as membrane transport, generation and transmission of nerve impulses, contraction of muscles and oxidative phosphorylation. It is essential for the maintenance of ribosomal structure and protein synthesis (Aikawa 1971). The human serum contains about 2-3 mg magnesium per 100 mL (Isselbacker *et al.* 1994), Seelig and Heggtveit (1974) reported that magnesium ions are essential for the maintenance of functional and structural integrity of the myocardium and magnesium deficiency induces cardiac necrosis and enhances susceptibility to cardiotoxic agents. Daily magnesium intake of 6 mg/ Kg body weight is recommended (Seelig 1964). Food and Nutrition Board (1968) recommends inclusion of magnesium in Recommended Dietary Allowance (RDA).

Sigel and Sigel (1990) reported that all traditional staples were rich sources of magnesium and 17 to 75% of the magnesium was lost during the culinary preparation of food items. Available magnesium may be missing from the food or lost before the uptake by the body and health suffers.

This study was carried out to measure the magnesium content in different food items and loss during preparation of food. Considering the importance of magnesium in physiology of heart and in controlling blood pressure of human body the present study was also conducted to measure the serum magnesium in a series of patients with cardiovascular / blood pressure diseases and to compare it with that of the normal person's serum.

MATERIALS AND METHODS

Procurement of Samples

Food samples were purchased from the local market.

Preparation of samples

The raw and cooked food samples were prepared by dry ashing method (Reith *et al.* 1974). Food items that were not homogeneous in nature were homogenized in deionized water.

Selection of the subjects

Blood samples from 30 patients (age range: 20-60 yrs) with cardiovascular disease and 30 (age range:20-60 yrs) with high blood pressure were collected from cardiology ward and medical ward of Mayo Hospital Lahore. Blood samples were also collected from 30 normal persons (age range: 20 - 60 yrs) at random and considered as control.

Blood sampling

The serum from blood samples for magnesium analysis was collected according to protocol suggested by Mauser and Khan (1989).

Magnesium analysis

Solutions of food materials and serum were analyzed for the trace element magnesium on a Hitachi Model 170-10 atomic absorption spectrophotometer equipped with hollow cathode lamp of magnesium. Metal content was calculated with standard, treated with same amount of reagent as for sample.

A stock solution of magnesium (0.1 mg/mL) was prepared (Pinta 1975) by dissolving 100 mg pure magnesium turnings in a minimum of HCl. Working solutions (0.05, 0.10, 0.15, 0.20 and 0.25 ppm) were

prepared from stock solution in a solution of strontium chloride (2.5 g/L) to avoid interference due to phosphate.

Statistical analysis

The data collected were subjected to statistical analysis by using the Analysis of Variance Technique and means were compared for their significance (Steel *et al*, 1999).

RESULTS AND DISCUSSION

Magnesium in food items

Food samples analyzed for magnesium are listed in Table 1. Food samples were selected and analyzed which were mostly consumed by adolescents and equally liked and consumed by middle and old age persons, as these foods are extremely popular among adolescents and may be a major source of magnesium in diets. Great variation in the distribution of magnesium in food was observed- Higher magnesium content was found in cereals, enriched and processed cereals, baked desserts and wheat products as compared to milk products, meats or vegetables (Table 1). Marier (1990) reported that whole grain cereals are the most abundant source of magnesium.

Loss of magnesium during cooking

Great variation in magnesium content was observed during cooking of food (Table 2). Magnesium content (440 mg/kg) was observed in raw eggs- Loss of 75.22 and 72.27 percent magnesium was observed when the eggs were hard boiled and scrambled respectively. Similarly loss of 53.49 percent magnesium was noted when wheat flour was cooked (chappatti). Loss of magnesium was less pronounced when potatoes were boiled (17.24%) but the loss significantly increased (37.24%) when the potatoes were baked (Table 2). Priestley (1979) reported that the behaviour of potato on heating is complex and depends on a number of factors including variety, maturity, agronomic effects, as well as processing conditions. The loss of magnesium during steaming and baking is due to heat, which brings about physiochemical changes at the surface of potato. High temperature (200- 300 °C) leads to loss of integrity of the cellular membrane allowing intercellular electrolytes to activate pectin methylesterase- This enzyme increases the release of free carboxyl groups which are available to form complexes with calcium and magnesium but remain within the cellwall resulting in decrease in free available magnesium. During boiling (100 °C) in water the same above action takes place but the release of free carboxyl groups is less to form complexes with calcium and magnesium. Thus free available magnesium is more.

Table 2. Effect of food preparation on magnesium loss

Food item	Magnesium (mg/Kg)	% Magnesium lost
Potatoes:		
Raw	290	0
Baked	182	37.24
Boiled	240	17.24
Peas:		
Fresh	330	0
Boiled	210	36.36
Cabbage (Chinese, white):		
Raw	170	0
Boiled	70	58.82
Carrot:		
Raw	120	0
Cooked	70	41.66
Wheat flour:		
Raw	643	0
Cooked (Chappatti)	299	53.49
Egg:		
Raw	440	0
Hard boiled	122	72.27
Scrambled	109	75.22

Table 3. Range of serum magnesium in normal persons and in patients with cardiovascular/blood pressure disease

No. of subject studied	Age	Magnesium mg/dL in serum of normal persons	Magnesium mg/dL in serum of patients with cardiovascular disease	Magnesium mg/dL in Serum of patients with high blood pressure
30	20-40	1.50- 1.60	0.17-1.27	0.25- 1.22
30	41-60	1.63- 1.77	1.33- 1.47	1.31- 1.48
30	61- above	1.83- 1.87	1.50- 1.57	1.49- 1.59

Table 1. Magnesium content of foodstuffs available in Pakistan

Food	Mg/Kg
Baked products	
Cake	
Sponge cake with cream filling	75 ± 4.04
Chocolate cake with fudge icing and cream filling	422 ± 3.83
Cookies	
Brownies	393 0.13
Caramel-peanut coated with chocolate	636 ± 0.02
Chocolate with crackers	412 ± 1.22
Chocolate sandwich with vanilla filling	508 ± 2.66
Beverages	
Coca Cola	17 ± 1.03
Pepsi Cola	5 ± 0.15
Seven Up	7 ± 0.47
Sprite	13 ± 1.15
Breakfast Cereals	
Corn, fruit-flavoured, nutrients added (Trix)	249 ± 3.60
Corn and oat Nutrients added Captain Crunch Honey Comb	372 ± 2.89 337 ± 1.67
Rice nutrients added (Rice Chex)	246 ± 0.07
Wheat flakes, nutrients added (wheaties)	1022 ± 2.33
Wheat and malted barley granules	670 ± 1.06
Cereals	
Wheat starch	15 ± 0.01
Wheat flour	643 ± 0.83
Popcorn	1562 ± 4.63
Egg	440 ± 3.73
Meat	
Beef	376 ± 2.63
Chicken	89 ± 0.11
Milk and milk products	
Whole fat dry milk	116 ± 0.07
Non fat dry milk	257 ± 1.13
Ice cream, chocolate	228 ± 1.77
Cream	68 ± 0.06
Spread, pasteurized, canned cheese	252 ± 1.04
Juices	
Orange juice	33 ± 0.11
Fruit mixture with vitamin C fortified	41 ± 0.68
Vegetables	
Cabbages, Chinese, raw	140 ± 2.30
Cabbage, red, raw	138 ± 3.60
Cabbage, white	135 ± 2.63
Orion, green, raw	206 ± 1.01
Peas, green fresh	3.30 ± 0.98
Potato	290 ± 3.18
Turnips, raw	110 ± 0.63
Turnips, cooked	67 ± 0.05

All samples were analyzed in triplicate

Marier (1990) reported that processing of foods contributes to decrease in magnesium content. Seelig (1964) recommended a daily magnesium intake of 6 mg/kg. So our diet should contain that required amount of magnesium but one of the factor that contributes to a lowered dietary intake of magnesium is the culinary preparation of food items during which 17 to 75% of the magnesium is removed (Marier 1990).

Magnesium content in serum of patients with reported cardiovascular disease

The magnesium content was low (range: 0.17-1.57mg/dL) in the serum of all age group of patients with reported cardiovascular disease as compared to normal persons (range: 1.50-1.87 mg/dL) (Table 3). Isselbacker *et al.* (1994) and Schamroth (1990) stated that cardiovascular effects of magnesium involved myocardial conduction and contraction. Its deficiency causes mechanical failure of heart. Aerobic oxidation of fatty acids, glucose and the synthesis of ATP in mitochondria provide energy in myocardial cells. Heart mitochondria contain the specific mitochondria isoenzyme of creatinine phosphokinase which plays an important role in intercellular energy transfer from ATP of the intermembrane space of mictochondria to cytoplasmic creatin with the formation of creatine phosphate. Magnesium ions play a regular role in the creatine phosphokinase reaction.

Seelig and Heggtveit (1974) and Manthey *et al.* (1981) reported low serum magnesium concentration in heart patients than the healthy normal persons. According to Seelig (1964) only 3% would be in negative magnesium balance, whereas low magnesium content induces cardiac necrosis and enhances susceptibility to cardiotoxic agent.

Magnesium content in serum of patients with reported high blood pressure disease.

Magnesium content was low (range; 0.25- 1.59 mg/dL) in patients as compared to normal persons (Table-3). Altura *et al.* (1984) and Rayssiguier *et al.* (1992) reported that deficiencies in serum magnesium were associated with high blood pressure. Magnesium is essential for oxidative phosphorylation and necessary for the replenishment of ATP to maintain strength of muscular contraction.

Magnesium ions influence the activity of the heart, it has been used for the stabilization of cardiac rhythmicity as well as for the regulation of blood pressure (Isselbacker *et al.* 1994, Schamroth 1990).

CONCLUSION

Magnesium ions are essential for maintenance of functional and structural integrity of myocardium and other related metabolic functions, Results indicate that cereals, enriched and baked desserts and wheat products etc. are rich sources of magnesium as compared to milk products, meats and vegetables. But a high percentage of magnesium is lost during the culinary preparation of food items. Thus inadequate dietary magnesium intake can cause diseases like cardiovascular and high blood pressure - So it is recommended that high magnesium food stuffs or magnesium supplements should be administered in daily intakes to reduce the disease risk factor.

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Physico-chemical changes in apples during different stages of maturity

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ABSTRACT

Nutritional quality and post-harvest losses in apples at different stages of maturity were studied. Total post-harvest losses reduced from 18 to 13% due to modernization of harvesting methods, better transportation and storage facilities. The maximum losses were observed at the retailer's shop, which were due to improper storage facilities. Physical characteristics i.e. shape, size, colour and taste of Amri, Kandhari, Kulu, Mashadi, Golden Delicious and Red Delicious apples showed variations. The shape and size varied from round oblique to round oblong. All the varieties had tough skin and sweet taste except Kulu. Proximate composition of apples at different stages of maturity showed that protein, fibre, ash, acidity, non-reducing sugars and ascorbic acid decreased whereas reducing sugars, total sugars, vitamin-A and mineral contents increased with maturity

Keywords: Apples, maturities, minerals, vitamins.

INTRODUCTION

Apple (*Pyrus malus*) is widely grown and consumed all over the world. The total area under cultivation in Pakistan is 47.70 thousand hectares with a total annual production of 315.40 thousand tonnes (GOP 2004). Good conservation of fresh produce is especially important because proportion of fruits, which is processed is very low. Aslam and Khan (1983) reported that post-harvest losses of 20 to 50% in perishable fruits and vegetables were due to inappropriate handling, transportation and storage facilities. Thus the total produce seldom reaches the consumer with full nutritive value. Salunkhe *et al.* (1974), Sehghal *et al.* (1975), Johson *et al.* (1984) and Khan *et al.* (1990) reported that apples and its juice were used by people of all ages for its high nutritional value and instant energy source. Castillo *et al.* (1997) and Podsedek *et al.* (2000) reported that apples were rich source of soluble sugars, minerals and vitamins.

The present investigation was undertaken to ascertain the nutritional losses and physical changes that may occur during different stages of maturity of the fruit after harvesting.

MATERIALS AND METHODS

Production statistics

Production statistics of apples were taken from the Pakistan Statistical Year Book (2004). The post-harvest losses of apples from the field to the retailer's shop were estimated through questionnaire from personals involved in the production, distribution and marketing.

Procurement of Apples

Six commercially important varieties of apples (Amri, Kandhari, Kulu, Mashadi, Golden Delicious and Red Delicious) were procured directly from the fields, orchids and wholesale market, brought to the laboratories and categorized as immature, mature and over-mature by visual examination. The damaged, soften and diseased fruits were discarded. The standard sized, sound fruits of different maturity were then analyzed for moisture, protein, fat, fibre, ash, acidity, carbohydrates, reducing and non-reducing sugars, calcium, iron and phosphorus according to AOAC (1999). Vitamin-C (ascorbic acid) was determined according to the method described by Bijaj and Gurdeep (1981). Vitamin-A was estimated by the method of Graig *et al.* (1960).

Statistical Analysis

The data obtained were analyzed statistically according to Steel *et al.* (1996).

RESULTS AND DISCUSSION

The total area under cultivation of apples during 2002-2003 was 47.70 thousand hectares with a total production of 315.4 thousand tonnes (Table 1).

Table 1. Production of apples in Pakistan

Year	Area under cultivation "000" hectares	Production in "000" tonnes
1980-81	11.40	99.20
1981-82	11.90	107.40
1982-83	12.90	114.10
1983-84	13.30	128.60
1984-85	14.80	128.10
1985-86	17.40	142.60
1986-87	18.60	166.00
1987-88	19.20	182.40
1988-89	21.80	215.10
1989-90	22.40	232.40
1990-91	22.80	243.00
1991-92	27.80	295.30
1992-93	31.40	339.00
1993-94	39.50	442.40
1994-95	40.40	533.10
1995-96	41.80	553.50
1996-97	43.50	568.50
1997-98	44.60	573.10
1998-99	45.90	589.30
1999-2000	51.70	377.30
2000-2001	58.20	438.90
2001-2002	48.70	367.20
2002-2003	47.70	315.40

Post-Harvest Losses

The post harvest losses in apples are experienced all over the world but the extent varies. The post harvest losses in appleas during harvesting, transportation and storage are reported in Table-2. The maximum losses in apples from field to retailer's shop were 12–18% during 1986. The losses decreased to 9–13% in 2003. The decreases in post-harvest losses are due to improvement in harvesting methods, better transportation facilities and improved packaging conditions. The results conforms with the findings of Coursey (1983) who reported that over-all losses of perishable plant fruits were extremely high and losses in developing countries were between 10–30%, which varied with commodity, location, and specific storage conditions. The maximum post-harvest losses 4.0 – 5.0% were at the retailer's shop during 1986. The conditions at the retailer's shop have also improved over the passage of time, thus reducing these losses to 3.0 – 3.5%. The results are comparable to the findings of Coursey (1983) and Skrupskis *et al.* (2000). Awad *et al.* (2000) reported that post-harvest losses of fruits could be reduced by better facilities of transportation and storage at low temperature.

Physical Characteristics

Different varieties of apples are produced in Pakistan. The prominent varieties i.e. Amri, Kandhari, Kulu, Mashadi, Golden Delicious and Red Delicious were studied (Table-3). Their shape and size varied from round oblique to round oblong and number of fruits per Kilogram ranged from 6–8. All the varieties had thin, tough skin and sweet taste except Kulu, which had thick skin and was slightly acidic in taste. A wide variation in colour was also observed ranging from green to yellow to red (Table-3).

Chemical Composition

Six commercial varieties of apples at different stages of maturity were analysed (Table-4). The under-ripe apples contained moisture (69.20 to 72.60%), fibre (12.70 to 14.60%), protein (2.50 to 3.40%), ash (2.20 to 2.70%) and acidity (7.10 to 9.20%). Except moisture, all values were higher than ripe and over-ripe apples. Non-reducing sugars decreased with maturity from 49.80 to 32.60% which was accompanied by an increase in reducing sugars from 26.10 to 46.40%. The increase may be due to the conversion of non-reducing sugars to reducing sugars or due to enzymic hydrolysis of fibrous materials in the ripe and over-ripe apples. Ketiku (1973) and Athanasopoulos *et al.* (2000) reported similar results in three cultivars of unripe, full-ripe and over-ripe

Table 2. Post-harvest losses in apples from field to retailer's shop

Years	Post-harvest losses % during						Total loss %
	Harvesting	Storage in the feld	Loading and transportation	Whole sale market	Transportation to retailer's shop	Retailer's shop	
1986	2.00 - 3.00	1.00 - 2.00	2.00 - 3.00	2.00 - 3.00	1.00 - 2.00	4.00 - 5.00	12.00 - 18.00
1995	2.00 - 3.00	1.00 - 2.00	1.00 - 2.00	2.00 - 2.50	1.00 - 2.00	4.00 - 4.50	11.00 - 16.00
2003	2.00 - 3.00	1.00 - 1.50	1.00 - 1.50	1.00 - 2.00	1.00 - 1.50	3.00 - 3.50	9.00 - 13.00

Table 3. Physical characteristics of commonly grown varieties of apple

Sr. No.	Variety	Shape	No./Kg	Skin	Colour	Seeds	Taste	Remarks
1.	Amri	Round conical-oblique	8	Thin very tough	Pale greenish	3 sided, oblong ovate	Sweet	Very good quality suitable for export
2.	Kandhari	Roundish turncate	6	Thin tough	Greenish to pale greenish	Flat, long ovate, smooth	Sweet without any trace of acidity	Good quality recommended for commercial plantation
3.	Kulu	Round conical to oblong conical	8	Thin tough	Pale greenish to waxen yellow	Large, plump, brown	Slightly sub-acidic sweet	Excellent keeping quality preferred for apple preserve and jellies
4.	Mashadi	Round oblique	8	Thin tough	Pale greenish	Light brown with acute apex	Sweet without any trace of acidity	Keeping quality fairly good
5.	Golden delicious	Oblate	6-8	Thin tough	Skin colour golden yellow covered with red stripes	Dark brown oblong	Sweet	Fine quality apple, good keeping quality
6.	Red delicious	Round oblong	6-8	Thin tough	Red in colour	Dark brown will acute apex	Sweet	Attractive variety for export

plantains. The acidity of under-ripe apples ranged from 7.10 to 9.20% and as the fruit ripens the amount of acidity decreases. Krishnaprakash *et al.* (1985) and Lopez *et al.* (2000) reported decrease in acidity of apples when harvested at different stages of maturity.

Vitamins and Minerals

Vitamins and minerals in apples at different stages of maturity are represented in Table-5. The ascorbic acid contents of un-riped apples ranged from 231.70 to 257.80 mg/100 g which decreased on ripening of the fruit. As the fruit ripens vitamin-A contents increases and the amount of ascorbic acid decreases. Kulu variety contained maximum amount of ascorbic acid (192.30 mg/100 g) and vitamin-A (0.96 mg/100 g), whereas it was minimum in case of Mashadi (Table-5). Golden Delicious contained maximum amount of vitamin-A (1.24 mg/100 g) at the over-ripe stage. The results are in agreement with the findings of Krishnaprakash *et al.* (1985) and Marcelle (1995) who reported that vitamin-A contents in all varieties of apples increase with maturity.

The minerals of apples increased with maturity (Table-5). The increase in calcium, iron and phosphorus contents was 29.70; 35.10 and 23.30 percent respectively. Golden Delicious variety (riped) contained the maximum amount of calcium (70.30 mg/100 gm), iron (9.40 mg/100 gm) and phosphorus (86.70 mg/100 gm). The variation in minerals at different stages of maturity are due to varietal differences and soil conditions. Present results are almost in agreement with the findings of Perring (1984).

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Table 4. Chemical composition of commonly grown varieties of apple at different stages of maturity

Sr.No.	Variety	Maturity Stage	Moisture (%)	Fibre (%)	Protein (%)	Ash (%)	Acidity (%)	Reducing sugar (%)	Non-reducing Sugar (%)	Total Sugars (%)
1.	Amri	Under-ripe	71.60 ± 1.10	14.60 ± 3.01	2.50 ± 0.98	2.40 ± 0.98	7.10 ± 1.11	26.10 ± 0.61	41.40 ± 1.67	67.50 ± 1.52
		Ripe	79.20 ± 0.98	7.90 ± 2.43	1.90 ± 0.71	1.80 ± 0.66	2.90 ± 1.35	34.30 ± 0.91	36.70 ± 1.55	71.00 ± 1.39
		Over-ripe	82.80 ± 1.02	7.10 ± 2.41	1.80 ± 0.32	1.70± 0.54	1.50 ± 1.27	36.70 ± 0.34	34.20 ± 1.32	70.90 ± 1.88
2.	Kandhari	Under-ripe	72.40 ± 2.65	13.70 ± 1.43	2.70 ± 0.46	2.30 ± 0.87	8.30 ± 1.89	26.90 ± 0.99	37.60 ± 1.46	64.50 ± 1.72
		Ripe	80.70 ± 2.40	7.30 ± 1.67	2.10 ± 0.57	1.70 ± 0.91	3.40 ± 1.55	33.60 ± 1.02	34.10 ± 1.11	67.70 ± 1.46
		Over-ripe	81.30 ± 1.98	6.20 ± 1.98	1.90 ± 0.43	1.60 ± 0.85	1.80 ± 1.63	35.30 ± 1.09	32.60 ± 1.01	67.90 ± 1.45
3.	Kulu	Under-ripe	69.90 ± 1.67	12.90 ± 1.55	2.80 ± 0.49	2.20 ± 0.32	9.20 ± 2.01	28.70 ± 0.32	47.60 ± 1.07	76.30 ± 1.33
		Ripe	80.80 ± 1.51	6.40 ± 1.50	1.90 ± 0.46	1.60 ± 0.61	4.10 ± 0.98	36.20 ± 1.76	43.10 ± 2.02	79.30 ± 1.67
		Over-ripe	82.60 ± 1.73	5.10 ± 1.70	1.70 ± 0.77	1.50 ± 1.07	2.40 ± 0.67	40.60 ± 2.01	39.20 ± 1.11	79.80 ± 1.69
4.	Mashadi	Under-ripe	71.80 ± 1.65	12.70 ± 1.69	2.60 ± 0.63	2.50 ± 0.98	7.80 ± 0.77	27.60 ± 1.77	44.60 ± 1.65	72.20 ± 1.31
		Ripe	82.60 ± 1.33	6.20 ± 1.34	1.80 ± 0.98	1.70 ± 1.15	2.80 ± 0.58	38.20 ± 1.53	41.80 ± 1.34	80.00 ± 1.27
		Over-ripe	84.00 ± 1.98	5.00 ± 1.87	1.50 ± 0.21	1.60 ± 0.34	1.90 ± 1.19	41.30 ± 1.01	39.20 ± 1.44	80.50 ± 1.29
5.	Golden Delicious	Under-ripe	72.60 ± 2.70	13.30 ± 1.67	3.40 ± 0.56	2.70 ± 0.59	8.10 ± 1.57	28.60 ± 0.99	49.80 ± 1.21	78.40 ± 1.56
		Ripe	82.90 ± 1.99	8.20 ± 1.43	2.40 ± 0.73	1.90 ± 0.99	3.00 ± 1.47	44.30 ± 0.31	39.20 ± 1.43	83.50 ± 1.43
		Over-ripe	84.50 ± 1.47	6.20 ± 1.91	1.90 ± 0.89	1.80 ± 1.71	1.80 ± 1.32	46.40 ± 1.51	36.70 ± 1.47	83.10 ± 1.01
6.	Red Delicious	Under-ripe	69.20 ± 1.99	14.20 ± 1.43	3.20 ± 0.77	2.40 ± 1.34	7.90 ± 1.02	27.70 ± 1.27	38.90 ± 1.21	66.60 ± 1.91
		Ripe	82.10 ± 2.01	8.40 ± 1.67	2.20 ± 0.31	1.70 ± 0.75	3.20 ± 1.50	35.10 ± 1.35	36.60 ± 1.36	71.70 ± 1.40
		Over-ripe	83.30 ± 2.43	6.80 ± 1.50	2.00 ± 0.29	1.60 ± 0.34	2.00 ± 1.10	38.60 ± 1.66	34.30 ± 1.47	72.90 ± 1.52

* Average of triplicate determinations
± Standard deviation values

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Table 5. Vitamin and mineral profile of commonly grown apples at different stages of maturity*

Sr. No.	Variety	Maturity Stage	Ascorbic Acid (mg/100 g)	Vitamin A (mg/100 g)	Calcium (mg/100 g)	Iron (mg/100 g)	Phosphorus (mg/100 g)
1.	Amri	Under-ripe	231.70 ± 0.67	0.76 ± 0.07	49.50 ± 1.11	6.20 ± 1.49	67.30 ± 2.03
		Ripe	172.30 ± 0.59	0.92 ± 0.34	67.30 ± 1.36	7.80 ± 1.73	77.90 ± 1.41
		Over-ripe	97.80 ± 1.43	1.10 ± 0.91	68.60 ± 1.49	8.10 ± 1.87	78.20 ± 2.39
2.	Kandhari	Under-ripe	242.60 ± 0.66	0.81 ± 0.11	58.90 ± 1.57	6.40 ± 1.63	68.20 ± 1.89
		Ripe	178.30 ± 0.34	0.93 ± 0.67	67.80 ± 1.32	8.20 ± 1.54	82.40 ± 1.99
		Over-ripe	102.90 ± 1.21	1.05 ± 0.43	68.20 ± 1.89	8.50 ± 1.99	83.60 ± 1.87
3.	Kulu	Under-ripe	257.80 ± 1.11	0.78 ± 0.41	54.90 ± 1.77	7.10 ± 2.10	70.10 ± 1.36
		Ripe	192.30 ± 2.01	0.96 ± 0.59	66.80 ± 1.54	8.70 ± 1.34	84.30 ± 1.77
		Over-ripe	120.70 ± 0.99	1.20 ± 0.32	67.10 ± 1.89	8.20 ± 1.60	85.20 ± 1.59
4.	Mashadi	Under-ripe	247.30 ± 0.86	0.77 ± 0.46	57.00 ± 1.32	6.30 ± 1.87	69.80 ± 1.93
		Ripe	165.40 ± 1.55	0.91 ± 0.69	64.50 ± 1.22	8.60 ± 1.39	85.60 ± 1.84
		Over-ripe	101.50 ± 1.32	1.08 ± 0.31	64.90 ± 2.06	8.70 ± 1.99	86.10 ± 1.69
5.	Golden Delicious	Under-ripe	249.70 ± 1.04	0.83 ± 0.29	61.80 ± 1.76	6.80 ± 1.07	68.80 ± 1.46
		Ripe	183.50 ± 1.87	0.95 ± 0.99	70.30 ± 1.57	9.40 ± 1.48	86.70 ± 1.82
		Over-ripe	107.20 ± 1.39	1.24 ± 0.81	71.00 ± 1.73	9.70 ± 1.06	87.40 ± 1.79
6.	Red Delicious	Under-ripe	237.40 ± 1.59	0.82 ± 0.74	56.90 ± 1.80	6.10 ± 1.64	72.60 ± 1.44
		Ripe	177.90 ± 1.62	0.93 ± 0.65	65.80 ± 1.49	8.80 ± 1.49	84.80 ± 1.99
		Over-ripe	99.40 ± 1.09	1.01 ± 0.97	66.00 ± 1.39	9.10 ± 1.88	85.60 ± 1.21

* Average of triplicate determinations
Standard deviation values

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