

## Effect of rice bran supplementation on quality of bread

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### ABSTRACT

This project was designed to evaluate the suitability of processed and treated rice bran for the supplementation of bread. Freshly milled rice bran was treated with acetic acid and cooked by passing through an extruder cooker. The treated and extruded rice bran was supplemented @ 5, 10, 15, 20, 25 and 30% in wheat flour for the production of bread. The bread was analyzed for different physicochemical parameters and subjected to sensory evaluation. The results showed an increase in crude protein from 11.87 to 12.94%, crude fat from 3.64 to 8.63%, crude fiber 0.62 to 2.15% and ash 1.52 to 4.18%. The sensory evaluation showed significant ( $P \leq 0.01$ ) differences in the scores for volume, color of crust, symmetry of form and character of crust. However, evenness of bake showed non-significant differences among various (PRB) breads. The breads supplemented with PRB @ 15% increased the sensoric scores. It can be concluded from the results that up to 15% PRB can be successfully incorporated in the bread to improve the sensoric and nutritional attributes.

**Key words:** Rice bran, PRB (Processed Rice Bran), supplemented bread, chemical composition, external characteristics and internal characteristics,

### INTRODUCTION

Rice bran is a good source of protein, lipids, dietary fiber, vitamins and minerals. The amino acid profile of rice bran has been generally reported to be superior to cereal grain proteins (Farrell 1994). The protein of rice bran is relatively of high nutritional value. As far as its nutrition is concerned, the protein efficiency ratio (PER) of rice bran has been reported to be in the range of 1.6 to 1.9 as compared to casein value of 2.5 while for its protein concentrates. PER values ranged from 2.0 to 2.5. The digestibility of rice bran protein has been found to be 73% as compared to 90% digestibility denoted for its protein concentrate. It is a good source of lysine and methionine and can be an effective tool to supplement the lysine and methionine deficient foods such as wheat, maize and sorghum to overcome the malnutrition problem prevailing among masses (Dale 1997).

Most recently nutraceutical properties of the rice bran are mainly used for poultry or animal feed as low quality ingredient. Many researchers have added rice bran to wheat flour at 15-30% in yeast bread and concluded that rice bran can be supplemented successfully up to 15% replacement level without affecting loaf weight, height or volume (Sharp and Kitchen 1990). Defatted rice bran increases dough yield, contributes to an attractive tan crumb and crust, does not disturb fermentation or mixing tolerance of dough, causes baked products to remain fresher,

more moist and adds significant amino acids, minerals and vitamins to baked goods (Lynn 1969). Hargrove (1990) discusses production, functional properties of rice bran and its applications in baked goods, pan cakes, cereals, granola type bars, snacks and extruded foodstuffs. Lima *et al.* (2002) highlights the functional properties of bread made with processed full fat and defatted rice bran and found it suitable for bread.

This situation demands that this healthy food ingredient may be incorporated in the daily diet. Hence the project was designed to find out the effect of rice bran supplemented wheat flour samples on the quality of bread.

### MATERIALS AND METHODS

#### Raw Materials

Rice bran of Basmati-385 was collected from Reem Rice Mills (Pvt.) Limited, Muridke, Sheikhupura, Pakistan soon after the milling. The wheat flour (straight grade flour) and other ingredients were purchased from local market.

#### Processing of Rice Bran

The processing of rice bran was carried out to inactivate their anti-nutritional factors without damaging the protein quality of rice bran. To achieve these objectives, the rice bran was mixed with 20% (w/v)

solution of 1% acetic acid. The rice bran was further subjected to physical treatments by passing through an extruder cooker maintained at a temperature of  $130\pm 2^\circ\text{C}$  for 10-15 sec. All the samples were dried to a moisture level less than 10% by using hot air oven.

#### Preparation of Supplemented Bread

The straight grade wheat flour was supplemented with processed rice bran (PRB) @ 5, 10, 15, 20, 25 and 30% according to following treatments

Treatments	Level of replacement
T <sub>1</sub>	Control
T <sub>2</sub>	5% PRB
T <sub>3</sub>	10% PRB
T <sub>4</sub>	15% PRB
T <sub>5</sub>	20% PRB
T <sub>6</sub>	25% PRB
T <sub>7</sub>	30% PRB

The bread was prepared by straight dough method from the processed rice bran supplemented flour samples according to the method of AACC (2000). The bread was evaluated for external and internal characteristics subjectively by a panel of 5 trained judges following the instructions of Larmond (1977).

#### Chemical Composition

The chemical composition of bread was tested for moisture, crude protein, crude fat, crude fiber, ash content and nitrogen free extract (NFE) according to the methods described in AACC (2000).

## RESULTS AND DISCUSSION

#### Chemical Composition of Bread

The processed rice bran (PRB) @ 5, 10, 15, 20, 25 and 30% was incorporated in wheat flour for the preparation of bread. The results obtained were tabulated and subjected to statistical analysis using analysis of variance techniques (Table 2).

The mean values for different chemical constituents have been presented in Table 1. The data revealed significant ( $P\leq 0.01$ ) differences existed among various treatments in crude protein, crude fat, crude fiber, ash and NFE. This indicated that inclusion of processed rice bran (T<sub>1</sub>-T<sub>7</sub>) at various proportions to wheat flour affected the proximate composition of breads (T<sub>1</sub>-T<sub>7</sub>), which were significantly different among each other.

**Table 1. Chemical composition of various processed rice bran supplemented breads**

Treatments	Crude Protein %	Crude Fat %	Crude Fiber %	Ash %	NFE %
T <sub>1</sub>	11.87e	3.64f	0.62e	1.52e	82.35a
T <sub>2</sub>	12.08de	4.31e	0.88de	2.13de	80.60ab
T <sub>3</sub>	12.29cd	5.47d	1.13cd	2.40d	78.71b
T <sub>4</sub>	12.51bc	6.35c	1.39bc	2.85cd	76.90b
T <sub>5</sub>	12.72ab	7.25b	1.65b	3.29bc	75.09b
T <sub>6</sub>	12.81ab	8.14a	1.96a	3.76ab	73.33c
T <sub>7</sub>	12.94a	8.63a	2.15a	4.18a	72.10c

Mean values sharing similar letters in a column are not significantly different

**Table 2. Mean squares for chemical composition of various processed rice bran supplemented breads**

Source	d.f.	Crude Protein	Crude Fat	Crude Fiber	Ash	NFE
Treatments	6	0.472**	10.715**	2.638**	0.945**	44.217**
Error	14	0.043	0.121	0.199	0.029	1.712

\*\* =  $P\leq 0.01$

The comparison of means by using Duncan Multiple Range Test techniques in Table 1, showed that the protein content of bread, in which 30% of PRB was incorporated (T<sub>7</sub>), showed significantly higher protein content (12.94%) than all the other breads (T<sub>1</sub>-T<sub>6</sub>), while T<sub>1</sub> showed significantly the lowest protein content (11.87%), where no PRB had been incorporated in bread. The addition of PRB to wheat flour increased the protein content from 11.87 to 12.94%. There were non-significant differences among T<sub>5</sub> (12.72%), T<sub>6</sub> (12.81%) and T<sub>7</sub> (12.94%) while T<sub>4</sub> (12.51%) was non-significant with T<sub>3</sub> (12.29%) and significantly different from T<sub>2</sub> (12.08%) and T<sub>1</sub> (11.87%) for protein content.

The addition of PRB to wheat flour increased the fat content of breads by 18.41, 50.27, 74.45, 99.18, 123.63 and 137.09%, respectively. Crude fat was significantly higher in T<sub>7</sub> and significantly lower in T<sub>1</sub> (control). Comparison of mean values (Table 1) for crude fat showed a progressive increase in the fat content on supplementation of PRB to wheat flour. The fat content was statistically significant among all treatments except T<sub>6</sub> and T<sub>7</sub> where it was non-significant. This increase in fat content was due to higher amount of fat in PRB than wheat flour.

The CF of bread increased from 0.62 to 2.15%. The comparison of mean values revealed statistically non-significant differences between T<sub>6</sub> & T<sub>7</sub> and T<sub>1</sub> & T<sub>2</sub>. Ash content of any food indicates the amount of minerals in it. The wheat flour contained 1.52% ash. On inclusion of PRB to wheat flour the ash content of

bread increased (Table 1) which was significantly higher in T<sub>7</sub> and significantly lower in T<sub>1</sub>.

Inclusion of PRB to wheat flour decreased the NFE of breads. Comparison of the mean values showed that amount of NFE was significantly higher in wheat flour (T<sub>1</sub>) bread than the PRB supplemented breads. The breads containing 5, 10, 15 and 20% PRB showed statistically non-significant differences for NFE but these were significantly different from all other breads. The mean NFE values of T<sub>6</sub> and T<sub>7</sub> were also found to be non-significantly different (Table 1).

The results of the present study are in line with the investigation of Taha *et al.* (1982) who worked on enrichment of wheat bread with rice bran. They concluded that protein content of bread increased significantly by the supplementation of rice bran to wheat flour as it is observed in the present study. Lynn (1969) reported that rice bran may be added at levels of 5 to 15% in various baked products (bread and cookies). It improved the lysine content of bread and gave a bland flavor and adds significant amount of amino acids, minerals and vitamins to baked goods.

The results regarding crude fat resemble with the earlier findings of Hussain (1985) who reported that the crude fat in pan bread was 1.2%. Zia-ul-Haq (2001) further supported the present investigation, who accomplished that commercial branded and unbranded breads contained fat content from 1.50 to 1.60% (as such basis).

The results correlate with the findings of Hussain (1985) who determined that pan bread manufactured from white flour had 1.5% crude fiber. Which is an excellent source of dietary fiber as reported by Sultan (1969). Krishnan *et al.* (1987) substituted oat bran with wheat flour and concluded that CF increased significantly by its supplementation. So the fiber content of bread increased by the incorporation of rice bran.

In the recent studies, Sharma and Chauhan (2002) substituted rice bran with wheat flour in proportions of 5-20% in breads and concluded that by the addition of rice bran to wheat flour increased the contents of proteins, lysine and dietary fiber in proportion to the level of supplementation. The CF contents of present study showed that dietary fiber was quite in range required for health.

The results of the present study also favor the conclusions made by Holland *et al.* (1991) who investigated that wheat flour with 70% extraction had 0.44% ash as compared to 0.92% in 85% extraction. In a similar study, Akhtar (1993) found 1.11% ash

content in 1% guar bread. In the present study ash content increased due to incorporation of rice bran which has higher quantity of ash (Saunders, 1990)

Hussain (1985) reported 51.5% NFE in pan bread. Later on Akhtar (1993) studied NFE (46.12%) in proximate composition of pan bread having 1% guar gum. The data was expressed on as such basis but in this study the results have been presented on dry basis which indicate some differences in results. The NFE found in the present studies is in the higher limits of Hussain (1985) and Akhtar (1993) which might be due to the incorporation of PRB.

## SENSORY EVALUATION OF BREAD

### External Characteristics of Bread

The data on sensory evaluation of external characteristics i.e. volume, color of crust, symmetry of form, evenness of bake and character of crust of experimental breads prepared by incorporation of PRB to wheat flour at levels of 0, 5, 10, 15, 20, 25 and 30% (T<sub>1</sub>-T<sub>7</sub>) were statistically analyzed, using analysis of variance techniques. The results showed significant (P<0.01) differences in the scores for volume, color of crust, symmetry of form and character of crust (Table 4). However, evenness of bake showed statistically non-significant differences among various PRB breads (T<sub>1</sub>-T<sub>7</sub>).

The scores assigned to volume of bread ranged from 6.63 to 8.31 among different treatments. The data presented in Table 5 showed that significantly higher scores were given to volume of bread prepared from 15% PRB (T<sub>4</sub>) followed by T<sub>3</sub>, T<sub>5</sub>, and T<sub>2</sub>. The breads of treatments T<sub>2</sub>, T<sub>3</sub> and T<sub>5</sub> yielded statistically non-significant difference in volume. Similarly the scores for T<sub>1</sub>, T<sub>6</sub> and T<sub>7</sub> were also non-significant among each other. The scores given to volume of bread was significantly lower in T<sub>7</sub> followed by T<sub>6</sub> and T<sub>1</sub> (Table 3).

**Table 3. Means for external characteristics of various processed rice bran supplemented breads**

Treatments	Volume	Color of crust	Symmetry of form	Evenness of bake	Character of crust
T <sub>1</sub>	6.81c	5.47ab	7.47bcd	8.48a	6.79abc
T <sub>2</sub>	7.25b	5.98a	8.11abc	8.71a	7.38ab
T <sub>3</sub>	7.58b	5.81a	8.52ab	8.54a	7.56a
T <sub>4</sub>	8.31a	5.84a	8.78a	8.66a	7.69a
T <sub>5</sub>	7.56b	4.83bc	7.51bcd	8.11a	6.85abc
T <sub>6</sub>	6.72c	4.75cd	7.23cd	8.03a	6.13bc
T <sub>7</sub>	6.63c	4.11d	6.82d	8.01a	5.84c

Mean values sharing similar letters in a column are not significantly different

The scores assigned to crust color of various breads ranged between 4.11- 5.98. The minimum scores for crust color were attained by the bread prepared from T<sub>7</sub> (30% PRB) and maximum scores were given to bread of T<sub>2</sub> (5% PRB). The scores for crust color were significantly higher of bread prepared from T<sub>2</sub> followed by T<sub>4</sub>, T<sub>3</sub> and T<sub>1</sub>, but these possessed non significant differences with each other. The scores for crust color was significantly lower in bread prepared from T<sub>7</sub> followed by T<sub>6</sub> but both of these treatments showed non significant difference for crust color.

The scores assigned by judges for symmetry of form ranged from 6.82 to 8.78 among breads of different treatments. The symmetry of form got significantly higher scores of bread prepared from T<sub>4</sub> (15% PRB) followed by breads of T<sub>3</sub> (10% PRB) and T<sub>2</sub> (5% PRB). The differences in symmetry of form scores among these treatments were found to be non significant between breads of T<sub>3</sub> and T<sub>2</sub>. However, significantly the lowest scores for symmetry of form were given to bread of T<sub>7</sub> (30% PRB) followed by breads of T<sub>6</sub> (25% PRB) and T<sub>5</sub> (20% PRB). The differences in scores of symmetry of form of bread among these three treatments were not significantly different.

The scores for evenness of bake of bread were statistically non-significant among different breads prepared from various treatments, indicating that incorporation of PRB up to 30% have no significant effect on evenness of bake of bread.

The scores given to breads for character of crust ranged from 5.84 to 7.69 (Table 3). While comparing the mean values for character of crust showed significantly the highest scores by bread of T<sub>4</sub> but it showed non-significant differences with respect to scores given to character of crust of breads prepared from T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>5</sub>. The scores assigned to character of crust were significantly the lowest of bread prepared from treatment T<sub>7</sub> followed by T<sub>6</sub>. This indicated that the character of crust was not affected to a great extent by incorporation of PRB to wheat flour up to 20%.

In the present study, the findings are comparable to those reported by Sharp and Kitchen (1990) who found that rice bran can be replaced up to 15% in white flour breads. Later on Sharma and Chauhan (2002) substituted rice bran with wheat flour and concluded that at higher proportion of rice bran substitution, bread loaf volume decreased and it is comparable with the present results which indicated

that by addition of 25 and 30% rice bran, the loaf volume decreased as compared to control T<sub>1</sub> where 100% wheat flour was used for bread preparation. The results of this study also resembles with the findings of Lima *et al.* (2002) who reported that functional properties of bread manufactured with supplementation of processed full fat rice bran for 10 and 20% replacement flour with PRB, the bread loaf volume increased 2% as compared to control and decreased 6% but in this investigation, loaf volume increased maximum for 15% replacement of wheat flour with PRB and decreased to minimum for 30% substitution.

During judging of baked bread, color of crust is very important criteria which provides information about bread raw material, its formulation and quality. For good product appeal or marketing, golden brown crust color is liked by the consumers.

The results for color of crust are in concordance with the findings of Lynn (1969) who concluded that defatted rice bran increased dough yield, contributed to an attractive tan crumb and crust. It did not disturb mixing or formation process of dough. By its substitution, baked products remained fresher with more moist condition. It also added amino acids, minerals and vitamins to baked goods.

The findings for symmetry of form, evenness of bake and character of crust are comparable to those reported by Taha *et al.* (1982) who worked on bread enrichment with rice bran and found that sensoric parameters were acceptable by its supplementation in wheat bread.

#### Internal Characteristics of Bread

The data on sensory parameters of internal characteristics (aroma, grain, color of crumb, taste and texture) of various breads prepared from wheat flour, supplemented with PRB, at 5, 10, 15, 20, 25 and 30% (T<sub>1</sub>-T<sub>7</sub>) levels were assessed by a panel of trained judges. The data were tabulated and analysis of variance techniques were applied and the results are given in Table 6.

The results for aroma and color of crumb showed statistically significant differences at  $P \leq 0.01$  percent level among various treatments i.e. T<sub>1</sub> to T<sub>7</sub>. Whereas the grain size, taste and texture were statistically significant at  $P \leq 0.05$  percent level. This indicated that all the internal characteristics based on sensory evaluation were significantly affected by the supplementation of wheat flour with PRB.

**Table 4. Mean squares for external characteristics of various processed rice bran supplemented breads**

Source	df	Volume	Color of crust	Symmetry of form	Evenness of bake	Character of crust
Treatments	6	1.099*	1.484**	1.525**	0.207 <sup>NS</sup>	1.510*
Error	14	0.319	0.150	0.316	0.259	0.491

\* =  $P \leq 0.05$ \*\* =  $P \geq 0.01$ 

NS = Non significant

The results in Table 5 showed that scores assigned to crumb color of bread were significantly the highest when wheat flour was supplemented with 0, 5, 10 and 15% PRB. However, these treatments were statistically identical with respect to crumb color of bread. The breads prepared from wheat flour supplemented with 20, 25 and 30% were ranked at the bottom with respect to crumb color.

**Table 5. Means for internal characteristics of various processed rice bran supplemented breads**

Treatments	Color of crumb	Aroma	Grain	Taste	Texture
T <sub>1</sub>	6.25ab	7.81a	8.17abc	6.18ab	8.13ab
T <sub>2</sub>	7.81a	8.03a	8.81ab	7.03a	8.55a
T <sub>3</sub>	7.42a	8.31a	8.93a	7.09a	8.97a
T <sub>4</sub>	7.31a	8.54a	9.11a	7.14a	8.92a
T <sub>5</sub>	5.15b	6.41b	7.23bc	5.84ab	6.73b
T <sub>6</sub>	5.02b	5.21c	6.95c	5.41b	6.64b
T <sub>7</sub>	4.95b	5.17c	6.78c	5.24b	6.55b

Mean values sharing similar letters in a column are not significantly different

**Table 6. Mean squares for internal characteristics of various processed rice bran supplemented breads**

Source	d.f.	Color of crumb	Aroma	Grain	Taste	Texture
Treatments	6	4.668**	6.336**	2.983*	2.080*	4.419*
Error	14	0.883	0.414	0.750	0.637	1.133

\* =  $P \leq 0.05$ \*\* =  $P \geq 0.01$ 

The scores assigned to aroma of bread ranged from 5.17 to 8.54. The significantly higher scores were assigned to the bread prepared from wheat flour with 15% supplemented PRB (T<sub>4</sub>) followed by 10% PRB (T<sub>3</sub>) and 5% PRB (T<sub>2</sub>). However, the differences among these treatments were non significant for aroma.

The scores assigned to aroma were significantly the lowest for the bread prepared from wheat flour supplemented with 30% PRB (T<sub>7</sub>) followed by supplemented with 25% PRB (T<sub>6</sub>). The differences

between these two treatments were also found to be non significant.

The results in Table 5 manifested that grain of breads prepared from wheat flour supplemented with 0, 5, 10 and 15% PRB got significantly higher scores than other breads. However, the scores for grain did not differ significantly among these breads.

The taste of bread prepared from various PRB supplemented breads ranged from 5.24 to 7.14 scores. Significantly the highest scores for taste was assigned to the bread supplemented with 15% PRB (T<sub>4</sub>) whereas significantly the lowest scores were assigned by the judges to the bread supplemented with 30% PRB (T<sub>7</sub>) followed by 25% PRB (T<sub>6</sub>) and 20% (T<sub>5</sub>). The differences for taste of bread scores among T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were found to differ non significantly.

The texture of bread prepared from wheat flour supplemented with 0, 5, 10 and 15% PRB got significantly higher scores. The scores given to texture of breads prepared from wheat flour supplemented with 20, 25 and 30% PRB were ranked at bottom. However, all these three treatments were statistically identical to control (0% PRB) with respect to texture of bread.

Krishnan *et al.* (1987) studied the effects of oat bran supplementation (10-15 %) in bread. Dietary fiber and protein increased significantly with oat bran supplementation up to 10%. Breads with 10% oat bran had better loaf volume, grain and texture than 15%. Absorption requirements in dough's increased with increase of bran levels. Aroma and taste of enriched rice bran bread increased with 2-acetyl-l-pyrroline (2AP) principal compound. Lima *et al.* (2002) reported that addition of 10% full fat rice bran to the bread had no detrimental effect on texture but a very slight hardening of the loaves occurred with 20% full fat rice bran when compared to the control i.e. texture profile analysis observed non-significant difference as far as cohesiveness and springiness but bread hardness, gumminess and chewiness increased with increased levels of rice bran and was higher for defatted rice bran bread than full fat rice bran bread.

The results of the present study are in close agreement to the above workers especially with Lima *et al.* (2002) who reported that up to 10% full fat rice bran showed no detrimental effect on bread characteristics. In the present study it has been observed that processed rice bran up to 15% did not show any negative effect on bread scores but showed improvement in sensory parameters than control.

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## Impact of storage on phytate content of fortified whole wheat flour

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### ABSTRACT

The present research was conducted to find out the changes in phytic acid (PA) during storage of iron and zinc fortified whole wheat flour. The whole wheat flour was fortified and packed in polypropylene woven bags and tin boxes. These were stored under controlled and ambient conditions for a period of sixty days to evaluate the effect of added forms of various iron and zinc sources on PA content. The results indicated that PA content decreased significantly from 1.55 to 0.88% in different fortified flours during the storage of 60 days. Packaging material however, did not affect the phytate content. Storage conditions also affected the PA content which remained higher in controlled storage as compared to ambient conditions. Iron and zinc fortificants decreased the PA content. Results of this experiment further indicated that PA content of whole wheat flour fortified with elemental iron stored under ambient conditions were less as compared to the flour, stored under controlled conditions. It is, therefore, concluded that iron and zinc fortificants decreased PA content in wheat flour during storage.

**Keywords:** Wheat flour, storage, phytic acid, fortification, iron, zinc

### INTRODUCTION

Iron and zinc deficiency is a serious global issue in the developing countries. In Pakistan, anemia has been recorded in 47% of the children and 30% of the adult females (Hamedani *et al.* 1987). The zinc deficiency has been reported in 54.2% pre school children (Paracha and Jamil, 2002). More than fifty percent of the total energy intake is derived from wheat flour Pakistan (OMNI, 1996). In its natural state, wheat is a good source of iron and zinc. Most of the minerals and vitamins are located in the bran. Though the bran is not removed in whole wheat flour and the milling losses of micronutrients are minimal but the phytic acid is also located mainly in the bran and its biodegradation products are well established chelators of iron and zinc.

Whole wheat flour contains phytate over 800mg /100g whereas enriched white flour (70-75% extraction rate) contained only 280mg phytate per 100g (Spiller 1993). The phytic acid in atta combines with minerals and makes it unavailable for absorption. (Sandberg *et al.* 1996). The amount of iron absorption is inversely related to the phytic acid content of flour (Brune *et al.* 1992).

An extensive research work has been carried out on its degradation through various processing methods

like soaking and fermentation. The inhibitory effects of phytic acid on the absorption of minerals through biological assay has also been well investigated but considerably fewer studies have focused on the fate of phytate and its interaction with extrinsic forms of minerals in stored fortified whole wheat flours to be used for making unleavened flat bread. The present research study was carried out to find out the changes in phytic acid content that may take place during storage of iron and zinc fortified whole wheat flour.

### MATERIAL AND METHODS

The whole wheat flour was produced from the wheat variety Inqulab 91. The wheat variety was procured from Post-Graduate Agricultural Research Station, University of Agriculture, Faisalabad. Four fortificants i.e. elemental iron, NaFeEDTA, zinc oxide and zinc sulfate were used as source of iron and zinc. The iron fortificants were obtained from Micronutrient Initiative (MI), Islamabad whereas zinc fortificants were received from Fortitech Inc., New York.

#### Level of fortification

The levels and combinations of fortificants used in this study are given as below:

Treatment	Fortificants	mg/kg	Treatment	Fortificants	mg/kg
T <sub>1</sub>	NaFeEDTA	40	T <sub>3</sub>	Elemental iron	40
	ZnSO <sub>4</sub>	20		Zn SO <sub>4</sub>	30
T <sub>2</sub>	NaFeEDTA	60	T <sub>4</sub>	Elemental iron	60
	Zn O	20		Zn O	30
T <sub>0</sub>	Control				

**Production of fortified whole wheat flour** The whole wheat flour was prepared through china Chakki equipped with micro feeder. The speed and feed rate of the china Chakki were adjusted and fortification process was carried out successfully.

#### Packaging and storage of fortified whole wheat flour

The fortified whole wheat flours were packed in polypropylene woven bags and tin boxes. The fortified flour was stored for a period of sixty days under controlled and ambient conditions with respect to temperature and relative humidity in these packing. A temperature of  $24 \pm 2^{\circ}\text{C}$  and relative humidity  $50 \pm 5\%$  were maintained in controlled storage conditions and during ambient storage conditions temperature remained  $40 \pm 2^{\circ}\text{C}$  and relative humidity ranged from 25 to 70 %. The temperature and relative humidity were recorded daily (morning & evening) both in controlled and ambient storage conditions.

#### Analytical Tests

The samples of fortified whole-wheat flours were analyzed for phytic acid content after every two weeks i.e. 0, 15, 30, 45 and 60 days. The phytic acid content in fortified whole wheat flour samples were determined by the method described by Haug and Lantzsch (1983). The solutions i.e. phytate reference solution, ferric solution and 2-2 Bipyridine solutions were prepared as required in the procedure. 0.5 ml of the extract was taken through pipette in the stoppered test tube. One ml of ferric solution was added and stoppered properly for heating in the water bath for 30 minutes. It was brought to room temperature after cooling in the ice water for 15 minutes. One ml of the supernatant was transferred in another test tube and added 1.5 ml of 2-2 Bipyridine solution in the test tube. Absorbance was recorded on spectrophotometer (Model CECIL-7200) at 519 nm against distilled water and phytic acid content was calculated accordingly through phytic acid standard curve.

#### Statistical Analysis

The data were analyzed statistically using analysis of variance technique. Four factor factorial experiment

was used as experimental plot as described by Steel *et al.* (1997). The values are given as means with their standard errors. Duncan's Multiple Range Test was applied to assess the difference between means (Duncan 1955).

## RESULTS AND DISCUSSION

The statistical results pertaining to phytic acid content of whole wheat flour samples (Table-1) showed that storage conditions, storage periods, and their interaction significantly affected phytic acid content of different fortified whole wheat flour samples. The treatments and its interaction with storage conditions had significant effect on the concentration of phytic acid in different fortified whole wheat flour samples. The results in Table 1 exhibited a highly significant interactive effect of storage period and treatment. Furthermore, interactive effect of storage conditions, storage periods and treatments was also found to be significant on the phytic acid content of the flour samples. The lowest phytic acid was found in whole wheat flour samples stored at ambient storage conditions as compared to controlled conditions (Fig. 1).

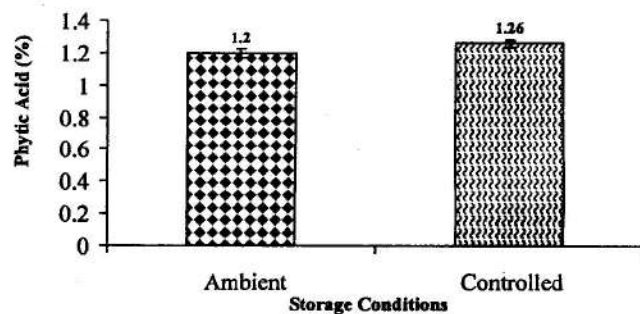


Fig. 1: Effect of storage conditions on phytic acid content of different whole wheat flours

The variation in storage temperature and relative humidity, the storage conditions affected phytic acid content as higher temperature and relative humidity promotes interaction of phytic acid with minerals and proteins present in the flour samples form insoluble complexes. As the temperature and relative humidity

ranged from 38 to 42°C and 25-70 %, respectively in ambient storage conditions, therefore, a significant change in phytic acid content was observed during storage ranging from 0.88 to 1.55 per cent (Fig-2).

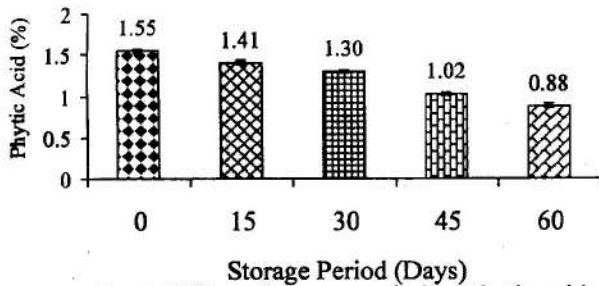


Fig. 2: Effect of storage period on phytic acid content of different whole wheat flours

A gradual decline in phytic acid content was observed during storage and this decline might be due to the interaction of various added salts, food components and to some extent the activity of native phytase with phytic acid over a certain time span. The treatment effect irrespective of storage conditions, storage periods and packaging material has been presented in Figure 3. It is obvious that phytic acid content of the fortified whole wheat flour samples was lower as compared to unfortified whole wheat flour samples. There was a significant effect of fortificant type and combination (Fig-3) as the treatments T<sub>1</sub> and T<sub>2</sub>

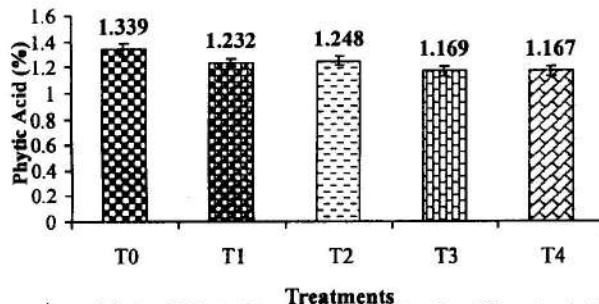


Fig 3 : Effect of treatments on phytic acid content of different whole wheat flours

differed significantly from treatments T<sub>3</sub> and T<sub>4</sub>. The phytic acid content in samples stored at controlled storage conditions was found to be significantly higher as compared to the flour samples stored at ambient conditions after 15 days of storage till the termination of the storage period (Fig-4).

The phytic acid degradation and its interaction with other elements might have been augmented under

ambient storage conditions where temperature and relative humidity was higher.

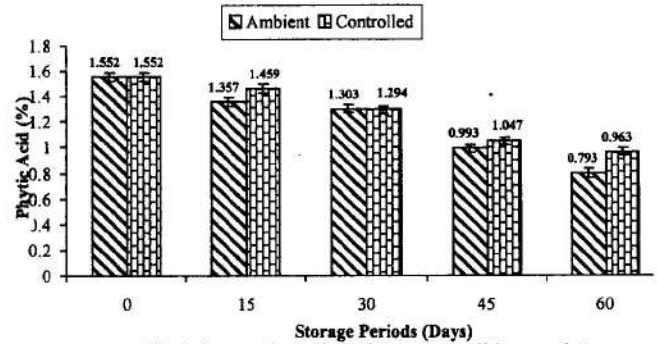


Fig 4: Interactive effect of storage conditions and storage periods on phytic acid content of different whole wheat flours

Table-1. Phytic acid content of different fortified whole wheat flours

Source of Variation	df	Sum of Squares	Mean Squares	F-Value
S.Condition(A)	1	0.301	0.301	11.3228**
Days (B)	4	18.483	4.621	173.7077**
AxB	4	0.333	0.083	3.1300**
Packaging(C)	1	0.007	0.007	0.2491 <sup>NS</sup>
AxC	1	0.006	0.006	0.2085 <sup>NS</sup>
BxC	4	0.013	0.003	0.1239 <sup>NS</sup>
AxBxC	4	0.019	0.005	0.1798 <sup>NS</sup>
Treatment(D)	4	1.196	0.299	11.2434**
AxD	4	0.350	0.087	3.2854**
BxD	16	0.711	0.044	1.6702**
AxBxD	16	1.079	0.067	2.5346**
CxD	4	0.049	0.012	0.4564 <sup>NS</sup>
AxCxD	4	0.186	0.046	1.7448 <sup>NS</sup>
BxCxD	16	0.286	0.018	0.6720 <sup>NS</sup>
AxBxCxD	16	0.451	0.028	1.0602 <sup>NS</sup>
Error	200	5.320	0.027	

\*\* Sig at P ≤ 0.01 \* Sig at P ≤ 0.05 <sup>NS</sup> Non-Sig. p < 0.05

The treatment effect was more pronounced when storage conditions were pooled (Fig-5).

However, the treatments T<sub>0</sub>, T<sub>1</sub> and T<sub>4</sub> did not affect the phytic acid content significantly in either storage condition. The treatments T<sub>2</sub> and T<sub>3</sub> were influenced by the storage conditions showing different behaviors.

The effect of packaging material was found to be significant on phytic acid content when the storage conditions were compared (Fig-6). The polypropylene.

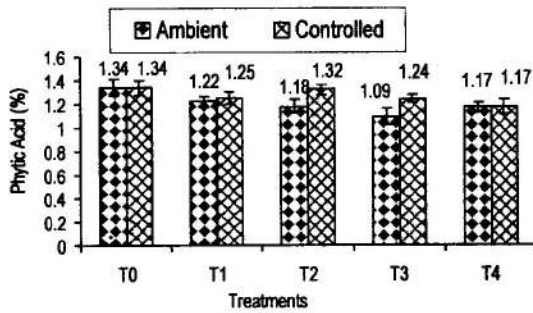


Fig 5 : Effect of storage conditions and treatments on phytic acid content of different whole wheat flours

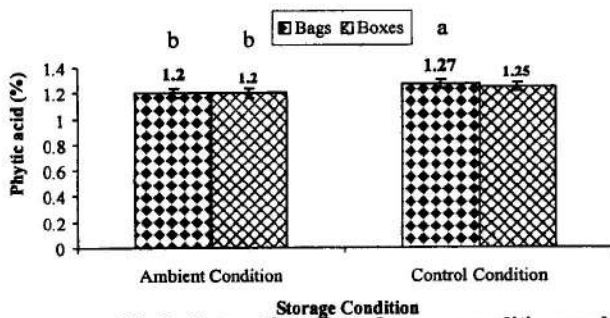


Fig 6 : Interactive effect of storage conditions and packaging materials on phytic acid content of different whole wheat flours

(elemental iron and zinc sulfate) at 60 days of storage period (0.82%). The treatment T<sub>3</sub> contained elemental iron which might have reacted with phytic acid biodegradation products more intensively as compared to rest of the fortificants.

Interactive effects of storage period, treatments and storage conditions have been shown in Table 3. The highest phytic acid content (1.73%) were observed in fresh fortified whole wheat flour samples stored under ambient storage conditions while the lowest phytic acid content (0.58%) was found in treatments T<sub>3</sub> at the termination of the storage period. It can be envisaged from the results that EDTA moiety in NaFeEDTA in treatments T<sub>1</sub> and T<sub>2</sub> might be acted as a chelating agent for minerals and did not let phytic acid to form complexes with iron in NaFe EDTA and other components of the flour.

INACG (1990) reported similar results stating that the major advantage of using NaFeEDTA over other iron fortificants is that it prevents iron from binding with the phytic acid present in many cereal and legume grains. Lynch *et al.* (1993) described that a proposed solution to the problem of phytic acid is the use of iron EDTA. Ranum (1999) explained that iron in NaFeEDTA is chelated with EDTA, a commonly used food additive; this prevents the iron from being bound to phytic acid.

There is no advantage in using EDTA in white flour or medium extraction flour used in yeast leavened bread making, but there does appear to be good justification

Table-2. Effect of storage periods and treatments on phytic acid content (%) of different fortified whole wheat flours

Treatment	Storage (Days)				
	0	15	30	45	60
T <sub>0</sub>	1.73a ± 0.051	1.61b ± 0.045	1.42 d-f ± 0.040	1.06 jk ± 0.045	0.88 m-o ± 0.033
T <sub>1</sub>	1.49 b-d ± 0.039	1.38d-g ± 0.040	1.35 e-g ± 0.042	1.01 j-l ± 0.031	0.93 l-o ± 0.029
T <sub>2</sub>	1.58 bc ± 0.049	1.34 fg ± 0.038	1.27 gh ± 0.038	1.11 ij ± 0.046	0.94 k-o ± 0.073
T <sub>3</sub>	1.49b-d ± 0.039	1.33 fg ± 0.038	1.25 gh ± 0.044	0.96 k-n ± 0.053	0.82 o ± 0.078
T <sub>4</sub>	1.47c-e ± 0.047	1.38 d-g ± 0.070	1.18 hi ± 0.036	0.97 k-m ± 0.027	0.83 no ± 0.042

Means (±SE) carrying similar alphabets a-o do not differ significantly)

The interactive effect of storage periods and bags gave significantly higher values for phytic acid content in controlled storage conditions treatments has been presented in Table 2. The highest concentration of phytic acid was found in the unfortified fresh whole wheat flour samples (1.73%) while the minimum phytic acid content was found in T<sub>3</sub>

to use it in atta flour used to make unleavened bread.

The concentration of phytic acid in the flour samples was in close agreement with findings of Toree *et al.* (1991) who stated that phytates constitute 1-2% of the weight of many cereals. Anjum and Walker (2002) observed 2.23 % phytic acid in whole wheat flour in Pakistani wheats.

Table-3. Effect of storage conditions, storage periods and treatments on phytic acid (%) of different fortified wheat flours

Treatment	Ambient condition						Controlled conditions					
	Storage (Days)						Storage (Days)					
	0	15	30	45	60	Mean	0	15	30	45	60	Mean
T <sub>0</sub>	1.73 a ±0.082	1.59a-c ±0.066	1.45 b-f ±0.057	1.13 j-o ±0.073	0.81 r-t ±0.033	1.34 <sup>A</sup> ±0.067	1.73a ±0.069	1.62 ab ±0.065	1.40 c-h ±0.059	1.00 n-r ±0.041	0.95 o-r ±0.043	1.34 <sup>A</sup> ±0.064
T <sub>1</sub>	1.49 be ±0.058	1.34 d-i ±0.055	1.36 d-i ±0.053	1.04m-q ±0.048	0.88 q-t ±0.037	1.22 <sup>C</sup> ±0.047	1.49 b-e ±0.058	1.43 b-g ±0.055	1.35 d-i ±0.070	0.98 n-r ±0.039	0.98 o-r ±0.039	1.25 <sup>BC</sup> ±0.047
T <sub>2</sub>	1.58 a-c ±0.062	1.31 e-j ±0.056	1.24 g-l ±0.048	1.01 n-q ±0.046	0.76 st ±0.094	1.18 <sup>CD</sup> ±0.058	1.58 a-c ±0.082	1.37 d-h ±0.055	1.32e-j ±0.059	1.21 h-n ±0.058	1.12 k-p ±0.053	1.32 <sup>AB</sup> ±0.039
T <sub>3</sub>	1.49 b-e ±0.058	1.31e-j ±0.055	1.27 f-k ±0.050	0.81 r-t ±0.040	0.58 u ±0.050	1.09 <sup>D</sup> ±0.067	1.49 b-e ±0.058	1.35 d-±0.054	1.24 g-l ±0.078	1.11 k-p ±0.044	1.06 l-q ±0.043	1.24 <sup>BC</sup> ±0.30
T <sub>4</sub>	1.47 b-e ±0.058	1.24 hl ±0.049	1.21 hm ±0.049	0.99 n-r ±0.039	0.94 p-s ±0.044	1.17 <sup>CD</sup> ±0.041	1.47 b-e ±0.079	1.53 b-d ±0.101	1.17 i-n ±0.055	0.95 o-r ±0.039	0.73 tu ±0.037	1.17 <sup>CD</sup> ±0.063

Means (±SE) in columns A-D carrying similar alphabets do not differ significantly (p<0.05)

Means (±SE) carrying similar alphabets a-u do not differ significantly (p<0.05)

Cheryan (1980) stated that at low pH and low cation concentration, phytate-protein complexes are formed due to direct electrostatic interaction, while at pH > 6 to 7, phytic acid-mineral-protein complex is formed which dissociates at high Na<sup>+</sup> concentrations. These complexes appear to be responsible for the decreased bioavailability of the complexed minerals and are also more resistant to proteolytic digestion at low pH.

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## Effect of polyols on quality and acceptability of frozen dough bread

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### ABSTRACT

Frozen dough products face a challenge of due is relatively short shelf life with decreasing quality characteristics. Some polyols have very efficient osmoprotectant properties. The mandate of this project was to improve the quality of frozen dough by incorporation of such polyols as sorbitol and mannitol. The dough was stored in the frozen form for up to two months. Bread characteristics were studied after every 15 days for external and internal characteristics. The frozen dough breads prepared from 0.5% mannitol and 1% sorbitol scored higher as compared to the other treatments. There was a significant gradual decrease in all sensory attributes with the increase in storage period. However, the breads received acceptable quality scores for up to 30 days storage due to polyols effect.

**Key words:** Frozen dough, polyols, surfactants, bread, dough storage

### INTRODUCTION

Due to rapid industrialization and urbanization of Pakistani society, the dietary habits are changing with time. The term "refrigerated dough" enjoys a special place in consumer's mind due to its unique combination of reliability, convenience and freshness. Refrigerated dough products find increasing appreciation among both retail consumers and the food service industry (Allenson 1982). The use of frozen dough has increased in bakeries, supermarkets and restaurants, as it allows easier and more profitable baking. In spite of progress being made in formulation, processing and freezing technology, frozen doughs often result in products with lower consumer acceptability due to stability problems.

It has been believed for a long time that there is no substitute for fresh baked goods. With lapse of time, a crispy crust of a fresh baked product develops a moist and leathery texture while the soft crumb becomes firm and dry. The fresh flavor is also lost within hours of baking, which has made bakers work midnight or early morning to provide the consumers with fresh bread on a daily basis. Transportation of baked products from large automated bakeries has also posed problems (Inoue and Bushuk 1991).

Quality is often poorer in baked goods prepared from frozen doughs than in fresh baking, as well as loaf volumes, are usually reduced. Mixed and moulded frozen dough that could be quickly transformed into freshly baked product was suggested as solution to the existing problem. The advantages of frozen dough made it popular since the 1970's. However, research

was initiated in this area due to the disadvantages which are its variable performance, increased expenses, and also loss of stability during extended frozen storage periods and freeze-thaw cycles during transportation (Berglund 1988).

Loss of stability refers to increase in proof time, decrease in loaf volume with poor bread characteristics, and loss of shelf life (Wolt and D'Appolinia 1984). The quality of a bread product baked from a frozen dough decreases with increasing frozen storage time (Inoue and Bushuk 1996; Nemeth *et al.* 1996).

Some polyols have very efficient osmoprotectant properties. It is of particular interest to study water-binding properties of polyols (mannitol and sorbitol) as humectants when protein and starch release the water in frozen dough systems. These polyols could potentially improve the quality of the frozen dough bakery products by reducing the water mobility during frozen storage.

Since the shelf life of frozen dough is less and bread produced from frozen dough also loses its acceptability after 72 hours. This situation demands exploring ways and means to improve shelf life and stability of the frozen dough bread.

### MATERIALS AND METHODS

**Procurement of raw material:** The commercial wheat flour and other ingredients i.e. sugar, salt, shortening, yeast were purchased from local market and polyols (sorbitol & mannitol) from a scientific store.

**Chemical analysis of flour:** The chemical analysis of flour was carried out for moisture, crude protein, crude fiber, crude fat, nitrogen free extracts, total ash as well as wet and dry gluten contents and pelshenke value as described in AACC (2000).

**Preparation and storage of dough:** Frozen dough was prepared by using different treatments of polyols (Table 1) by the straight-no-time dough formulation procedure as described by Inoue and Bushuk (1991). After mixing the dough was rounded, and rested in a fermentation cabinet for 10 minutes at 30°C and 70-75 % relative humidity. Then dough was moulded manually and immediately placed in polyethylene zip bags and stored in freezer at -10°C.

**Table-1. Treatments used in the study**

Treatments	Polyols (%)	
	Sorbitol	Mannitol
T <sub>0</sub>	-	-
T <sub>1</sub>	0.1	-
T <sub>2</sub>	0.5	-
T <sub>3</sub>	1.0	-
T <sub>4</sub>	1.5	-
T <sub>5</sub>	2.0	-
T <sub>6</sub>	-	0.1
T <sub>7</sub>	-	0.5
T <sub>8</sub>	-	1.0
T <sub>9</sub>	-	1.5
T <sub>10</sub>	-	2.0

T<sub>0</sub> = Acts as control

#### Bread preparation from frozen dough

##### Recipe

Flour	100g	Sugar	4g
Salt	1g	Shortening	5g
Ascorbic acid	100ppm	Yeast	3g
Polyols	as per treatment		

Water according to water absorption capacity of flour

##### Procedure

After every 15 days, thawing of the dough was carried out by keeping the dough at room temperature for 45 minutes. Then mixing of thawed dough was done for 1-1.5 minutes in order to homogenize. After molding dough was shifted into the greased pans and placed in proofer at 30°C and 85% relative humidity till definite loaf height. The proofed loaves were transferred in the oven for baking at 220°C for 15-20 minutes.

**Sensory evaluation of frozen dough bread:** The breads made from frozen dough were evaluated for sensory attributes after every 15, 30, 45 and 60 days storage interval by a panel of five judges for external

and internal characteristics as described by Land and Shepherd (1988).

**Statistical analysis:** The data obtained from sensory evaluation of bread were subjected to statistical analysis to determine the level of significance between quality parameters of different treatments by using completely randomized design and means were compared according to the appropriate methods described by Steel *et al.* (1997).

**Results and Discussion:** The chemical analysis of wheat flour showed that flour contained moisture, ash, crude protein, crude fat, crude fiber and nitrogen free extract as 11.33, 0.54, 12.46, 1.67, 0.34 and 84.97%, respectively. The wet and dry gluten contents of flour are shown in Table-2.

**Table2. Composition of wheat flour**

Characteristics	Percentage
Moisture	11.33
Crude Protein	12.46
Ash	0.54
Crude Fiber	0.34
Fat	1.67
Nitrogen free extracts	84.97
Wet gluten	30.24
Dry Gluten	9.59

The mean scores for different treatments indicated that loaf volume of bread was significantly affected by the addition of polyols (Table 3). The maximum score for volume was recorded in T<sub>7</sub> (0.5% mannitol) while the minimum score was obtained by T<sub>0</sub>. The mean scores for volume of breads ranged from 5.48 to 6.96 among different treatment and the mean scores across different storage intervals varied from 4.95 to 7.35. The scores for crust color of breads range from 4.80 for control (T<sub>0</sub>) to 6.20 for 2% sorbitol (T<sub>5</sub>) among different treatments., while during 60 days storage scores at different intervals of 60 varied from 4.80 to 5.96 (Table-4; Fig. 1).

**Table-3. External characteristics of bread prepared from frozen dough**

Treatments	Volume	Crust color	Symmetry of form	Evenness of bake
T <sub>0</sub>	5.48d	4.80e	2.60b	2.24a
T <sub>1</sub>	6.04c	5.28cde	3.00ab	2.32a
T <sub>2</sub>	6.32bc	5.48bcd	3.16ab	2.40a
T <sub>3</sub>	6.76ab	5.64abcd	3.36a	2.48a
T <sub>4</sub>	6.28bc	5.92abc	3.20ab	2.44a
T <sub>5</sub>	6.12c	6.20a	3.12ab	2.36a
T <sub>6</sub>	6.44abc	5.16de	3.24ab	2.40a
T <sub>7</sub>	6.96a	5.32bcde	3.52a	2.56a
T <sub>8</sub>	6.60abc	5.44bcd	3.24ab	2.40a
T <sub>9</sub>	6.40abc	5.68abcd	3.20ab	2.36a
T <sub>10</sub>	6.24bc	5.96ab	3.08ab	2.32a

Mean values for treatments carrying same letters in a column are not significantly different

**Table-4. Effect of storage on external characteristics of bread prepared from frozen dough**

Days	Volume	Crust color	Symmetry of form	Evenness of bake
0 day	7.35a	6.82a	4.05a	2.91a
15 day	6.80b	6.18b	3.56ab	2.65a
30 day	6.44bc	6.04b	3.16bc	2.56a
45 day	6.12c	4.96c	2.71cd	2.02a
60 day	4.95d	3.67d	2.29d	1.80b

Mean values for treatments carrying same letters in a column are not significantly different

Different treatments significantly affected the symmetry of form of frozen dough bread. The mean value for T<sub>7</sub> (3.52) was ranked at the top followed by T<sub>3</sub> (3.36) while T<sub>0</sub> with mean score of 2.60 were placed at the lowest position. The mean score of storage up to 60 days ranged from 2.29 to 4.05. The scores for evenness of bake of breads ranged from 2.24 to 2.56 among treatment. However, scores for evenness of bake varied from 1.80 to 2.91 for storage of dough. Maximum mean score was obtained by T<sub>7</sub> containing 0.5% mannitol while lowest score was for T<sub>0</sub>. Table-5 shows the mean scores of treatments regarding internal characteristics, while Table-6 and Fig 2 indicates the different mean scores at different storage intervals.

**Table-5. Internal characteristics of bread prepared from frozen dough**

Treatments	Aroma	Grain	Crumb color	Taste	Texture
T <sub>0</sub>	7.24b	10.08d	7.16c	12.16d	10.40d
T <sub>1</sub>	7.44ab	10.60cd	7.48abc	12.80c	11.24c
T <sub>2</sub>	7.52ab	10.96abc	7.64abc	13.04bc	11.56bc
T <sub>3</sub>	7.68ab	11.24ab	7.80abc	13.28abc	12.00ab
T <sub>4</sub>	7.76ab	10.84bc	7.92ab	13.56ab	11.76abc
T <sub>5</sub>	7.96a	10.68bc	8.12a	13.92a	11.52bc
T <sub>6</sub>	7.32ab	11.12abc	7.40bc	12.72cd	11.60abc
T <sub>7</sub>	7.40ab	11.52a	7.48abc	12.84c	12.24a
T <sub>8</sub>	7.60ab	11.20abc	7.60abc	13.04bc	11.88abc
T <sub>9</sub>	7.64ab	10.96abc	7.76abc	13.32abc	11.76abc
T <sub>10</sub>	7.80ab	10.84bc	8.04ab	13.60ab	11.48bc

Mean values for treatments carrying same letters in a column are not significantly different

It is obvious that the scores for aroma of breads ranged from 7.24 to 7.96 among different treatments. However the score varied from 6.91 to 8.25 during different storage intervals. T<sub>5</sub> containing 2% sorbitol got significantly higher score followed by T<sub>10</sub> while the lowest score was obtained by T<sub>0</sub> (control). The results

for grain indicated that T<sub>7</sub> scored best throughout the 60 days of frozen storage with mean value (11.52) followed by T<sub>3</sub> and the least score for grain was found to be (10.08) for T<sub>0</sub>. The mean score for storage intervals ranged from 8.98 to 12.75. Statistical analysis regarding the crumb color revealed significant effect of treatment on scores where T<sub>5</sub> containing 2% sorbitol showed the best results followed by T<sub>10</sub> containing 2% mannitol while T<sub>0</sub> obtained lowest mean score. Mean scores ranged from 7.16 to 8.12 for treatments while for storage these were 6.87 to 8.31.

**Table-6. Effect of storage on internal characteristics of bread prepared from frozen dough**

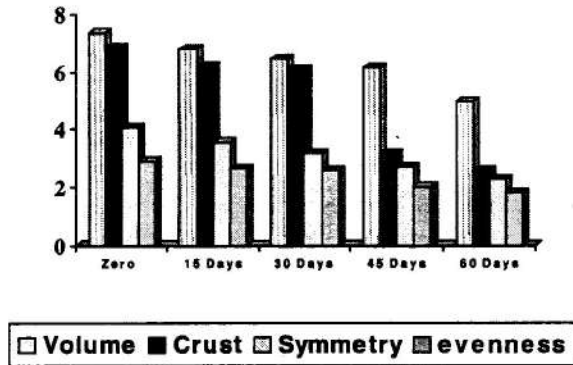
Days	Aroma	Grain	Crumb color	Taste	Texture
0 day	8.25a	12.75a	8.31a	14.89a	13.05a
15 day	7.96ab	11.95b	8.09ab	14.13b	12.47b
30 day	7.49bc	11.09c	7.65bc	13.35c	11.73c
45 day	7.27c	9.80d	7.44cd	12.31d	11.16c
60 day	6.91c	8.98e	6.87d	10.91e	9.51d

Mean values for treatments carrying same letters in a column are not significantly different

Taste of frozen dough bread was also affected significantly in all treatments. Mean scores ranged from 12.16 to 13.92 while 10.91 to 14.89 for different storage intervals up to 60 days. The mean scores for texture ranged from 10.40 to 12.24 among treatments

and 9.51 to 13.05 for storage up to 60 days of frozen dough storage. T<sub>7</sub> (0.5% mannitol) followed by T<sub>3</sub> (1% sorbitol) ranked at the top with highest mean scores respectively while T<sub>0</sub> remained at lowest position with lowest scores. The results found in this study were in

Fig. 1. Effect of storage on external characteristics of bread prepared from frozen dough.



close agreement to the findings of Rayas (2000) and Farooq (1996).

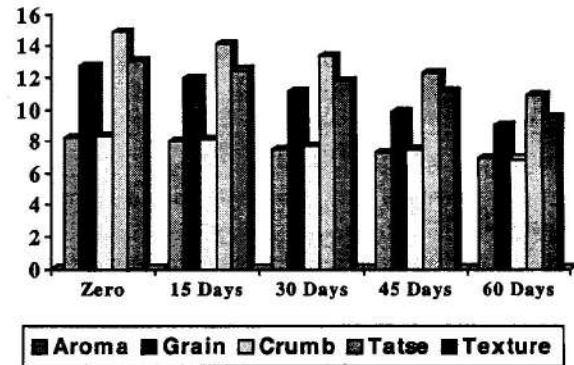
### CONCLUSIONS

The external characteristics of frozen dough bread such as volume, colour of crust, symmetry of form, evenness of bake and character of crust as well as internal characteristics like grain, colour of crumb, aroma, taste and texture were evaluated subjectively after 0, 15, 30, 45 and 60 days. It was revealed that the difference in polyol treatments and different storage periods had significant effect on all these parameters. The interactions between the storage intervals and different treatments were non-significant for internal and external characteristics of the frozen dough breads. The frozen dough breads having mannitol got significantly higher scores as compared to the breads containing sorbitol. The frozen dough breads having 0.5% mannitol and 1% sorbitol in the recipe got higher scores as compared to the other treatments. There was a significant gradual decrease in all the parameters of frozen dough breads with the increase in storage period. However, the breads obtained acceptable scores up to the 30 days frozen storage due to different treatments of polyols. From these studies it may be concluded that addition of 0.5% mannitol and 1.0% sorbitol in frozen doughs can improve the quality and stability of the bread.

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Fig. 2. Effect of storage on internal characteristics of bread prepared from frozen dough.



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## Biological evaluation of chemically preserved *Agaricus bitorquis* mushroom

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### ABSTRACT

Citric acid (0.1%), acetic acid (0.3%), sodium chloride (5%), potassium metabisulfite (0.05-0.09%), ascorbic acid (0.1%) and sodium benzoate (0.05-0.09%) and potassium sorbate (0.1%) were used for the extension of shelf life of mushrooms. Biological evaluation was done to check the efficacy of mushroom protein after 90 days of preservation. Protein efficiency ratio (PER), net protein utilization (NPU), feed efficacy (FE), biological value (BV), net protein ratio (NPR) and protein digestibility (PD) were determined. The data was analyzed statistically and the results were interpreted.

**Keywords:** Mushroom, preservation, acetic acid, citric acid, protein, biological value.

### INTRODUCTION

Mushrooms are highly perishable and deteriorate within short period of time after harvest. These mushrooms require great deal of attention during storage, marketing and processing at the post-harvest stages as some of the problems encountered are discoloration, weight loss, flavor loss, shriveling and browning (Doores *et al.* 1987).

From the quality point of view mushrooms have to be processed immediately after harvesting. Different doses of sodium benzoate (SB) and potassium meta bisulphited (KMS) were used to increase the shelf life of mushrooms. Mushrooms preserved in this manner are very economical and can be transported to far off distant places conveniently, to be used for further processing and preservation (Sandhu and Aggarwal 2001).

No doubt the mushrooms are good source of protein but method of food preservation poses a potential problem in digestion of protein. All the amount of protein which is taken is not digested. In addition to the preservation, the present research was designed to study efficacy of chemically preserved mushrooms. The absorption and bioavailability of protein using Albino male rats was also estimated.

### MATERIALS AND METHODS

Following two groups of chemicals were used for preservation,

#### Fixed Chemicals

- Citric acid 2-4% for adjusting the pH to 4 - 4.5
- Ascorbic acid 0.1% as an antioxidant.
- Acetic acid 0.3% as preservative.

- Sodium chloride 5% as preservative
- Potassium sorbate 0.1% as preservative.

#### Variable chemicals

To find out minimum effective dose of combined effect of chemical preservatives, the lower permissible limit of KMS and SB 0.1 % was further subdivided into nine variable combinations randomly as shown in Table-1.

Table-1. Doses of chemical treatments

Treatment	KMS %	SB%
T <sub>1</sub>	0.08	0.08
T <sub>2</sub>	0.08	0.06
T <sub>3</sub>	0.06	0.08
T <sub>4</sub>	0.06	0.06
T <sub>5</sub>	0.07	0.07
T <sub>6</sub>	0.07	0.09
T <sub>7</sub>	0.07	0.05
T <sub>8</sub>	0.09	0.07
T <sub>9</sub>	0.05	0.07
T <sub>10</sub> (Fresh)	--	--

SB = Sodium benzoate

KMS = Potassium meta bisulfite

#### Preparation of mushroom powder

The mushrooms were blanched for four minutes in water to inactivate enzymes and filled in glass jars along with chemicals. The jars were made air tight at about 90 °C. The lids were internally lined with aluminum foil to prevent any leakage or corrosion. After 90 days storage period, the mushrooms were taken out of the steeping solution and dried in an air oven at 105 °C till a constant weight was obtained. The dried mushroom powder was used for preparation of diet.

## BIOLOGICAL EVALUATION

The albino rats were purchased from National Institute of Health (Veterinary Division) Islamabad, and brought to fruit and vegetable laboratory at the Institute of Food Science and Technology, University of Agriculture Faisalabad.

The diet of the rats was prepared by mixing preserved mushroom powder to study the protein efficacy, along with an inert carrier like maize starch as reported by Warner (1962).

Mushroom diet was fed to rats for 10 days. Biological study was conducted by the method of Miller and Bender (1955). Weaning was done at 30 days of age. The rats were put on the stock diet for seven days prior to the experiment. Clean water was made available during whole study period. Body weight of rats was recorded on daily basis. The faecal material of each cage was collected daily and dried to a constant weight for nitrogen determination. At the end of the trial all the rats were killed with over dose of chloroform and their skull and abdominal cavities were opened. The carcasses of each group including the intestinal contents were weighed before and after drying at 105°C to a constant weight. The dried carcasses were ground in the domestic mincer and were stored in airtight bottles till the estimation of body nitrogen. The nitrogen contents of each diet, faeces and carcasses were determined by Kjeldhal's method.

## RESULTS

### Feed Efficiency

Feed Efficiency of *Agaricus bitorquis* varied from 0.45

Table-2. Analysis of variance for parameters of efficacy study of *Agaricus bitorquis* mushroom after 90 days

SOV	df	Feed Efficiency	Protein digestibility	Net Protein Utilization	Biological Value	Protein Efficiency Ratio	Net Protein Ratio
Treatment	9	0.055**	0.176*	285.410**	0.029**	0.263**	0.630**
Error	21	0.003	0.173	25.634	0.003	0.013	0.030
Total	30						

\*\* Highly Significant \* Significant

(T<sub>1</sub>) to 0.82 (T<sub>7</sub>) and the differences among the means were highly significant. Fresh and treatment T<sub>7</sub> had significantly higher (P<0.01) feed efficiency ratio than all the other treatments. All the nine treatments differed highly significantly from one another in feed efficiency.

### Protein Digestibility

Protein digestibility of *Agaricus bitorquis* varied from 95.90% (T<sub>5</sub>) to 96.72% (T<sub>7</sub>). The differences among the means were significant. Treatment T<sub>7</sub> had protein digestibility higher than all other treatments.

### Net Protein Utilization

Net protein utilization of the *Agaricus bitorquis* varied from 42.10 (T<sub>5</sub>) to 74.41 (T<sub>1</sub>). The differences among the means were highly significant (P<0.01). Treatment T<sub>1</sub> had significantly higher net protein utilization.

### Biological Value

Biological value of *Agaricus bitorquis* varied from 0.44 (T<sub>5</sub>) to 0.76 (T<sub>1</sub>). The differences among the means were highly significant. Treatment T<sub>1</sub> had significantly higher biological value.

### Net Protein Ratio

Net protein ratio of the *Agaricus bitorquis* varied from 1.87 (T<sub>9</sub>) to 2.63 (T<sub>1</sub>). The differences among the means were highly significant. Net protein ratio for treatment T<sub>2</sub> and T<sub>3</sub>, and T<sub>5</sub> and T<sub>6</sub> were observed to be similar as is evident from Table 3.

### Protein Efficiency Ratio

Protein efficiency ratio of the *Agaricus bitorquis* varied from 0.95 (T<sub>9</sub>) to 1.85 (T<sub>1</sub>). The differences among the means were highly significant. Treatment T<sub>1</sub> had significantly higher protein efficiency ratio than all other treatments.

## DISCUSSION

Digestibility varies in test animals considerably (Moughan and Donkoh, 1991). Digestibility of treatment T<sub>7</sub> was higher than all other treatments. The results of digestibility in the present study are in agreement with the previous findings of Sarwar *et al.*

(1989). The digestibility was found to be higher than the findings of Thayumanavan and Manicham (1980). They showed digestibility as 84.1 in *P. sajor caju*. Flegg and Maw (1976) concluded that digestibility of *P. florida* (79.07) has been found to be higher than that of spinach protein (73%) but poorer than that of meat (99%). In the present study, it was found that digestibility was significantly affected by chemical preservation. Duncan's multiple range test was applied to differences of means.

Table-3. Mean values for parameters of efficacy study

Parameters/ Treatments	Feed Efficiency	Protein Digestibility	Net Protein Utilization	Biological Value	Protein Efficiency Ratio	Net Protein Ratio
T <sub>1</sub>	0.45h	96.19cde	74.41a	0.76a	1.85a	2.63a
T <sub>2</sub>	0.51g	96.19cde	62.94e	0.65c	1.62b	2.35c
T <sub>3</sub>	0.51g	96.55ab	64.81d	0.65c	1.62b	2.34c
T <sub>4</sub>	0.54f	96.50abc	69.39b	0.71b	1.53c	2.26d
T <sub>5</sub>	0.56e	95.90e	42.10h	0.44f	1.49cd	2.27d
T <sub>6</sub>	0.69c	96.40abcd	63.39de	0.68bc	1.21e	2.00e
T <sub>7</sub>	0.82a	96.72a	57.74f	0.59d	1.01f	1.93f
T <sub>8</sub>	0.57d	96.12de	48.27g	0.50e	1.46d	2.42b
T <sub>9</sub>	0.78b	96.62ab	68.96b	0.71b	0.95g	1.87g
T <sub>10</sub> (Fresh)	0.51g	96.28bcd	66.76c	0.74a	1.62b	2.60a

The result showed that digestibility of diet containing treatment T<sub>7</sub> was significantly different from other groups.

Net protein utilization values of different experimental diets are given in Table 3. Net protein utilization is in agreement to the findings of Thayumanavan and Manicham (1980) 75.1 %, and in this study it varied from 42.10 (T<sub>5</sub>) to 74.41 (T<sub>1</sub>), these variations may be due to the deleterious effects of chemicals on protein. Net protein utilization of the fresh mushroom (73.76) and that of T<sub>1</sub> (74.41) are similar. Analysis of variance showed a highly significant difference between different groups of experimental diet, as presented in Table-2.

Duncan's multiple range test revealed that net protein utilization of diet containing treatment T<sub>1</sub> (0.08 % KMS and 0.08% Sodium benzoate) and fresh mushroom (96.28%) were significantly different from other groups.

Biological values calculated from net protein utilization and digestibility is shown in the Table-3. Biological values of T<sub>10</sub> and T<sub>1</sub> were similar (0.76 and 0.74 respectively). Biological values observed in this study were similar to those observed by Thayumanavan and Manicham (1980) and Udipi and Punekar (1980). This difference may be due to chemicals used, substrate used for cultivation, environment and damaging effect of heat on amino acids during the baking of the starch containing the mushroom powder.

Duncan's multiple range test showed that the difference in biological values of diet containing treatment T<sub>1</sub> (0.08 % KMS and 0.08 % Sodium benzoate) and fresh mushroom was non-significant but differed significantly from other groups. A non-significant difference was observed in diets containing

T<sub>2</sub> (0.08 % KMS and 0.06% sodium benzoate) and T<sub>3</sub> (0.06% KMS and 0.08% sodium benzoate).

Net protein ratio of the diet containing treatment T<sub>1</sub> was higher than the other diets. Statistical analysis showed highly significant differences among various treatments. Animals on diet containing 0.08 % KMS and sodium benzoate differed significantly from other treatments. However, a non-significant difference was noticed between diets containing treatments T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, T<sub>5</sub>. It was observed that net protein ratio of diet containing treatment T<sub>1</sub> and fresh mushroom powder is same.

Protein efficiency ratio of the *Agaricus bitorquis* varied from 0.95 (T<sub>9</sub>) to 1.85 (T<sub>1</sub>). The analysis of variance (Table-2) indicated highly significant differences among the means for PER values of various experimental diets. Duncan's multiple range test showed that PER values of diet containing treatment T<sub>1</sub> (0.08 % KMS and 0.08 % sodium benzoate) differ significantly from other groups. A non-significant difference was observed in diets containing T<sub>2</sub> (0.08 % KMS and 0.06% sodium benzoate), T<sub>3</sub> (0.06% KMS and 0.08% sodium benzoate) and fresh. The results of the present study are in agreement to the findings of Gupta *et al.* (1981).

Feed efficiency of the diet containing treatment T<sub>7</sub> (0.82) was highest and lowest for diet containing treatment T<sub>1</sub> (0.45). It shows that rats consumed significantly the lowest feed per unit weight gain on diet containing treatment T<sub>1</sub> (0.08 % KMS and Sodium benzoate).

## CONCLUSIONS

On the basis of these findings, it is concluded that diet containing 0.08% KMS and 0.08% sodium benzoate (T<sub>1</sub>) showed best results, and protein was less damaged due to inhibition of enzyme activity. The mushroom, thus, preserved has better nutritive status that may be used to make up the protein deficiency of

the people. These mushrooms are likely to be less expensive than those preserved by other techniques like canning and refrigeration.

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## Comparative aspects of pectin extraction from peels of different varieties of citrus fruit

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### ABSTRACT

Comparative aspects of pectin extraction from peels of different varieties of citrus fruit were studied. The conditions for extraction of pectin from the peels of Feutral, Musambi, Malta, and Kinnow were optimized according to the standard procedures. pH, soaking temperature and time significantly affected the extraction of pectin. The Feutral yielded maximum pectin followed by Mosambi, 18.5%, Malta; 15.29% and Kinnow 14.01% respectively. Methoxyl contents of Feutral, Musambi, Malta and Kinnow were 9.96%, 18.5%, 15.29% and 14.01%. The equivalent weights were 1209, 1044, 943 and 777 respectively. These chemical characteristics of pectin indicated that quality of pectin for commercial applications was acceptable.

**Keywords:** Pectin, citrus fruit peels, extraction, chemical quality.

### INTRODUCTION

Pectin is extensively utilized by the food processors for conversion of low-grade fruits into quality products like jam, jelly, Marmalade and candies. Fruits and vegetable waste materials are usually used for the extraction of pectin by soaking the fruit and vegetables wastes in acidic solution at different pH (Baltaga 1962; Srirangarajan and Shrikhande 1979; Jain *et al.* 1984). Muralikrishna and Tharanathan (1994) extracted 1.43-5.37% pectin by soaking pulse husk in hydrochloric acid and EDTA solution at 70°C, whereas extracted 9-10% good quality pectin with extracted each from potato wastes (Chen *et al.* 1999). Industrial pectin of good quality was prepared from orange peels and peach pomace (Rouse and Crandall 1976; Pagan *et al.* 1999). Attempts were also made to extract pectin from orange peels with microbial enzymes (Elian *et al.* 1984).

This study was carried out to optimize the conditions for maximum extraction of pectin from peels of different varieties of citrus fruit (Feutral, Musambi, Malta and Kinnow).

### MATERIALS AND METHODS

Fully matured and fresh citrus fruits (Feutral, Musambi, Malta and Kinnow) were procured from the local market in Lahore. The peels of these fruits were passed through shredding machine to separate albedo (Pectin rich) and flavedo (oil & pigment) portions. The albedo portion was minced mechanically and washed with cold water to remove the adhering and other mucilages. The washed and minced albedo was then dried in a cabinet dryer at 65°C to reduce moisture content to 5-6%. The dried material was finally ground to 80 mesh size and

defatted using hexane solvent.

### EXTRACTION OF PECTIN FROM CITRUS PEELS

Twenty-five gram of the dried ground sample of citrus peels (Feutral, Musambi, Kinnow & Malta) were mixed with water of pH (2.5, 3), keeping substrate to water ratio 1 :40 (W/V). The desired pH of the mixture was adjusted with 0.1M sulphuric acid on pH meter (PYE UNICAM Model 1292) and then incubated at different temperatures (70°C – 80°C – 90°C) for different time periods (60 – 120 minutes) with frequent stirring. After incubation, the contents were filtered through cheese cloth and pectin from the filtrate was precipitated with 95% ethanol. The obtained pectin was dried in a vacuum oven at 40°C to constant weight and ground finally to very fine powder to study chemical quality characteristics. Yield was calculated as dried pectin/100g dried citrus peels.

Anhydrogalacturonic acid content, equivalent weight and methoxyl contents were determined as quality characteristics of citrus peel pectin by the standard methods of Owens *et al.* (1952). Triplicate determinations were performed for all parameters studied and standard deviations (SD) were calculated according to the method of Steel *et al.* (1997).

### RESULTS AND DISCUSSION

Data presented in Table 1 show that pH of the solution played a great role in the extraction of pectin from different varieties of citrus peels. At pH 2.5 and 3.0, pectin yield was 14.01 and 12.56% from Kinnow peels, 18.50 and 15.92% from Musambi peels, 15.29 and 13.77% from Malta peels and 18.64 and 16.86% from Feutral peels respectively on soaking the peels powder of citrus at 80°C for 120°C. It is apparent from

these results that maximum pectin yield was obtained by soaking the peels in a solution of pH 2.5. However, decline in pectin yield was observed at pH 3.0 at all temperatures 70°C-90°C after 120 minutes extraction.

Extraction of pectin was also affected by changing the temperature ranging from 70°C-90°C (Table 1). Maximum amount of pectin was obtained by soaking ground peels of different varieties of citrus. On increasing the temperature from 80°C-90°C reduction in the yield of pectin was observed. Maximum pectin yield was 14.01, 18.50, 15.29 and 18.64% on soaking ground peels of Kinnow, Musambi, Malta and Feutral in acidic solution of pH 2.5 for 120 minutes. Decrease in pectin yield at higher temperature (90°C) could be attributed to break down of pectin molecules as already observed by Chang *et al.* (1994).

Soaking time is another factor which affect the extractability of pectin. It apparent from the results mentioned in Table 1 that maximum amount of pectin was obtained at all pH after 120 minutes. No further increase in pectin yield was observed after 120 minutes extraction time (Results are not shown after 120 minutes). These results are consistent with findings of earlier workers who reported that prolonged extraction and higher temperatures adversely affected the yield of pectin (Turmucin *et al.*

on soaking the ground peels at 80°C for 60 and 120 minutes respectively. Rouse and Crandall (1976) extracted 11.0, 8.15, and 6.35% of 150 grade pectin from lemon, mango and grape fruit peels at pH 1.6 respectively whereas Zhao *et al.* (1995) extracted 20% pectin from orange peels by precipitation with ferric salt. The difference between our results and reported in literature may be due to variations in raw material particle size, and extraction method and varietal difference.

Table 2 summarizes the chemical characteristics of pectin obtained by soaking peels of different varieties of citrus fruit in acetic solution of pH 2.5 at 80°C for 120 minutes. The moisture and ash contents of pectin obtained from Kinnow, Musambi, Malta and Feutral ranged from 9.35-10.01% and 7.14-8.11% respectively.

These values are in agreement of reported value of Sarfraz (1976) and Altaf (1980) who found 10.28-10.85% moisture and 6.47-8.92% ash contents in pectin extracted from orange peels. Anhydrogalacturonic acid and methoxyl content values ranged from 68.99-72.80% and 7.79-9.96% while equivalent weight values were from 783-1209 for these four varieties of citrus peels. These results

**Table 1. Effect of pH, temperature and time period of extraction on the yield of pectin (percentage) from different varieties citrus**

	pH 2.5						pH 3.0					
	70°C		80°C		90°C		70°C		80°C		90°C	
	60 min	120 min	60 min	120 min	60 min	120 min	60 min	120 min	60 min	120 min	60 min	120 min
<b>Kinnow</b>	12.24 ±0.3	13.14 ±0.3	13.61 ±0.4	14.01 ±0.2	10.90 ±0.3	11.19 ±0.3	10.61 ±0.4	11.01 ±0.3	11.66 ±0.2	12.56 ±0.4	10.20 ±0.2	10.41 ±0.4
<b>Musambi</b>	16.81 ±0.4	17.14 ±0.2	18.10 ±0.2	18.50 ±0.4	15.40 ±0.3	16.93 ±0.2	14.60 ±0.4	14.84 ±0.4	14.21 ±0.3	15.92 ±0.2	73.10 ±0.3	13.98 ±0.3
<b>Malta</b>	13.08 ±0.2	15.26 ±0.4	13.67 ±0.4	15.29 ±0.4	12.86 ±0.3	14.05 ±0.2	11.95 ±0.3	13.17 ±0.4	12.21 ±0.3	13.77 ±0.2	11.80 ±0.4	12.29 ±0.3
<b>Feutral</b>	16.90 ±0.2	17.82 ±0.4	17.7 ±0.3	18.64 ±0.3	16.2 ±0.4	17.17 ±0.3	15.45 ±0.3	15.85 ±0.3	16.24 ±0.4	16.86 ±0.2	14.18 ±0.2	14.97 ±0.4

Mean Values ± S.D. Triplicate determinations.

1983; Chang *et al.* 1994). At pH 2.5, 13.61 and 14.01% from Kinnow, 18.10 and 18.50% pectin from Musambi, 13.67 and 15.29% pectin from Malta and 17.70 and 18.64% pectin from Feutral was obtained

were within the range of reported values for anhydrogalacturonic acid (68.5-75.0%) and methoxyl content of (8.4-9.7% of good quality orange peel pectin (Zafirris and Oreopoulou 1992).

**Table 2. Chemical quality characteristics of pectin obtained from different varieties citrus at pH 2.5, 80°C for 120 minutes**

Variety of Citrus	Moisture (%)	Ash (%)	Methoxyl Content (%)	Equivalent Weight (%)	Anhydrogalacturonic Acid (%)
Kinnow	9.87±1.7	7.77±1.3	7.79±2.1	783±1.6	72.80±2.0
Musambi	9.77±1.9	7.14±1.4	9.77±1.8	1044±1.5	68.99±1.9
Malta	9.35±2.3	7.56±1.5	8.52±1.7	943±1.0	74.00±1.8
Feutral	10.01±1.1	8.11±2.0	9.96±1.6	1209±1.0	71.00±1.5

Mean Values ± S.D. Triplicate determinations.

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## Effects of processing and storage on the physicochemical and sensory properties of Okra (*Hibiscus esculentum*)

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### ABSTRACT

Sliced okra pieces prints were processed by sulphiting and blanching. Treated and untreated (control) samples were stored under refrigeration (4–6°C), frozen (-4°C) and ambient temperatures (30 ± 2°C). The ascorbic acid content, pH, titratable acidity, microbial load, viscosity and sensory properties of the samples were monitored every three weeks for 15 weeks. Within the total storage period, frozen blanched and sulphited okra lost 38% of its ascorbic acid, while the frozen untreated sample lost 66%. Both treated and untreated samples stored at refrigeration temperature lost 79% and 83% ascorbic acid respectively. The losses in ascorbic acid in treated and untreated solar dried samples were the almost same, 68% and 66%. Frozen samples retained their viscosity better than refrigerated and solar dried ones. The pH of frozen and solar dried samples decreased with storage time while titratable acidities increased. The reverse was the case for refrigerated samples. Overall acceptability of blanched, sulphited and frozen okra was comparable to those of the fresh samples.

**Keywords:** Blanching, drying, okra, refrigeration, sulphiting, frozen, storage, viscosity.

### INTRODUCTION

Okra (*Hibiscus esculentum* L. Moench) is a herbaceous vegetable crop of the family Malvaceae (Yoyock *et al.* 1988). Annual, biennial and short-lived perennial varieties exist (Dupriez and Deleener, 1989). Both, the leaves and elongated fruits are used in soup and stew preparations in Nigeria unripe and tender okra as well as young shoots and leaves are eaten as vegetables (FAO 1988). In ethno medicine, the plant is used as blood volume extender in the treatment of peptic ulcer and the relief of hemorrhoids (Kordyias 1990). It is also important in neutralizing the acidic substances produced in the course of digestion of meats. It is also a good source of protein, oil, ascorbic acid, water soluble vitamins and minerals in the diet (Karakoltsidis and Constantinides 1975; Addo 1983; FAO 1988). However, its high respiration rate and attendant perishability makes post-harvest processing and storage very obvious. The present study highlights the effect of simple processing and storage methods on the ascorbic acid retention, physicochemical and sensory properties of okra.

### MATERIALS AND METHODS

#### Preparation of Samples

Freshly harvested okra (late dwarf variety) was procured from Ezira, Anambra State, Nigeria. The okra pods were sorted trimmed, washed and air-dried before they were sliced to 3mm thickness.

A quantity, 15kg of sliced okra was blanched at 98°C for 2 min. The blanched sliced okra pods were then

dipped for 1min in 500ppm of sodium metabisulphite solution and allowed to stand for 2 hours (Echetama 1991). The unblanched, unsulphited sliced pods (untreated samples) were used as control. Both the treated and untreated samples were divided into 3 portions each.

The first portion was dried to 9% moisture content in a see-saw solar drier, packed in polyethylene bag, sealed and stored in a dessicator. The second portion was stored in a deep freezer at -4°C while the third portion was stored in a refrigerator at 4-6°C. Samples were withdrawn from the six treatments and analysed every three weeks.

#### Determination of Ascorbic Acid Content

A quantity of sample weighing 5g from each treatment group was homogenized in 50mL TCA/EDTA solution (drawn from 500mL solution made by dissolving 60g TCA and 4.653g EDTA in distilled water) for 3 min. The homogenate was centrifuged at 5000g for 10 min. A portion 20mL of the supernatant was used for determination of ascorbic acid content using the AOAC standard procedures.

#### Determination of Physicochemical Properties

The pH of the samples was determined every three weeks using the pH meter (Model 7020). Samples (5g portions) were homogenized in 50mL distilled water, centrifuged at 5000g for 10 min and the supernatant was used for the determination of pH of the extract.

The titratable acidity was determined by titrating 25mL of sample extract against 0.1M NaOH using 2 drops of 0.02% phenolphthalein indicator. The specific gravity was determined by comparing the weight of a density bottle filled with the extract with the weight of the same bottle filled with distilled water.

The viscosity of extracted samples after homogenization and centrifugation (5000g for 10min) was determined using ball bearing of known variable diameters. The times for the fall of the ball bearings were recorded using a stop watch. The Stokes law equation was used to calculate the terminal velocity in m/s for each ball.

#### Microbial Count

The microbial count was carried out every three weeks using the serial dilution and plate count method as described by Cruickshank *et al.* (1975).

#### Determination of Sensory Characteristics

Sensory tests were carried out on salted cooked samples on a weekly basis by a ten-member trained panel using the nine point Hedonic scale. Sensory parameters were compared with those of salted cooked fresh samples. The panelists rated the samples for color, flavor, viscosity and overall acceptability.

### RESULTS

Fresh okra had ascorbic acid content of 46.6mg/100g, pH 6.3, titratable acidity of 0.23, viscosity of 29.84 NSM<sup>2</sup>, microbial load of  $1.63 \times 10^3$  cfu/ml and 88.4%

moisture. The samples used for shelf-life studies were (a) Blanched, sulphited and frozen (BSF), (b) Unblanched, unsulphited and frozen (UUF), (c) Blanched, sulphited and refrigerated (BSR), (d) Unblanched, unsulphited and refrigerated (UUR), (e) Blanched, sulphited and solar dried (BSSD) and (f) Unblanched, unsulphited and solar dried (UUSD). The solar dried samples were stored at ambient temperature ( $30 \pm 2^\circ\text{C}$ ). For the samples in frozen storage, blanching combined with sulphiting led to a much better retention of ascorbic acid (Table 1). However, in the refrigerated samples, the ascorbic acid retention after 15 – week storage period was much lower. The ascorbic acid retention in the solar dried samples stored at ambient temperature was better than those stored at refrigeration temperature. Solar drying reduced the moisture content of samples to about 9% thereby possibly limiting the deteriorative reactions. The pH of frozen and solar dried samples decreased on storage, with a concomitant increase in titratable acidity (Table 2, 3). Refrigerated samples showed increasing pH values and decreasing titratable acidity values within the 15 – week storage period.

There was only about 10 – 11% loss in the viscosity of both sulphited and unsulphited refrigerated samples (Table 5). The marked loss in viscosity was recorded for the solar dried samples due to poor retention of mucilage. The blanching – sulphiting treatments did not affect the viscosity properties as much as the

Table 1. Ascorbic acid content of treated and untreated sliced okra under storage

Ascorbic acid content of okra (mg/100g) on storage in weeks						
Sample	0	3	6	9	12	15
BSF	46.20±0.33 <sup>a</sup>	38.50±0.29 <sup>a</sup>	35.90±0.19 <sup>a</sup>	32.60±0.08 <sup>a</sup>	30.90±0.18 <sup>a</sup>	28.60±0.08 <sup>a</sup>
UUF	46.80±0.22 <sup>a</sup>	30.14±0.16 <sup>c</sup>	21.60±0.16 <sup>b</sup>	19.10±0.14 <sup>b</sup>	18.20±0.08 <sup>b</sup>	16.00±0.14 <sup>b</sup>
BSR	46.30±0.36 <sup>a</sup>	37.00±0.08 <sup>b</sup>	20.20±0.08 <sup>bc</sup>	15.00±0.00 <sup>d</sup>	12.10±0.08 <sup>a</sup>	9.70±0.00 <sup>f</sup>
UUR	46.70±0.36 <sup>a</sup>	36.40±0.16 <sup>b</sup>	18.60±0.00 <sup>cd</sup>	14.70±0.08 <sup>d</sup>	11.60±0.12 <sup>a</sup>	8.10±0.10 <sup>f</sup>
BSSD	32.20±0.08 <sup>c</sup>	26.10±0.18 <sup>d</sup>	17.00±0.22 <sup>d</sup>	15.80±0.08 <sup>c</sup>	13.60±0.08 <sup>d</sup>	10.20±0.09 <sup>d</sup>
UUSD	36.70±0.09 <sup>b</sup>	30.00±0.15 <sup>c</sup>	17.40±0.08 <sup>d</sup>	16.10±0.14 <sup>c</sup>	14.80±0.22 <sup>d</sup>	13.10±0.04 <sup>c</sup>
LSD	4.0	0.62	2.5	0.34	0.68	0.32

Means followed by the same letters in any column are not significantly different ( $P < 0.05$ )

BSF = Blanched – sulphited frozen samples

UUF = Unblanched – unsulphited frozen samples

BSR = Blanched – sulphited refrigerated samples

UUR = Unblanched – unsulphited refrigerated samples

BSSD = Blanched – sulphited solar dried samples

UUSD = Unblanched – unsulphited solar dried samples.

**Table 2. pH of treated and untreated sliced okra under storage**

Sample	pH during on storage in weeks					
	0	3	6	9	12	15
BSF	6.39±0.01 <sup>bc</sup>	6.14±0.00 <sup>c</sup>	5.80±0.01 <sup>c</sup>	5.40±0.04 <sup>c</sup>	5.10±0.02 <sup>c</sup>	4.80±0.01 <sup>b</sup>
UUF	6.40±0.02 <sup>abc</sup>	5.90±0.01 <sup>e</sup>	5.60±0.01 <sup>e</sup>	5.20±0.02 <sup>d</sup>	5.00±0.02 <sup>d</sup>	4.70±0.02 <sup>b</sup>
BSR	6.38±0.04 <sup>c</sup>	6.50±0.02 <sup>b</sup>	6.62±0.02 <sup>b</sup>	7.10±0.03 <sup>b</sup>	7.28±0.05 <sup>b</sup>	7.40±0.03 <sup>a</sup>
UUR	6.41±0.01 <sup>ab</sup>	6.68±0.02 <sup>a</sup>	7.00±0.00 <sup>a</sup>	7.28±0.02 <sup>a</sup>	7.40±0.03 <sup>a</sup>	7.42±0.02 <sup>a</sup>
BSSD	6.39±0.03 <sup>bc</sup>	6.20±0.01 <sup>c</sup>	5.70±0.02 <sup>d</sup>	5.20±0.01 <sup>d</sup>	5.00±0.02 <sup>d</sup>	5.00±0.00 <sup>b</sup>
UUSD	6.42±0.02 <sup>a</sup>	6.00±0.03 <sup>d</sup>	5.30±0.01 <sup>f</sup>	5.10±0.00 <sup>e</sup>	4.90±0.03 <sup>e</sup>	4.80±0.01 <sup>b</sup>
LSD	0.029	0.066	0.039	0.084	0.079	0.51

Means followed by the same letters in any column are not significantly different ( $P < 0.05$ )

BSF = Blanched – sulphited frozen samples

BSR = Blanched – sulphited refrigerated samples

BSSD = Blanched – sulphited solar dried samples

UUF = Unblanched – unsulphited frozen samples

UUR = Unblanched – unsulphited refrigerated samples

UUSD = Unblanched – unsulphited solar dried samples.

**Table 3. Titratable acidity of treated and untreated sliced okra under storage**

Sample	Titratable acidity (%) values of sliced okra in weeks					
	0	3	6	9	12	15
BSF	0.22±0.01 <sup>a</sup>	0.27±0.01 <sup>a</sup>	0.29±0.01 <sup>b</sup>	0.32±0.00 <sup>a</sup>	0.35±0.01 <sup>a</sup>	0.41±0.01 <sup>a</sup>
UUF	0.21±0.01 <sup>ab</sup>	0.28±0.02 <sup>a</sup>	0.30±0.02 <sup>b</sup>	0.34±0.01 <sup>a</sup>	0.35±0.01 <sup>a</sup>	0.39±0.01 <sup>ab</sup>
BSR	0.20±0.00 <sup>abc</sup>	0.12±0.01 <sup>b</sup>	0.09±0.01 <sup>a</sup>	0.04±0.00 <sup>b</sup>	0.03±0.00 <sup>b</sup>	0.02±0.00 <sup>c</sup>
UUR	0.19±0.02 <sup>bc</sup>	0.10±0.2 <sup>b</sup>	0.07±0.01 <sup>c</sup>	0.03±0.00 <sup>b</sup>	0.02±0.00 <sup>b</sup>	0.02±0.00 <sup>c</sup>
BSSD	0.21±0.03 <sup>ab</sup>	0.26±0.03 <sup>a</sup>	0.31±0.00 <sup>b</sup>	0.33±0.02 <sup>a</sup>	0.36±0.01 <sup>a</sup>	0.37±0.02 <sup>b</sup>
UUSD	0.18±0.02 <sup>c</sup>	0.27±0.02 <sup>a</sup>	0.30±0.02 <sup>b</sup>	0.34±0.01 <sup>a</sup>	0.37±0.02 <sup>a</sup>	0.37±0.01 <sup>b</sup>
LSD	0.023	0.028	0.027	0.023	0.028	0.029

Means followed by the same letters in any column are not significantly different ( $P < 0.05$ )

BSF = Blanched – sulphited frozen samples

BSR = Blanched – sulphited refrigerated samples

BSSD = Blanched – sulphited solar dried samples

UUF = Unblanched – unsulphited frozen samples

UUR = Unblanched – unsulphited refrigerated sample

UUSD = Unblanched – unsulphited solar dried samples.

storage conditions. Table 6 shows the sensory properties of okra samples stored under different conditions. The blanched, sulphited and frozen sample compared favourably with fresh okra in all the sensory quality attributes measured. Blanching and sulphiting treatments had no improvement in the sensory properties of solar dried okra.

## DISCUSSION

The values of ascorbic acid obtained from this study were similar to those obtained by various researchers (Mark *et al.* 1977; Addo 1983). Varietal and environmental factors were known to affect the ascorbic acid content of fruits and vegetables. However in the current study, there appeared to be meaningful effect of processing and preservation

methods on the vitamin C content of okra. Solanke and Awonorin (2002) have noted that loss of most nutrients in vegetables depends on the processing method used. Frozen storage appears to be the best method of retaining vitamin C in okra when compared with other preservation methods used in this study (Table 1). Blanching has been reported to inactivate ascorbic oxidase in vegetables which degrades ascorbic acid to hydroascorbic acid. Combination of blanching and frozen storage can help in the retention of ascorbic acid during storage of vegetables (Omodara and Aworh, 2001). Sulphiting reduced microbial load of some food spoilage microorganisms in the treated okra samples. Frozen storage enhanced the effects of these two processing

**Table 4. Microbial load of treated and untreated sliced okra under storage**

Sample	Microbial load (cfu/mL) x 10 <sup>3</sup> on storage in weeks					
	0	3	6	9	12	15
BSF	1.90±0.05 <sup>b</sup>	1.80±0.04 <sup>d</sup>	1.60±0.02 <sup>c</sup>	1.40±0.03 <sup>d</sup>	1.20±0.04 <sup>d</sup>	1.10±0.01 <sup>c</sup>
UUF	1.90±0.04 <sup>b</sup>	1.90±0.02 <sup>c</sup>	1.60±0.02 <sup>c</sup>	1.50±0.02 <sup>c</sup>	1.30±0.03 <sup>cd</sup>	1.20±0.02 <sup>c</sup>
BSR	1.90±0.04 <sup>b</sup>	2.30±0.03 <sup>b</sup>	2.70±0.04 <sup>b</sup>	3.16±0.01 <sup>b</sup>	3.40±0.03 <sup>b</sup>	4.00±0.08 <sup>b</sup>
UUR	1.90±0.03 <sup>b</sup>	2.40±0.02 <sup>a</sup>	3.10±0.01 <sup>a</sup>	4.90±0.05 <sup>a</sup>	6.50±0.08 <sup>a</sup>	7.20±0.08 <sup>a</sup>
BSSD	2.00±0.02 <sup>a</sup>	1.90±0.01 <sup>c</sup>	1.70±0.01 <sup>c</sup>	1.50±0.04 <sup>c</sup>	1.40±0.02 <sup>c</sup>	1.20±0.02 <sup>c</sup>
UUSD	1.90±0.00 <sup>b</sup>	1.90±0.03 <sup>c</sup>	1.70±0.02 <sup>c</sup>	1.50±0.03 <sup>c</sup>	1.30±0.02 <sup>cd</sup>	1.20±0.02 <sup>c</sup>
LSD	0.080	0.074	0.200	0.063	0.160	0.178

Means followed by the same letters in any column are not significantly different ( $P < 0.05$ )

BSF = Blanched – sulphited frozen samples

UUF = Unblanched – unsulphited frozen samples

BSR = Blanched – sulphited refrigerated samples

UUR = Unblanched – unsulphited refrigerated samples

BSSD = Blanched – sulphited solar dried samples

UUSD = Unblanched – unsulphited solar dried samples.

treatments and possibly lead to a better ascorbic acid complete inactivation of enzymes and microbes that

**Table 5. Viscosity of treated and untreated sliced okra under storage**

Sample	Viscosity (NSM <sup>2</sup> ) and Storage in weeks on storage in weeks					
	0	3	6	9	12	15
BSF	29.10±0.22 <sup>a</sup>	28.80±0.22 <sup>a</sup>	28.50±0.08 <sup>a</sup>	27.60±0.04 <sup>b</sup>	26.90±0.16 <sup>a</sup>	26.00±0.25 <sup>a</sup>
UUF	29.20±0.11 <sup>a</sup>	29.70±0.14 <sup>a</sup>	28.30±0.04 <sup>b</sup>	27.20±0.07 <sup>b</sup>	26.80±0.18 <sup>a</sup>	25.90±0.11 <sup>a</sup>
BSR	29.10±0.15 <sup>a</sup>	28.70±0.08 <sup>a</sup>	27.00±0.00 <sup>c</sup>	24.60±0.08 <sup>d</sup>	23.00±0.14 <sup>b</sup>	20.80±0.14 <sup>b</sup>
UUR	29.20±0.08 <sup>a</sup>	28.70±0.22 <sup>a</sup>	26.80±0.04 <sup>d</sup>	25.00±0.09 <sup>c</sup>	23.10±0.15 <sup>b</sup>	21.00±0.13 <sup>b</sup>
BSSD	17.00±0.09 <sup>b</sup>	15.00±0.08 <sup>c</sup>	14.10±0.04 <sup>f</sup>	13.30±0.08 <sup>f</sup>	12.20±0.19 <sup>d</sup>	11.00±0.05 <sup>c</sup>
UUSD	17.40±0.08 <sup>b</sup>	16.20±0.09 <sup>b</sup>	15.00±0.03 <sup>e</sup>	13.70±0.04 <sup>e</sup>	12.80±0.11 <sup>c</sup>	11.20±0.11 <sup>c</sup>
LSD	0.47	0.56	0.15	0.20	0.58	0.50

Means followed by the same letters in any column are not significantly different ( $P < 0.05$ )

BSF = Blanched – sulphited frozen sliced okra

BSR = Blanched – sulphited refrigerated sliced okra

UUF = Unblanched – unsulphited frozen sliced okra

UUR = Unblanched – unsulphited refrigerated sliced okra

retention.

During storage, the values of pH (Table 2) and titratable acidity (Table 3) in both solar dried and frozen products gradually fell and rose respectively. And also there were no distinct differences in titratable acidity values in three weeks of storage for the blanched – sulphited and unblanched – unsulphited products during the 15 – week storage. The more acidic environment provided in these products would no doubt have imparted antiseptic properties thereby extending the shelf-life of the products. From the results obtained, the blanching and sulphiting treatments in the present study did not achieve

enhance ascorbic acid degradation. While the low temperature of frozen storage and low moisture content of solar dried samples reduced deteriorative reactions, the effect of refrigeration temperatures was comparatively minimal. Refrigeration storage did not reduce appreciably the high rate of multiplication of spoilage microorganisms in okra (Table 4). However, the combination of blanching and sulphiting comparatively reduced the rate of proliferation of microorganisms in refrigerated okra samples.

The corresponding increase and decrease in the pH and titratable acidity respectively of refrigerated samples (Table 2, 3) could be

Table 6. Sensory scores of treated and untreated sliced okra after 15 – week storage

Quality attributes				
Sample	Color	Flavor	Drawing strength	Overall acceptability
BSF	7.7±0.8 <sup>a</sup>	7.3±0.6 <sup>ab</sup>	7.6±0.7 <sup>ab</sup>	7.1±0.6 <sup>ab</sup>
UUF	5.8±0.5 <sup>b</sup>	5.8±1.0 <sup>bc</sup>	6.5±0.5 <sup>bc</sup>	6.3±0.5 <sup>bc</sup>
BSR	6.1±1.0 <sup>b</sup>	6.1±0.7 <sup>bc</sup>	6.9±1.0 <sup>ab</sup>	6.5±0.7 <sup>b</sup>
UUR	4.8±0.3 <sup>bc</sup>	4.4±0.4 <sup>c</sup>	6.0±0.8 <sup>bc</sup>	4.9±0.5 <sup>cd</sup>
BSSD	3.7±0.6 <sup>cd</sup>	3.0±0.5 <sup>d</sup>	2.7±0.4 <sup>d</sup>	2.7±0.4 <sup>e</sup>
UUSD	3.8±0.4 <sup>c</sup>	3.3±0.4 <sup>d</sup>	2.8±0.5 <sup>d</sup>	3.4±0.5 <sup>de</sup>
FO	8.6±0.8 <sup>a</sup>	8.1±0.7 <sup>a</sup>	8.4±0.7 <sup>a</sup>	8.6±0.5 <sup>a</sup>
LSD	1.50	2.6	1.8	2.0

Means followed by the same letters in the same column are significantly different from one another ( $P < 0.05$ )

BSF = Blanched – sulphited frozen sliced okra

BSR = Blanched – sulphited refrigerated sliced okra

BSSD = Blanched – sulphited solar dried sliced okra

FO = Fresh okra

UUF = Unblanched – unsulphited frozen sliced okra

UUR = Unblanched – unsulphited refrigerated sliced okra

UUSD = Unblanched – unsulphited solar dried sliced okra.

explained by the high metabolic rate of proliferating spoilage microorganisms especially in the unblanched–unsulphited and refrigerated sample. Frozen storage has been shown to promote retention of viscoelastic properties of okra (Aworh *et al.* 1980; Olorunda and Tung, 1977). Blanching – sulphiting treatment of okra appeared to have no advantage in terms of viscosity in frozen storage as there was no significant difference between the viscosity of frozen blanched – sulphited and unblanched – unsulphited okra towards the end of 15 – week storage period (Table 5). The drastic reduction in the viscosity of solar dried okra samples could be due to deterioration caused by spoilage microorganisms at the early stage of drying as solar drying took longer time to accomplish. The microbial load in the frozen and solar dried samples reduced within the 15 – week storage period explaining the effect of decreasing pH (Table 2) and increasing titratable acidity (Table 3). Also this could be attributable to non-availability of moisture in these samples. On the other hand, the increased microbial load and activities in the refrigerated samples were probably because water was available in the liquid form (Table 4). Blanched – sulphited samples had sensory attributes that were comparable in all aspects ( $P < 0.05$ ) with the fresh sample. Solar dried samples had the poorest sensory quality attributes, followed by the

unblanched – unsulphited and refrigerated sample (Table 6).

### CONCLUSIONS

Processing treatments of blanching and sulphiting in combination with frozen storage led to better retention of ascorbic acid content, physicochemical properties and sensory attributes of sliced okra than refrigerated storage and solar drying, followed by ambient temperature storage.

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## Studies on monitoring pH responses during preservation of mushrooms (*Agaricus bisporus*, *A. bitorquis* and *Pleurotus ostreatus*) in steeping solution

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### ABSTRACT

The pH of three chemically preserved mushroom varieties was monitored during 90 days storage. The steeping solution containing variable amounts of chemical preservatives were used to study the affect of pH on preservation (browning) of mushrooms. The pH was determined after 30 days interval. Low acid mushrooms were made acidic by adding food grade acids as acetic acid, and citric acid to reduce the pH below 4.5. The chemical treatments affected the pH of mushrooms significantly while storage had non-significant effect on pH of brine solution. The highest mean pH value (4.27) was observed in *A. bisporus*, while the lowest in *P. ostreatus* (4.18). However, during three months storage a gradual decrease in pH was recorded in both the mushrooms.

**Key word:** *Pleurotus ostreatus*, *Agaricus bitorquis*, *Agaricus bisporus*, steeping solution.

### INTRODUCTION

The ability of foods to support the growth of food poisoning bacteria is measured by their pH value. The pH 4.5 is critical in food processing because below this value, *Clostridium botulinum*, the most dangerous and heat resistant food poisoning bacterium, is unable to grow.

Foods with a pH greater than 4.5 are termed low acid and require pressure-cooking to make them shelf stable. For acid foods pressure equipment is not required. Mushrooms are low acid food which can be made acidic by adding sufficient food acids such as acetic acid, and citric acid to reduce the pH below 4.5. To minimize the risk of spoilage, the pH should be maintained as low as possible and preferably not more than 4.5. To achieve effective processing for mushrooms, with pH 4.0, the filling temperature may be adjusted to 85°C and preferably between 90° and 95°C. In the presence of oxygen, rapid browning occurs due to the enzymatic oxidation of phenols to orthoquinones, which rapidly polymerize to form brown or black pigments, such as melanins. The most important factors that determine the rate of enzymatic browning of vegetables and fruits are the concentration of active polyphenoloxidase (PPO), phenolic compounds, pH, temperature and oxygen availability to the tissue. L-ascorbic acid is used in processing of foods as an oxygen scavenger (Bauernfeind 1982; Newsome 1987). The optimum pH of PPO activity varies with enzyme source and the substrate over a relatively wide range. In most cases, the optimum pH range of PPO is between 4 and 7.

The adjustment of pH with acids to 4 or below can be used to control browning as long as the acidity can be tolerated taste wise. The temperature stability of PPO varies with species and cultivars. The enzyme is relatively heat labile and activity is completely destroyed at 80°C (Vamos-Vigyazo, 1981).

### MATERIALS

#### Procurement of Raw Materials

Commercially available, freshly harvested button (*A. bitorquis* and *A. bisporus*) and oyster mushrooms (*P. ostreatus*) grown on cotton waste were procured from commercial mushroom farms situated at Sheikhpura (Pearl Mushroom Farm) and Faisalabad (Zahid Mushroom Farm). The culture of *A. bitorquis* (AGS W20) was originally obtained from HRI Wellsbourne, a famous research institute of UK, and revived at the culture bank of Institute of Horticultural Sciences, Mushroom Research Laboratory, University of Agriculture, Faisalabad, Pakistan.

The research was conducted in the fruit and vegetable research laboratory, Institute of Food Science and Technology, University of Agriculture, Faisalabad.

Other materials such as acetic acid, ascorbic acid, potassium sorbate, citric acid, potassium metabisulfite, sodium benzoate and sodium chloride were purchased from local scientific stores. The chemicals for physico-chemical analyses were purchased from Sigma-Eldrich Fine Chemicals, Faisalabad.

## METHOD OF PRESERVATION

### Washing and Blanching of Mushrooms

To avoid rapid browning of mushrooms during preparation, the mushrooms were cut into 2-3mm thick slices in a solution containing 0.1% citric acid and 0.3% potassium metabisulfite. The mushroom slices were blanched in boiling water for 4 minutes to inactivate enzymes and to prevent flavor and weight loss. Then the mushrooms were cooled to room temperature with water to avoid nutrient and flavor losses (Colin and Lucas 1979).

### Preparation of Steeping solution and Chemical Preservation

Steeping solution was prepared by dissolving calculated doses of chemicals to keep their concentration constant throughout the study period by using the formula stated by Bennett (1951). The steeping solution contained sodium chloride 5%, acetic acid 0.3%, ascorbic acid 0.1%, potassium sorbate 0.1%. Citric acid (2-4%) was used to adjust the pH of steeping solution to 4.0 using a pH meter (Hanna model 8520, Italy). The doses of acetic acid, common salt, ascorbic acid and potassium sorbate were kept constant while potassium metabisulfite and sodium benzoate were used in the steeping solution as independent variables according to the models generated by Response Surface Methodology (Minitab version.11.12.32, 1996).

### Variable values: potassium metabisulfite & sodium benzoate

T <sub>1</sub>	(0.06% KMS, 0.06% SB)
T <sub>2</sub>	(0.06% KMS, 0.08% SB)
T <sub>3</sub>	(0.08% KMS, 0.06% SB)
T <sub>4</sub>	(0.08% KMS, 0.08% SB)
T <sub>5</sub>	(0.07% KMS, 0.07% SB)
T <sub>6</sub>	(0.07% KMS, 0.07% SB)
T <sub>7</sub>	(0.07% KMS, 0.07% SB)
T <sub>8</sub>	(0.07% KMS, 0.07% SB)
T <sub>9</sub>	(0.07% KMS, 0.05% SB)
T <sub>10</sub>	(0.07% KMS, 0.09% SB)
T <sub>11</sub>	(0.05% KMS, 0.07% SB)
T <sub>12</sub>	(0.09% KMS, 0.07% SB)
T <sub>13</sub>	(0.07% KMS, 0.07% SB)
T <sub>14</sub>	(0.07% KMS, 0.07% SB)

KMS= Potassium metabisulfite  
SB= Sodium Benzoate

Coded and original levels of independent variables design matrix were made according to the Two Factors with Blocks for Fitting Second-Order

Response Model. Constant Values: Citric acid 2-4%, Acetic acid 0.3%, sodium chloride 5%, Ascorbic acid 0.1%, Potassium sorbate 0.1%.

### Method of Packing Mushroom

The mushrooms with steeping solution were packed in pre warmed glass jars with an equal quantity of mushrooms and steeping solution at 90° to 95°C, internally lined with aluminium foil to avoid any reaction between steeping solution and lid of the jar. The glass jars were cooled in air and put in a horizontal position at least for 5 minutes. Samples were stored at ambient temperature (27-30°C) for analyses.

### Determination of pH of Steeping Solution

The pH of the steeping solution of chemically preserved mushrooms was estimated using a pH meter (Hanna Model 8520, Italy) at 20°C at 30 days intervals up to 90 days.

### Statistical Analyses

Data were analyzed by using Analysis of Variance and Response Surface Techniques (Gacula and Singh 1984; Steel *et al* 1996; Minitab version 11.12.23, 1996).

## RESULTS AND DISCUSSION

Mushrooms were allowed to reach at equilibrium for 3 days and zero day readings were recorded. The pH of chemically preserved mushrooms was monitored during the whole course of study period after 30 days interval to ensure proper preservation of mushrooms. The data obtained were subjected to statistical analyses. The analyses of variances for pH are presented in Table-1 and the mean values are presented in Tables-2, 3 and 4. Reduction in pH was very minute due to the adjustment of pH with acids in the brine according to the formula (Bennett 1951). The analyses of variances reveal that pH of mushrooms was highly significantly different between samples. The treatments affected the pH value of mushrooms significantly while storage had non-significant effect on pH of brine solution. The interaction between storage and chemical treatments was also non-significant. Results of interaction between mushrooms, periods and treatments were also non-significant. The highest mean value (4.27) for pH was obtained with *A. bisporus*, while the lowest mean value (4.18) was obtained with *P. ostreatus*. *A. bitorquis* and *A. bisporus* were found statistically similar. A general decreasing trend in pH over 90 days storage period was observed.

It was observed during these studies that addition of

citric acid in appropriate amount not only assisted in proper preservation but also improved the taste and color (Tsai *et al.* 1984; Girdhari *et al.* 1998) of mushrooms. The amount of acid after reaching equilibrium in the steeping solution was within the tolerable limit and showed no microbial spoilage. Adjustment of pH within the limit 4.0 to 4.5 helped in proper processing/sterilization of mushroom.

Similar findings were reported by Iqbal (1996) who stated that addition of acids had discernible lethal effect on microorganisms as well as on enzyme inhibition. During his studies a gradual decrease in pH from 3.40 to 3.13 was observed during one month storage in brine solution.

**Table-1 Analyses of variances for pH of chemically preserved mushrooms during 90 days storage period**

SOV	DF	pH
Mushrooms (M)	2	0.452**
Storage periods(S)	3	0.511**
M x S	6	0.124 <sup>NS</sup>
Treatments (T)	13	0.085 <sup>NS</sup>
M x T	26	0.086 <sup>NS</sup>
S x T	39	0.063 <sup>NS</sup>
M x S x T	78	0.054 <sup>NS</sup>
Error	336	0.059
Total	503	

\*\*= Highly Significant; \*= Significant; NS=Non-Significant

**Table-2 Effect of different treatments on pH value of preserved mushrooms during 90 days storage period**

Mush/Treat	<i>P. ostreatus</i>	<i>A. bitorquis</i>	<i>A. bisporus</i>	Means
T <sub>1</sub>	4.14	4.32	4.37	4.28
T <sub>2</sub>	4.16	4.29	4.26	4.24
T <sub>3</sub>	4.18	4.30	4.21	4.23
T <sub>4</sub>	4.19	4.33	4.25	4.25
T <sub>5</sub>	4.15	4.21	4.08	4.15
T <sub>6</sub>	4.18	4.24	3.98	4.13
T <sub>7</sub>	4.16	4.26	4.36	4.26
T <sub>8</sub>	4.14	4.34	4.25	4.24
T <sub>9</sub>	4.16	4.13	4.27	4.19
T <sub>10</sub>	4.14	4.31	4.36	4.27
T <sub>11</sub>	4.13	4.25	4.26	4.21
T <sub>12</sub>	4.18	4.16	4.39	4.24
T <sub>13</sub>	4.37	4.21	4.36	4.31
T <sub>14</sub>	4.16	4.25	4.38	4.26
Means	4.18b	4.26a	4.27a	

Mean values showing same letters in columns are statistically non-significant (P>0.05)

**Table-3 Effect of different storage periods on pH value of preserved mushrooms during 90 days storage period**

Days/Treat	0	30	60	90	Means
T <sub>1</sub>	4.33	4.29	4.25	4.23	4.28
T <sub>2</sub>	4.28	4.25	4.23	4.19	4.24
T <sub>3</sub>	4.25	4.24	4.23	4.21	4.23
T <sub>4</sub>	4.31	4.25	4.23	4.21	4.25
T <sub>5</sub>	4.31	4.26	4.22	3.82	4.15
T <sub>6</sub>	4.31	4.23	4.18	3.81	4.13
T <sub>7</sub>	4.34	4.26	4.23	4.21	4.26
T <sub>8</sub>	4.28	4.25	4.22	4.21	4.24
T <sub>9</sub>	4.31	4.19	4.14	4.11	4.19
T <sub>10</sub>	4.33	4.27	4.25	4.22	4.27
T <sub>11</sub>	4.31	4.20	4.19	4.15	4.21
T <sub>12</sub>	4.32	4.27	4.22	4.16	4.24
T <sub>13</sub>	4.30	4.26	4.21	4.48	4.31
T <sub>14</sub>	4.35	4.29	4.23	4.18	4.26
Means	4.31a	4.25ab	4.22bc	4.16c	

Mean values showing same letters in columns are statistically non-significant (P>0.05)

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## Physico-chemical indices of red variety of seabuckthorn (*Hippophae rhamnoides*)

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### ABSTRACT

This study was carried out to evaluate the physico-chemical composition of seabuckthorn red berry variety wildy grown in Northern Areas of Pakistan, Skardu, Shigar, Khaplu and Hunza. The analysis of the fruit was carried out in the form of whole berries, pulp and seed. The parameters studied were pH, acidity, sugar, protein, ascorbic acid, moisture, total soluble solids (TSS), ash and oil. Mean moisture content were 62.05, 67.55 and 16.56% in whole berries, pulp and seeds, respectively. The ash levels from four sites were found in the range of 3.12-3.26 (whole), 0.91-1.34 (pulp) and 1.76-1.92% (seed). Maximum mean concentrations of various parameters determined were 11.37 (TSS), 13.33 (crude oil), 20.07 (crude protein), 1.18 (titratable acidity) and 38.12 mg/100g (ascorbic acid). Highest total and reducing sugars were 9.38 and 7.98% in the pulp while maximum non-reducing level was observed in seed (5.42%).

**Key word:** Seabuckthorn berries, ascorbic acid, oil content, reducing sugars, total soluble solids.

### INTRODUCTION

The multipurpose shrub tree, Seabuckthorn, belongs to the family Elaeagnaceae. The most important specie is *Hippophae rhamnoides* L (Linn). It is a unique and valuable plant currently cultivated in various parts of the world. The natural habitat of seabuckthorn extends widely in China, Mongolia, Russia, and most parts of North Europe. It can withstand extreme temperatures from -43°C to 40°C and is considered to be drought resistant (Li and Shroeder 1999). *Hippophae rhamnoides* Linn, sub-species *Turkestanica* is found in Chitral and Northern Areas of Pakistan. Normally it is spread throughout the Karakorum and Himalayan ranges at altitudes of 1500-3500 m. According to a Chinese seabuckthorn expert, Professor Rongsen, Pakistan produces 1200-2500 tons seabuckthorn annually (Khan 1999). *Hippophae rhamnoides* fruits and juices were found to be rich in proteins, carbohydrates, organic acids, amino acids, globulins and albumins. Fruit drinks were among the earliest seabuckthorn products developed in China. Seabuckthorn based juice is also popular in Germany and Scandinavia (Huang *et al.* 1991). The berries also appeared to be a natural source of Vitamin A, B, B<sub>1</sub>, B<sub>2</sub>, C, E, K and P, carotenes and flavonoids. Vitamin C content of seabuckthorn is 3-6 times higher than that of Kiwi fruit (Solonenko and Shishkina 1983; Rongsen 1992; Beveridge *et al.* 1999). About 27 mineral elements are present in seabuckthorn, among these the Al, Ca, Fe, Mg, P, Mn, Ti, Si, As and Ba contents are found in high quantity (Tigong 1988).

Based on scientific research, seabuckthorn has become an important medicinal and nutritional product. Although the oil content of seabuckthorn is not much as compared to most oil crops, but its nutritive and medicinal values are much more than those of most oil crops, because it contains a lot of fat-soluble bioactive substances (Guoli 1987). Shyrko and Radzyuk (1989) presented data on the quality and chemical composition of fruit of 20 varieties of seabuckthorn while Heilscher and Lorber (1999) compared the composition and nutritive value of berries of this plant to those of other fruits including apple and oranges.

The aim of this study was to analyze the physico-chemical contents of red variety of seabuckthorn wildy grown in Northern Areas of Pakistan. The outcome of this work will bring awareness regarding the nutritional and economic importance of this fruit among the growers, government departments and private investors in these areas.

### MATERIALS AND METHODS

#### Collection and preparation of samples

Fully ripened, sound and healthy seabuckthorn berries of red variety were collected from four different localities of Northern Areas i.e. Skardu, Shigar, Khaplu and Hunza. Whole berries were washed crushed with electric blender and were tested for physico-chemical characteristics. Pulp and seeds of

berries were also analyzed for proximate composition separately.

#### Physico-chemical Analysis

Levels of selected parameters (moisture, ash, pH, total soluble solids, oil, protein, acidity, ascorbic acid reducing, non-reducing and total sugars) were determined by standard methods (AOAC 1984). Crude oil was estimated by intermittent Soxhlet extraction apparatus while crude protein was determined by Kjeldahl method. pH was measured by using Inolab Digital pH Meter. The total titratable acidity was analyzed by titrating against standard alkali solution. Total soluble solids (TSS) were detected using hand refractometer at room temperature. Sugar was determined by Lane Eynon method and ascorbic acid by the titrametric method.

#### RESULTS AND DISCUSSION

Samples of red variety of seabuckthorn berries, collected from four different locations (Skardu, Shigar, Khaplu and Hunza) were analyzed for selected parameters. Results presented in the tables are the mean values of three independent readings. In all cases, data are statistically different ( $P < 0.05$ ) from each other. Proximate composition of the berries from four locations was averaged and are given in Table-1. Red variety contained 62.05, 67.55 and 16.56% moisture, 3.04, 1.20 and 1.83% ash, 13.37, 5.37 and 7.95% crude oil, 3.54, 2.92 and 19.57% crude protein, 3.15, 3.15 and 4.33 pH, 0.98, 1.18 and 0.79% titratable acidity, 6.71, 7.98 and 1.48% reducing sugar, 1.66, 1.40 and 5.42% non reducing sugar, 78.38, 9.38 and 6.89% total sugar, 26.88, 38.12 and 7.29mg/100g ascorbic acid in whole berries, pulp and seed respectively. Total soluble solids (TSS) contents were 11.12 and 11.37% Brix in whole berries and pulp, respectively. Zhiban (1987) studied different parts of seabuckthorn and found that the dry content of pulp, seed and whole berries were 22.9, 82.8 and 26.0% respectively.

Results of moisture, ash, pH and TSS are presented in Table 2. As shown in Table II, maximum moisture content (72.12%) in whole berries was observed in samples collected from Skardu location while minimum level (52.50%) was found in the sample collected from Hunza location. In pulp highest moisture level (73.42%) was found also at Skardu location while lowest level (63.80%) was observed at Hunza site. Minimum moisture concentration (13.83%) was recorded in seeds from Hunza location while maximum moisture value of 19.41% was observed in seeds from Skardu location. The varieties and locality means for whole berries of seabuckthorn fruit were non significant, while for pulp both had significant

effect on moisture contents, whereas the means of locality for seed were non significantly different but varieties have significant effect on moisture content. The results shown here slightly differ from those of Zhiban and Yansheng (1987) who recorded moisture 74.0, 77.1 and 17.2% in whole fruit, pulp and seed respectively. The slight decrease in moisture contents in our observations might be due to storage effect on berries, which might lead to moisture loss during transportation.

Tabl-1 Proximate composition of red berry variety of seabuckthorn (on average basis)

Parameters	Whole	Pulp	Seed
Moisture (%)	62.05	67.55	16.56
Ash (%)	3.04	1.20	1.83
pH	3.15	3.15	4.33
T.S.S (%)	11.12	11.37	-
Crude Oil (%)	13.33	5.37	7.95
Crude Protein (%)	3.54	2.92	19.57
Titratable Acidity (%)	0.98	1.18	0.79
Ascorbic Acid (mg/100g)	26.88	38.12	7.29
Reducing Sugar (%)	6.71	7.98	1.48
Non Reducing Sugar (%)	1.66	1.40	5.42
Total Sugar (%)	8.38	9.38	6.89

The data in Table-2 show the highest ash content 3.26% at Hunza location while lowest level (3.12%) was found at Shigar and Khaplu locations in whole berries. The ash content in pulp was found maximum at Hunza location i.e. 1.34%, while minimum level of 0.91% was recorded at Skardu site. In seed the maximum ash concentration (1.92%) was observed for Hunza location while minimum (1.76%) was found in samples collected from Skardu. Analysis of variance at  $\alpha = 0.05$  for ash percentage of whole berries and pulp shows significant effect on whole berries, pulp and seed but non significant effect on location, while for seed both variety and location shows non significant effect. This result slightly differs from data obtained by Shyrko and Radzyuk (1989) who reported ash percentage in fruit as 0.32-0.48%. The slight increase in ash in our observation might be due to genotype, geographical location, soil condition and climate.

Table-3 indicates that in the whole berries of seabuckthorn, high pH value (3.10) was observed for Khaplu and Shigar location while lowest pH (3.08) was observed in Skardu samples. Similarly, lowest pH (3.13) in pulp was detected at Skardu location while highest value of 3.17 was found at Shigar location. In seed, maximum pH i.e. 4.74 was found at Shigar location while minimum pH of 4.10 in samples

obtained from Khaplu was noticed. Statistical analysis have significant effect, while in whole berries, pulp and seed shows non-significant effect. This result is in agreement with Beveridge *et al.* (1999) who recorded pH of 2.7. This slightly increase high pH might be due to high sugar content of the subspecies *turkistanika* studied in present work, than other species (Rongsen, 2003).

As shown in Table-2, the highest TSS level (12.5°Brix) in whole berries was recorded at Hunza location, while lowest value of 9.5°Brix was at Skardu. In pulp the maximum TSS (12.25°Brix) was found at Hunza location while minimum (10.25°Brix) was observed at Khaplu location. When statistically analyzed, it was observed that there was significant difference among localities and 3 parts of berries, while pulp shows no significant effect for both the factors. Our observation is in agreement with the reported (Rongsen, 2003) range of TSS in seabuckthorn is 10-13°Brix.

The crude oil content in whole berries was found to be maximum (14.75%) at location Khaplu while minimum (12.26%) at Skardu site (Table-3). Also in pulp the maximum oil content of 6.46% was detected in Khaplu location while minimum concentration of 4.83% was recorded in samples obtained from Skardu. The highest oil percentage (8.42) was observed in seed at Shigar location while lowest (7.43) was observed at Skardu location. Statistical analysis of variance for crude oil revealed that whole berries, pulp and seed showed significant effect among the four types of berries. While whole berries and pulp have significant effect for location instead of seed that showed non-significant effect for locality. This result is in agreement with the data of Rongsen (2003) who determined the oil from seabuckthorn and stated that the oil contents were 7.8-17.85% in seed and 2.46-18.45% in pulp.

Regarding protein content, highest value (4.38%) was found in berries grown at Skardu location while lowest content (3.15%) at Khaplu location in whole berries (Table-3). Maximum protein level (3.27%) in pulp was observed at Shigar while minimum value of 2.75% was at Hunza site. In seed maximum protein content i.e. 21.37% was at Shigar location and minimum in Khaplu location i.e. 18.58%. Statistical analysis of variance shows that means of protein for seed is significant for both location and varieties, while for pulp significant effect was observed while location are non significant. For whole berries localities show non-significant effect while location shows significant effect. Our results are slightly different from Ma

of variance shows that the means for pulp and seed Zhiben and Yansheng (1987) who reported 1.20, 0.4 and 19.6 % protein in whole berries, pulp and seed of seabuckthorn subspecies *Sinensis*. This increase in our observation may be due to difference in ecological zone, species or climatic conditions.

Titrateable acidity of seabuckthorn (Table-3) revealed highest percent titrateable acidity (1.20) in red variety at Skardu location, while lowest (0.87) was found at Hunza. In pulp the maximum acidity (1.34%) was found at Shigar and Shigar, while minimum (1.00%) was detected at Khaplu location. Seed shows highest content (1.13%) at Hunza location and lowest level of 0.46% at Skardu. Analysis for variance shows that seed has significant effect for location, while pulp has significant effect for four types of berries but non-significant effect for location. For whole berries non-significant effect is obtained for both of variety and location. The result in Table III shows slightly low acidity than reported by Jalakas *et al.* (2003), who recorded the acidity in range of 2.1-3.0%. The slight decrease in acidity may be due to high sugar content of *turkistanika* sub species.

Whole berries contain high ascorbic acid (35.54 mg/100g) at Khaplu location, while low content (21.23) was observed at Skardu location (table-3). Ascorbic acid percentage in pulp was found maximum (43.52) at Khaplu location, while minimum (33.56) at Skardu location. When seed was analyzed, it showed maximum vitamin C (8.03) at Shigar location, while minimum content (6.71) was observed at Skardu site. When statistically analyzed the locality means showed significant effect for whole berries, pulp and seed. This result is in agreement with Yao *et al.* (1992) who reported that vitamin C concentration varied from 28-201 mg/100g of berries among bushes.

Levels of reducing, non-reducing and total sugars are given in Table-4. It is evident that maximum total sugar content (9.34%) was in Hunza location, while minimum (7.58%) at Skardu location in whole berries. Pulp contained the highest total sugar (9.95) at Hunza location while minimum (8.89%) was observed at Khaplu location.

Total sugar percentage in seed shows highest value of 7.24 at Shigar location, while lowest level of 6.60 was recorded at Skardu location. Statistical analysis shows significant effect for all except locality means in seed that is non significant. Similar findings were reported by Zhiben and Yansheng (1987) who recorded 6.29, 7.17 and 5.84% total sugar in whole fruit, pulp and seed of seabuckthorn, respectively.

Tabl-2: Mean levels of moisture, ash, pH and total soluble solid (TSS) in red variety of seabuckthorn

Location	Whole berries	Pulp	Seed
<b>Moisture (%)</b>			
Skardu	72.12	73.42	19.41
Shigar	60.06	65.36	16.48
Khaplu	63.52	67.62	16.54
Hunza	52.50	63.80	13.83
<b>Means</b>	<b>62.05</b>	<b>67.55</b>	<b>16.56</b>
<b>Ash (%)</b>			
Skardu	2.67	0.91	1.76
Shigar	3.12	1.25	1.87
Khaplu	3.12	1.32	1.80
Hunza	3.26	1.34	1.92
<b>Means</b>	<b>3.04a</b>	<b>1.20</b>	<b>1.83a</b>
<b>pH</b>			
	<b>Whole berries</b>	<b>Pulp</b>	<b>Seed</b>
Skardu	3.08	3.13	4.18
Shigar	3.10	3.17	4.74
Khaplu	3.10	3.16	4.10
Hunza	3.09	3.15	4.31
<b>Means</b>	<b>3.15</b>	<b>3.15</b>	<b>4.33</b>
<b>TSS (%)</b>			
Skardu	9.5	11.25	-
Shigar	11.25	11.75	-
Khaplu	11.25	10.25	-
Hunza	12.5	12.25	-
<b>Means</b>	<b>11.12a</b>	<b>11.37</b>	<b>-</b>

\*Figures with different letters are statistically different ( $P < 0.05$ ) from each other.

The slight difference may be due to species (Rongsen, 2003). Whole berries of seabuckthorn at Hunza contained high percentage of reducing sugar (7.95%) while minimum (5.65%) was observed from Skardu location. In pulp the highest reducing sugar value of 8.85% was observed at location of Hunza, while lowest one (7.24%) was at Khaplu. Reducing sugar content in seed was found highest (1.64%) at Hunza site, while lowest (1.32%) at Khaplu location. Statistical analysis shows significant result (at  $\alpha = 0.05$ ) both for four types of berries and location in whole berries and pulp, while with respect to seed, all four types showed significant effect, where as the localities have non significant differences. Our result is in agreement with Ma Zhiben and Yansheng (1987), who determined reducing sugars in whole berries, pulp and seed as 6.05, 6.95 and 1.60%, respectively. This slight increase may be due to species and altitude (Rongsen, 2003). Non-reducing sugar content in whole berries was high in Skardu location (1.93%) while low content was observed at

Hunza location (1.39%). In pulp maximum non-reducing sugar (1.80%) was found at Shigar location, while minimum (1.05%) was found grown in Skardu. When seed was analyzed highest non-

reducing sugar (5.82%) was found at Shigar location, while lowest was observed at Skardu location (5.07%). Statistical analysis shows non-significant differences for localities and in all cases that is whole berries, pulp and seed. This result is in agreement with Ma Zhiben and Yansheng (1987) who reported 0.24, 0.22 and 4.24% in whole fruit, pulp and seed respectively. The slight increase may be due to species (Rongsen 2003).

### CONCLUSIONS

From the results of this investigation it can be concluded that moisture, acidity, ascorbic acid and total and reducing sugars were high to low in the order of pulp, whole berries and seed, while the ash and crude oil contents were in the sequence of

**Table-3. Mean levels of crude oil, crude protein, titrateable acidity and ascorbic acid in red variety of seabuckthorn**

Location	Whole berries	Pulp	Seed
<b>Crude oil (%)</b>			
Skardu	12.26	4.83	7.43
Shigar	13.67	5.25	8.42
Khaplu	14.75	6.46	8.29
Hunza	12.64	4.97	7.67
<b>Means</b>	<b>13.33b</b>	<b>5.37b</b>	<b>7.95b</b>
<b>Crude protein (%)</b>			
Skardu	4.38	2.92	21.35
Shigar	3.44	3.27	21.37
Khaplu	3.15	2.76	18.58
Hunza	3.22	2.75	18.98
<b>Means</b>	<b>3.54</b>	<b>2.42b</b>	<b>20.07b</b>
<b>Titrateable acidity (%)</b>			
Skardu	1.20	1.20	0.46
Shigar	0.98	1.34	0.73
Khaplu	0.89	1.00	0.86
Hunza	0.87	1.20	1.13
<b>Mean</b>	<b>0.98</b>	<b>1.18b</b>	<b>0.79b</b>
<b>Ascorbic acid (mg/100)</b>			
Skardu	21.23	33.56	6.71
Shigar	28.75	40.16	8.03
Khaplu	35.54	43.52	7.50
Hunza	22.02	35.24	6.94
<b>Means</b>	<b>26.88b</b>	<b>38.12b</b>	<b>7.29b</b>

\*Figures with different letters are statistically different ( $P < 0.05$ ) from each other.

**Table-4. Percent mean levels of total, reducing and non-reducing sugars in red variety of seabuckthorn**

Location	Whole berries	Pulp	Seed
<b>Total sugar (%)</b>			
Skardu	7.58	8.99	6.60
Shigar	8.39	9.72	7.24
Khaplu	8.21	8.89	6.99
Hunza	9.34	9.95	6.76
<b>Means</b>	<b>8.38</b>	<b>9.38</b>	<b>6.89</b>
<b>Reducing sugar (%)</b>			
Skardu	5.65	7.94	1.53
Shigar	6.55	7.92	1.42
Khaplu	6.7	7.24	1.32
Hunza	7.95	8.85	1.64
<b>Means</b>	<b>6.71a</b>	<b>7.98a</b>	<b>1.47a</b>
<b>Non-reducing sugar (%)</b>			
Skardu	1.93	1.05	5.07
Shigar	1.84	1.80	5.82
Khaplu	1.51	1.65	5.67
Hunza	1.39	1.10	5.12
<b>Means</b>	<b>1.66</b>	<b>1.40</b>	<b>5.42</b>

\*Figures with different letters are statistically different ( $P < 0.05$ ) from each other.

whole berries>seed>pulp. Concentration of crude protein and non-reducing sugar were highest maximum in the seed and lowest in pulp. Highest pH was observed in seed while lower in whole berries and pulp portions.

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## Modification of ice-cream characteristics by addition of peach and non-nutritive sweetener

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### ABSTRACT

The cream was prepared by the addition of fresh and non nutrition sweet era. The study showed significant changes in physico-chemical and sensory parameters in ice-cream samples prepared with 40% peach fruit and 15% non-nutritive sweetener or 42% peach fruit and 16% non-nutritive sweetener and 44% peach fruit and 15% sucrose were found. Physico-chemical changes were measured in terms of moisture, total solids, ash, pH, acidity, over-run, lactose, fat and sucrose. Sensory analysis showed decline in various attributes such as appearance, flavor and taste. Same trend was observed in respect of texture and overall quality.

**Key word:** Ice cream, peach fruit, non-nutritive sweetener, physico-chemical analysis, sensory analysis.

### INTRODUCTION

A quality Ice-cream is the balanced blend of components like milk, sweeteners, stabilizers, emulsifier, flavoring and other ingredients such as egg products, colorings and starch hydrolysates. Peach (*Prunus persica*) is an important fruit crop grown in Pakistan. It is rich source of vitamin A and potassium. Peach contains water 89.1%, phosphorus 19 mg, calcium 9 mg, potassium 202 mg, vitamin-A 1330 International units, ascorbic acid 7 mg and food energy 38 calories per 100g. In peach glutamic acid is present in the amount of 143 mg/100 mg.

The ratio of fruit flavor concentration to sugar in ice-cream mix could be critical. When fresh fruits are used the concentration ranged from a low of 10% of the mix as for raspberry and pineapple and up to 25% for apple, apricot and peach.

Saccharine, aspartame, acesulfame-k and sucralose are used as non-nutritive sweeteners in ice-cream. Ice-cream mix formulation contains more than 12% and more than 7% by weight of sucrose and fats respectively as well as skim milk, glucose, saturated fat, stabilizer and emulsifier. Flavors give ice-cream variety and consumer appeal. Vanilla is still the most popular flavor followed by chocolate, strawberry and a very large number of fruit, nut and other combinations.

New variety of ice-cream with appetizing flavor notes and chewy eating sensation could be manufactured by utilizing peach fruit. There is predominant effect of osmotically extracted jambolana color and aroma on ice-cream quality.

Peach ice cream can be of great economic importance. The main objective of this research was to develop a new type of ice cream by the addition of peach and non-nutritive sweetener to the formulations. This paper reports the studies on the preparation of ice cream with different ratios of peach and non-nutritive sweetener.

### MATERIALS AND METHODS

The raw materials such as milk, cream, skim milk peach fruit, flavor, color were purchased from the local market. All the ingredients were weighed using analytical balance according to the formulation. Milk was measured volumetrically. The dry ingredients such as sugar and skim milk powder were mixed together and sprinkled over the liquid ingredients along with manual stirring and these ingredients were also blended in the blender.

#### Formulation of Ice-cream

Ice-cream mix formulation applied was as under:

Milk solids-not-fat	11.0%
Fat	10.0%
Sugar (sucrose)	15.0%
Non nutritive sweetener	15.0%
Peach Flavor	0.03%
Peach Fruit	40-44%

#### Details of treatments

T<sub>1</sub> = Ice cream with 40% fruit and 15% non-nutritive sweetener

T<sub>2</sub> = Ice cream with 42% fruit and 16% non-nutritive sweetener

T<sub>3</sub> = Ice cream with 44% fruit and 15% sucrose

### Preparation of Ice cream

The ice-cream mix was pasteurized at 72°C for 30 minutes to destroy pathogenic organisms (such as *Bacillus tuberculosis*, *Brucella abortus* and *Streptococcus mastitis*). Pasteurization melts the fat globules and aids in texture and body of the ice cream. After pasteurization the ice-cream mix and peach slurry were homogenized in an electric homogenizer. The process of homogenization was repeated to get the fat globules of desired size. Homogenization is known to check creaming and improving appearance of the ice cream. Then the mix was cooled down to 4°C in a deep freezer and stored at 4°C for 5-6 hr for ageing. Performance of the mix during freezing is improved by this treatment. If ageing is not done then loose stand up and very wet product on drawing from freezing machine is obtained. This was done to give time for the fat to crystallize and protein and stabilizer to bind water. Ageing also increases the viscosity of the mix. After ageing, the mix was frozen at -5°C in the freezing chamber of electrically operated batch type ice-cream machine. The ice cream was packed in disposable 100 ml cups and immediately transferred to deep freezer at -20°C. The storage studies were conducted for a period of 30-days. Physico-chemical and sensory testing of prepared ice cream samples were conducted at 0, 15, 30 days intervals. The temperature of the products during storage was maintained at -20°C.

### Physico-chemical analysis

Moisture and acidity were determined by the method given by Egan *et al.* (1987). In moisture analysis each sample (5.0 g) was taken in a dried dish containing sand and flat-ended rod. To assist spreading of the sample 5ml water was added and then dried on a boiling water bath and kept for at least 2 hr in an oven at 98-100°C. Acidity was determined by taking 10 g sample of ice-cream in a dish and adding distilled water to make volume up to 20 ml. then a few drops of phenolphthalein indicator were added and the material was titrated against 0.1 M NaOH to a light pink end point. Total solids, ash and pH were analyzed according to methods described by AOAC (1990). In total solids analysis a sample of about 2 gram was weighed into round, flat bottom dish and heated on steam bath for 30 minutes and then, in hot air oven for 3.5 hours at 98-100°C. Ash contents were determined by taking 3-5 grams of prepared samples in a China dish. The material was then placed in furnace at

550°C until carbon free ash was obtained. The pH of ice cream was determined with a pH meter. Each sample was mixed thoroughly and then taken in the beaker in which electrodes were placed and reading recorded. Over-run of the ice cream samples were determined by using the method given by Varnam and Sutherland (1994). First of all empty graduated

cylinder was weighed. Then ice-cream was melted in cup and graduated cylinder was filled with this melted ice-cream after that ice-cream sample was taken in the graduated cylinder and weighed. In lactose analysis Fehling's solution (10 mL) comprising of Fehling A and B was taken in conical flask and titrated against the 3 % ice-cream sample solution.

The fat contents of ice cream were estimated by Gerber method. For this purpose, ice cream was brought to the room temperature. A measured quantity i.e. 10 mL of concentrated sulphuric acid was taken into the cream butyrometer to which 5.0g of the sample was carefully added. Then 5ml of hot distilled water was added followed by addition of 1ml amyl alcohol. In sucrose analysis, 40 mL of 10% ice-cream solution was taken in a conical flask and 5 ml of concentrated HCl was added and cooled in water bath for 10 minutes. It was then neutralized with 50% NaOH solution. The volume was then made 200 mL and Fehling's A and B solutions 10 mL were titrated against it. All the ice-cream samples were organoleptically rated for appearance, taste, flavor, texture and overall acceptability by a panel of 3 judges using the 9 point hedonic scale.

### RESULTS AND DISCUSSION

The moisture did not significantly change during storage but significant change was observed between treatments. T<sub>1</sub> ranked first and T<sub>3</sub> third because T<sub>1</sub> contained fewer amounts of fruit and T<sub>3</sub> contained more fruit. Results are also deviating from standard significantly. Samples have no effect during storage period but effect was observed between treatments when analyzed for total solids T<sub>1</sub> ranked third and T<sub>3</sub> ranked first due to variable quantities of fruit. Samples deviated from the standard significantly. Significant change was observed both between treatments and between storage periods when samples were analyzed for ash. T<sub>1</sub> ranked third and T<sub>3</sub> ranked first in ash content and there was an increasing trend during storage period. Some of the samples deviated from the standard significantly but some did not. When samples were analyzed for pH, no significant change was observed during storage period but change was observed between treatments. T<sub>1</sub> ranked first and T<sub>3</sub> ranked third. Results did not deviate from standard

Table-1. Physico-chemical analysis of peach ice cream

Physico-Chemical parameters of Ice-cream	Storage period	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
	Interval in days	MV	MV	MV
Moisture (%)	0	68.05± 0.42	67.0±0.14	65.7± 0.42
	15	67.80± 0.70	67.2± 0.70	65.8± 0.14
	30	68.0± 0.14	67.0± 2.12	65.7± 0.98
Total solids (%)	0	31.95± 0.42	33.0± 0.14	34.3 ± 0.42
	15	32.2± 0.35	32.8± 0.70	34.2± 0.14
	30	32.0± 0.07	33.0± 2.12	34.3± 0.98
Ash (%)	0	0.66 ± 0.00	1.00 ± 0.00	1.30± 0.24
	15	0.90± 0.00	1.10± 0.70	1.38± 0.02
	30	1.00 ± 0.14	1.20 ± 0.46	1.46 ± 0.23
pH	0	6.00 ± 0.00	5.80 ± 0.00	5.60 ± 0.00
	15	5.90 ± 0.00	5.60 ± 0.00	5.40 ± 0.00
	30	6.00 ± 0.00	5.70 ± 0.00	5.50 ± 0.00
Acidity (%)	0	0.49 ± 0.02	0.59 ± 0.01	0.62 ± 0.03
	15	0.52 ± 0.02	0.61 ± 0.02	0.64 ± 0.02
	30	0.56 ± 0.02	0.63 ± 0.02	0.68 ± 0.03
Over-run (%)	0	85.65 ± 0.50	99.29 ± 0.69	81.87 ± 0.60
	15	84.65 ± 0.63	97.50 ± 0.70	79.75 ± 0.63
	30	83.40 ± 0.84	96.67 ± 0.60	78.60 ± 0.84
Lactose (%)	0	5.43 ± 0.09	5.50 ± 0.07	5.65 ± 0.21
	15	5.41 ± 0.06	5.51 ± 0.05	5.63 ± 0.02
	30	5.43 ± 0.06	5.50 ± 0.05	5.65 ± 0.01
Fat (%)	0	10.4 ± 0.57	10.8 ± 0.42	11.2 ± 0.28
	15	10.3 ± 0.42	10.8 ± 0.35	11.3 ± 0.14
	30	10.4 ± 0.49	10.8 ± 0.35	11.2 ± 0.35
Sucrose (%)	0	2.0± 0.21	2.5± 0.14	15.7 ±0.21
	15	2.2± 0.21	2.6± 0.14	15.8 ± 0.21
	30	2.0± 0.21	2.5± 0.21	15.7 ± 0.21

significantly. When samples were analyzed for acidity significant changes were observed between treatments as well as during storage period. T<sub>1</sub> ranked third and T<sub>3</sub> ranked first.

Acidity increased during storage period. Results did not deviate from standard significantly. When samples were analyzed for over-run, a decreasing trend was observed during storage period and T<sub>1</sub> ranked third and T<sub>3</sub> ranked first. In case of lactose analysis significant change was observed between treatments and no change was observed during storage period. T<sub>1</sub> ranked third and T<sub>3</sub> ranked first. Results did not deviate from standard significantly. When samples were analyzed for fat, change was observed between treatments but no change was observed during storage period. T<sub>3</sub> ranked first and T<sub>1</sub> ranked third. Results deviated from standard significantly. When samples were analyzed for sucrose no change was

observed during storage period but change was observed between treatments. T<sub>3</sub> ranked first and T<sub>1</sub> ranked third because T<sub>3</sub> contained sucrose and rest of treatments contained non-nutritive sweetener. Results deviated from standard significantly (Table-1)

The flavor significantly changed during storage period as well as between treatments. The flavor decreased during storage period T<sub>1</sub> ranked first and T<sub>3</sub> ranked third. Appearance of the sample decreased during storage period and there was also significant difference between treatments. T<sub>3</sub> ranked first and T<sub>2</sub> ranked third. Taste of the samples decreased during storage period. T<sub>2</sub> ranked first and T<sub>1</sub> ranked third. Texture decreased during storage period, T<sub>2</sub> ranked first T<sub>3</sub> ranked third.

In overall acceptability T<sub>1</sub> ranked first and T<sub>3</sub> ranked third. Overall acceptability decreased during storage period (Table-2).

Table-2. Sensory parameters of ice cream

Sensory evaluation parameter of ice-cream	Storage period	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Flavor (H.R.S)	Interval in days	MV	MV	MV
	0	7.00 ± 2.64	6.30 ± 1.15	6.00 ± 1.00
	15	6.20 ± 2.36	5.80 ± 1.15	5.50 ± 1.00
	30	5.70 ± 2.36	5.30 ± 1.15	5.00 ± 1.00
Appearance (H.R.S)	0	6.60 ± 1.52	6.30 ± 0.57	7.50 ± 1.00
	15	6.50 ± 1.80	6.40 ± 0.57	7.00 ± 1.00
	30	6.30 ± 2.08	6.30 ± 0.57	7.50 ± 1.00
Taste (H.R.S)	0	6.30 ± 2.88	6.70 ± 0.57	6.30 ± 0.57
	15	5.50 ± 2.59	5.80 ± 0.28	5.80 ± 0.57
	30	5.00 ± 2.59	5.30 ± 0.28	5.30 ± 0.57
Body/Texture (H.R.S)	0	6.60 ± 2.51	7.00 ± 1.00	5.60 ± 1.52
	15	6.20 ± 2.51	6.50 ± 1.00	5.20 ± 1.52
	30	5.60 ± 2.51	6.00 ± 1.00	4.00 ± 1.52
Overall acceptability (H.R.S)	0	7.60 ± 1.15	6.30 ± 0.57	6.00 ± 1.00
	15	7.20 ± 1.15	5.80 ± 0.57	5.50 ± 1.00
	30	6.70 ± 1.15	5.30 ± 0.57	5.00 ± 1.57

HRS = Hedonic Rating Scale

## CONCLUSIONS

A new variety of ice cream particularly with sweet sensation and appetizing flavor notes can be manufactured by utilizing different ratios of peach fruit. It is felt that use of different ratios of peach fruit may result in a product, which has better acceptance. Non-nutritive sweetener can be incorporated in two types of ice cream instead of sucrose. Inclusion of peach and non-nutritive sweetener in ice cream can be done to improve nutritional value of ice cream and it is also a good step towards diet ice cream.

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## Rice harvesting and threshing

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### ABSTRACT

Rice harvesting and threshing methods practiced in the world with special reference to Pakistan have been discussed. Rice harvesting and threshing is done manually in Punjab whereas in Sindh, harvesting is done manually and threshing by tractors as well as bullocks treading. The reconditioned wheat combines are also used for rice harvesting in Punjab. These are insufficient in number and hardly cover 40 percent rice area in Punjab. These are not properly adjusted for rice harvesting and are operated by poorly trained operators. This results in sizable field loss and internal damage to harvested rice grains and hence affects its milling quality. The head feeding combine harvesters based on Kubota design with rubber tracks have been found suitable for fine varieties rice for maximum grain threshing with least internal damage and leaving paddy straw in un-bruised form. Therefore, in order to minimize post harvest losses (both in quantity and quality), there is urgent need to educate rice farmers on; harvesting their rice crop at proper maturity stage, encourage farm machinery rental individuals/companies through incentives by the local governments to increase their combine fleet and their timely availability, import of Kubota head feeding combines initially in reconditioned form and then their local manufacturing, and training of operators on proper use and maintenance of farm' machinery including combines.

**Key words:** Rice, harvesting, threshing, reaper-windrower, thresher, combine, losses

### INTRODUCTION

Rice is an important crop for local consumption and export. Generally it is grown on an area of 2.3 million ha with production of about 4.8 million tons. The country earns about Rs. 30 billions foreign exchange through its export. Harvesting and threshing play a significant role in realizing the full benefit of raised crop by reducing post-harvest losses as well as improving quality of milled rice. In Pakistan, the harvesting and threshing is done manually except in Punjab where 40 percent area is harvested by combines. There is a sizeable grain loss and damage during harvesting and threshing with traditional manual practices and by use of old and reconditioned wheat combines by poorly trained operators. With the migration of rural labor to the cities for better employment opportunities, there is acute shortage of labor during peak rice harvesting and threshing period. This causes delay in rice harvesting and threshing and thus increases both quantitative and qualitative post-harvest losses. Because of insufficient number of combines and difficulty in manual threshing of Super Basmati rice which is occupying 70 percent Basmati area in Punjab, the paddy growers of Super Basmati prefer to get their crop combined on their availability in their areas even if it is not fully matured and thus suffer loss due to lower price of their produce. In this paper, the effect of harvesting method and harvesting time, grain moisture content, threshing method and time on yield losses and grain quality has been discussed.

### RICE HARVESTING

Harvesting of rice generally refers to all operations carried out in the field till the crop is transported to the threshing plate form except for combining and strip harvesting. These include cutting the rice stalk or reaping the panicles, either laying out the paddy-on-stalk or stacking it to dry, bundling and transport. The rice is harvested manually and by using mechanical means. The manual harvesting has been mainly practiced in developing countries and least developed countries. Whereas mechanical harvesting using reaper-windrower, reaper-binder, combine harvester and stripper harvester is employed in developed countries. A. Manual Harvesting Methods a. Panicle reaping. This is accomplished by using a hand-held cutting tool or knife (called *yatab* in the Philippines and *ani-ani* in Indonesia and *kae* in Thailand). A quarter-circle blade fixed cross-wise on a wooden, grip-sized handle is passed between the index and the middle fingers which grab the panicle stems and execute the cutting action by pressing the panicle stems against the blade. The method is still used in areas where the traditional varieties are grown which are resistant to shattering, an important feature when handling and transporting. The bundles of panicles from the field to the house. The labor required for panicle reaping (240 labor-hour/hectare) is at least four times more than hand sickle harvesting. It is advantageous over the stalk cutting by sickle when fields are flooded or terraced, as in the hilly areas that are inaccessible by

wheeled vehicle. The carrying capacity of transport labor is more than that when the straw is cut long by sickle. In addition, it is an income source for the landless rural folks. Long-stalk cutting by sickle. The stalk is cut by sickle about 10-15 cm above the ground. There are many variations in sickle design, depending on the socio-cultural acceptance of the harvesting labor. After cutting, the stalks are laid in small bundles on the stubble to dry their ears for two or three days. In some places in Thailand the bundles are sized such that each one will give about 10 kg of paddy and laid up on the field for a few days to dry up. The reaping efficiency depends upon the plant density and variety, degree of lodging, the soil condition and the skill of the harvester. Lodged paddy and saturated soils may reduce the cutting rate by 50 %. This method is efficient than panicle reaping as it requires 60-80 labor hours to harvest one hectare of rice crop. Additional 100-200 labor-hours are required for manual gathering and binding of one hectare harvested crop.

### B. Mechanical Harvesting Methods

Unless labor in harvesting has become scarce in a locality due to industrialization or migration to employment from rice areas, rice harvesting will continue to be done with the sickle method in most developing countries. In the Philippines, the income or share in kind (usually 1/6 of the harvested paddy) gained by a manual harvester is high compared with other field operations. In times of calamity as in a typhoon where the rice crop is lodged and soaked, a farmer-owner is sometimes constrained to share up to 1/2 of the harvest to the harvesters rather than lose the crop altogether. The following mechanized harvesting methods are used depending upon the custom and the suitability of the machine to the soil conditions and the crop being harvested, the local custom, affordability of the machine, and other socio-economic factors.

(a) **Wind-rower.** This cuts and unloads paddy only laterally. These machines have theoretical work capacity varying from 4 to 8 hl/ha but need big labor force (100 to 200 h/ha) for manual gathering and binding of the paddy.

(b) **Reaper-binder.** This had once been popular in Japan but is being replaced by the combine. The machine cuts and bundles the stems together and lays them in the field in one operation. Equipped with a cutting bar and a gathering and binding device, these machines do good work even in harvesting lodged paddy (20°-30° angle to the ground). Depending on their construction features (adjustability of height, width of cutter), the work capacity of these machines vary from 5 to 20 h/ha with grain losses lower than 2 percent. In addition to the above methods, combines

and strippers are also used to simultaneously harvest and thresh paddy and have been described under paddy threshing section.

### HANDLING OF HARVESTED CROP

The gathering and bundling of the harvested crop (by manual labor with sickle and reaper windrower) needs 100-200 labor hours. Each additional handling step causes losses (Naphire, 1997) which varied from 1-2 % (Samson and Duff, 1973), and 2-7 % (Toquero and Duff, 1974). In-field transport which includes bundling of the cut stalks causes 0.11 to 0.35 % losses. Field stacking of the harvested crop can incur losses ranging from 0.11 to 0.76 %. The longer the stack is left in the field, particularly where the grain moisture content is high, the greater is the degree of loss. Heating of the harvested crop stack causes yellowing of the rice grains due to attack of micro-organisms and fermentation.

### FACTORS AFFECTING GRAIN LOSSES

#### Harvesting Time

Table 1. Effect of Harvesting Time on Grain Losses

Harvesting Time	Grain Losses (%)
One week earlier than maturity	0.8
At maturity	3.4
One week after maturity	5.6
Two week after maturity	8.6
Three week after maturity	40.7
Four week after maturity	60.5

Source: Almera 1997 (Taken from IRRI Table 3.1.1 authored by Ray Latin and edited by AGSI/FAO) web site [www.fao.inpho/compend/text/ch](http://www.fao.inpho/compend/text/ch)

Table 2. Harvesting losses related to condition of ripeness of rice.

Harvesting system	3 days before normal stage %	Normal stage for traditional %	3 days after normal stage %	5 days after normal stage %
Traditional hand Cut	6.00	8.70	10.50	12.00
Reaper-binder	1.00	3.10	1.20	5.80
Combine harvester	2.00	3.10	1.20	5.80

Source: Hilangalantileke (Taken from IRRI Table 3.1.4 authored by Ray Lantin and edited by AGSI/FAO) web site [www.fao.inpho/compend/text/ch](http://www.fao.inpho/compend/text/ch)

Proper time is important in harvesting the crop as losses increased with delay in harvesting. Recommended harvesting time of rice is one week before the maturity date. Harvesting systems and time of harvesting profoundly affect the extent of losses. In

case of traditional system of harvesting, the harvesting losses are minimal at 3 days before normal stage (ripeness) and increase linearly as the harvesting is delayed. In case of reaper binder, the losses are least at 3 days before normal stage and then increase but their pattern is inconsistent. But in case of combine harvester, the minimum loss is at 3 days after normal stage (Tables 1 and 2). Premature cutting of the rice keeps the grain from reaching maturity, and can cause serious losses in the quality of the product. Furthermore immature grains due to too early harvest result in high percentage of broken and low milling recovery. Maximum head rice recovery was obtained when the rice crop harvested at 35 days after 50 % flowering at moisture content ranging from 20-30 %. The recovery reduced with delay in harvesting beyond this time. Harvesting 33-39 days after 50 % flowering gave significantly higher head rice recovery than 27 30 days or 42 days after flowering (Table 3) (Ali et al 1993 and Salim and Sagar, 2003). Delayed harvesting also exposes the crop to insects, rodents and birds, in addition to increased risks of lodging and grain shattering. The ideal is to be within the window of optimum harvest period.

Table 3

**Table 3. Effect of Harvesting Intervals after 50 % flowering of Basmati in Pakistan**

Harvesting interval (days)	Moisture (%)	Total milled rice (%)	Head rice (%)
27	27.8	68.2 c	49.6
30	25.3	69.1 b	52.8 bc
33	22.9	70.2 a	54.5 a
36	20.3	70.4 a	54.6 a
39	17.9	70.4 a	53.8 ab
42	15.5	70.3 a	81.9 c

In a column, means followed by a common letter are not significantly different at 5 % level by DMRT Source: M. Salim, and MA. Sagar 2003)

The indicators of optimum harvest of grains are as follow:

- The variety has reached the particular date of maturity or number of days after heading, i.e. 28 to 34 days
- Eighty percent (80 percent) of the grains or the upper portion of the panicle has changed from green to straw color;
- At least 20 percent of the grains at the base are already in hard dough stage;
- The hulled grain is clear and hard

#### Moisture Content

There is effect of moisture content at harvesting time on grain losses and milling quality of rice. Excess grain moisture can alter final characteristics of the rice. On the other hand, too low a moisture content can cause

the panicles shattering. Short- and medium-grain varieties are generally harvested at 20 to 24 % moisture content and long-grain varieties at 18 to 21 % moisture content. Studies on the relationship between grain moisture at harvest and total milled rice and head rice revealed that there was no visible effect of grain moisture at harvest on the total milled rice quantity. But the head rice recovery increases with increase in grain moisture at harvest time (Fig. 1). The harvesting can be delayed up to 19 % moisture content without inducing losses due to breakage during milling because of recent developments in rice (Bemo, et al, 2002). A recent study conducted by PHMP (Table 4) (Ahmad, T. et al, 2004) revealed that there was no difference of harvesting stages on head rice recovery and broken rice at 5 % level. The head rice recovery increased with harvesting stage up to 2 green grains and after that the effect of harvesting stage was inconsistent.

**Table 4. Effect of harvesting stage on head and broken rice of Super Basmati in Pakistan**

Harvesting stage (no. of green grains)	Milling yield (%)	Head rice recovery (%)	Broken rice (%)
F0	69.5	41	28.5
1	68.9	45.5	23.4
2	69.3	48.9	20.4
3	68.5	47.5	21
4	69	49.8	19.2
5	68.5	47.8	20.7

Source: Tanveer 2004

Similar trend was observed in case of broken rice. In Pakistan, reaping is done manually. Saw-edge sickles with a blade length of 25 cm are used for cutting stems. Stems of IR varieties are usually cut at a height of 10 cm from the ground. While Basmati varieties are cut at a height of 20-30 cm from the ground. In Sindh Province, reaping of rice starts from the end of September and continues until the beginning of December. Its peak time is around the middle of November. In Punjab Province, the reaping time for IRRI varieties rice is from the end of October to the middle of November, and for Basmati varieties from the beginning of November to the beginning of December. The locally developed Reaper windrower (based on IRRI design) could not be introduced in the country because of its inability to handle lodged paddy crop and non-availability of suitable paddy thresher. The wheat combines with paddy kit are gaining popularity in Punjab due to shortage of labor during peak paddy harvesting time. Approximately 30-40 % paddy area mostly with Basmati varieties is presently combined in Punjab. The results of loss assessment study conducted by JICA in Pakistan on manual reaping of paddy (Anonymous, 1986) indicated:

- a) The average loss for rice plants reaped for green fodder in Sindh was 0.5 %.
- b) The average loss was 0.5 % for IR-6 in both Sindh and Punjab when dried for short period of time in the field. But it was 0.28 and 0.15 % in Punjab and Sindh, respectively when dried in the field for 5 days.
- c) The average loss was 3 % (very large as compared with IR-6) with Basmati in Punjab because of its easy threshing and low lodging resistance.

### RICE THRESHING

Paddy threshing involves the detachment of paddy kernels or grains from the panicles through rubbing action, impact; and stripping. The rubbing action occurs when paddy is threshed by trampling by humans, animals or tractors. The impact method is the most popular method of threshing paddy. Most mechanical threshers primarily utilize the impact principle for threshing, although some stripping action is also involved. The third type, stripping has also been used in paddy threshing. Some impulsive stripping occurs ordinarily with impact threshing in conventional threshing cylinders. Paddy threshers may either be hold-on or throw-in type on the basis of paddy feeding method. In the hold-on type, paddy straws are held stationary while threshing is done by the impact on the particle from cylinder bars, spikes or wire loops. In the throw-in type, whole paddy stalks are fed into the machine and a major portion of the grain is threshed by the initial impact of the bars or spikes on the cylinder. The initial impact also accelerates the straw and further threshing is accomplished as the moving particles hit the bar and the concave. In the throw-in type of thresher, large amounts of straw pass through the machine. Some designs utilize straw walkers to initially separate the loose grain from the bulk of straw and chaff.

**Manual Threshing.** In this method, threshing is accomplished by either treading, beating the panicles on tub, threshing board or rack, or beating the panicles with stick or flail device. The pedal-operated thresher (Fig 2) consists of a rotating drum with wire loops, which strip the grains from the panicles when fed by hand. It can also be operated by women and can be used in hilly or terraced areas because of its portability. Machines driven by a manual device or a pedal are often used to improve yields and working conditions during threshing. By means of the handle or pedal, a big drum fitted with metal rings or teeth is made to rotate. The rice is threshed by hand-holding the sheaves and pressing the panicles against the rotating drum. The speed of the threshing-drum must be kept at about 300 revolutions per minute (rpm). The hand-held sheaves must all be of the same length with the panicles all laid in the same direction, and the grains

must be very ripe and dry. The machine must be continuously and regularly fed, but without introducing excessive quantities of product. If the paddy obtained contains too many un-threshed panicles and plant residues, a second threshing must be followed by an effective cleaning of the product. Use of these threshing machines may require two or three workers. Depending on the type of machine, the skill of the workers and organization of the work, yields can be estimated at a maximum of 100 kg/in.

**Power Threshing.** Treading of the harvested crop under tractor tires (Fig 3) is a method used in some Asian countries. The popularity of this method can be attributed to its convenience and the lack of suitable tractor PTO-driven threshers. The grain is separated from the straw by hand and then cleaned by winnowing. Most, if not all powered paddy threshers are equipped with one of the following types of cylinder and concave arrangement: (a) rasp bar with concave (b) spike tooth and concave (c) wire loop with concave (d) wire loop without concave. Testing carried out at International Rice Research Institute (IRRI), Philippines indicated that the spike-tooth cylinders performed well both with the hold-on and the throw-in methods of feeding and its threshing quality is less affected by changes in cylinder speed. In the axial-flow thresher, the harvested crop is fed at one end of the cylinder/concave and conveyed by rotary action on the spiral ribs to the other end while being threshed and separated at the concave. Paddles at the exit end throw out the straw and the grain is collected at the bottom of the concave after passing through a screen cleaner. Several versions of the original IRRI design of the axial-flow thresher have been developed in most countries to suit the local requirements of capacity and crop conditions. Thus, there are small-sized portable ones and tractor PTO-powered and engine-powered ones. Many custom operators in Asia use the axial flow threshers to satisfy the threshing and grain cleaning requirements of rice farmers. There is a need to dry the harvested rice in the field for better performance of the threshers. However, in order to maintain the high quality of the harvested grains, it should be threshed immediately after harvesting. Avoid field drying and stacking for several days as it affects grain quality due to over drying. Stacked grains of high moisture content results in discoloration or yellowing.

**Combine:** The small combine has become popular in Japan since the 1960s. The Republic of Korea has also manufactured it commercially since the early 1980s. It is gradually being introduced in other Asian countries but primary hurdle to adoption is the high initial cost and adaptability to local conditions. The self-propelled machines have cutting widths of 50 to 150 cm and have capacities of about 0.5 ha/h (NAPHIRE

1997). Thailand has local versions of large combines popular in developed countries and is being adopted because of the increased costs and scarcity of labor. As a rice-exporting country, Thailand attempts to mechanize rice production and processing operations. Vietnam may also adopt mechanized methods because of economies of scale. Although Malaysia is a net importer of rice, it depends on modified large combines imported second-hand mainly from Europe to harvest its rice crop. Large combines are being used in commercial rice production in countries like Brazil and Uruguay in Latin America and in Europe and the USA. Their introduction and field use in some African countries through aid programs is under much criticism as to their appropriateness in situations where ready and efficient repair and maintenance facilities and services are not available. In California rice is harvested by large, self-propelled grain combines which cut the entire plant and separates straw from grain internally (Fig. 4).

The following situations hinder the adoption of combine harvesters.

(a) Low income, inability to raise capital, reluctance to change traditional methods, poor

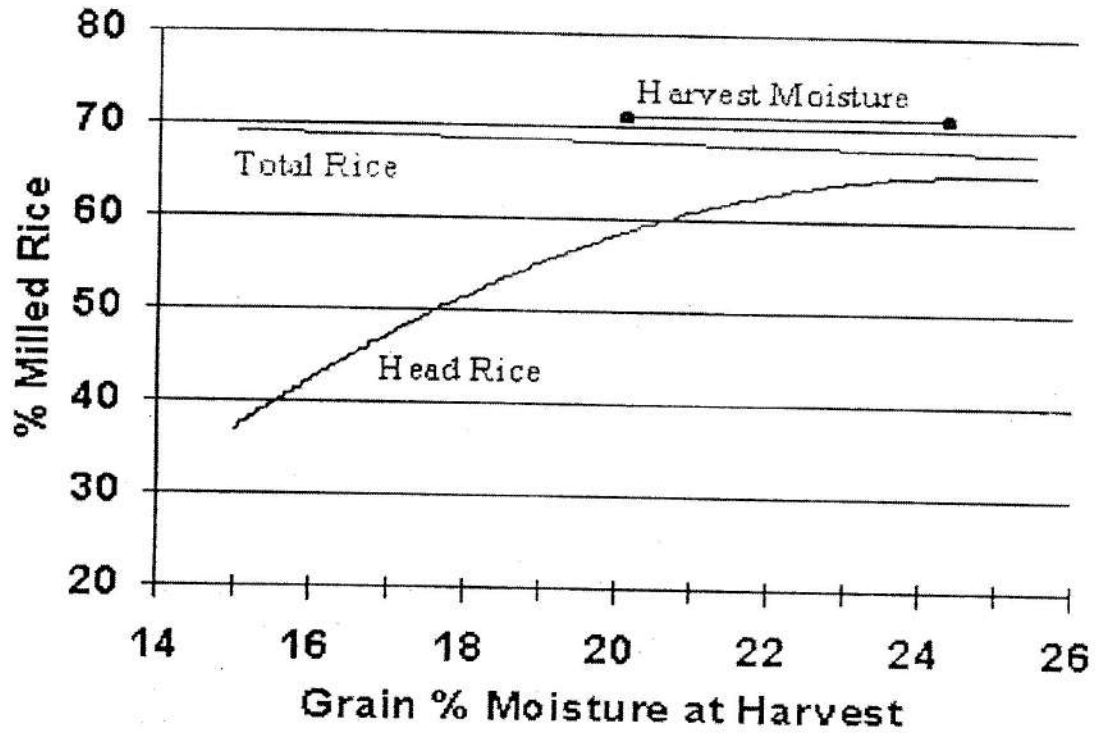
**Mechanical aptitude and the desire to save straw for uses other than farm.**

(b) Small 1 and holding, very small plot size with high bunds, poor water control, inadequate ground support and poor traffic ability for powered harvesting equipment, and lack of access of roads to the fields. In case of combine-harvesters, these should be equipped with tracks, rather than wheels, so that harvesting can be done even on very wet ground. (c) Excessive moisture content at harvest time, uneven ripening, severe lodging and entangling of paddy (specially the traditional long-stalked varieties), and high-shattering and low grain straw ratio varieties. (d) The rice husks contain silica, which gives them a highly abrasive quality that provokes rapid wear on the moving parts of the machines.

**Stripper Harvester.** This is an innovation item the International Rice Research Institute (IRRI), Philippines which adapted the rotary stripping comb principle developed by the Silsoe Research Institute in Silsoe, U. K. The rice stripper ideally works with a variety which is non-lodging, of medium stature with erect panicles, and have low to medium shattering. A high grain: straw ratio is advantageous in achieving high harvesting productivity. The IRRI-designed pedestrian stripper-gatherer has undergone several field trials in more than 20 rice-producing countries since 1994 and the reactions to the machines were mostly favorable, except when the machine has to be used in wet or soft fields where traction is a problem. Efforts however, are

needed from the national institutions in the various countries to extend the machine to farmers or to harvesting custom operators and to modify the machines to suit local soil and crop conditions. The local manufacturers must first be trained in its fabrication and in the provision of efficient and reliable after-sales services. The attempt to make a small and ride-on combine version of the machine has been beset by traction and floatation problems in wet and soft soils. The design and development activities on it have been discontinued or suspended by IRRI. There is still a lack of functionally and economically suitable equipment for tropical conditions due to inadequate research, development and thorough field-testing activities in the developing countries in mechanical harvesting. The high cost of imported equipment and the requirement of good machinery management must compete with relatively low-cost labour. In Pakistan, both IRRI designed axial-flow and Korean hold-on paddy threshers were evaluated, adapted and got locally developed by the Farm Machinery Institute (FMI) of the Pakistan Agricultural Research Council (P ARC), Islamabad. But these were not accepted because of their low output. Recently a larger capacity (1.5 ton/hour) version of tractor operated paddy thresher built on IRRI axial flow concept was imported from Thailand by the FMI. It was evaluated on IRRI paddy variety and demonstrated extensively in Sindh. On its acceptability among large IRRI paddy growers in Sindh, it has been got locally developed. Large and self propelled combines imported from the West were also introduced in the country for wheat harvesting in early Eighties. With increase in prices of these combines, reconditioned combines are being brought in the country and are available at approximate price of Rs 1.2 million. These are also being used for paddy harvesting since mid Ninety. The Japanese head feeding combines brought under KR-2 Grant in late Ninety are also used for paddy harvesting. Presently 30-40 percent of paddy area in Punjab (Pakistan) is harvested by combines particularly Super Basmati (difficult to thresh by manual labour). The harvesting charges are Rs. 2500 and 6200/ha for reconditioned and Japanese head feeding combines, respectively. The higher charges for Japanese head feeding combines are due to little grain breakage and saving of paddy straw by their use. The manual harvesting and threshing of paddy charges varies from Rs. 3500 to 4500 per hectare. Like reaping of rice, there are not much studies conducted in the country on threshing of rice. The results of loss assessment study conducted by JICA in Pakistan on mechanized reaping and threshing of paddy (Anonymous, 1986) indicated: a) The average reaping loss with reaper was 0.3 %. b) The average reaping loss with auto combine and combine were 1.1 and less than 0.3 %, respectively c)

The total loss including of collecting and transporting and threshing with auto combine and combine were Figure 1.  
**Effect of Grain Moisture Content at Harvest on Milling and Head Rice Recovery**



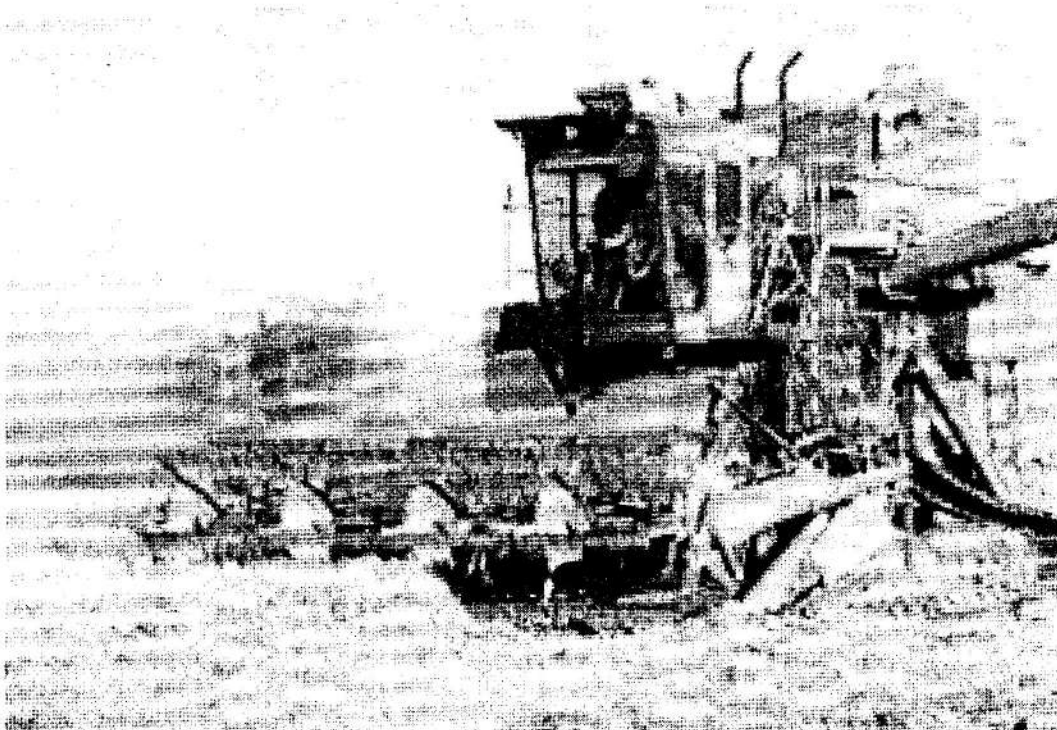
**Figure 2. Small Pedal Operated Rice Thresher**



**Figure 3. Rice Threshing by Tractor**



**Figure 4. Typical self-propelled rice combine harvester.**



4.0 and less than 1.3 %, respectively. d) The quality loss including non-husked and increased ratio of cracked kernels with auto combine and combine were 4.1 and 3.0 %, respectively. In another study conducted by PHMP in collaboration of Rice Program and FMI, NARC; Rice Institute, Kala Shah Kaku (Ahmad, T. et al, 2004) on "Effect of paddy harvesting method on rice quality and head rice recovery" have indicated: a) There is statistical significant effect of harvesting methods on milling yield in tons of head rice recovery (%) and broken rice (%) at 5 % level b) The mean value of head rice recovery (49.5%) of manual harvesting and threshing method was higher than conventional and head feeding combines harvesting. The mean value of head rice recovery was higher in head feeding combine harvesting (46~5%) than conventional combine harvesting (44.9%). c) A positive correlation of 0.74 was found between harvesting stage and moisture contents of harvested paddy grains. d) There was not any statistically significant difference found of harvesting stages on head rice recovery (%) and broken rice (%) at 5 % level. The head rice recovery increased with harvesting stage up to 2 green grains and after that the effect of harvesting stage is inconsistent. Similar trend was observed in case of broken rice.

#### RECOMMENDATIONS

1) The Local Government should facilitate the availability of threshers and combines to the rice growers on rental basis by encouraging private farm machinery rental individuals/companies with soft term loans. 2) The head feeding combine harvesters based on Kubota design with rubber tracks are suitable for fine varieties rice for maximum grain threshing with least internal damage and leaving paddy straw in un-bruised

form. Efforts are needed on the part of MINF AL to facilitate private sector through their import in reconditioned form initially and then encourage their local manufacturing. 3) The combine operators should be trained in efficient use and proper maintenance of combines and other rice harvesting and threshing machinery. 4) Awareness campaign should be launched for the rice farmers by Provincial Agricultural Extension Departments on timely harvesting by adopting proper post harvest measures to improve quality of milled rice for maximum head rice recovery.

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