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Use of Natural Preservative in Bread Making

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Abstract: This study was carried out to increase the shelf life of bread by using different additives at ambient temperature. The Wheat flour used was also chemically analyzed and results depicted 12.1% moisture, 11.8% crude protein, 0.52% ash and 1.3% crude fat. Shelf life of breads was depicted from moisture and yeast and mould count. The results for moisture analysis showed that it was in range of 16.75-37.13%. among different treatments T₆ having a combination of 0.32% suhanjina, 3% lecithin and 0.1% ascorbic acid retained maximum moisture and minimum yeast and mould count at the end of storage. Most mould isolated belonged to the genera *Aspergillus*, *Penicillium* and *Actinomyces*.

Key words: Suhanjina (*Moringa oleifera*), shelf life, preservation, bread making, lecithin, ascorbic acid

Introduction

Bread is the most important commercial product of wheat flour consumed as staple food by most of the wheat eating people. In Pakistan bread follows chapatti both in its production and consumption pattern. Moreover, the demand of bread as an essential break fast item is increasing day by day (Latif, 1996). Moreover, small baking plants lack technical expertise and hence often face problems while using the flour of varying characteristics for bread preparation (Chaudhary, 1991).

Baking industry in Pakistan has made tremendous progress during the past decade. Bread is prepared at the small scale by some modern bakery plants including Vita Bread Ltd., Dawn Bread Ltd., Rohi Bread Ltd., Wonder Bread Ltd., Bunny Bread Ltd., etc. Pan bread is the major product of these bakery plants (Chaudhary, 1991).

Unfortunately the bread being marketed by some of these bakery plants lacks in quality and has short shelf life. Hence a substantial loss is borne by the producers from unsold loaves. Since, bread is an important part of our daily diet; therefore, ways and means should be explored to improve the quality and shelf life.

Some stabilizers have extended the shelf life of bakery bread by two days, retain the sensory properties (Staszewska and Janik, 1977) and improve water retention capacity, modify texture, volume and cell structure of the products (Brummer, 1977). The major type of microbial spoilage of baked bread is usually caused by molds and *Rizopus stolonifer* (Jay, 1990). In this research bread is prepared by using a natural plant extract to increase the shelf life of bread. This plant "suhanjina" *Moringa oleifera* is reported to have so many benefits in different studies carried out in different countries.

Moringa seeds are effective against skin-infecting bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. They contain the potent antibiotic and fungicide terygosperrin. The

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alkaloid spirachin (a nerve paralytant) has been found in the roots. Even when free of bark, the condiment in excess may be harmful. A decoction of the flowers is used as a cold remedy. The gum is diuretic, astringent and abortifacient and is used against asthma. Oil of Ben is used for hysteria, scurvy, prostate problems and bladder troubles. The roots and bark are used for cardiac and circulatory problems, as a tonic and for inflammation. The bark is an appetizer and digestive (Anonymous, 1986).

The cytotoxicity and antimicrobial activity of different *Moringa oleifera* seeds extracts were tested against *Scenedesmus obliquus* (green algae), *Escherichia coli* ATCC 13706, *Pseudomonas aeruginosa* ATCC10145, *Staphylococcus aureus* NAMRU 3 25923, *Bacillus sterothermophilus* (bacterial strains) and *Herpes simplex* virus type 1 (HSV 1) and Polio virus type 1 (sabin vaccine). Fixed oil extracted from the seeds was found to activate the growth of *Scenedesmus obliquus* with high algal biomass production. Aqueous methanolic extracts (LC50 207.5 mg L⁻¹) and water extract (LC50 287.5 mg L⁻¹) has a cytotoxicity effect on *Scenedesmus* growth. On the other hand, the antibacterial effects of fixed oil, aqueous methanolic extract and water extract concentrations showed a fluctuation in its effects. Although, *P. aeruginosa* was more resistant to all *M. oleifera* extracts, *B. sterothermophilus* was more sensitive than other organisms to all extracts. The effect of aqueous methanolic extract and fixed oil on HSV1 was highly similar, 52.22 and 45.2%. Very low activity was observed for fixed oil on PV1 (Anonymous, 2004).

Five vegetables traditionally consumed among South-Asian migrants in Bradford (Yorkshire, UK) were tested for their Free Radical Scavenging Activity (FRSA) in the DPPH (1,1-diphenyl-2-picrylhydrazil radical) screening assay (using extracts prepared both by cold maceration and also by boiling the plant in the solvent under reflux) and for their *in vitro* non-enzymatic inhibition of bovine brain lipid peroxidation. In both anti-oxidant assays a strong activity was shown by extracts derived from okra (*Abelmoschus esculentus*, Malvaceae) fruits and charungli (*Caralluma edulis*, Asclepiadaceae) aerial parts. Extracts from bitter melon (*Momordica charantia*, Cucurbitaceae) and angular loofah (*Luffa acutangula*) showed a significant difference in the FRSA between the extract obtained by using cold maceration and that prepared by boiling the plant in the solvent under reflux, suggesting the chemical composition of the plant changed during the heating process, leading to an increase in the amount of anti-oxidant components. These findings confirm the big interest of the nutraceutical sciences in extracts of *Caralluma edulis*, whose phytochemistry and phytopharmacology should be investigated further in order to detect possible phytotherapeutical uses for the prevention of Ageing Related Diseases (ARDs) and Alzheimer Disease (AD) (Ansari *et al.*, 2005).

Use of chemicals to increase the shelf life also causes so many other side effects on bread quality so we have to use such preservative that increase shelf life without any harmful effect on bread quality. The shelf life of breads produced now days is very limited of about three days. Keeping in view the current status of bread industry the present study was designed and to compare the effect of different food additives on the quality of bread to access the suitability among the tested additives to extend the shelf life of bread.

Materials and Methods

The present study was carried out in the Department of Food Technology, University of Arid Agriculture Rawalpindi Pakistan. All work completed in approximately one year.

Collection of Raw Materials

All the raw material was purchased from the local market of Rawalpindi. It included wheat flour, yeast, sugar, salt, shortening, stabilizers and preservatives. The chemical composition of wheat flour with respect to protein, fat, ash and moisture was estimated according to AACC (1983) methods.

Table 1: Elaboration of treatments

Treatments	CMC	Calcium propionate	Bran	Suhanjna	Lecithin	Ascorbic acid
T ₀	-	-	-	-	-	-
T ₁	0.5%	0.15%	-	-	-	-
T ₂	-	0.15%	3%	-	-	-
T ₃	0.15%	-	-	0.32%	-	-
T ₄	-	0.15%	-	0.32%	3%	0.1%
T ₅	0.5%	-	-	0.32%	3%	0.1%
T ₆	-	-	-	0.32%	3%	0.1%
T ₇	0.5%	0.15%	-	0.32%	3%	0.1%

Preparation of Bread

Bread was prepared according to the methods as described in AACC (1983). Different preservatives, stabilizers and their combinations were used to extend the shelf life of the bread. The treatments are elaborated in Table 1.

Chemical Analysis

Breads were analyzed for moisture content, crude protein percentage, crude fat percentage and ash according to AACC (1983) methods.

Microbial Analysis and Bread Quality Evaluation

The yeast and mould growth on crust and crumb of breads were examined visually and counted by modified method of Fox (1993).

Sensory Evaluation

A panel of five judges carried out sensory evaluation. A nine point hedonic scale was used for sensory evaluation as described by Larmond (1977).

Statistical Analysis

Data obtained for given parameters was statistically analyzed using the Analysis Of Variance (ANOVA) technique and the Least Significance Difference (LSD) to compare the means according to Steel and Torrie (1980).

Result and Discussion

Chemical Analysis of Wheat Flour and Bread

The data showed 12.5% moisture, 11.5% crude protein, 0.52% ash and 1.3% crude fat in wheat flour (Table 2). The result of wheat flour analysis negate the findings of Qamar (1994) who used the flour of particular wheat variety i.e., Inqlab in his research.

The breads were also chemically analyzed for moisture, fat, protein and ash contents. The results revealed that moisture, crude protein, crude fat and ash of breads ranged from 36.57-40.41, 6.12-8.84, 2.47-2.94 and 1.02-1.85, respectively (Table 2). Among different treatments T₆ showed the maximum tested parameters regarding chemical analysis that is 40.41, 8.84, 1.85, 2.94% moisture, crude protein, ash and crude fat respectively because of the best combination of additives used for this treatment.

These results of chemical composition of bread are in line with those of Rehman and Mudassar (2003) who studied the effect of CMC and Carrageenan gum on shelf life of bread.

Table 2: Proximate analysis of flour and breads having different levels of additives

Characteristics (%)	Flour	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Moisture	12.1	36.57	38.25	37.54	41.17	39.81	38.45	40.41	39.54
Crude protein	11.8	6.12	6.12	7.41	8.22	8.41	8.05	8.84	8.63
Ash	0.52	1.02	1.26	1.37	1.19	1.13	1.54	1.85	1.76
Crude fat	1.3	2.47	2.76	2.58	2.41	2.07	2.61	2.94	2.33

*All values are the mean of three replications

Table 3: Effect of additives on the yeast and mould count of bread

Treatments	Storage time (days)					
	0	2nd	4th	6th	8th	10th
T ₀	90	1.27×10 ²	5.37×10 ²	0	0	0
T ₁	87	92	2.57×10 ²	3.15×10 ²	0	0
T ₂	94	1.14×10 ²	2.71×10 ²	3.32×10 ²	0	0
T ₃	0	1.08×10 ²	2.49×10 ²	3.05×10 ²	3.50×10 ²	0
T ₄	0	0	54	1.09×10 ²	1.78×10 ²	2.27×10 ²
T ₅	0	0	48	97	1.72×10 ²	2.05×10 ²
T ₆	0	0	24	83	1.48×10 ²	2.03×10 ²
T ₇	0	0	37	93	1.67×10 ²	2.24×10 ²

* All values are the mean of three replications

Moisture Percentage

It was observed from Table 4 that there is a continuous decrease in moisture content with the passage of time. Moisture content of the control treatment T₀ can be measured up to 4th day of storage at ambient temperature later on it was spoiled. The results further revealed that maximum moisture content was recorded when Suhanjna (0.32%) was used with the combination of lecithin (3%) and ascorbic acid (0.12%) in T₆.

It was observed from the data behavior of treatments was also effected by the storage interval (Table 5). Maximum value was obtained at zero day of storage while minimum value was observed in 10th day of storage and there was a significant difference among all storage intervals.

These results are in accordance with findings of Rehman and Mudassar (2003) who had reported that with the use of additives CMC (1%) and Carrageenan gum (0.1%), the moisture content of bread ranges from 31.14-35.83%.

Yeast and Mould Count

The results for mould colonies during storage at ambient temperature have shown that there is a large variation among different treatments. It is clear from Table 3 that Yeast and Mould Count increases with the passage of time mould colonies appeared on treatments T₄, T₅, T₆ and T₇ at 4th day storage of storage.

The highest Yeast and Mould Count was found in T₀ followed by T₂ and T₁, while T₆ (0.32% Suhanjna, 3% lecithin, 0.1% ascorbic acid) superceded all other treatments with least Yeast and Mould Count 10th day of storage.

The results are in line with findings of Masood *et al.* (2001) who observed the maximum number of colonies 2×10²cfu g⁻¹ with the use of additives like Calcium propionate (0.15%), Lactic acid (0.10%) and Acetic acid (0.10%).

Sensory Evaluation of Bread

Volume of Bread

The comparison of arithmetic means for volume of breads depicts that there was a positive effect of additives (Suhanjna, CMC, Calcium Propionate, Lecithin, Bran and Ascorbic acid) on volume. The means of both treatments and storage intervals are arranged in Table 4.

Table 4: Effect of additives on the moisture content and sensory characteristics of the breads (treatment means)

Treatments	Moisture	Volume	Crust colour	Evenness of bake	Symmetry of form	Character of crust
T ₀	16.75e	2.900d	2.483f	0.750e	0.850g	0.750g
T ₁	23.39d	4.300c	3.183e	0.933d	1.083f	1.167fe
T ₂	22.54d	4.467c	3.267e	1.283c	1.433e	1.310e
T ₃	28.97c	5.433b	4.700d	1.567b	1.817d	1.619d
T ₄	34.58b	6.733a	5.817c	2.133a	2.267c	2.200c
T ₅	35.38b	6.767a	5.750c	2.136a	2.467b	2.150c
T ₆	35.67b	6.833a	6.750a	2.133a	2.733a	2.683a
T ₇	37.13a	6.933a	6.367b	2.217a	2.267c	2.367b

Table 4: Continued

Treatments	Break and shred	Bread grain	Crumb colour	Taste	Mastication	Texture	Aroma
T ₀	1.250e	3.333e	3.000d	5.000d	3.917f	6.167d	3.417g
T ₁	1.717d	4.500d	4.667c	6.667c	5.333e	8.500c	4.917e
T ₂	1.750d	4.750d	4.583c	7.333c	5.583e	8.333c	4.500f
T ₃	2.208c	6.000c	6.000b	9.333b	7.000d	10.83b	6.333d
T ₄	2.683b	7.417b	8.583a	12.25a	8.583c	13.67a	7.833c
T ₅	2.861a	7.667ab	8.667a	12.00a	9.000ab	14.00a	8.556b
T ₆	2.933a	7.750a	8.750a	12.67a	9.333a	14.17a	8.833a
T ₇	2.900a	7.750a	8.667a	12.31a	8.722bc	13.97a	8.500b

Table 5: Effect of storage interval on the moisture content and sensory characteristics of the breads (storage interval means)

Treatments	Moisture	Volume	Crust colour	Evenness of bake	Symmetry of form	Character of crust
D ₁	38.97a	7.287a	6.113a	2.100a	2.350a	2.137a
D ₂	37.47b	7.200a	5.963b	2.100a	2.350a	2.141a
D ₃	34.45c	6.650b	5.613c	1.862b	2.162b	2.112a
D ₄	28.96d	5.438c	4.787d	1.637c	1.913c	1.862b
D ₅	20.30e	3.787d	3.475e	1.188d	1.350d	1.331c
D ₆	15.65f	2.950e	2.787f	0.977e	1.063e	1.100d

Table 5: Continued

Treatments	Break and shred	Bread grain	Crumb colour	Taste	Mastication	Texture	Aroma
D ₁	2.844a	7.625a	8.063a	11.73a	8.813a	13.60a	8.000a
D ₂	2.819a	7.406ab	7.875a	11.75a	8.688a	13.50a	7.938a
D ₃	2.715b	7.250b	7.625b	11.50a	8.500a	13.13a	7.667b
D ₄	2.313c	6.344c	6.750c	9.938b	7.438b	11.63b	6.875c
D ₅	1.675d	4.563d	5.125d	7.250c	5.513c	8.500c	5.063d
D ₆	1.362e	3.688e	4.250e	6.000d	4.354d	6.875d	4.125e

Table 4 revealed that minimum value (2.900) was found in T₀ while maximum (6.933) in T₆ followed by T₇ (6.833), T₄ (6.767) and T₅ (6.733). It is clear from Table 4 that there is non significant difference among T₆, T₇, T₄ and T₅ but these differ significantly from others.

Storage also effects the treatments as D₁ (zero day) and D₂ (2nd day) show best score than D₆ (10th days) that was least (Table 5). The storage means decreased with the increase of storage period. Maximum values were observed at zero and 2nd day of storage.

These results are in agreement with those of Pylar (1988) who had studied the effect of ascorbic acid, amylase, lecithin sugar and skimmed milk.

Colour of Crust

Comparison of the means of the different treatments showed that the maximum value for colour of crust is obtained in T₆ (6.750) followed by T₇ and T₄ while minimum value was observed in T₀ (control). Table 4 further revealed that T₆ significantly differs from other treatments but there is non significant difference among T₄, T₅ and T₂ and T₁.

Storage intervals also effect the treatments as D₁ (zero day) show highest result and D₆ (10th day) remained least (Table 5). The values for the colour of crust of control and treated samples revealed that the values of all treatments decreased significantly at 4th and 6th day of storage as compare to T₆ that's value decreased slowly till the end of the storage.

The previous studies carried out by Pylar (1988) and Latif (1996) showed that malt supplementation in wheat flour improved crust colour so the improvements in crust colour in present study is in conformity with previous ones.

Evenness of Bake

Table 4 revealed that T₆, T₇, T₅ and T₄ are statistically similar but maximum values were observed in T₆ (0.32% Suhanjna, 3% lecithin, 0.1% ascorbic acid) followed by T₅, T₇ and T₄, while all these are significantly different from others and minimum values were observed in control and T₁.

There was also a significant effect of storage on the treatments and in this regard D₁ (zero day) and D₂ (2nd day) show maximum score. While minimum value of storage intervals mean was observed in D₆ as shown in Table 5.

These results are comparable with findings of Rehman and Mudassar (2003) who studied the effect of CMC (1%) and Carrageenan gum (0.1%) on shelf life and evenness of bake of bread.

Symmetry of Form

Table 4 showed that maximum value was observed in T₆ followed by T₅, T₄ and T₇ and T₆ got top position The minimum value was observed in the control. There was a non significant difference among T₄ and T₇ but were significantly different from others. Moreover all other treatments showed a significant difference.

The best score in case of storage intervals effect was found in D₁ (zero day) and D₂ (2nd day) and this score decreases with the increase in storage period.

The similar results were reported by Taboada and Santiesteban (2000) who stated that bread having 1% CMC gave better symmetry of form.

Character of Crust

The comparison of mean values indicates that highest value was obtained by T₆ then comes T₇, T₄, T₅, T₃ and T₂ (Table 4).

All values fell statistically in different groups except T₄ and T₅ yet T₆ is better than all. Storage intervals also effect the treatments and Table revealed that intervals D₁ (zero day), D₂ (2nd day) and D₃ (4th day) had highest score with respect to the storage interval. Minimum value was obtained in D₆ (10th day) that was the end of storage (Table 5).

These results are comparable with the findings of Rao *et al.* (1985) who reported that guar gum can improve the character of crust.

Break and Shred

This revealed that the results of all treatments are significantly different among each other except T₆, T₇ and T₅. Means of these treatments were non significant with each other and were better as compared to other treatments (Table 4).

Maximum value (2.933) was in T₆, while minimum in control.

Time interval of storage also affected the break and shred. It was excellent at D₁ (zero day) and D₂ (2nd day) while later on it reduced gradually during storage A gradual decrease was observed in all treatments throughout the storage period of 10 days at ambient temperature (Table 5).

These results are comparable with findings of Pylar (1988) who narrated the effect of preservatives and malt supplementation on bread break and shred of bread.

Grain of Bread

The arithmetic means are in range of 3.333 to 7.750. The comparison of means indicated the highest scores were given to T₆ (0.32% Suhanjna, 3% lecithin, 0.1%) while control T₀ showed a minimum value. The difference between T₆, T₇ and T₅ is non significant while these differs from other treatments significantly (Table 4).

The score for bread grain decreased with the storage period. The deterioration rate was lower among D₁ (zero day), D₂ (2nd day) and D₃ (4th day) but greater in D₆ (10th day) (Table 5).

These results are in line with the findings of Ozer and Altan (1995) who reported that ascorbic acid, potassium bromate, alpha amylase enzyme and lecithin have remarkable improving effect on grain of bread.

Colour of Crumb

Table 4 and 5 depicts the both treatment and storage means. This explains that T₆ got maximum score of 8.833 followed by T₅ and T₇ and minimum was observed in control.

Although time intervals were significant but D₁ (zero day) and D₂ (2nd day) showed some better results as compared to D₃ (4th day) and D₄ (6th day) while D₅ (8th day) and D₆ (10th day) remained at least. The decrease of score in T₁ is sharp at 4th day of storage, while all other treatments exhibit a gradual trend of decrease.

The results of present study are in line with the findings of Goncharov and Sokolov (1977) who reported the improvement in crumb colour by the use of Amylolytic lactic acid bacteria.

Taste

Table 4 shows that T₆ having mean score of 12.67 got top position. Table 4 further revealed that T₆, T₇, T₄ and T₅ are non significant to each other while these differ significantly from other treatments.

Time intervals also affect the storage of bread. The taste score was maximum at D₁ (zero day) and lowest at D₆ (10th day) as depicted from Table 5.

These results are supported by Tarar (1999) who noted the improvement in bread taste by using acidulants and their salts.

Mastication

The Table 4 and 5 revealed that treatments T₆ and T₅ have highest score yet T₆ retained maximum score till 10th days storage. The treatment and storage interval means in Table 4 shows that T₆ got maximum score of 9.333 for mastication followed by T₅ and T₇ while least score was observed in control (T₀).

It further revealed that T₆ and T₅ fell statistically in same group but these differ from others. The mastication of bread was significantly better upto D₃ (4th day) after that deterioration rate was very fast. The mastication score of all treatments decreases with the increasing storage period except T₆ which value decreased only at 8th day of storage.

The results are in close agreement with those reported by Latif (1996) who investigated that addition of malt improves the chewiness and mastication of breads.

Texture

Comparison of the means of the different treatments showed that the maximum value for texture is obtained in T₆ followed by T₅ and T₇ while minimum value was observed in T₀ (control) (Table 4).

Table 4 further revealed that T₆ significantly differs from other treatments but there is non significant difference among T₆, T₅, T₇ and T₄. The top score was found in T₆ that has value of 14.17.

Storage intervals also effect the treatments as D₁ (zero day) show highest result and D₆ (10th day) remained least.

Texture

Comparison of the means of the different treatments showed that the maximum value for texture is obtained in T₆ followed by T₅ and T₇ while minimum value was observed in T₀ (control) (Table 4).

Table 4 further revealed that T₆ significantly differs from other treatments but there is non significant difference among T₆, T₅, T₇ and T₄. The top score was found in T₆ that has value of 14.17.

Storage intervals also effect the treatments as D₁ (zero day) show highest result and D₆ (10th day) remained least (Table 5).

Aroma

The Table 4 and 5 shows that the aroma of treatment T₆ remained good and acceptable upto 10th day of storage at ambient temperature. It is evident from Table 4 that T₆ having mean score of 8.833 got top position.

Table 4 further revealed that T₇ and T₅ are non significant to each other while these differ significantly from other treatments, rest of the treatments exhibit significant difference.

Time intervals also affect the storage of bread. The taste score was maximum at D₁ (zero day) and D₂ (2nd day) and minimum at D₆ (10th day) (Table 5).

These results are in line with the results of Latif (1996) Wilfred (1960) who observed the improvements in taste and aroma of bread by the use of malt. Similar results were found by Masood *et al.* (2001) who reported to had good aroma by the use of additives like Calcium propionate (0.15%), Lactic acid (0.10%) and Acetic acid (0.10%).

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