Pak. J. Biochem. Mol. Biol., 2012; 45(2): 81-84

Studies on the shelf life enhancement of traditional leavened bread

*Sajid Hussain[1] and Khalid Jamil[2]**
[1]Department of Food Science and Technology, University of Karachi, Karachi, Pakistan
[2]Food and Marine Resources Research Center, PCSIR Laboratories Complex, Karachi, Pakistan

Abstract: Chemical preservatives (calcium propionate and glycerol propionate) were added to wheat flour during mixing at a level of 0.14% (on flour wt basis). Sodium propionate, calcium acetate and potassium sorbate were incorporated in to spray mixture and then sprayed/coated during various production stages of leavened bread. Wheat flour was analyzed for chemical and rheological characteristics. The bread was stored at 29 C° and evaluated microbiologically at successive intervals of 24, 48, 72, 96 and 120 hours. Treatment T4 (Coating of pan+Spray on dough+Slicer+Packaging) proved to be the most effective against control of fungus and bacteria (no colonies were observed up to 96-hours storage) followed by T6 (Spray on packaging material). As regards sensory evaluation results, volume, symmetry of form, crust character and grain size of bread were significantly affected by treatments. While, crust character, crumband crust color, texture, aroma and taste of bread were affected by storage time. It was therefore concluded that leavened bread treated by spray mixture at various production stages has remarkable potential against microorganisms and retains good sensory characteristics up to 96 hours storage.

Keywords: Rheological characteristics, microorganisms, leavened bread, sensory evaluation.
Received: September 20, 2011 **Accepted:** March 28, 2012
***Author for Correspondence:** jamilpcsir57@yahoo.com

INTRODUCTION

Due to its superior nutritional, sensorial, and textural characteristics bread has always been one of the most popular and appealing food products[1]. However, during the last decades, major changes in the way food products are prepared, distributed, and retailed, led to the development of alternative approaches or novel methods for the preparation and preservation of bakery product[2]. The main ingredients of bread are flour, water, salt, sugar, shortening and yeast etc. Most of these bread ingredients are supportive to growth of micro-organisms and multiplication at various stages of bread production, processing and packaging.

The chief types of microbial spoilage for baked bread are usually moldiness and ropiness, termed "mold" and "rope". Chief molds involved in the spoilage of bread are so called "bread mold", *Rhizopus nigricans*, with the white cottony mycelium and black dots of sporangia; the green spored *Penicillium expansum* or *P. Stolonifer*; *Aspergillus niger* with its greenish to black conodial heads. The shelf life of intermediate-moisture baked goods is determined by mold growth. These molds are destroyed during baking stage. However, recontamination can occur during cooling and packaging and cause growth to take place[3,4]. Ropy spoilage is caused mainly by *Bacillus subtilis* and Bacillus licheniformis. The spores of these microorganisms contaminate raw materials such as flour, bread improvers, yeast etc, and have ability to survive at backing temperatures[5].

Since, bread is an important food of our daily diet; therefore, ways and means should be explored to increase its shelf life. Microbial spore numbers can be controlled by ensuring raw material quality, improving the hygienic conditions of mixing and baking halls and cooling of production and storage environments. Once the food passes out the processing stage, its keeping qualities and the extent to which it will retain its intended characteristics is dependent on the microenvironment in the package. In many intermediate moisture bakery products, chemical preservatives (antimicrobial agents) and humectants are often added to prevent growth of spoilage fungi and bacteria. These include the salts of propionic, sorbic, and acetic acids. These antimicrobial agents are highly active in the range of 5-6 pH (pH of bread is 5.6) Moreover, these antimicrobial chemicals also act as pH controlling agents, texturizers, antioxidants and firming agents.[6] Keeping in view all these facts, this research project was designed to enhance the shelf life and observe the sensory characteristics of leavened bread by using antifungal and antibacterial agents (in flour and spray mixture) at various stages of bread production such as mixing, panning, slicing and packaging.

MATERIALS AND METHODS

The commercial wheat flour samples and chemicals were purchased from local market and scientific stores of Karachi.

Proximate analysis

The wheat flour samples were analyzed for moisture, crude protein, crude fat, crude fiber and ash contents according to the procedure of AACC[7].

Farinographic studies

The wheat flour samples were passed through Brabender Farinograph to assess the rheological properties of dough according to AACC[7].

Bread production

The wheat flour was mixed in Hobart mixer to optimum dough development. Chemical additives

(calcium propionate and glycerol propionate) were added in flour during mixing at the level of 0.14%. Sodium propionate, calcium acetate and potassium sorbate were incorporated in to spray mixture. The mixture was sprayed/ coated according to the plan as given in table 1.

Table 1: Plan for treatments.

Treatments	Application
T0	Without any spray
T1	Coating of pan (2 ml spray mixture)
T2	Coating of pan+Spray on dough (2+2=4ml spray mixture)
T3	Coating of pan+Spray on dough+Slicer (2+2+2=6ml spray mixture)
T4	Coating of pan+Spray on dough+Slicer+ Packaging (2+2+2+2=8ml spray mix)
T5	Spray on slicer (2 ml spray mixture)
T6	Spray on packaging material (2 ml spray mixture)

Fermentation, dividing and moulding

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

Proofing

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

Baking

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

Cooling, slicing and packaging

The baked breads were cooled to 35ºC and sliced with the help of slicer. The cooled and sliced breads were packed manually in polyethylene bags.

Microbiological analysis

Bacterial count was made on nutrient medium by applying plate count method[8]. On the other hand counting of molds was done by pour plate method on Sabouraud agar medium[9].

Sensory evaluation

A panel of judges was used for sensory evaluation of bread to examine its external and internal characteristics[10].

Statistical analysis

The data for each parameter was subjected to statistical analysis as described by Steel and Torrie[11].

RESULTS AND DISCUSSION

The results regarding chemical analysis of flour samples are given in table 2. These results have close similarity with the finding as reported by Naseem et. al. who analyzed six wheat varieties and found protein contents 8.35-13.68%, moisture 9.8-11.23% and ash 0.86-1.33%[12].

Farinographic studies

The results regarding farinographic study are given in table 3. The results indicated that flour samples have 61.3-63.2 ml water absorption, 7.9-9.7 (min.) dough stability, 45-60 B.U. tolerant index and 70-95 B.U. softening of dough. These results are in line with the previous findings that analyzed seven wheat flours for farinographic studies and found water absorption (54.05-57.38%), dough stability(4.11-9.17 minutes), tolerance index (35-110 BU) and softening of dough (46.67-116.67 BU)[13].

Table 2: Chemical composition of wheat flours.

Flour samples	Moisture %	Crude Protein %	Fat %	Crude fiber %	Ash %	NFE %
A	11.10	12.82	1.38	0.47	0.79	73.44
B	11.18	12.56	1.10	0.72	0.87	73.57
C	11.65	11.34	1.12	0.69	0.96	74.24

Table 3: Farinographic characteristics of wheat flours.

Flour sample	Water absorption	Dough time (min)	Dough Stability (min)	Tolerant index	Softening of dough
A	63.2	4.8	9.7	50 B.U.	80 B.U.
B	62.4	5.20	8.3	45B.U.	70 B.U.
C	61.3	4.15	7.9	60 B.U.	95 B.U.

Microbiological analysis

Results regarding colony count of bacteria are shown in table 4. Maximum numbers of bacterial colonies ($6.8x10^3$) were observed in treatment without any spray (T0) at 120 hours storage. It is obvious from results that treatment T4 is the most effective treatment against bacterial spoilage of bread and has bacterial count ($1.2x10^2$) at maximum storage time.

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

Treatments	24 hours	48 hours	72 hours	96 hours	120 hours
T_0	5×10^2	1.9×10^3	3.8×10^3	5.1×10^3	6.8×10^3
T_1	-	4×10^2	2.4×10^3	4.2×10^3	4.8×10^3
T_2	-	4×10^2	2.1×10^3	3.8×10^3	5.2×10^3
T_3	-	-	-	7×10^2	1.9×10^3
T_4	-	-	-	-	1.2×10^2
T_5	-	-	-	$8x\ 10^2$	2.1×10^3
T_6	-	-	-	3×10^2	1.7×10^3

The results regarding fungal count are presented in Table 5. The results indicate that maximum fungal colonies ($1.9x10^4$) appeared in T0. While, in treatment (T4), first colony was observed at 120 hours of storage. The results pointed out that packaging material and slicer are the main source of contamination in bread after baking.

These findings are supported by Al-Mohizea et.al. who found that microbial load of the air and relative humidity inside the package contribute a major role in bread spoilage[14].

Sensory evaluation

The data regarding effect of treatments and storage on external characteristics i.e., volume, color of crust, symmetry of form, evenness of bake, characteristics of crust, and internal parameters (i.e. aroma, taste, texture, crumb color and grain) of leavened bread is presented in table 6 and 7 respectively. It is obvious from the data that symmetry of form, character of crust, volume and grain of leavened bread were significantly affected by treatments. Storage time significantly affected the aroma, taste, texture, crust and crumb color. While, treatments did not affect crust and crumb color, aroma, texture and taste of leavened bread.

CONCLUSION

The chemical additives showed a positive effect on the shelf life and sensory attributes of traditional leavened bread. On the other hand, storage negatively affected texture, aroma and color of crust and crumb. Among the treatments, T4 did not show any visible sign of microbes up to 96 hours of storage and was better than other treatments with respect to overall acceptability of leavened bread. It is obvious from the study that bread treated by spray mixture during panning, proofing, slicing and packaging processes prolonged shelf life and remarkable resistance against fungus and bacteria.

Table 5: Fungal colony count (CFU/gram) at various storage intervals.

Treatments	24 hours	48 hours	72 hours	96 hours	120 hours
T0	-	7×10^2	2.8×10^3	4.6×10^3	1.9×10^4
T1	-	5×10^2	1.9×10^3	4.3×10^3	5.1×10^3
T2	-	3×10^2	1.9×10^3	3.9×10^3	4.4×10^3
T3	-	-	-	3×10^2	3.2×10^3
T4	-	-	-	-	6×10^2
T5	-	-	-	3.1×10^2	3.4×10^3
T6	-	-	-	1×10^2	2.1×10^3

These results are supported by Zulfiqar who observed that storage times (0, 24, 48, 72 and 96 hours) significantly affected internal characters like grain, color of crumb, aroma, taste and texture.). He found that the external characters like volume, color of crust, symmetry of form, evenness of bake and character of crust of bread were significantly affected by storage time (0, 24, 48, 72 and 96 hours)[15].

Table 6: Effect of treatments on external and internal characteristics of bread.

Treatments	Symmetry of form	Character of crust	Color of crust	Color of crumb	Evenness of bake	Volume	Grain	Aroma	Texture	Taste	Mean score
T0	3.10	2.88	6.34	6.60	2.24	6.68	9.74	5.66	10.42	14.24	6.79
T1	3.18	3.12	6.18	6.76	2.14	6.60	10.72	5.80	11.80	14.12	6.48
T2	2.96	2.82	6.34	6.90	2.22	6.44	10.84	5.94	11.36	14.02	6.98
T3	3.38	3.00	6.46	6.98	2.18	6.16	10.00	5.68	11.18	14.04	6.90
T4	3.40	3.00	6.32	7.02	2.28	5.46	9.70	5.88	11.38	14.32	6.87
T5	3.18	2.90	6.56	6.76	2.24	6.02	10.40	5.78	11.30	14.24	6.93
T6	3.34	2.76	6.18	6.78	2.32	6.06	10.32	5.56	11.60	13.96	6.88
Mean±SD	3.22± 0.16	2.92± 0.12	6.34± 0.13	6.82± 0.14	2.23± 0.05	6.20± 0.41	10.24± 9.45	5.75± 0.13	11.29± 0.43	14.13± 0.13	-

Table 7: Effect of storage on external and internal characteristics of bread.

Storage interval	Symmetry of form	Character of crust	Color of crust	Color of crumb	Evenness of bake	Vol	Grain	Aroma	Texture	Taste	Mean score
24 hours	3.61	3.10	6.84	7.15	2.31	6.48	10.50	6.32	12.14	15.09	7.35 ±4.1
48 hours	3.38	2.92	6.70	7.00	2.28	6.35	10.47	6.00	11.73	14.69	7.15 ±4.03
72 hours	3.22	2.88	6.42	6.83	2.27	6.14	10.32	5.87	11.43	14.26	6.96 ±3.93
96 hours	3.08	2.85	6.02	6.64	2.17	6.14	10.02	5.47	10.80	13.99	6.71 ±3.82
120 hours	2.94	2.85	5.79	6.15	2.11	5.85	8.25	5.11	10.47	12.66	6.20 ±3.40
Mean± SD	3.24 ±0.26	2.92 ±0.10	6.33 ±0.47	6.75 ±0.38	2.22 ±0.08	6.19 ±0.2	9.91 ±0.94	5.75 ±0.47	11.31 ±0.67	14.13 ±0.92	

REFERENCES

1. Cauvain SP and Young LS. Technology of bread making. Gaithersburg, MD.: Aspen Publishers. 1999.
2. Franke I, Wijma E and Bouma K. Shelf life extension of pre-baked buns by an active packaging ethanol emitter. *Food Addit.Contam.*, 2002; 19: 314-322
3. Smith JP, Hosino J and Abe Y. Interactive packaging involving sachet iechnology. In: Acive Food Packaging. Rooney ML, Ed., London: Blackie Academic and Professional. 1995.
4. Soares NFF, Rutishauser DM, Melo N, Cruz RS and Andrade NJ. Inhibition of microbial growth in bread through active packaging. *Package. Technol. Sci.*, 2002; 15: 129-132.
5. Rosenquist H. The role of *Bacillus species* in spoilage of wheat bread. PhD thesis, Department of dairy and food science, The Royal Veterinary and Agricultural University, Copenhagen. 1996.
6. Marin S, Guynot ME, Neira P, Bernadó M, Sanchis V and Ramos AJ. Risk assessment of the use of sub-optimal levels of weak-acid preservatives in the control of mould growth on bakery products. *Int. J. Food Microbiol*., 2002; 79: 203-211.
7. AACC. Approved Methods of American Association of Cereal Chemists. The Amer. Associ. Cereal Chemists, St. Paul, Minnesota. 2005.
8. Harrigan WF and McCance MC. Laboratory Methods in Food and Diary microbiology. Academic Press, London. 1976.
9. Beneke ES. Medical mycology lab manual. Burgess Publ. Co., Minneapolis, Minnesota. 1962.
10. Land DG and Shepherd R. Scaling and Ranking Methods In "Sensory Analysis of Foods". (J.R. Riggot(ED). Elsevier Applied/Science, New York. 1988.
11. Steel RGD and Torrie JH. Principles and procedures of statistics. McGraw Hill Book Co., New York. 1996.
12. Naseem T, Bhatti MS, Ahmed A and Khalid N. Suitability of some Pakistani wheat varieties for pizza baking. *J. Agric. Res*., 2011; 49
13. Amjad M, Safdar MN, Mumtaz A, Naseem K, Raza S and Khalil S. Comparison of different wheat varieties grown in Punjab for leavened flat bread (naan) production. *Pak. J. Nutr.*, 2010; 9: 146-150.
14. Al-Mohizea IS, Mousa EI and Fawzi NM. Microbiological studies on two common types of bread in Saudi Arabia I. *Cereal Foods World*, 1987; 32: 610-612.
15. Ali Z, Anjum FM and Zahoor T. Sensory characteristics of bread prepared from lactic acid obtained from corn cobs, an agricultural waste. *Pak. J. Agri. Sci.*, 2007; 44: 539.