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Effect of Water Activity (A_w) Moisture Content and Total Microbial Count on the Overall Quality of Bread

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ABSTRACT

A study was conducted to know the effect of moisture content, water activity (a_w) and microbial count on over all quality of industrial and local bread i.e. S1, S2, S3, S4, S5 and S6 for a period of five days. These samples were stored at (12 – 25^{0}C) and were evaluated for a_w, moisture content and total microbial count with an interval of 24 h. The initial moisture content of S1, S2 and S3 was in the range of 36.6 to 45.49% which increased to 43.07 to 48.02% after five days. While in S4, S5 and S6 the initial moisture content was in range of 36.05 to 43.07 which increased to 41.29 to 49.26% after five days. The a_w value for S1, S2 and S3 ranged between 0.9425 to 0.97 which increased from 0.977 to 0.9975. Minimum microbial growth was observed in S2 (0 - 57 cfu / g) while maximum (0 – 197 cfu / g) was recorded in case of S4. During sensory evaluation S1, S2 and S3 obtained maximum mean scores for color, flavour and texture during the period of storage. The effect of treatments and storage period was significant ($p< 0.05$) on moisture content, a_w and microbial growth on the samples.

Key Words: Bread water activity; Moisture; Microbial activity

INTRODUCTION

Bread is a first major food of all human beings from the time life came in to existence. Bread history is older than the history of any other food as it is clearly mentioned in the Bible that Abraham said to his wife Sarah, “Quickly make ready three measures of fine meal; kneed it and make bread.” The Egyptian and Babylonians knew baking and brewing more than 100 years before Christian era. In 168 B.C., the Roman baker used a stone or clay mixer to prepare his bread from fermented dough (Pomeranz, 1987).

Bread is a rich source of carbohydrates, protein, vitamins and supplies half of calories to over 50% of world population. The carbohydrates of bread range from 45 - 58%. The protein content is generally around 6%. The amount of fat is 0.5 – 2% and is increased significantly by using shortening in the formula. There are 1.5 - 3.0% minerals in the bread. Thiamin (0.46 mg/100 g), Riboflavin (0.29 mg/100 g), Niacin (4.39 mg/100 g) and Choline (202 mg/100 g) are the major vitamins presents in bread (Pomeranz, 1987a). A slice (11.4 g) of bread gives us energy of 29 k calories. From per 100 g of bread we can obtain 254 k calories energy (Kent, 1975).

Moisture content varies in the products baked with different techniques. For this reason, they are susceptible to microorganisms which decrease their shelf life. According to Chichester *et al*. (1963) water activity is defined as the ratio of the water vapour pressure of the food to the vapour pressure of pure water at the same temperature.

Mathematically it can be written as $a_w = P/P°$. P is the Vapour pressure of the food; P° is the Vapour pressure of the pure water.

Water activity has marked effect on the growth of microorganisms. Actually the need for a meaningful term to describe the behaviour of microorganisms in environments with reduced moisture helped to establish the term water activity (Breene *et al.*, 1988).

Labuza *et al*. (1972) reported that reducing water activity below 0.7 prevents microbial spoilage. Although, the food would not spoil from microorganisms, other deteriorative reactions can still occur. To successfully preserve a food product, water activity would have to be lowered to a range where the rate of deteriorative reactions is minimized. Chief molds involved in the spoilage of bread are *Rhizopus nigricans* with its white cottony mycelium and black dots of sporangia, the green spored *Pencillium expansum* or *Pencillium stoloniferum and Aspergillus niger* with its greenish or purplish brown to black conidial heads and yellow pigments diffusing into the bread. Ropiness of bread is fairly common in home baked bread, especially during hot weather. Ropiness is caused by *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus panis.* The spores of these species can withstand high temperature during baking (Frazier & Westhoff, 1978)

As it is known that edible products prepared from flour (like bread, bun and cookies) are susceptible to microorganisms in open environment and these products act as a carrier of microorganisms when they are consumed causing different diseases. This study was initiated to make a comparison for better quality with respect to microbial load, moisture content and water activity, so that consumers may get a nutritive, more hygienic and shelf stable product at their homes.

MATERIALS AND METHODS

Breads S1, S2, S3 (from registered baking industries) and breads S4, S5 and S6 were collected from the local market of Peshawar for different physicochemical analysis. Water Activity of all the samples was determined by methods described by Landrock and Proctor (1951). Samples (in small plastic cups) were equilibrated against the saturated salt solution at 20°C. Water activity of the samples was determined by recording data on water loss or gain per gram of samples. Moisture content was determined by using air oven method (105±1°C) recommended by A.O.A.C. (1984). Selected samples were analyzed for total microbial count by the total plate count method (Diliello, 1982).

Organoleptic evaluation. Selected samples of the products were evaluated for color, flavor and texture by a panel of judges. Taste is the sensation of taste buds located on tongue; flavor is a combination of taste, odor and feel (Mayer, 1960). Sensory evaluation was carried out by 9-point Hedonic Scale as described by Larmond (1977).

Statistical analysis. All the data was statistical analyzed using CRD using as prescribed by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Products prepared by flour are considered as major diet for all human beings. Mold and bacteria usually deteriorate these products. Each microorganism has its own range of a_w for growth under a given environmental conditions. Therefore an unfavorable a_w of food would result in the reduction of growth rate and delay in initiation of growth of spores. (Frazier & Westhoff, 1978).

Moisture content. Results regarding moisture content of breads showed that S1 had moisture content 36.64% when brought from the market on first day. Its moisture content increased to 38.91, 39.1, 41.39 and 43.07%, respectively during five days of storage. Sample S2 showed an increase from 45.49 to 48.02%. For sample S3 this increase was from 44.28 to 46.57%, respectively during storage period. Similar increase in moisture content was observed in S4 (from 36.05 to 41.29%) and S5 (from 37.62 to 49.26%) respectively when stored for five days. S6 showed 43.07% moisture content which increased to 44.28, 45.54, 46.53 and 47.52%, respectively (Table I). Internal difference between different breads showed that bread having low moisture was better than the bread having high moisture content. The overall mean moisture content of different breads showed that S4 had lowest moisture content *i.e.* 37.93% followed by S1 (40.23%), S5 (44.84%), S3 (45.26%), S6 (45.38%) and S2 (46.61%) during storage (Table I).

Table I. Moisture content (%) of breads

Bread	0 Day	Day 1	Day 2	Day 3	Day 4	Mean
S1	36.6	38.91	39.1	41.39	43.07	40.23b
S2	45.49	45.5	46.7	47.29	48.02	46.61a
S3	44.28	44.84	45.0	45.59	46.57	45.26a
S4	36.05	36.45	37.67	38.19	41.29	37.93c
S5	37.62	43.29	46.0	48.02	49.26	44.84a
S6	43.07	44.28	45.5	46.53	47.52	45.38a
Mean	40.85d	42.21cd	43.3bc	44.50ab	45.62a	

Figures mentioned with same small letters are not statistically different ($p<0.05$)

Statistical analysis showed that storage had a significant effect on moisture content of bread. These results are in agreement with the New Zealand Institute for Crop and Food Research Limited (2001), Pomeranz (1987a), Haas *et al.* (1975). These authors found a range of moisture content of bread between 35-45% for one day storage. According to Sanina *et al.* (1996), bread quality is also affected by dough moisture content. Ridge analysis with the aid of a generalized large range multiplier method was used to establish that the optimum whey concentration and moisture content for improved bread quality are 16.6 and 46.2%, respectively.

Water activity. Results showed that water activity (a_w) of all bread samples increased during 5 days storage period. The a_w of S1 after storing increased from 0.9425 to 0.9575, 0.96, 0.97 and 0.977 during five days storage period (Fig. 1). S2 and S3 had a_w of 0.9475, 0.97, which increased to 0.9675, 0.98, 0.9725, 0.985, 0.98, 0.9875, 0.9825 and 0.9975 during storage period (Fig. 2 & 3). While in S4 the increase was from 0.9625 to 0.97, 0.9733, 0.9825 and 0.985, respectively (Fig. 4). Sample S5 showed a_w value of 0.955 which increased to 0.9663, 0.9725, 0.975 and 0.99, respectively (Fig. 5). The a_w of S6 also increased from 0.9725 to 0.9825, 0.9875, 0.995 and 0.9975, respectively (Fig. 6). The comparison between industrial and local breads showed that bread having low a_w was better than the bread having high a_w. The overall mean values of a_w showed that S1 had lowest a_w values i.e. (0.9614) followed by S2 (0.97), S5 (0.972), S4 (0.975), S3 (0.984) and S6 (0.987) (Table II).

Table II. Water activity of breads

Bread	0 Day	Day 1	Day 2	Day 3	Day 4	Mean
S1	0.9425	0.9575	0.96	0.97	0.977	0.9614f
S2	0.9475	0.9675	0.9725	0.98	0.9825	0.97e
S3	0.97	0.98	0.985	0.9875	0.9975	0.984b
S4	0.9625	0.97	0.9733	0.9825	0.985	0.975c
S5	0.955	0.9663	0.9725	0.975	0.99	0.972d
S6	0.9725	0.9825	0.9875	0.995	0.9975	0.987a
Mean	0.958e	0.971d	0.975c	0.982b	0.988a	

Figures mentioned with same small letters are not statistically different ($p<0.05$)

Statistical analysis showed that storage had a significant increase in the a_w values of all bread samples during five days storage. These results are in agreement with John (2001), Haas *et al* (1975). These authors found a_w of bread between 0.95 - 0.96. In another study, Esakie *et al.* (1996) reported that the water activity of high voltage electric field treated bread was 0.987 ± 0.0056 being higher than that of untreated bread by 0.011 (*i.e.* 0.976), where Fett (1973) observed that white bread after one day of storage had a_w 0.942 – 0.95.

Microbial count. No microbial colony was observed during the first two days of storage in S1 while during last three days there were 25, 30 and 89 cfu/g (colony forming unit per gram). While in S2 and S3 no colony was formed during

Fig. 1. Water activity of S1

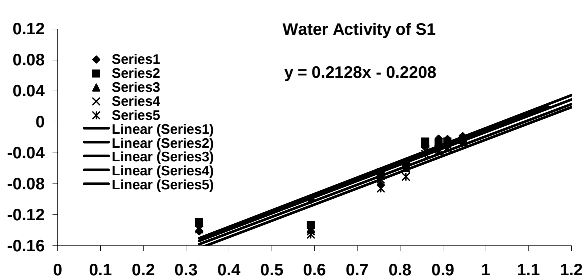


Fig. 2. Water activity of S2

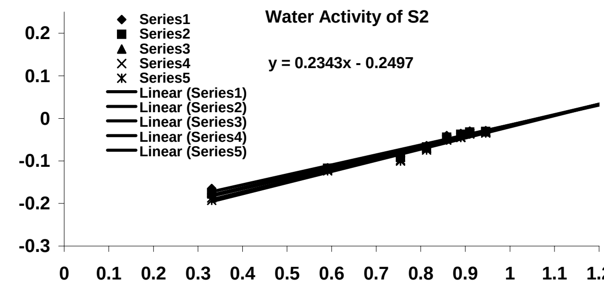


Fig. 3. Water activity of S3

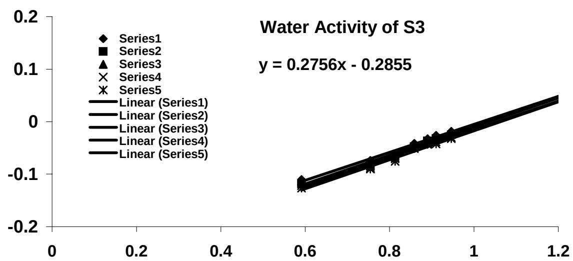


Fig. 4. Water activity of S4

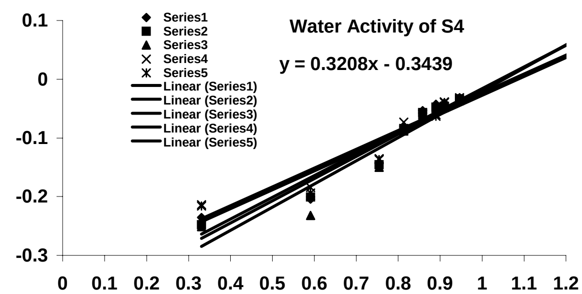


Fig. 5. Water activity of S5

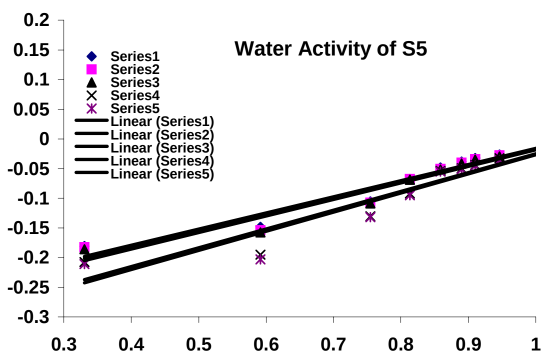
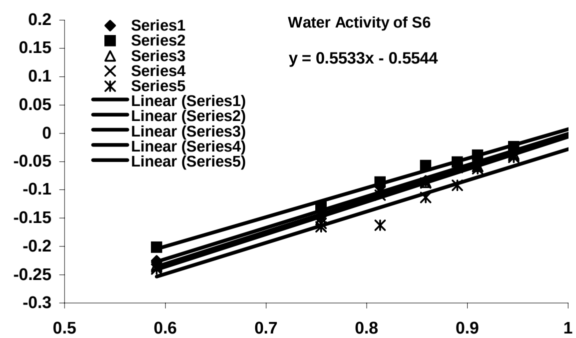


Fig. 6. Water activity of S6



water gain or loss/g

Water Activity

Series 1=Data of day1, Series 2= Data of day2, Series 3 = data of day3, Series 4= Data of day 4, Series 5= Data of day 5

first day of storage but in remaining 4 days of storage there were 4, 16, 19, 29, 37, 33, 57 and 77 cfu/g. Similarly no colony was determined in S4, S5 and S6 during first day. In sample S4 it was 0, 31, 144 and 197 after 4 days of storage. In S5 it was 0, 9, 26, 50 and 69 cfu/g and in S6 it was 41, 60, 71 and 99 cfu/g (Table III).

Results showed that minimum overall mean values of microbial count were observed in S2 i.e. 23.4 cfu/g followed by S1 (28.8), S5 (30.8), S3 (31.0), S6 (54.2) and S4 (74.4) cfu/g during storage (Table III). Analysis of variance showed that with the passage of time there was a significant increase in microbial population on all breads. These results are in agreement with the findings of Collins *et al.* (1991) who isolated two hundred and two (202) Bacillus strains from ropey bread and were characterized according to colony morphology on Reinforced Nutrient Agar medium. In another study Bailey *et al.* (1993) reported that rope spoilage of bread by *Bacillus Subtilis* and *Bacillus licheniformis* caused economic losses to the baking industries. Baked bread stored at 30°C got Bacillus counts of 107 cfu/g after three days of storage.

Table III. Microbial Count

Bread	0 Day	Day 1	Day 2	Day 3	Day 4	Mean
S1	0	0	25	30	89	28.8bc
S2	0	4	19	37	57	23.4c
S3	0	16	29	33	77	31.0b
S4	0	0	31	144	197	74.4a
S5	0	9	26	50	69	30.8b
S6	0	41	60	71	99	54.2ab
Mean	0 d	11.667c	31.667bc	60.883b	98.0a	

Table IV. Mean score of judges for color

Bread	0 Day	Day_1	Day_2	Day_3	Day_4	Mean
S1	7.7	7.0	6.7	6.2	5.3	6.58b
S2	8.0	7.8	7.3	6.7	5.9	7.14a
S3	7.5	7.1	6.5	5.7	5.2	6.4c
S4	7.0	6.7	6.2	5.7	5.1	6.14d
S5	7.6	6.9	6.5	6.0	5.1	6.42bc
S6	7.6	6.8	6.5	5.9	5.1	6.38c
Mean	7.567a	7.05b	6.617c	6.033d	5.283e	

Table V. Mean score of judges for flavour

Bread	0 Day	Day_1	Day_2	Day_3	Day_4	Mean
S1	7.8	7.4	6.9	6.1	5.4	6.72bc
S2	8.2	7.8	7.4	6.7	5.8	7.18a
S3	7.7	7.2	6.8	6.4	5.5	6.72bc
S4	7.4	7.1	6.7	6.1	5.5	6.56c
S5	7.8	7.5	6.7	6.0	5.2	6.64c
S6	7.8	7.5	6.9	6.5	5.6	6.86b
Mean	7.783a	7.417b	6.9c	6.3d	6.5e	

Table VI. Mean score of judges for texture

Bread	0 Day	Day_1	Day_2	Day_3	Day_4	Mean
S1	8.0	7.6	7.0	6.2	5.5	6.86ab
S2	8.1	7.7	7.2	6.5	5.4	6.98a
S3	7.9	7.6	7.0	6.4	5.4	6.86ab
S4	7.5	7.2	6.7	6.1	5.1	6.52c
S5	7.4	6.8	6.7	6.0	5.1	6.4c
S6	7.7	7.5	7.0	6.1	5.7	6.8b
Mean	7.767a	7.4b	6.933c	6.217d	5.367e	

Figures mentioned with same small letters are not statistically different ($p<0.05$)

Sensory Evaluation

Color. S1 got a mean score of 7.7 for color. It showed a decreasing to 5.3 after five days of storage. The mean score for S2 and S3 was 8.0 and 7.5 which decreased to 5.9 and 5.2, respectively after five days of storage. S4 also showed decrease in its value from 7 to 5.1. While similar decrease was observed in S5 and S6 (7.9 and 6.9 to 5.1) after five days of storage at room temperature. The overall mean score was maximum in case of S2 (7.14) followed by S1 (6.58), S5 (6.42), S3 (6.4), S6 (6.38) and S4 (6.14) (Table IV).

Statistical analysis showed that storage had a significant effect on color of all the breads. Carson *et al*. (2000) conducted sensory test on bread made from 50% sorghum based composite flour by six trained panelists. A descriptive test was used to identify the characteristics of aroma, flavour and texture of bread. Sorghum composite bread resulted in an over all acceptability mean score of 6.9 on a nine point hedonic scale. In another study, Pomeranz (1987a) reported that color, texture and flavour are sensory criteria mostly used by the consumer to evaluate acceptability of bread.

Flavour. Results showed that during storage S1 got a mean value 7.8 for flavour, which decreased to 5.4 after five days of storage. The mean value for S2 and S3 was 8.2 and 7.7 which decreased to 5.8 and 5.5, respectively after five days storage. A steady decrease was observed in the mean values for S4, S5 and S6. Their mean values were 7.4, 7.8 and 7.8 which decreased to 5.5, 5.2 and 5.6, respectively. The overall mean values for different breads showed that S2 had maximum mean value (7.18) followed by S6 (6.86), S1 and S3 (6.72), S5 (6.64) and S4 (6.56) (Table V).

Statistical analysis showed that storage had a significant effect on loss in flavour of all bread samples. According to Carson *et al*. (2000), sensory test on bread made from 50% sorghum based composite flour by six trained panelists showed that the sorghum composite bread resulted in an over all acceptability mean score of 6.9 on a nine point hedonic scale. In another study Pomeranz (1987a) reported that color, texture and flavour are sensory criteria mostly used by the consumer to evaluate acceptability of bread.

Texture. Results indicated that S1 got mean value of 8 for texture which decreased to 5.5 while in S2 it was from 8.1 to 5.4, in S3 it was from 7.9 to5.4 Similar decrease was observed in mean score for texture of bread in S4, S5 and S6. Their initial mean score was 7.5, 7.4 and 7.7, which decreased to 5.1, 5.1 and 5.7, respectively. Data showed that bread with high score for texture was better than the low scored bread. The overall mean score for different breads showed that S2 had maximum score for texture i.e. 6.98 followed by S1 and S3 (6.86), S6 (6.8), S4 (6.52) and S5 (6.4) (Table VI).

Statistical analysis showed that storage had a significantly negative effect on texture of all bread samples. According to Carson *et al*. (2000), sensory test on bread made from 50% sorghum based composite flour, by six trained panelists resulted in an over all acceptability mean score of 6.9 on a nine point hedonic scale. In another study Pomeranz (1987a) reported that color, texture and flavor are sensory criteria mostly used by the consumer to evaluate acceptability of bread.

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