The Pharma Innovation Journal 2022; 11(3): 531-537

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(3): 531-537

www.thepharmajournal.com
Received: 08-12-2021
Accepted: 18-01-2022

Shashikumar JN
Department of Food Science and Technology Agricultural College, Hassan University of Agricultural Sciences, Bangalore, Karnataka, India

Udaykumar Nidoni
Department of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur, Karnataka, India

Ramachandra CT
Department of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Bangalore, Karnataka, India

Corresponding Author:
Shashikumar JN
Department of Food science and Technology Agricultural College, Hassan University of Agricultural Sciences, Bangalore, Karnataka, India

Influence of active packaging materials on microbial characteristics of wheat flour bread during storage

Shashikumar JN, Udaykumar Nidoni and Ramachandra CT

Abstract
The influence of active packaging materials namely, ethanol emitter, oxygen absorber and moisture absorber on microbial characteristics of resfined wheat flour (RWF) and whole wheat flour (WWF) bread during storage was investigated. Initial proximate composition of the RWF and WWF breads was determined. The TPC, yeast and moulds and *Bacillus* spp. of fresh RWF bread and WWF bread were 1.2 $\times 10^4$ and 1.3 $\times 10^4$ cfu/g; 1.2 $\times 10^3$and 1.3 $\times 10^3$ cfu/g; 1.1 $\times 10^4$ and 1.1 $\times 10^4$ cfu/g, respectively. The results revealed that the control samples of RWF and WWF bread crossed the safe limit of TPC (7 log cfu/g), yeast and moulds (4 log cfu/g) and *Bacillus* spp. (5 log cfu/g) on 4th day, whereas the microbial counts in RWF and WWF bread samples packed in combination of ethanol emitter (E), oxygen absorber (O) and moisture absorber (M) were found within the safe limit even after 12 and 6 days of storage, respectively. The shelf life of RWF bread and WWF bread packaged with active packaging materials enhanced 15 and 4 days, respectively more than the control.

Keywords: Wheat flour bread, shelf-life, ethanol emitter, oxygen absorber, moisture absorber

Introduction
Bread is a basic dietary item dating back to the Neolithic era, which is prepared by baking in oven. The first bread was prepared around 10,000 years BC or over 12,000 years ago, which might have been developed by deliberate experimentation with water and grain flour (Mondal. and Datta. 2007) [9]. According to the Ministry of Food Processing Industries, Government of India, the present processed food market, accounts for about 32 per cent of the total food market. Eighty five per cent of the total bread produced in India is by the unorganized sector and the size of the bread market is estimated at Rs. 15.00 billion. The demand for bread has been rising from Rs. 6.42 billion in 1990-91 to Rs. 21.10 billion in 2009-2010 (Smitha 2013) [14].

The consumers prefer to consume bread freshly, but the bread remains fresh for just few hours after baking. The main factors that cause bread properties to deteriorate are microbial spoilage and chemical or physical changes during storage (Pateras 2007) [11]. In bread, it is reported that 60 per cent of spoilage is attributed to moulds (*Penicillium* spp. and *Aspergillus niger*) whereas, yeasts accounted for about 15 per cent. Besides the repelling sight of visible growth, fungi are responsible for off-flavour development, the production of mycotoxins as well as allergenic compounds. These compounds may be formed even before mould growth is visible (Nielsen and Rios 2000) [10]. Even though, the moulds are destroyed during baking, recontamination occurs during cooling and subsequent packaging causing the above problems (Galic *et al.* 2009) [6].

Traditional packaging concepts are limited in their ability to prolong the shelf-life of bakery products. The use of vacuum packaging is limited in the baking industry due to its crushing effect on delicate products (Simth, 1994) [13]. Modified Atmosphere Packaging (MAP) has been used for shelf-life extension of a large variety of foodstuffs including bakery products such as wheat bread, rye bread, hot-dog bread and soy bread. The problem associated with the MAP of bakery products is that it is very difficult to reduce the oxygen content within the package to a very low level due to a large number of pores in the bread matrix which tend to trap oxygen (Latou *et al.* 2010) [8].

Active packaging techniques for packaging of bread have been reported for enhancing its shelf-life. Very few researchers have studied the use of ethanol emitters and oxygen absorbers for extending the shelf-life of bread. The studies reported encouraging results showing reduction in rate of reaction and improvement in shelf-life. The use of moisture absorbers have also been reported for enhancing shelf-life of highly perishable foods such as fish and meat products. Since the bread has significant moisture to support the growth of moulds, the use of moisture absorber might help in extending the shelf life of the bread. Keeping in view of the above facts, the research topic entitled "Influence of active packaging materials on microbial characteristics of wheat flour bread" was undertaken the Department of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur, Karnataka (India).

Material And Methods

Raw Materials

Refined wheat flour (RWF) bread and whole wheat flour (WWF) bread were procured from local bakery unit of Raichur, Karnataka (India).

Proximate Composition of Fresh RWF and WWF Breads

The proximate compositions *viz.*, moisture content (No. 945.43), carbohydrates (No. 975.14), crude protein (No. 950.36), crude fiber (No. 962.09E), crude fat (No. 922.06) and total ash (No. 925.23) of fresh RWF and WWF bread samples were determined by following the respective methods described in (AOAC, 2005) [1]

Packaging Materials

Active packaging materials in the form of sachets namely, moisture absorbers (M) and oxygen absorbers (O) were procured from M/s. Dry Air Technologies, Tamil Nadu (India) and ethanol emitters (E) from M/s. Freund Corporation, Shinjuku, Tokyo, (Japan) LDPE bags of 200 gauge thickness were purchased from local market of Raichur.

Microbiological Analysis

Microbial analysis was carried out for every four and two days of interval for all the treatments of RWF bread and WWF breads, respectively. The bread samples were weighed aseptically (10 g) and homogenized in a stomacher for 60 s at room temperature (28±2 °C) with 90 ml sterile distilled water. From this, 1 ml of the solution was accurately pipetted out into test tube containing 9 ml of sterile distilled water (10^{-1}) and serially diluted until 10^{-4} dilution were reached. One ml of aliquot each from10^{-4} for total plate counts, 10^{-3} dilutions for yeast and mould, 10^{-4} for *Bacillus* spp. were transferred to the sterile petri plates for the enumeration of microbial load (Latou *et al.* 2010) [8]. Plates were triplicated for each dilution. Approximately, 15-20 ml of molten and cooled total plate count agar medium (45 °C) for Total Plate Count agar medium (TPC), Potato Dextrose Agar medium (PDA) for yeast and moulds and Bacillus medium (BC) (2 g peptone, 2 g yeast extract, 5 g dextrose and 15 g agar in 1000 ml distilled water) for *Bacillus* spp. were added into the petri plates and were rotated clockwise and anticlockwise directions on the flat surface to have a uniform distribution of colonies. After the solidification of agar, the plates were inverted and incubated at 30 °C for 2 days for TPC and BC and 25 °C for 3 days for PDA. The colonies were counted after the incubation period and the number of colony forming units per gram (cfu/g) of sample were calculated by applying the following formula (Costa *et al*. 2011) [3]

$$\text{TPC/yeast and mould/}\textit{Bacillus}\text{ spp. (cfu/g)} = \frac{\text{Mean number of cfu} \times \text{Dilution factor}}{\text{Weight of the sample (g)}} \quad (1)$$

Where, Dilution factor is the reciprocal of the dilution (*e.g.*, $10^{-3}=10^{3}$)

Treatment Details

The selected breads (RWF bread and WWF bread) and the active packaging materials packaged in low density poly ethylene bags (200 gauge) were stored to study their shelf-life. The treatment details are given in Table 1.

Shelf-life Evaluation of Bread Samples

The shelf-life in terms of number of days of RWF bread and WWF bread was evaluated based on the microbiological analysis during storage period and the safe limit of the microbial load in bread samples. According to the International Commission on Microbiological specifications for foods (ICMSF) and the European Food Safety Authority (EFSA), the safe limit for different microbial loads in the food/bread sample are given below (Latou *et al.* 2010) [8].

- Total plate count (TPC) : < 7 log cfu/g
- Yeast and moulds : < 4 log cfu/g
- Bacillus spp. : < 5 log cfu/g

Statistical Analysis

All the experiments in the present investigation were conducted in triplicate and mean values were reported. General Factorial Completely Randomised Design (CRD) was used to analyse the data. After proper analysis, data were accommodated in the tables as per the needs of objectives for interpretation of results.

Results and Discussion

Proximate Composition of Fresh RWF and WWF breads

The proximate composition of fresh RWF and WWF breads are presented in Table 2. From the table, it is seen that, the moisture contents, carbohydrate contents, crude protein, crude fiber contents, crude fat content and total ash content of fresh RWF bread and WWF bread were 31.58 and 32.43 per cent (w.b.); 51.75 and 50.07 per cent; 9.92 and 10.23 per cent; 2.56 to 2.95 per cent; 3.04 and 3.07 per and 1.15 and 1.25 per cent respectively.

Total Plate Count

The results of increase in TPC of selected bread samples packed with different active packaging materials during storage are presented in Fig. 1. From the figure, it is observed that, there was a slight difference of 0.1 per cent in initial TPC of fresh RWF and WWF breads. This variation might be due to the microbial load carried with the ingredients used in the preparation of breads. It is also observed that, the TPC of the bread samples increased during the storage period irrespective of the type of bread and treatment combinations. The increase in TPC of bread samples might be due to migration of moisture, degradation of protein, fat which favoured the growth of TPC (Salminen *et al* 2010) [12]. From the figure, it can also be seen that the TPC values in B1T7 and B2T7 were within the safe limit (7 log cfu/g) up to 16 and 8 days of storage.

At the end of 20th day of storage, the lowest increase in TPCs (85.18% and 98.63%) with respect to the initial values of were observed in B1T7 and B2T7, whereas highest increase (98.71% and 98.79%) were found in treatments B1T0 and B2T0 respectively. The lower growth of TPC in B1T7 sample might be due to the antimicrobial activity of ethanol emitted by ethanol emitter, anoxic environment created by the oxygen absorber and reduction of moisture content by moisture absorber that inhibited the growth of TPC. Similar results of increase in TPC of bread sample during storage period have been reported by (Salminen *et al.* 1998; Nielsen. and Rios 2000; Franke *et al.* 2010; Guynot *et al.* 2003) [12, 10, 5, 7]. The results of less growth in TPC due to the effect of ethanol emitter and oxygen absorber have been reported by (Latou *et al.* 2010) [8] for sliced wheat bread.

Yeast and Moulds

The influence of active packaging materials on yeast and moulds of RWF bread and WWF bread during storage period are presented in Fig. 2. From the figure, it is observed that, there was a slight difference of 0.85 per cent in initial yeast and moulds of fresh RWF and WWF breads. It is also observed that, the yeast and moulds of the bread samples increased during the storage period irrespective of the type of bread and treatment combinations. The increase in yeast and moulds of bread might be due to migration of moisture, degradation of protein, fat which favoured the growth of yeast and moulds (Salminen *et al*. 1998; Nielsen. and Rios 2000; Franke *et al*. 2010; Guynot *et al*. 2003) [12. 10, 5, 7]. From the figure, it can also be seen that the yeast and moulds were within the safe level (4 log cfu/g) up to 16 and 8 days of storage in B1T7 and B2T7 (Fernandez *et al.* 2006) [4]

At the end of 20th day of storage, the lowest increase in yeast and moulds (89.28% and 96.67%) with respect to the initial values of treatments was observed in B1T7 and B2T7, whereas highest increase (96.85% and 97.55%) were found in treatments B1T0 and B2T0, respectively, this might be due to the antimicrobial activity of ethanol emitted by ethanol emitter, anoxic environment created by the oxygen absorber and reduction of water activity by moisture absorber that inhibited the growth of yeast and moulds. Similar results of increase in yeast and moulds of bread samples during storage period have been reported by (Salminen *et al*. 1998; Nielsen. and Rios 2000; Franke *et al*. 2010; Guynot *et al*. 2003) [12, 10, 5, 7]. The results of decrease in yeast and moulds due to the effect of ethanol emitter and oxygen absorber have been reported by (Latou *et al.* 2010) [8] for sliced wheat bread.

***Bacillus* spp.**

The increase in the values of *Bacillus* spp. of selected bread samples packed with different active packaging materials during storage period are presented in Fig. 3. From the figure, it is observed that, the *Bacillus* spp. of the bread samples increased during the storage period irrespective of the type of bread and treatment combinations. The increase in *Bacillus* spp. of bread samples might be due to migration of moisture, degradation of protein, fat and increase in water activity which tend to increase in bacterial growth counts (Latou *et al.* 2010) [8]. From the figure, it can also be seen that the *Bacillus* spp. in B_1T_7 and B_2T_7 were within the safe limits (5 log cfu/g) up to 16 and 8 days of storage.

At the end of 20th day of storage, the lowest increase in *Bacillus* spp. (82.53 per cent and 97.11%) with respect to the initial values of treatments was observed in B_1T_7 and B_2T_7, whereas highest increase (96.86% and 97.11%) were found in treatments B_1T_0 and B_2T_0, respectively, this might be due to the antimicrobial activity of ethanol emitted by ethanol emitter, anoxic environment created by the oxygen absorber and reduction of water activity by moisture absorber that inhibited the growth of *Bacillus* spp. Similar results of increase in *Bacillus* spp. of bread samples during storage period have been reported by (Salminen *et al*. 1998; Nielsen. and Rios 2000; Franke *et al*. 2010; Guynot *et al*. 2003) [12, 10, 5, 7]. The results of decrease in *Bacillus* spp. due to the effect of ethanol emitter and oxygen absorber have been reported by (Latou *et al.* 2010) [8] for sliced wheat bread.

Shelf-life of RWF bread and WWF bread during storage

The influence of active packaging materials on shelf-life of RWF bread and WWF bread and the safe limit for different microbial loads are presented in Fig. 4. From the figure, it is observed that, among the treatments, the highest shelf-life (16 and 8 days) in treatments B1T7 and B2T7, whereas the lowest shelf-life (4 days) was found in treatments B1T0 and B2T0. The enhanced shelf-life of bread samples might be due to the antimicrobial activity of ethanol emitter, anoxic environment created by the oxygen absorber and reduction in relative humidity of the surrounding air by moisture absorber that reduced the rate of reaction and prevented the growth of microorganisms during the storage period. Similar results of increase in shelf-life due to the effect of ethanol emitter and oxygen absorber have been reported by (Latou *et al.* 2010) [8] for sliced wheat bread and the effect of moisture absorber on shelf-life of mushroom has been reported by (Villaescusa, and Gil. 2003) [15]. The shelf-life of RWF bread (B1T7) was enhanced 15 days more than that of the control sample (B1T0), whereas in case of WWF bread (B2T7), it was increased to 4 days more than the control (B2T0).

It is also noticed from the figure that, the effect of active packaging materials was predominant for RWF bread than WWF bread. The variation in the effect might be due to the difference in basic ingredients used for the preparation of the respective breads. The lesser shelf-life of WWF bread might be due to relatively higher initial moisture content and higher crude fat content compared to RWF bread. The composition resulted in higher rate of reaction(metabolic activity) and increased growth of microbes in WWF bread samples leading to the shorter shelf-life, similar results have been reported by (Butt *et al.* 2002) [2] for wheat flour.

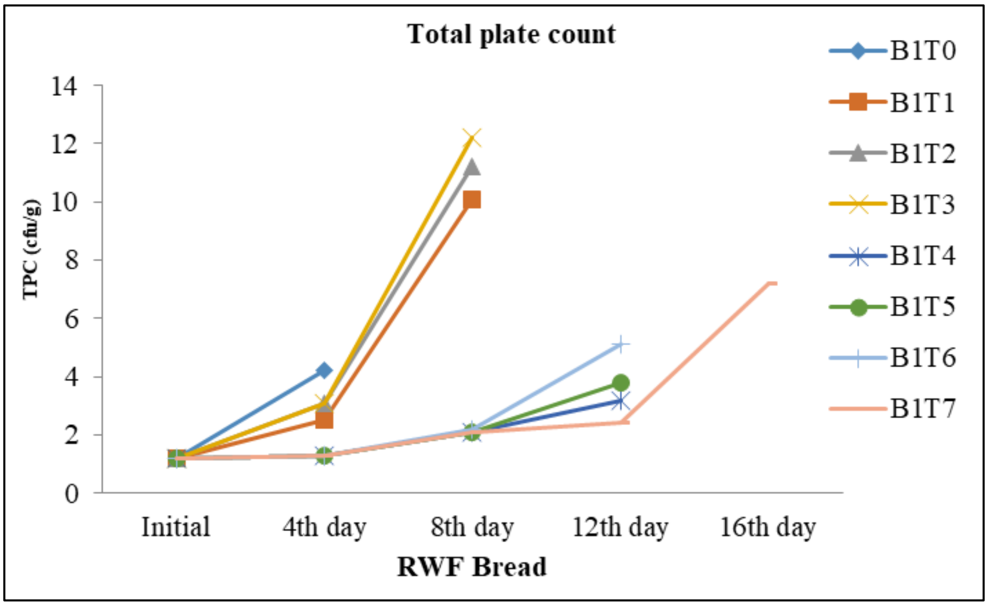
Table 1: Treatment details of active packaging of selected bread samples

B_1T_0	RWF bread + Without active packaging material (control)	B_2T_0	WWF bread + Without active packaging material (control)
B_1T_1	RWF bread + Ethanol emitter	B_2T_1	WWF bread + Ethanol emitter
B_1T_2	RWF bread + Oxygen absorber	B_2T_2	WWF bread + Oxygen absorber
B_1T_3	RWF bread + Moisture absorber	B_2T_3	WWF bread + Moisture absorber
B_1T_4	RWF bread + Ethanol emitter + Oxygen absorber	B_2T_4	WWF bread + Ethanol emitter + Oxygen absorber
B_1T_5	RWF bread + Ethanol emitter + Moisture absorber	B_2T_5	WWF bread + Ethanol emitter + Moisture absorber

B_1T_6	RWF bread + Oxygen absorber + Moisture absorber	B_2T_6	WWF bread + Oxygen absorber + Moisture absorber
B_1T_7	RWF bread + Ethanol emitter + Oxygen absorber + Moisture absorber	B_2T_7	WWF bread + Ethanol emitter + Oxygen absorber + Moisture absorber

Table 2: Proximate composition of fresh RWF and WWF breads

Proximate composition (%)	Bread samples	
	RWF	WWF
Moisture	31.58	32.43
Carbohydrates	51.75	50.07
Crude protein	9.92	10.23
Crude fiber	2.56	2.95
Crude fat	3.04	3.07
Total ash	1.15	1.25



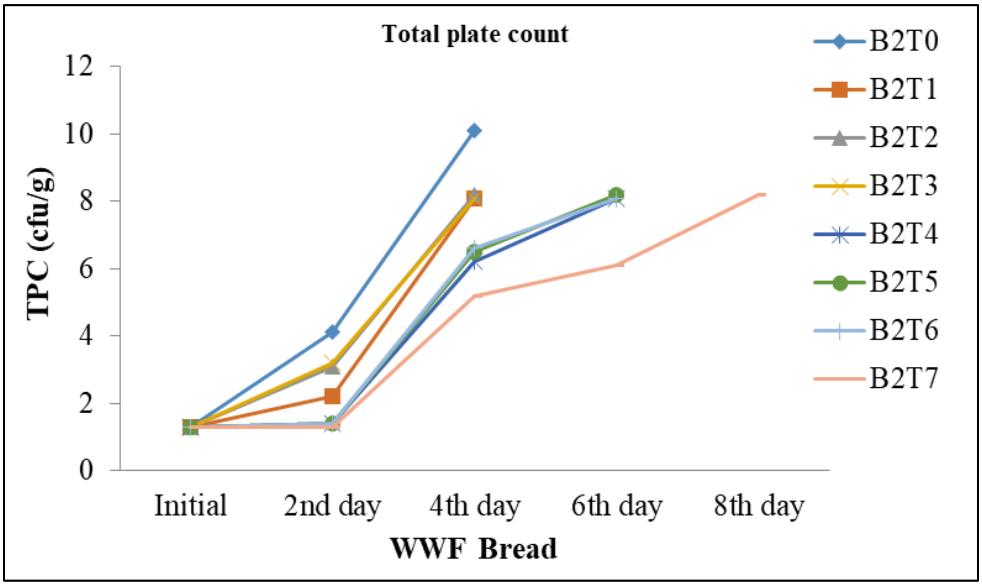


Fig 1: Influence of active packaging materials on TPC of RWF bread and WWF bread during storage

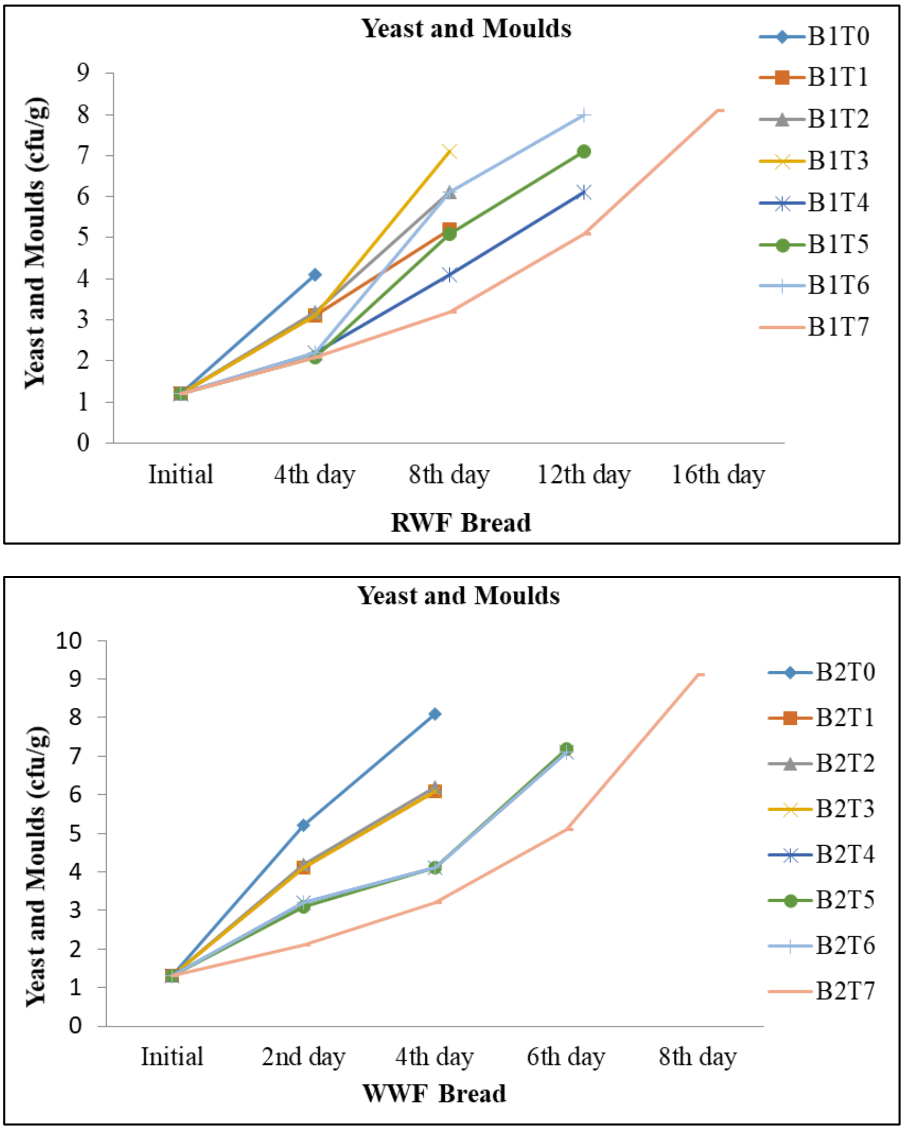
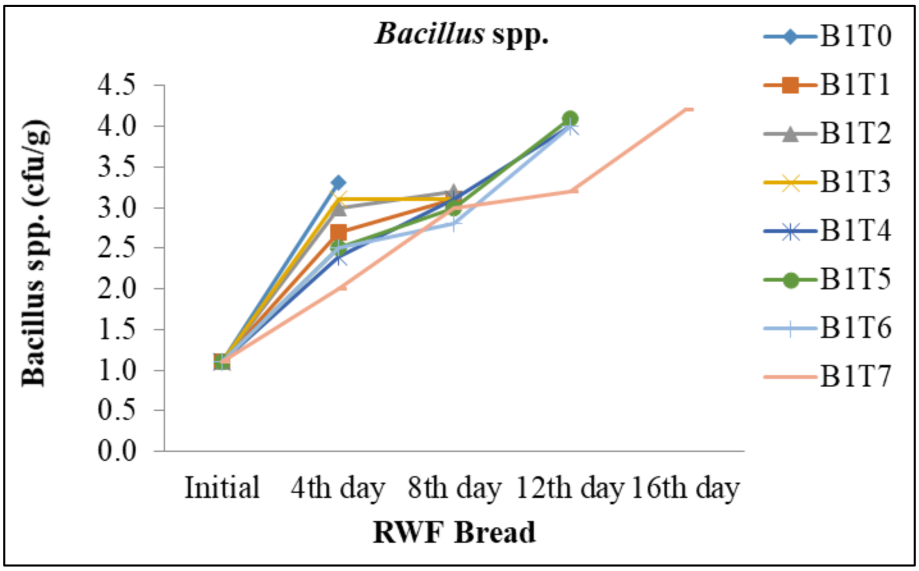


Fig 2: Influence of active packaging materials on yeast and moulds of RWF and WWF bread during storage



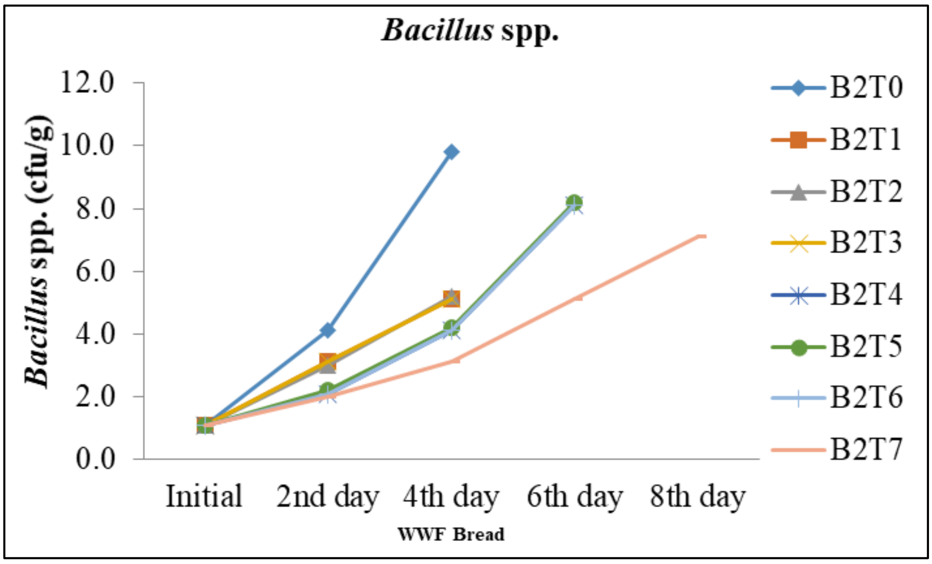


Fig 3: Influence of active packaging materials on *Bacillus* spp. of RWF bread and WWF bread during storage

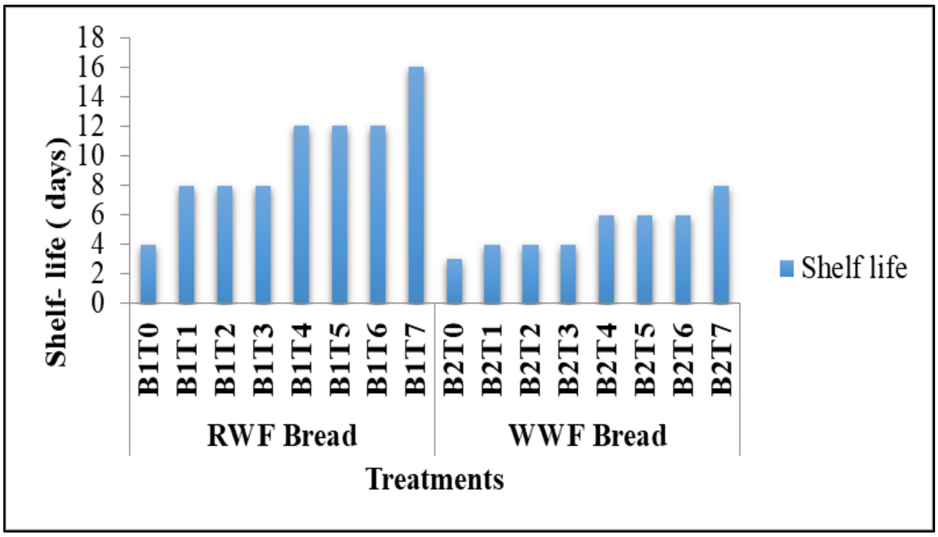


Fig 4: Influence of active packaging materials on shelf-life of RWF bread and WWF bread

Conclusions

RWF bread and WWF bread packed in a combination of ethanol emitter, oxygen absorber and moisture absorber (B_1T_7 and B_2T_7) were found to inhibit the growth of microbes effectively compared to the control. Active packaging materials used for packaging of selected breads influenced in enhancing the shelf-life. Maximum shelf-life of 16 and 8 days were obtained for RWF and WWF breads packed in B_1T_7 and B_2T_7, respectively.

References

1. AOAC, Official Methods of Analysis (16th Edition), Association of Official Analytical Chemists, Washington, DC. 2005.
2. Butt MS, Muhammad N, Saeed A, Kamran S. "Effect of moisture and packaging on the shelf-life of wheat flour," Journal of Food Safety, 2002;4(1):1-6.
3. Costa C, Conte A, Buonocore GG, Delnobile MA. "Antimicrobial Silver Montmorillonite Nanoparticles to Prolong the Shelf-Life Of Fresh Fruit Salad," International Journal of Food Microbiology. 2011;148(1):164-167.
4. Fernandez U, Vodovotz Y, Courtney P, Pascall M. Extended shelf-life of soy bread using modified atmosphere packaging, Journal of Food Protection., 2006;69(1):693-698.
5. Franke I, Wijma E, Bouma K. "Shelf-Life Extension of Pre Baked Buns by an Active Packaging Ethanol Emitter," Journal of Food Additives contaminants. 2010;19(3):314-322.
6. Galic K, Curic D, Gabric D. "Shelf-Life of Packaged Bakery Goods- A Review," Food Science and Nutrition., 2009;49:405-426.
7. Guynot ME, Sanchis V, Ramos AJ, Marin S. Mold Free Shelf-Life Extension of Bakery Products by Active Packaging, Food Microbiology and Safety. 2003;01:86.
8. Latou E, Mexis SF, Badeka AV, Kontominas MG. "Shelf Life Extension of Sliced Wheat Bread Using Either an Ethanol Emitter or an Ethanol Emitter Combined with an Oxygen Absorber as Alternatives to Chemical Preservatives, Journal of Cereal Science. 2010;52(1):457-465.
9. Mondal A, Datta AK. Bread Baking- A Review, Journal of Food Engineering. 2007;86(4):465-474.
10. Nielsen PV, Rios R. "Inhibition of Fungal Growth on Bread by Volatile Components from Spices and Herbs

and The Possible Application in Active Packaging, With Special Emphasis on Mustard Essential Oil," International Journal of Food Microbiology. 2000;60:219-229.

11. Pateras I. "Bread Spoilage and Staling," Technology of Breading. 2007, 275-298.
12. Salminen A, Latva K, Randell K, Hurme E, Linko P, Ahvenainen R. "The Effect of Ethanol and Oxygen Absorption on The Shelf-Life of Packed Sliced Rye Bread," Packaging Technology and Science. 1998;9(1): 29-42.
13. Simth JP. Modified Atmosphere Packaging for Bakery Products, American Institute of Baking. 1994;16:1-9.
14. Smitha N. Assessing Customer Satisfaction and Brand Awareness of Branded Bread, Journal of Business Management. 2013;12(2):13-18.
15. Villaescusa R, Gil MI. Quality Improvement of Pleurotus Mushrooms by Modified Atmosphere Packaging and Moisture Absorbers, Postharvest Biology and Technology. 2003;28:169-179.