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ORIGINAL ARTICLE

Influence of modified atmosphere packaging and potassium sorbate on microbiological characteristics of sliced bread

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Abstract Effects of modified atmosphere packaging (MAP) and potassium sorbate (PS) on total viable count (TVC) and yeast and mould counts (YMC) in sliced bread during storage were investigated. Gas combinations of air (control), 100% N_2 (A), 70% N_2:30% CO_2 (B), 50% N_2:50% CO_2 (C), 30% N_2:70% CO_2 (D) and 100% CO_2 (E) and PS concentrations of 0, 0.15 and 0.30% were tested during 21 days of storage at ambient conditions (20±2 °C and 60±2% RH). At the end of 21 days in all samples both with and without PS, the lowest YMC were in E. In air packed control without PS and with 0.15% PS, mould developed after 14 days storage. In addition to this, none of samples in all MAP treatments presented signs of mold at the end of the storage period (21 days). Similarly, E was the most effective treatment for the inhibition of bacteria. Also, it is concluded that 100 CO_2 atmospheres in MAP treatments and 0.15% PS addition to bread dough were sufficient for YM growth inhibition in sliced bread, in terms of human health. However, TVC was under 3 log cfu/g in only sample packaged with E and containing 0.30% PS until day 14.

Keywords Bread · Modified atmosphere packaging · Potassium sorbate · Microbiological characteristics

Introduction

In general, moulds and bacteria are responsible for the microbial spoilage of bakery foods. Commonly, a shelf-life of 3–4 days may be expected when they are unpreserved. Apart from the repelling sight of visible growth, fungi are responsible for off-flavour and production of mycotoxins and allergic compounds. These compounds may be formed even before the mould growth is visible (Ponte and Tsen 1979; Legan 1993; Ponte et al. 1993; Nielsen and Rios 2000; Fernandez et al. 2006; Pagani et al. 2006). Bacteria are the other potential contaminants observed in bakery products. They have higher nutritional needs than moulds and their growth is highly restricted by low water activity and low pH (Ponte et al. 1993).

Microbial contamination is caused mainly by the micro-organisms naturally present in the ingredients used in manufacturing of bakery foods, or from external sources such as air and packaging materials to which the products are exposed after processing (Ponte and Tsen 1979). Of late consumers prefer for products without preservatives. Therefore, challenge for the food industry is to fulfill these demands with minimum change in food quality and

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maximum security, without using chemical preservatives (Gutiérrez et al. 2009). The use of weak organic acids such as propionic, benzoic and sorbic, as also investigation on the packaging material, or modified atmosphere packaging (MAP) have been the main choice for satisfying the market demands to extend the shelf-life of bakery products (Legan 1993; Ponte et al. 1993; Fernandez et al. 2006; Pagani et al. 2006).

MAP is used to reduce the amount of oxygen present in the headspace of the product. The oxygen level inside the package limits the shelf life of the product in terms of aerobic microorganisms (Ponte et al. 1993). Although there have been number of researches related with the MAP technique on different foods, limited research have been reported on the MAP technique to prolong shelf-life of bread (Nielsen and Rios 2000; Suhr and Nielsen 2005; Fernandez et al. 2006). Shelf-life for many bakery foods can be extended by modifying the gas concentration inside the package. Shelf-life of perishable food products such as bakery foods in normal atmospheres is mainly limited by two factors: atmospheric oxygen and the growth of aerobic spoilage microorganisms (Rodriguez et al. 2000). By using MAP, oxygen concentration can be decreased to 2%. For this reason there is an increasing demand for storage of bread in modified atmospheres, which is most often composed of CO_2 alone or mixtures of CO_2 and N_2 (Seiler 1989; Del Nobile et al. 2003). Carbon dioxide is the most important gas in gas packaged bakery products. It is both fungistatic and bacteriostatic and prevents insect growth in packaged and stored food products. Nitrogen is an inert, tasteless gas, and is used as a filler gas. Because of its insolubility in water, the presence of nitrogen in MAP food can prevent pack collapse that occurs when high concentrations of CO_2 are used (Ponte et al. 1993; Smith and Simpson 1995; Kotsianis et al. 2002). The general recommendation for MAP of bakery products has been a mixture of 60% CO_2:40% N_2, but specific gas mixtures should be used for each type of product and mixtures have variations from 0 to 100% CO_2 balanced with N_2 (Farber 1991; Suhr and Nielsen 2005).

Several researchers have experimented with bakery products under MAP, generally with positive results (Black et al. 1993; Piergiovanni and Fava 1997; Rodriguez et al. 2000). Many studies have been conducted to extend the shelf life of bakery products by optimizing MAP (Kotsianis et al. 2002). The increase in shelf life of bakery products using MAP can be attributed to its residual effect on molds such as *Penicillium* and *Aspergillus* and on the yeasts (Rodriguez et al. 2000). The preservatives are used to prevent or minimize the microbial growth (Ponte et al. 1993). By using MAP technique, the need of chemical preservatives can be reduced or eliminated while maintaining a desired shelf life for the packaged food product (Fernandez et al. 2006).

In this study, it was aimed to investigate the effects of MAP and potassium sorbate on TVC and YM growth to prolong the shelf-life of sliced wheat bread. For this, different combinations of gases like air, N_2 and CO_2 and different concentrations of potassium sorbate were tried.

Materials and methods

For bread manufacturing, wheat (*Triticum durum*) (Type 550) flour containing 12% protein (db), 0.55% ash (db), 14% moisture and 27.2% wet gluten was used and supplied from the Toru Flour Milling Co., Ltd (Bandırma / Turkey). Commercial compressed bakers' yeast (1.5%, w/w, flour basis) and salt (1.5%, w/w, flour basis) were used to prepare bread doughs.

Breadmaking procedure The experimental bread dough was manufactured by adding water (60%), baker's yeast (3%), salt (1.5%) and potassium sorbate (0, 0.15 and 0.30%). Dough was mixed for 10 min in high-speed mixer (Diasno-Sp12d, Germany). Final dough temperature was 25±2 °C. The dough was rested in bulk for 15 min, scaled into 350 g portions, moulded, placed in pan and put into the proofer set to 30 °C and 85% RH for 120 min. The pan size was 98 mm × 280 mm × 80 mm. The baking was carried out at 240 °C for 20 min. The oven (Werner-Pfleiderer, Carat 6.8, Germany) was pre-steamed before (0.3 l water) and after loading (0.7 l water) via the injection of water. The loaves were left to cool at room temperature (20±2 °C). After cooling, the loaves were sliced and packaged by MAP technique.

Packaging Sliced wheat bread samples were packaged in expanded amorphous polyester/ ethylene vinyl alcohol copolymer/polyethylene trays (density 1.26–1.28), thickness range 190–1,000 μm, comparable thickness 750 μm (Neo Plastica / Klöckner Pentaplast, Germany). A film (77 μm thickness) consisting of polyamide/polyethylene and having O_2 transmission rate of 3.5 cm^3 / m^2 / 24 h (23 °C, 0% RH) with antifog property was used to cover or seal the package (Neo Plastica / Klöckner Pentaplast, Germany). The sliced wheat bread samples were placed into trays, and trays were vacuumised, filled with mixed gas and sealed by means of a Multivac R-230 packaging machine (Germany) equipped with a MAP mix 9000 gas-mixer (PBI–Dansensor A/S, Ringsted, Denmark). The trays were packed using different gas combinations. Six gas concentrations tested included: air (control), 100% N_2 (A), 70% N_2:30% CO_2 (B), 50% N_2:50% CO_2 (C), 30% N_2:70% CO_2 (D) and 100% CO_2 (E). Food-grade quality CO_2 and N_2 with purity 99.7% and 99.9%, respectively were used. All packaged bread samples were stored at 20±2 °C and 60±2% R.H.

Microbiological analysis The microbial analysis were performed in triplicate after 1, 7, 14 and 21 days of storage. The samples of sliced wheat bread were weighed aseptically (10 g) and homogenized in a Stomacher (Masticator, IUL Instruments, Spain) for 60 sec at room temperature (20±1 °C) with 90 ml sterile maximum recovery diluent (Oxoid CM0733). Decimal dilutions were prepared by using maximum recovery diluent. YMC were examined with the method given by Rodriguez et al. (2003) and Pascall et al. (2008). Rose Bengal Chloramphenicol Agar (Oxoid CM0549 supplemented with SR0078) was used for yeasts and moulds and incubated at 25 °C for 5 days. Plate Count Agar (Oxoid CM0325) was used for total viable counts (TVC) and incubated at 30 °C for 48 h. Microbial counts were expressed as log cfu/g.

Measurement of headspace O_2 concentration The O_2 concentrations in the package headspaces were monitored using a digital analyzer (PBI, Dansensor, Check Pointer O_2/CO_2, Ringsted, Denmark) in the defined storage period. The gas compositions were measured in triplicate for each sample. The samples were analyzed at 1, 7, 14 and 21 days of storage (Rodriguez et al. 2003).

Statistical analysis The data were compared using analysis of variance with respect to different levels of atmospheres and potassium sorbates. All tests were performed at least in triplicate. When significant differences were found ($p \leq 0.01$), the least significant difference test was used to determine the differences among treatment means, JMP IN 6.0.0, Statistical Discovery from SAS 2005, Institute Inc. software was used to perform the statistical analyses.

Results and discussion

Headspace oxygen concentration The headspace O_2 concentration was less than 1% during the whole storage period for the sliced bread samples packaged in MAP with different gas combinations, except air. During storage, the concentration of O_2 in the headspace decreased compared with the initial gas concentration in all samples, except B (Fig. 1). Similar results were obtained by Rasmussen and Hansen (2001), who found that the concentration of CO_2 in the headspace decreased by 2–5% compared with the initial gas concentration. The headspace O_2 contents of control samples packaged in air with 0.15% PS and without PS

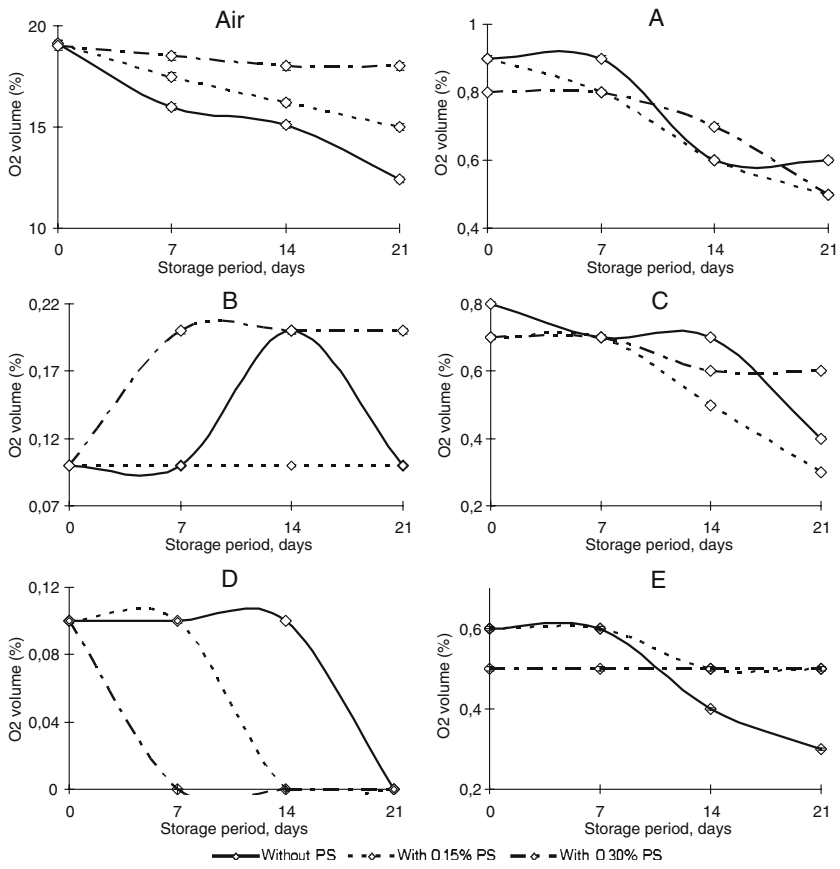

Fig. 1 Changes in headspace oxygen concentration in sliced bread sample packed with modified atmosphere (n=3). **a**: 100% N_2, **b**: 70% N_2+30% CO_2, **c**: 50% N_2+50% CO_2, **d**: 30% N_2+70% CO_2 and **e**: 100% CO_2

Table 1 Yeast and moulds counts (YMC) of sliced breads packaged with air and modified atmospheres

Days	Atmosphere	Y+M counts log cfu/g		
		O% PS	0.15% PS	0.30% PS
1	Air (Control)	1.1	1.0	0.80
	A	1.2	1.3	0.65
	B	1.0	1.2	0.90
	C	1.0	1.1	0.83
	D	1.6	1.0	0.83
	E	1.0	0.9	0.77
7	Air (Control)	6.4	3.5	1.3
	A	4.7	3.5	1.5
	B	2.7	2.2	1.3
	C	1.7	1.4	1.0
	D	2.9	1.2	1.0
	E	1.9	1.0	1.0
14	Air (Control)	6.6	6.3	2.9
	A	5.1	3.7	1.8
	B	3.6	3.1	2.1
	C	3.7	2.6	2.1
	D	5.6	2.3	2.0
	E	3.0	2.2	1.7
21	Air (Control)	6.8	6.3	3.4
	A	5.1	3.8	2.5
	B	4.7	3.5	2.1
	C	4.1	3.2	2.4
	D	6.6	2.8	2.5
	E	3.5	2.5	2.1

PS Potassium Sorbate

(n=3) A–E: As in Fig. 1

decreased during the storage period. These results can be explained by mould metabolism and were parallel with the yeasts and moulds counts as reported by Rodriguez et al. (2003). In contrast, headspace O_2 concentration remained almost at the same level until the end of storage period (21 days) in control (air) containing 0.30% PS due lack of mould growth in this sample (Fig. 1).

Table 3 LSD analysis for YMC of sliced bread samples containing two levels of potassium sorbate (PS) and packaged with five different gas combinations

Source of variation		Day 1	Day 7	Day 14	Day 21
PS	PS 0%	1.155^{a}	3.384^{a}	4.631^{a}	5.126^{a}
	PS 0.15%	1.100^{b}	2.125^{b}	3.345^{b}	3.661^{b}
	PS 0.30%	0.794^{c}	1.182^{c}	2.096^{c}	2.524^{c}
MAP	Air (control)	0.977^{ab}	3.740^{a}	5.262^{a}	5.551^{a}
	A	1.055^{ab}	3.223^{b}	3.521^{b}	3.782^{c}
	B	1.066^{a}	2.068^{c}	2.922^{d}	3.435^{d}
	C	0.977^{ab}	1.365^{e}	2.803^{e}	3.245^{e}
	D	1.133^{a}	1.698^{d}	3.301^{c}	3.936^{b}
	E	0.888^{b}	1.287^{f}	2.335^{f}	2.674^{f}

Means within a column followed by different superscripts are significantly different (p<0.01)

A–E: As in Fig. 1

Yeast and mould counts (YMC) during storage After 7 days of storage, YMC were lower than 3 log cfu/g in all MAP treatments without PS, except treatment A (Table 1). On the contrary, after 14 days, YMC exceeded 3 log cfu/g limit in all MAP samples without PS, except treatment E. At the end of the storage period, none of the samples without PS were lower than 3 log cfu/g. After 21 days, in the samples containing 0.15% PS, the lowest YMC were determined in D and E, which were 2.8 and 2.5 log cfu/g, respectively. At the end of storage, the samples containing 0.30% PS, YMC were below 3 log cfu/g in all MAP treatments, while YMC was 3.4 log cfu/g in sample packed in air (control). At the end of the storage, the best treatment was E, because it reduced YMC under 3 log cfu/g in the samples with 0.15% and 0.30% PS (2.5, 2.1 log cfu/g, respectively). The normal microbiological load for baked goods on routine quality control tests in the baking industry must be lower than 3 log cfu/g for moulds and yeast (Fernandez et al. 2006).

Other researchers have also made similar observations relating to high CO_2 concentration (El Halouat and Debevere 1997; Rodriguez et al. 2000; Del Nobile et al. 2003; Fernandez et al. 2006). Although we used the

Table 2 Variance analysis for YMC of sliced bread samples containing two levels of PS and packaged with five different gas combinations

Source of variation	DF	Day 1		Day 7		Day 14		Day 21	
		SS	F	SS	F	SS	F	SS	F
PS (A)	2	0.681	21.1158**	21.963	104960.5**	28.919	93513.67**	30.633	73847.84**
MAP(B)	5	0.067	2.0650 ns	9.377	44814.28**	9.358	30259.19**	8.617	20773.97**
AxB	10	0.095	2.9614**	2.373	11340.15**	1.996	6455.568**	1.6778	4044.846**
Error	36	1.1602667		0.00753		0.01113		0.01493	

ns not significant

**Significant at $p \leq 0.01$

Table 4 Total viable counts (TVC) of sliced breads packaged with air and modified atmospheres

Days	Atmosphere	TVC log cfu/g[a]		
		O % PS	0.15% PS	0.30%PS
1	Air (Control)	3.5	2.6	2.0
	A	2.7	1.6	1.0
	B	2.2	2.0	1.8
	C	2.0	1.9	1.9
	D	2.1	2.2	1.3
	E	2.1	1.8	1.0
7	Air (Control)	6.0	5.1	4.1
	A	7.1	4.7	4.0
	B	6.4	5.3	3.5
	C	6.3	4.7	4.6
	D	5.3	4.8	3.0
	E	5.0	4.5	2.1
14	Air (Control)	7.2	5.2	5.6
	A	7.1	5.3	4.7
	B	6.7	6.3	4.2
	C	6.8	5.8	5.2
	D	5.7	5.3	3.1
	E	5.1	4.7	3.0
21	Air (Control)	7.5	5.5	5.4
	A	6.8	5.7	5.1
	B	6.9	5.8	4.2
	C	6.9	5.8	5.1
	D	6.6	5.8	3.7
	E	5.8	4.8	3.2

(n=3), A–E : As in Fig. 1

preservative, we think that, this inhibitory effect can be related with high CO_2 concentration in the headspace of the samples. Because, in E without PS, YMC was below 3 log cfu/g after 14 days storage. CO_2 has greater inhibitory effect on mould growth in the bakery products than bacteria and yeast (Rodriguez et al. 2000). In MAP the microbial growth rate diminished because of the bacteriostatic effect of CO_2 coupled with a decrease in O_2 from the package head space. In fact, the microbial shelf life of bags containing high concentration of CO_2 had a longer shelf-life (Del Nobile et al. 2003).

Table 6 LSD analysis for TVC of sliced bread samples containing two levels of Potassium sorbate (PS) and packed with five different gas combinations

Source of variation		Day 1	Day 7	Day 14	Day 21
PS	PS 0%	2.427[a]	6.009[a]	6.460[a]	6.729[a]
	PS 0.15%	2.016[b]	4.832[b]	5.448[b]	5.567[b]
	PS 0.30%	1.498[c]	3.563[c]	4.298[c]	4.448[c]
MAP	Air (control)	2.668[a]	5.060[c]	5.993[a]	6.115[a]
	A	1.771[e]	5.262[a]	5.714[d]	5.838[c]
	B	2.012[b]	5.038[d]	5.738[c]	5.641[d]
	C	1.911[c]	5.181[b]	5.945[b]	5.937[b]
	D	1.896[d]	4.387[e]	4.741[e]	5.351[e]
	E	1.624[f]	3.881[f]	4.282[f]	4.605[f]

Means within a column followed by a different superscripts are significantly different (p<0.01)

Variance analysis for YMC of sliced bread samples containing two levels of PS and packed with five different gas combinations, showed that the effects of PS usage, MAP treatments and their interaction were all significant ($p\leq0.01$) at days 7, 14 and 21 (Table 2). According to LSD, at 7, 14 and 21 days of storage, the PS addition significantly ($p\leq0.01$) reduced YMC compared to sample without PS. MAP treatments also significantly ($p\leq0.01$) reduced YMC compared to control (air packed) (Table 3).

Monitoring of mould during storage In control with 0.15% PS and without PS, mould developed after 14 days storage (results not shown). But no mould was observed in control with 0.30% PS after 21 days. In addition, none of the samples in all MAP treatments presented signs of mould growth at the end of the storage period (21 days). The number of days without mould was prolonged when samples were packed under modified atmospheres. The mould-free shelf life was extended to 21 days in all MAP treatments with and without PS while the shelf-life of sliced

Table 5 Variance analysis for TVC of sliced bread samples containing two levels of PS and packed with five different gas combinations

Source of variation	DF	Day 1		Day 7		Day 14		Day 21	
		SS	F	SS	F	SS	F	SS	F
PS (A)	2	3.00	29251.35**	26.938	124331.4**	21.045	41027.80**	23.4184	23770.58**
MAP (B)	5	1.183	8874.189**	2.696	12443.48**	4.584	8937.199**	2.677	2717.76**
AxB	10	0.386	2894.314**	0.832	3838.162**	0.864	1686.053**	0.534	542.533**
Error	36	0.00480		0.0078		0.0184		0.0354	

PS Potassium Sorbate

**Significant at 0.01 probability level

bread in air packaging (control) with 0.15% PS was 14 days. In this case, it can be said that MAP treatment without PS is enough for preventing mould growth Carbonic atmospheres would represent good substitutes for chemical preservatives. Atmospheres with the highest CO_2 concentrations are ideal. In order to be make this effective, high concentrations of N_2 almost 100% are required. If O_2 concentration in the headspace exceeds 1%, the antimicrobial effect of N_2 is lost and moulds begin to develop, even at very low concentrations of O_2 (Rodriguez et al. 2000).

Total viable counts (TVC) during storage In general, the TVC increased during the storage period (Table 4). Lowest TVC were obtained from E with and without PS**.** The normal microbiological load for baked goods on routine quality control tests in the baking industry is ≤3 log cfu/g for moulds, yeast, and aerobic plate counts. In the present study, TVC was lower than 3 log cfu/g in only E with 0.30% PS at the end of 14 days (Table 4). Briefly, E was the most effective MAP treatment for the inhibition of bacteria. Similarly, Rasmussen and Hansen (2001) demonstrated that packaging in modified atmospheres with increased levels of CO_2 can be used to extend the microbial shelf life of bread during storage.

According to variance analysis, when compared with air treatment (control), MAP treatments, PS addition and their interaction caused significant ($p \leq 0.01$) changes to inhibit bacterial growth (Table 5). The results of LSD test showed that the PS addition significantly ($p \leq 0.01$) reduced TVC compared to sample without PS during storage. MAP treatments also significantly ($p \leq 0.01$) reduced TVC compared to control (air packed) at 14 and 21 days of storage (Table 6).

Conclusion

Samples without PS did not show mould growth at the end of storage when packed under modified atmospheres. In contrast, mould developed in air packed samples without and with 0.15% PS after 14 days storage. The best MAP treatment was 100% CO_2 atmosphere, because it reduced TVC and YMC in the samples with and without PS at the end of storage compared to air treatment. We concluded that 100% CO_2 atmosphere with 0.15% PS addition was sufficient for yeast and mould growth inhibition in sliced bread packaged with modified atmosphere. In addition to this, E (100% CO_2) treatment and 0.30% PS addition were required to reduce TVC below 3 log cfu/g.

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