Journal of Cereal Science 52 (2010) 457–465

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

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

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ARTICLE INFO

Article history:
Received 26 May 2010
Received in revised form
12 July 2010
Accepted 21 July 2010

Keywords:
Wheat bread
Shelf life extension
Ethanol emitter
Oxygen absorber

ABSTRACT

In the present study, the effect of active packaging [ethanol emitter (EE) or ethanol emitter combined with an oxygen absorber (EE + OA)] on shelf life extension of sliced wheat bread stored at 20 °C was investigated. Bread containing commercial preservatives (WP) and no preservatives (WOP) were taken as controls. Microbiological, physicochemical and sensory changes occurring in the product as a function of treatment and storage time were monitored for 30 days. Counts for yeasts and molds were 5.1, 3.8, 2.0 and 2.0 log cfu/g and for *Bacillus cereus* 4.7, 2.5, 2.3 and 2.0 log cfu/g for WOP, WP, EE and EE + OA treatments respectively after 30 days of storage. Initial pH 6.3 and 6.4, for WP and WOP samples remained fairly constant irrespective of specific treatment. Aroma quality deterioration during storage was due to the loss of volatile compounds and the formation of "off-flavors" through lipid oxidation. Neither the EE nor the EE + OA had an adverse effect on initial odor, taste and texture of bread. Based on sensory (texture) and microbiological data, shelf life was ca. 4 days for samples WOP; 6 days for samples WP; 24 days for samples containing the EE and at least 30 days for samples containing the EE + OA.

1. Introduction

Bread has always been one of the most popular food products due to its superior nutritional and sensory characteristics being consumed daily (Galić et al., 2009). Generally the shelf life of bread is limited by several deterioration processes including fungal growth (Nielsen and Rios, 2000), the loss of moisture and staling (Del Nobile et al., 2003). According to Legan and Voysey (1991) in a study performed on bakery products and their ingredients, 60% of spoilage was attributed to molds (*Penicillium* spp and *Aspergillus niger*) whereas yeasts accounted for only 15%. Besides the repelling sight of visible growth, fungi are responsible for off-flavor development, the production of mycotoxins as well as allergenic compounds. These compounds may be formed even before mold growth is visible (Nielsen and Rios, 2000). However even though molds are destroyed during baking, recontamination may occur during cooling and subsequent packaging causing the above problems (Galić et al., 2009).

In general, modified atmosphere packaging (MAP) has been used for shelf life extension of a large variety of foodstuffs including bakery products such as wheat bread (Rodríguez et al., 2000), rye bread, hot-dog bread (Nielsen and Rios, 2000) and soy bread (Fernandez et al., 2006). A 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 (Galić et al., 2009). One approach to overcome this problem is to use oxygen absorbers inside the package. Oxygen absorbers have been used to prevent discoloration of meats, inhibit rancidity in high fat foods and mold growth in high water activity products (Berenzon and Sam Saguy, 1998).

Besides oxygen absorbers, various companies manufacture and sell products that release ethanol vapor into the packaged headspace inhibiting the growth of molds, yeasts and bacteria. Ethanol vapor generators may be combined with oxygen absorbers. Ethanol emitters and/or oxygen absorbers have also been used for the shelf life extension of bakery products such as sliced rye bread (Salminen et al., 1996) and durum wheat bread (Del Nobile et al., 2003).

To the best of our knowledge there is no data available in the literature regarding the combined use of an ethanol emitter and an oxygen absorber for the preservation of sliced wheat bread. Based on the above, the objective of the present work was to study the effect of either an ethanol emitter (EE) or an ethanol emitter combined with

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doi:10.1016/j.jcs.2010.07.011

an oxygen absorber (EE + OA) for extending the shelf life of sliced wheat bread. A second objective was to test the experimental PET-SiOx//LDPE laminate, a high barrier material, for the preservation of sliced wheat bread.

2. Experimental

2.1. Packaging and storage

Wheat bread was produced according to standard commercial practices by a local bread manufacturer using wheat flour, extraction rate 70%, bakers' yeast and water. In case of commercial samples, the following additives were used at a 1% total concentration in wheat flour as dough improving agents: ascorbic acid (E300), calcium carbonate (E170) and diacetyl-tartaric esters of fatty acids (E472e). Baked loaves were cooled down to room temperature for 60 min, transferred to the laboratory within 30 min and sliced 2 cm thick. Bread composition was determined using official AOAC methods (protein: 8.0%, fat: 1.7%, starch: 47.9%, sugars: 1.8%, fiber: 2.7%, moisture: 39.0%).

Four lots of samples were prepared: Lot 1 comprised the control samples (samples without chemical preservatives, WOP); Lot 2 consisted of commercial control samples with chemical preservatives (WP). In lot 3 an ethanol emitter sachet (EE) was added to the inner surface of the package of samples WOP while, in lot 4, a sachet combining an ethanol emitter and an oxygen absorber (EE + OA) were used (Freund Ind. Co, Japan). All samples (150 g) were packaged in high barrier PET-SiOx//LDPE pouches, 62 μm in thickness and 4.0 mL/(m^2 day atm) in oxygen permeability at 75% RH, 25 °C. Pouches were heat-sealed using a BOSS model N48 thermal sealer (BOSS, Bad Homburg, Germany). Size of OA/EE was based on product weight and water activity ($a_w = 0.95$, measured using a Novasina a_w meter, Lachen, Switzerland). Samples were then stored at ambient temperature (20 ± 1 °C intermittently exposed to daylight/dark) for a period up to 30 days.

Three separate pouches from each treatment were removed for analysis on day: 0, 3, 6 and 9 of storage for WOP samples; sampling was continued up to day 15 for WP samples and up to day 30 for the samples containing the EE and EE + OA.

2.2. Microbiological analysis

The following groups of microflora were determined according to official protocols (APHA, 2001): TVC, Yeasts/Molds, Enterobacteriaceae, *Bacillus cereus* and *Clostridium* spp. Anaerobic conditions were achieved by the use of Anaeropack® GENbox Jars combined with Pack-Anaero oxygen absorbers. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. In addition, the selectivity of each medium was checked routinely by Gram staining and microscopic examination of smears prepared from randomly selected colonies from all of the media.

2.3. Physicochemical analysis

2.3.1. Determination of the headspace gas composition

The headspace O_2 composition was measured using a Dansensor CheckMate 9900 gas space analyser (PBI Dansensor, Ringsted, Denmark) after 4, 8, 12, 16 and 24 h of pouch sealing to determine the time required for the oxygen absorber to reduce the oxygen concentration inside the pouch to below 0.01%. Headspace ethanol was determined by gas chromatography using an HP5890 GC equipped with a FID. A Supelcowax™-10 column (30 m × 0.32 mm i.d. × 0.5 μm film thickness) (Supelco, Bellefonte, USA) was used, operated isothermally at 70 °C with a helium flow rate of 1 ml/min. The injector was operated in split mode (split ratio 10:1) at 260 °C. The detector temperature was 280 °C. Ethanol concentrations were reported as volume %.

2.3.2. Semi quantitative determination of volatile compounds

Volatiles were determined according to the method of Mexis et al. (2009) using 1 g of sliced bread (crust and crumb) and 20 μl of 4-ethyl-2-pentanone as an internal standard. A Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber 50/30 mm was used for volatiles' separation.

2.3.3. pH determination

pH was determined using the method of AOAC (1995) after appropriate modification (Goulas and Kontominas, 2005).

2.3.4. Texture

Objective texture analysis was performed using an Instron Universal Testing Machine, model 4411 (Instron Corp., Bucks, UK). Disks of bread crumbs (25 mm diameter × 15 mm in thickness) were cut from the central part of each bread slice.

The bread samples were analyzed in a double compression cycle under the following conditions: crosshead speed 100 mm/min, specimen compression depth 60% using a 5 cm diameter stainless steel compression plunger.

The texture parameters considered were hardness (peak force of the first compression cycle in N) and springiness (ratio of the time duration of force input during the second compression to that during the first compression, dimensionless) (Bianchi et al., 2008). Data were recorded using the Bluehill software.

2.4. Sensory evaluation

Sensory evaluation (acceptability test) was carried out by a 51 member untrained panel (31 females and 20 males) consisting of faculty and graduate students of the Department of Food Chemistry, University of Ioannina. Panelists were chosen using the following criteria: ages between 22 and 60, non-smokers, without reported cases of food allergies who consume bread daily. Sensory data were collected between day 0 and day 30 of storage. After each sampling day, samples were held at 0 °C until sensory evaluation at the end of storage. Approximately 10 g of bread samples were placed in small plastic cups, coded with 3-digit random numbers and tightly capped. Along with each set of four test samples, a reference sample was presented to panelists consisting of sliced bread taken from the same loaf that had been packed under nitrogen and stored in the dark at 0 °C up to 30 days. The samples were allowed to stand for half an hour prior to evaluation to allow equilibration of volatiles in the cup headspace. Sensory attributes evaluated included odor, texture and taste. Scoring was carried out on paper ballots using a 9 point hedonic scale where: 9 = extremely like and 1 = extremely dislike for the evaluation of odor and taste and 9 = very soft and 1 = very hard for evaluation of texture. A score of 5 was taken as the lower limit of acceptability for odor, taste and texture. Triplicate samples were evaluated for each treatment on each sampling day. Bread slices were considered unacceptable if one of triplicate samples showed visible signs of mold growth or odor, taste or texture received a score less than 5.

2.5. Statistical analysis

The experiment was replicated twice with different bread samples on different occasions ($n = 2 \times 3 = 6$). Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g) and were subjected to analysis of variance using the software SPSS 16 for windows. Results are reported as mean values ± standard