Active Packaging Influence on Shelf Life 
Extension of Sliced Wheat Bread

Sandra Muizniece-Brasava, Lija Dukalska, Irisha Murniece, Ilona Dabina-Bicka, Emils Kozlinskis, Svetlana Sarvi, Ralfs Santars, Anna Silvjane

Abstract—The research object was wheat bread. Experiments were carried out at the Faculty of Food Technology of the Latvia University of Agriculture. An active packaging in combination with modified atmosphere (MAP, CO₂ 60% and N₂ 40%) was examined and compared with traditional packaging in air ambient. Polymer Multibarrier 60, PP and OPP bags were used. Influence of iron based oxygen absorber in sachets of 100 cc obtained from Mitsubishi Gas Chemical Europe Ageless® was tested on the quality during the shelf of wheat bread. Samples of 40±4 g were packaged in polymer pouches (110 mm x 120 mm), hermetically sealed by MULTIVAC C300 vacuum chamber machine, and stored in room temperature +21.0±0.5 °C. The physiochemical properties — weight losses, moisture content, hardness, pH, colour, changes of atmosphere content (CO₂ and O₂) in headspace of packs, and microbial conditions were analysed before packaging and in the 7th, 14th, 21st and 28th days of storage.

Keywords—Active packaging, wheat bread, shelf life.

I. INTRODUCTION

FOOD choice is a complex function of sensory characteristics (taste, odour and texture), and non sensory characteristics such as physiochemical properties, familiarity, food-related expectations, attitudes and health claims [1], [2]. Consumers today demand high-quality products in various innovative forms and for competitive prices [3]–[7].

Since distant ages bread has been one of the most popular food products. Both before and at this time most bread is eaten fresh because the raw materials are ready available. Nowadays many bread sorts are bought in supermarkets and are expected to stay fresh for several days, though it requires extra demands for longer shelf life. At the time being the extension of bread often has been provided by adding several chemical preservatives, however public demand for more natural foods has increased. The shelf life of bread could be extended through sanitation and improved hygiene in the bakery plant, using traditional preservatives or by natural antimicrobial substances from plants – either alone or in combinations with modified atmosphere packaging (MAP) or active packaging. The spoilage of bakery products is mainly caused by moulds and yeasts, only occasionally by bacteria. The spoilage fungi are fastidious organisms, and only 2–3 days may be expected for products like unserved wheat bread. Fungi are responsible for of-flavour formation and production of mycotoxins and allergic compounds, which may be formed even before growth is visible [8], [9].

The factors governed the rate of freshness loss during the storage could be divided into two groups – microbial attack and a result of series of slow physical and chemical changes which lead to progressive firming up the crumb, commonly referred as styling. Mould spoilage of bread is due to post-processing contamination. Bread loves fresh out of the oven are free of moulds or mould spores due to the thermal inactivation during baking process. Bread becomes contaminated from the mould spores present in the atmosphere surrounding loves during cooling, slicing, packaging and storage [10]. The diffusion of moisture in food materials is of fundamental importance for processing and storage. The transport of moisture into or from food materials is an important factor in controlling food quality, chemical reactions and microbial growth during storage [11]. One way to slow down moisture transport is to use barrier between the domains of a food material. Barrier packaging films protect the food from air whereas edible films inhibit moisture migration between different moisture domains within a confection [12].

Relative humidity plays an important role in food product development, storage and packaging. Packaging is a medium between product manufacturers and consumers [13], packaging has a significant role in the food supply chain [14]. The main functions of packaging are to extent the shelf life, and maintain the quality and safety of the packed goods. An effective packaging must prevent the transmission of oxygen, light and water vapour, and microbial growth to retard quality deterioration of packaged goods [22], [15]. For the time being the application of vacuum and modified atmosphere packaging technologies appeared successful in extending the shelf-life and quality of the food [16]–[18]. Modified atmosphere packaging (MAP) is known as longstanding method to extend the microbial shelf-life of bread [19].

Elevated CO₂ concentration in the headspace of packages has shown both bacteriostatic and fungistatic properties and will hinder the growth of certain aerobic organisms [20]. Therefore the storage of bread in modified atmosphere generally consisting of pure CO₂ or mixture of CO₂ and N₂ can be interesting however the influence of gas mixture on the physico-chemical quality changes occurring during storage of bread is problematic as published results are contradictory [19]–[22]. The application of modified atmospheres packaging (MAP) of sliced bread with different αw, moisture content and pH values, with or without preservative added (calcium propionate) and at different storage temperatures, has been studied with the aim of establishing the effect of MAP on the...
shelf-life of the selected product. By increasing the CO₂ concentration to 50%, the increases in shelf life of the samples with added preservative were 167% and 195% at 22–25°C and 15–20°C, respectively. [23]. The latest investigations of different gas combinations of modified atmosphere packaging (MAP) and potassium sorbate effects on total viable count (TVC) and yeast and mould counts (YMC) in sliced bread during storage showed that none of samples in all MAP treatments presented signs of mould at the end of the storage period (21 days) [24]. Reduction of oxygen is one of the primary requirements for quality maintenance of perishable foods, nevertheless in case of modified atmosphere application the remaining concentration of O₂ in head space could not be provided less than 0.5%, therefore an active packaging could be used. Active packaging, however, allows packages to interact with food and the environment and play a dynamic role in food preservation. Developments in active packaging have led to advances in many areas, including delayed oxidation and controlled respiration rate, microbial growth, and moisture migration [25]. Ethanol has been shown to extend the shelf life of bread, cake and pizza when sprayed onto product surfaces prior to packaging. Sachets containing encapsulated ethanol release its vapour into the packaging headspace thus maintaining the protective effect. These ethanol systems, approved for use in Japan, extend the mold-free shelf life of various bakery products. [26]. A complete study and comparison of the efficiency of three types of packaging systems: active packaging with cinnamon essential oil, modified atmosphere packaging (MAP) and the combination of both was carried out for extending the shelf-life of gluten-free sliced bread. The results showed that active packaging considerably increases the shelf life of packaged food, so it could be an attractive option to extend the shelf life. [27]. The food industry has seen great advances in the packaging sector since its inception in the 18th century with most active and intelligent innovations occurring during the past century. These advances have led to improved food quality and safety. While some innovations have stemmed from unexpected sources, most have been driven by changing consumer preferences. The new advances have mostly focused on delaying oxidation and controlling moisture migration, microbial growth, respiration rates, and volatile flavors and aromas [25]. The objective of this work was to evaluate the application of traditional Multibarrier 60 and OPP film without and with oxygen scavengers incorporated for wheat bread packaging in modified atmosphere (MAP) consisting of 60% carbon dioxide CO₂ and 40% nitrogen. (E 290), as control selecting air packaging of wheat bread in PP pouches.

II. MATERIALS AND METHODS

A. Experimental design

Experiments were carried out in the laboratories of Department of Food Technology, Latvia University of Agriculture. The object of the research was wheat bread produced in Latvia. Ingredients of wheat bread: wheat-flour, water, unskimmed milk, butter, sugar, salt, yeast, emulsifiers E472e, E320, ascorbic acid and enzymes. Dimensions of one slice of bread in average was 100 x 70 x 15 mm, mass 25 ±2 g.

B. Packaging and storage of samples

Wheat bread was packed in conventional Multibarrier 60 (lamine, APA/TIE/PA/EVH/PAP/TIE/ PE/PE, oriented polypropylene (OPP) and polypropylene (PP) polymer film pouches. Samples were packed in modified atmosphere packaging (MAP) and air ambience (control). Modified atmosphere consisting of carbon dioxide CO₂ (E 290) 60% and nitrogen N₂ (E 941) 40% was used. For shelf life extension the use of both usual MAP conditions as well as with oxygen scavenger commitment in the pouch was investigated. For reduced oxygen packaging (ROP) creation (O₂ – 0%) in pouches an iron based oxygen scavenger sachets of 100 cc obtained from Mitsubishi Gas Chemical Europe Ageless™ were used. The samples were hermetically sealed by MULTIVAC C300 vacuum chamber machine and stored at the room temperature of +21±0.5 °C, (controlled by MINILog Greisinger electronic) and about 40% RH for 28 days under day and night conditions. A characteristic of packaging materials used in experiments is shown in the Table 1 and structure of performed experiments – in Fig. 1.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Packaging material</th>
<th>Composition</th>
<th>Thickness, μm</th>
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<tbody>
<tr>
<td>1.</td>
<td>PP</td>
<td>Single layer, transparent</td>
<td>40±2</td>
</tr>
<tr>
<td>2.</td>
<td>OPP</td>
<td>Single layer, transparent</td>
<td>40±2</td>
</tr>
<tr>
<td>3.</td>
<td>Multibarrier 60</td>
<td>APA/TIE/PA/EVH/PA/TIE/PE/PE, transparent</td>
<td>60±2</td>
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The materials for experiments were selected with different water vapour transmission rate and various thicknesses in order to assess whether hardening can be ascribed by water loss from bread or from redistribution of the moisture inside the product, or by combination of moisture loss and redistribution. Two slices of bread were placed in each package. Size of each pouch was 110 x 120 mm, the total product mass in each package – 50±4 g. The results were reported as an average value of all determinations. Samples were analyzed before packaging (day 0) and in the 7th, 14th, 21st, and 28th day of storage, five measurement repetitions of each sample were performed.

![Fig. 1 Structure of performed experiments](image-url)
C. Physical, chemical, and microbial analysis

The following mechanical and physical characteristics were analyzed:

- The dynamics of gas composition in a hermetically sealed pouch headspace at the storage time was measured as a percentage of oxygen and carbon dioxide by a gas analyser OXYBABY® V O₂/CO₂.

- Moisture content was determined by ISO 6496:1999 as accordant to the storage time by verified balance KERN (Germany) with precision ±0.001 g; mass loss calculation (%) were determined by weighing packed samples on the electronic scales, by standard LVS ISO 1442: 1997.

- Hardness of the wheat bread samples was analysed by using “TA.XT.plus Texture Analyser” equipment (Stable Micro Systems Ltd., Surrey, UK) with the measuring probe P/25 (25 mm dia., cylinder aluminium, supplied with the Texture Analyser). The thickness of the bread sample slices was 15 mm. The measuring parameters of the bread samples were following: pre-test speed 1 mm s⁻¹; test speed 1 mm s⁻¹; post test speed 10 mm s⁻¹; penetrating distance of 4 mm into the bread crumb. The measurement is triggered automatically at 0.05 N. The system was equipped with a compression cell of 50 kg and software Texture Exponent 32. Hardness was measured as the maximum penetration force (N) reached during tissue breakage. The maximum force required for sample compression was calculated as an average of 10 measurements with the precision of the standard deviation < 0.05.

- pH was measured by JENWAY 3510 pH-meter, standard method LVS ISO 5542:2010.

- Microbial analyses: samples for microbiological testing were prepared by dilution method in conformity with standard LVS EN ISO 6887-1:1999 and 6887-4:2044. TPC (total plate count) – determined in conformity with standard LVS EN ISO 4833:2003 A; yeast and mould plate count – determined in conformity with standard LVS ISO 21527-2:2008. Plate counts evaluated as decimal logarithm of colony forming units (CFU) per gram of a product (log10 CFU·g⁻¹).

- Colour of wheat bread was measured in CIE L*a*b* colour system using Tristimulus Colorimeter, measuring Hunter colour parameters by Colour Tec PCM/PSM. Colour values were recorded as L* (brightness) – the vertical coordinate runs from L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) – the horizontal coordinate, that runs from -a* (green) through grey to +a* (red) and b* (-b, blueness, +b, yellowness) – another horizontal coordinate, that runs from -b* (blue) through grey to +b* (yellow) [28]. The measurements were repeated on different randomly selected locations at the surface of each sample. For evaluation of colour change, the total colour difference (ΔE*), was calculated between measurements before packaging of bread samples and during the storage time according to equation (1):

  \[
  \Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}.
  \]  

  Where:

  - \(L^*, a^*, b^*\) – value of bread sample colour components measured before packaging;
  - \(L_0^*, a_0^*, b_0^*\) – value of bread sample colour components measured after storage time.

E. Statistical analysis

The results were processed by mathematical and statistical methods. Statistics on completely randomized design were determined using the General Linear Model (GLM) procedure SPSS, version 16.00. Two-way analyses of variance (p<0.05) were used to determine significance of differences between weight losses, moisture content, hardness, pH, colour changes of atmosphere content (CO₂ and O₂) in headspace of packs, and microbial conditions by different packed samples.

II. RESULTS AND DISCUSSION

Significant differences in carbon dioxide (CO₂) content during the 28 days storage among all wheat bread samples packed in different kinds of materials were found (p<0.05), (Fig. 2).

The changes of carbon dioxide content during the all storage time in Multibarrier 60 pouches without oxygen scavenger (sample 2) and with incorporated O₂ scavenger, 100 cc (sample 3) were not significant (p>0.05), although the content of CO₂ in pouches with incorporated oxygen scavenger was somewhat less (by 6%) caused by chemical reactions between FeO and CO₂, consequently a salt FeCO₃ formed.

In OPP pouches with and without oxygen scavenger the CO₂ content at the 1st day began to fall down step by step and after 5 days the carbon dioxide decreases till 0.1% (Fig.2), similar it is in the control sample (sample 1). This phenomenon can be explained with packaging material barrier properties – high CO₂ permeability.

Presumably, the permeability of CO₂, as we can see in the Fig. 3, was mutually connected with simultaneous increase of oxygen content in pouches. The concentration of carbon dioxide in PP (control) pouches was not more than 1.0±0.1%.
The oxygen content (O₂) in Multibarrier 60 packaging during 28 storage days increased on average till 2.4±0.5%. (Fig.3). In the packages made of Multibarrier 60 with incorporated oxygen scavenger during 28 storage days the O₂ content increased on average till 1.8±0.3%. During 28 days storage in all pouches made from investigated Multibarrier 60 material the O₂ content did not disparse (p<0.05). In return in the OPP and PP pouches the content of O₂ during all experiment period was disparate from Multibarrier 60 packages – it was similar as in the surrounding environment after some storage days. The changes of oxygen content during the storage time in OPP pouches without oxygen scavenger (sample 2) and with incorporated O₂ scavenger, (sample 3) were significant (p<0.05).

Hardness changes in wheat bread samples stored in various packaging materials are presented in Fig. 5. The wheat bread became harder irrespective of used packaging technology and material type. Presumably the major hardening reason can be water vapour migration through the packaging material during the storage, which promotes hardening. The initial hardness of all samples, was 4.14±0.11 N. Mouth feel, texture and eating qualities are adversely affected by the loss of moisture [26].

![Fig. 5 The dynamics of hardness changes of wheat bread during storage](image)

Initial moisture content of wheat bread was 33.81±0.10%. As we can see in Fig. 4, the moisture content decrease during 28 storage days was various, influenced by different water vapour permeation of packaging materials. Significant difference in moisture content values at the end of storage among wheat bread samples packed in Multibarrier 60 without and with incorporated oxygen scavengers was not found (p>0.05), as well as the changes of moisture content during the storage were in little importance. In OPP and PP it decreased till 28±0.5% after 7 storage days, and the moisture content differed (p<0.05) from those packed in previously mentioned packaging materials.

Mouth feel of tested samples during the investigated storage time was different. Evaluating from the view point of product hardening, as the best variant for wheat bread packaging we have found transparent Multibarrier 60 film with incorporated oxygen scavenger – after 28 storage days showing increase in the hardness of packed product from 4.14±0.11 N till 15.5±1.87 N. At the same time in Multibarrier 60 film without oxygen scavenger the increase of hardness was somewhat higher – till 18.39±1.63 N during the storage time – by 18.5% higher. It could be a potential influence of chemical reactions between FeO and CO₂, and salt FeCO₃ formation which delays the bread hardening. On the contrary the increase of wheat bread hardness packed in PP, after 7 storage days was from 4.14±0.11 till 15.09±1.17 N (p<0.05), and this bread was not useable for eating, and moulds growth started as well. Significant difference of pH values among investigated wheat bread samples after 28 days storage was not found (p>0.05), and the increase of pH was not notable (Fig. 6).

![Fig. 6 The dynamics of pH value of wheat bread during storage](image)
Growth of microorganisms in wheat bread was affected by packaging material, and packaging method as well (Fig. 7–8). Evaluation of the experimentally obtained results is concerned with the fact, that for the time being in Latvia any document recommending total plate count (TPC), yeast and mould allowable plate count in pastr y-cooks does not exist.

Therefore, in this experiment we have followed the recent regulation of the Cabinet of Ministers of Latvia No. 292 “For food contamination” being in force from 02.08.1999, as well as considered previously accomplished microbiological studies. In this research we have supposed, that TPC could not be allowed more than log10 cfu·g\(^{-1}\), and yeast and mould plate count – not more than log10 cfu·g\(^{-1}\) 2.0.

Microbial analyses of wheat bread samples packed in OPP pouches (MAP) without oxygen scavenger incorporation in the pouches were accomplished only till 11 storage days, because the samples got spoiled. The samples packed in PP (air ambience) were microbiologically analyzed only during 7 days storage, because samples got spoiled too.

The results demonstrated preference of active packaging realised with oxygen scavenger incorporation in the pouches as an effective method to prevent microbial growth during storage and enhancing the shelf life of wheat bread till 14 days.

Total plate count after 11 storage days in packages with oxygen scavenger in OPP pouches (sample 5) was 5.6 log10 cfu·g\(^{-1}\), which is considerably less than in the same material packed samples without oxygen scavenger (sample 4) – 6.3 log10 cfu·g\(^{-1}\).

Total plate count after 14 storage days in packages with oxygen scavenger in Multibarrier 60 pouches was 4.5 log10 cfu·g\(^{-1}\), which is considerably less than in the same material packed samples without oxygen scavenger – 5.5 log10 cfu·g\(^{-1}\).

The initial yeast plate count of wheat bread was 5.22 ± 0.01 log10 cfu·g\(^{-1}\) (Fig. 8), which during storage time increased different.

Yeast plate count in wheat bread samples packed in Multibarrier 60 film pouches during 28 storage days did not exceed 5.60 log10 cfu·g\(^{-1}\), as we had supposed previously, however the mould count was influenced by active packaging – the incorporated oxygen scavenger slowed down the mould growth (p<0.05).

The initial value of wheat bread colour component L* was 78.2 ± 0.5 units, which in all investigated packaging situations in polymer films during 15 storage weeks step by step decreased till 75±2.5 units (Fig 9). It means – the colour became lighter and did not differ among samples.

The values of a* meaning (-a) – greenness, (+) – redness, are graphically represented in (Fig. 10). The initial a* value of wheat bread samples was -4.1 ± 0.5, which in Multibarrier 60 film packaging both without and with oxygen scavengers gradually increased on average till -3.4 ±0.4 units, and after storage of 28 days there was not observed substitution influence of oxygen scavengers on the a* value.
The $a^*$ value of samples packed in PP pouches in air ambiance were not significant ($p>0.05$) from previously mentioned samples in Multibarrier 60 packaging, already after one week storage increasing till -3.8±0.5 units, which as well, similarly like $L^*$ value changes, could be explained by moisture loss during storage.

![Graph of $a^*$ values vs. storage time](image)

Fig. 10 The changes of $a^*$ values during the storage time
1 – PP pouches; 2 – Multibrier 60 pouches (60% CO$_2$; 40% N$_2$); 3 – Multibrier 60 pouches (60% CO$_2$; 40% N$_2$) +O$_2$ scavenger, 100 cc; 4 – OPP pouches (60% CO$_2$; 40% N$_2$); 5 – OPP pouches (60% CO$_2$; 40% N$_2$) +O$_2$ scavenger, 100 cc.

The values of $b^*$ meaning (-$b$) – blueness, (+$b$) – yellowness, are graphically represented in Fig. 11. The initial $b^*$ value of wheat bread samples was 15.2±0.5 units. The changes of $b^*$ value during the all storage time in Multibarrier 60 pouches without oxygen scavenger (sample 2) and with incorporated O$_2$ scavenger, 100 cc (sample 3) were significantly different si ($p<0.05$). The least decrease were observed in Multibarrier 60 packaging without incorporated oxygen scavenger – only till 14.8±0.8 units, still in the same polymer with oxygen scavenger the decrease in $b^*$ value was – till 13.1±0.3, it means the results among samples after 28 storage days differed.

![Graph of $b^*$ values vs. storage time](image)

Fig. 11 The changes of $b^*$ values during the storage time
1 – PP pouches; 2 – Multibrier 60 pouches (60% CO$_2$; 40% N$_2$); 3 – Multibrier 60 pouches (60% CO$_2$; 40% N$_2$) +O$_2$ scavenger, 100 cc; 4 – OPP pouches (60% CO$_2$; 40% N$_2$); 5 – OPP pouches (60% CO$_2$; 40% N$_2$) +O$_2$ scavenger, 100 cc.

To describe the product overall colour change during the storage time, the influence of packaging materials and oxygen scavenger on the total colour difference ($\Delta E^*$) has been calculated by equation (1) (Fig. 12.). The presence of oxygen scavengers in packaging during 28 storage days substantially influence the colour difference of wheat bread samples packed in Multibarrier 60 film pouches ($p<0.05$). In return comparing the samples packed in OPP without and with oxygen scavenger we can not found disparity in colour difference ($p>0.05$).

Total colour difference ($\Delta E^*$) substantially differed of those samples packed in different packaging materials during storage time, what could be explained by influence of moisture loss during storage as well as by oxygen presence in packs.

![Graph of $\Delta E^*$ vs. storage time](image)

Fig. 12 The total colour difference ($\Delta E$) of wheat bread during the storage time
1 – PP pouches; 2 – Multibrier 60 pouches (60% CO$_2$; 40% N$_2$); 3 – Multibrier 60 pouches (60% CO$_2$; 40% N$_2$) +O$_2$ scavenger, 100 cc; 4 – OPP pouches (60% CO$_2$; 40% N$_2$); 5 – OPP pouches (60% CO$_2$; 40% N$_2$) +O$_2$ scavenger, 100 cc.

IV. CONCLUSION

Obtained results show, that the investigated conventional Multibarrier 60 film is applicable for wheat bread long-term storage in packaging maintaining quality, among them the active packaging in Multibarier 60 pouches (MAP, 60% CO$_2$; 40% N$_2$) with incorporated oxygen scavenger could be considered as the best variant. Packaging in OPP pouches can maintain quality of wheat bread during storage time only till 7 days as many as in usually used PP pouches and could be used only for bread short-term packaging on the supermarket shelves.

ACKNOWLEDGMENT

This research has been prepared within the framework of the Project Formation of the research group in food science Contract Nr.2009/0232/1DP/1.1.1.2.0/09/ APIA/V1AA/122.

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