

NITROGEN ATMOSPHERE AND NATURAL ANTIOXIDANTS EFFECT ON MUESLI OXIDATION DURING LONG-TIME STORAGE

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Abstract. The effects of natural antioxidants from raspberry and black currant seeds and modified atmosphere packaging on muesli oxidative stability measured by monitoring volatile lipid oxidation products were evaluated. The effectiveness toward lipid oxidation was investigated during 10 months storage at ambient temperature. Both ethanolic extracts as well as nitrogen atmosphere influenced lipid oxidation rate in muesli measured by volatile compounds content. The most abundant lipid derived volatile compounds was hexanal. After storage, its concentration changed from 802 $\mu\text{g}/\text{kg}$ to 9.8 mg/kg in muesli stored in air atmosphere, whereas in muesli stored in nitrogen atmosphere with raspberry seed extract addition it raised to 3.1 mg/kg . Although, both natural antioxidants rich in phenolic compounds, were effective towards lipid oxidation, the strongest inhibiting effect had modified atmosphere packaging. The addition of ethanolic extracts did not fortify its positive effect. Total concentration of volatile compounds in muesli after 10 months of storage was 19.6 mg/kg when stored in air and 13.7 and 11.8 mg/kg when stored with raspberry and black currant seeds extract addition respectively, while 9.8 mg/kg when stored in nitrogen atmosphere without antioxidants, and 9.7 and 9.9 mg/kg when stored with antioxidants mentioned above.

Key words: muesli, red raspberry extract, nitrogen atmosphere packaging, SPME

INTRODUCTION

Muesli and breakfast cereals are dry foods with relatively high lipids content. Because of low water activity they are stable against microbial growth, however, enzymatic and chemical reactions can occur and results in deterioration [Labuza 1980], which is generally the result of lipid oxidation. This process influences the shelf-life of a product decreasing its nutritional value, and is responsible for the formation of off-flavours and also potentially toxic substances during storage [Uchida 2000], making the product undesirable to consumers. That is why food industry tries to develop methods,

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which could delay lipids oxidation and extend shelf-life of products. Therefore, food packaging has an outstanding importance once it protects the food from microbial and chemical contamination, as well as from oxygen, water vapour and light [Callegarin et al. 1997]. Packaging techniques, including modified atmosphere, oxygen barrier packaging material, active packaging [Lee and Krochta 2002, Larsen et al. 2003] and the addition of natural and synthetic antioxidants [Stashenko et al. 2002, Wessling et al. 2000], have been investigated. Modified atmosphere packaging (MAP), which is considered an effective method of food preservation, is based on lowering the amount of oxygen in the package, which could be replaced by another gas; for example nitrogen. Packaging in nitrogen atmosphere was used to control oxidation in extruded oats by Larsen et al. [2003]. It was found that during 3 months of storage no rancid odours or flavors developed when the oxygen transmission rate (OTR) of the packages was low or the extruded oat were stored in darkness. However, extruded oats exposed to light in packages with medium and high OTR developed the highest degree of rancidity as measured by paint odour. Authors also indicate that oxygen concentration had the 2nd largest effect on the development of rancidity, next to light. Moreover, shelf-life of the product could be successfully extended by the addition of natural antioxidants. Plant extracts containing polyphenols are known to exhibit antioxidant activity towards lipid oxidation in different food systems [Shahidi and Wanasundara 1992]. There are evidence, that ethanolic extracts from defatted seeds of raspberry have a high antioxidant activity comparable to BHA [Pacholek and Małecka 2005]. Additionally, Samotyja and Małecka [2007] indicate that blackcurrant seeds extract greatly inhibited hydroperoxide breakdown and hexanal formation in lipids substrate, while Pacholek and Małecka [2000] indicated positive effect as well in model system as in rapeseed oil during heating. Besides the positive effect towards lipid oxidation, raspberry and black currant seeds extracts showed significant effect in decreasing oxyphytosterols content in peanuts [Małecka et al. 2003]. Furthermore, the combined effect of modified atmosphere packaging and addition of natural antioxidant have been extensively investigated in meat products [Sanchez-Escalante et al. 2003, Djenane et al. 2002].

As food flavour is one of the most important attributes participating food quality and influence consumer acceptance, it can undergo great changes during storage. Therefore, early detection of lipids oxidation products is so important in prevention of unfavourable changes. The state of lipid oxidation has often been quantified in terms of the peroxide value or the content of secondary oxidation products. Since hexanal is one of the most abundant products of lipids oxidation and its concentration increased during storage, it was found to be a good marker of oxidative rancidity in food [Jensen and Risbo 2007, Jensen 2005].

The major purpose of this paper was to evaluate the influence of modified atmosphere packaging and natural antioxidants from raspberry and black currant seeds on oxidative stability of muesli during long-time storage. Muesli oxidative stability was monitored by determination of volatile lipid oxidation products.

MATERIALS AND METHODS

Muesli samples

Muesli containing oat flakes (30%), wheat flakes (30%), corn flakes (10%), hazelnuts (10%), raisins (5%), sunflower seeds (5%) and flax-seeds (5%) was prepared in the laboratory. All muesli ingredients were purchased in a local store, with minimum three months of shelf-life. In storage experiment muesli samples were packaged using Multivac A-300 in batches of 100 g each in foil bags, made from laminate oriented polyamide/polyethylene with the dimension of 15×21 cm, and gas permeability at 23°C in $\text{cm}^3/\text{m}^2/24$ h: $\text{O}_2 - 45$, $\text{CO}_2 - 200$, water vapor: 2-3. To evaluate the effect of modified atmosphere and natural antioxidants samples were stored during 10 months at ambient temperature in two different atmospheres: air and nitrogen (98% N_2 , 2% O_2) with the 0.3% addition of ethanolic extract of raspberry seeds (RS) or black currant seeds (BCS). The extract containing 2370 mg/100 g d.m. and 340 mg/100 g d.m. phenolic compounds respectively, using caffeic acid as a calibration standard was added by spraying thin layer at the surface of product. Analyses were carried out once a month to evaluate the effect of storage conditions, each sample was analysed in triplicate.

Oxidative stability determination

Oxidative stability was evaluated by analysing samples for secondary oxidation product formation. Volatile lipid derived compounds were isolated by HS-SPME and analysed using Hewlett Packard HP 5890 II coupled to a quadrupole mass detector HP 5971 (Hewlett Packard, Palo Alto, CA) fitted with a DB-5 capillary column ($25 \text{ m} \times 0.2 \text{ mm} \times 0.33 \text{ }\mu\text{m}$). Helium was the carrier gas at a flow rate of 0.6 mL/min. The inlet temperature was 260°C and the transfer line was set to 280°C. The oven temperature was 40°C for 1 min, followed by increase of 4°C/min to 160°C and 10°C/min to 280°C. Injection port was set to a splitless mode (1 min). The procedure used was based on our previously elaborated method [Klensporf and Jeleń 2005]. The three-phase fiber (DVB/CAR/PDMS) was used in all analyses. The headspace sampling was performed for 30 min at the 50°C, the 10% addition of water was used. All analyses were repeated at least three times. The quantitation was done based on response factors of particular compounds and internal standard pyrazine- d_4 . All samples were spiked with 100 μL of pyrazine- d_4 solution providing concentration 2 $\mu\text{g/g}$ sample. The MS response factors were determined for all standards mentioned in materials section at two different concentrations (50 ppb, 500 ppb), and averaged.

RESULTS AND DISCUSSION

Oxidative stability of muesli

The progress of lipid oxidation measured by volatile compounds content in muesli increased with time in all experimental storage conditions. However, depending on the storage atmosphere and natural antioxidants addition, the total concentration of volatiles after 10 months was significantly diverse. Additionally, samples stored for 10 months

in air not only have the highest concentration of volatiles, but also the most differential volatiles profile (Fig. 1). The concentration of lipid derived volatile compounds is given in Table 1, which for clear presentation contains data taken every two months. The predominant group of fatty acids derived components were volatile aldehydes such as hexanal, pentanal, heptanal, octanal and nonanal. Hexanal being a good marker of oxidative rancidity in cereal products [McEwan et al. 2005, Jensen and Risbo 2007, Moltenberg et al. 1996], was also a most abundant volatile compound in muesli stored at room temperature for 10 months. Depending on storage conditions its concentration varied significantly. In freshly prepared muesli (control sample) hexanal content was estimated at the level of 802 $\mu\text{g}/\text{kg}$, whereas in samples stored in air atmosphere its concentration increased to 9.8 mg/kg (Fig. 2). The largest increase occurred after 5th month of storage, when the concentration changed over 5.9 mg/kg . Slightly lower increase of hexanal concentration was observed in muesli samples stored in air with the addition of RS extract, its concentration was 6.2 mg/kg . The replacement of air in foil

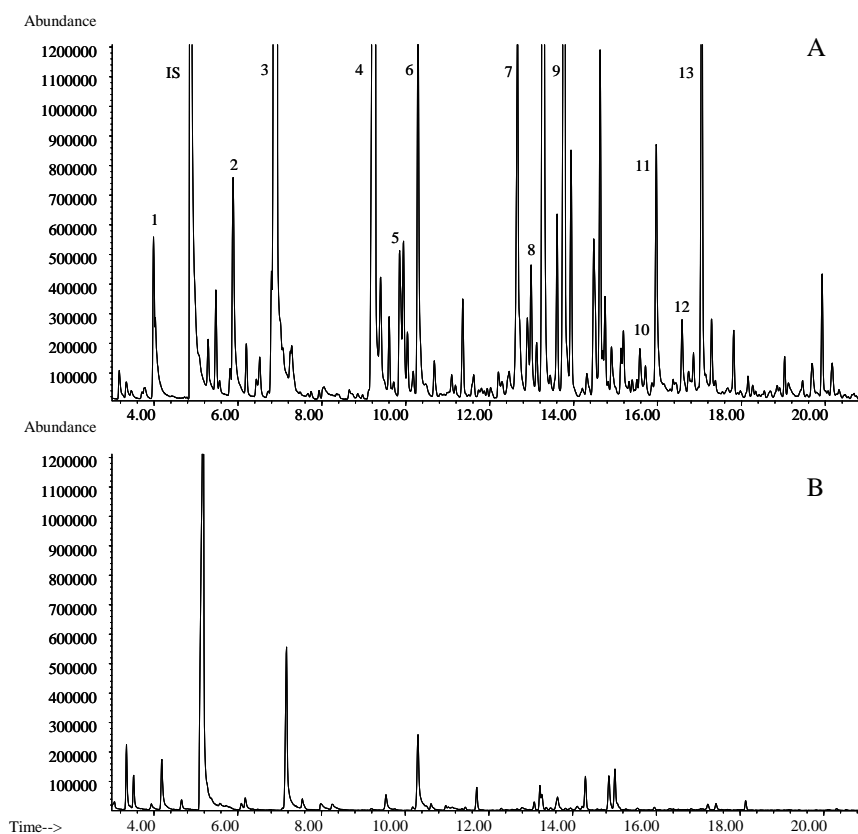


Fig. 1. Volatile profile of muesli stored at ambient temperature in air atmosphere: A – muesli stored for 10 months, B – control sample – freshly prepared muesli. IS – internal standard: pyrazine d_4 , 1 – pentanal, 2 – 1-pentanol, 3 – hexanal, 4 – 1-hexanol, 5 – 2-heptanone, 6 – heptanal, 7 – 1-heptanol, 8 – 1-octen-3-ol, 9 – octanal, 10 – E-2-octenal, 11 – 1-octanol, 12 – 2-nonanone, 13 – nonanal

Table 1. Secondary oxidation products (SOP) concentration in muesli stored at ambient temperature for 10 months, $\mu\text{g}/\text{kg}$

Compound	Storage experiment	Storage time, SOP, $\mu\text{g}/\text{kg}$					
		0	2	4	6	8	10
Pentanal	I	nd	120 \pm 8 ^c	150 \pm 5 ^d	120 \pm 5 ^b	183 \pm 5 ^d	320 \pm 12 ^c
	II		49 \pm 6 ^a	66 \pm 5 ^b	97 \pm 8 ^b	126 \pm 10 ^b	204 \pm 2 ^b
	III		100 \pm 8 ^b	119 \pm 11 ^c	144 \pm 1 ^c	164 \pm 5 ^c	190 \pm 10 ^b
	IV		nd	27 \pm 4 ^a	66 \pm 2 ^a	79 \pm 4 ^a	94 \pm 9 ^a
1-pentanol	I	55 \pm 5	187 \pm 10 ^c	147 \pm 9 ^b	215 \pm 15 ^c	152 \pm 13 ^b	696 \pm 51 ^c
	II		90 \pm 9 ^b	163 \pm 13 ^b	118 \pm 8 ^b	179 \pm 4 ^c	529 \pm 30 ^b
	III		180 \pm 9 ^c	173 \pm 15 ^b	213 \pm 20 ^c	135 \pm 5 ^{ab}	711 \pm 53 ^c
	IV		69 \pm 5 ^a	45 \pm 4 ^a	67 \pm 5 ^a	110 \pm 11 ^a	424 \pm 21 ^a
Hexanal	I	802 \pm 82	2 304 \pm 131 ^{bc}	2 817 \pm 255 ^b	4 855 \pm 404 ^b	6 317 \pm 320 ^c	9 849 \pm 572 ^c
	II		1 867 \pm 186 ^{ac}	1 453 \pm 55 ^a	3 642 \pm 373 ^a	2 965 \pm 242 ^b	6 192 \pm 109 ^b
	III		2 577 \pm 108 ^b	2 593 \pm 233 ^b	3 588 \pm 87 ^a	2 051 \pm 112 ^a	3 394 \pm 199 ^a
	IV		1 506 \pm 104 ^a	1 300 \pm 52 ^a	3 496 \pm 347 ^a	1 846 \pm 110 ^a	3 132 \pm 178 ^a
1-hexanol	I	57 \pm 6	724 \pm 73 ^c	870 \pm 64 ^d	1 161 \pm 68 ^a	924 \pm 68 ^b	3 436 \pm 220 ^c
	II		373 \pm 38 ^a	500 \pm 2 ^b	1 853 \pm 92 ^b	1 139 \pm 135 ^b	2 485 \pm 82 ^b
	III		531 \pm 35 ^b	696 \pm 59 ^c	1 146 \pm 36 ^a	678 \pm 61 ^a	1 666 \pm 147 ^a
	IV		430 \pm 46 ^{ab}	301 \pm 26 ^a	1 371 \pm 80 ^c	626 \pm 39 ^a	1 572 \pm 103 ^a
2-heptanone	I	nd	48 \pm 4 ^b	54 \pm 3 ^b	65 \pm 6 ^{ab}	130 \pm 1 ^b	253 \pm 12 ^{ab}
	II		42 \pm 1 ^a	23 \pm 1 ^a	87 \pm 4 ^c	104 \pm 4 ^a	218 \pm 6 ^a
	III		43 \pm 1 ^a	46 \pm 5 ^b	66 \pm 5 ^b	146 \pm 7 ^c	248 \pm 17 ^a
	IV		43 \pm 4 ^{ab}	50 \pm 1 ^b	54 \pm 1 ^a	195 \pm 7 ^d	296 \pm 28 ^b
Heptanal	I	nd	79 \pm 8 ^a	154 \pm 12 ^b	203 \pm 19 ^a	308 \pm 14 ^a	861 \pm 54 ^b
	II		nd	164 \pm 6 ^b	337 \pm 47 ^b	367 \pm 12 ^b	592 \pm 13 ^a
	III		nd	99 \pm 6 ^a	226 \pm 18 ^a	424 \pm 18 ^c	860 \pm 10 ^b
	IV		nd	111 \pm 3 ^a	249 \pm 6 ^a	388 \pm 20 ^{bc}	668 \pm 43 ^a
1-heptanol	I	nd	nd	nd	201 \pm 3 ^b	182 \pm 15 ^b	828 \pm 32 ^c
	II				314 \pm 2 ^c	337 \pm 22 ^c	614 \pm 26 ^b
	III				115 \pm 5 ^a	125 \pm 7 ^a	267 \pm 26 ^a
	IV				123 \pm 2 ^a	182 \pm 5 ^b	289 \pm 22 ^a
Octanal	I	nd	49 \pm 3 ^b	202 \pm 9 ^c	405 \pm 9 ^c	1031 \pm 33 ^d	1663 \pm 138 ^b
	II		32 \pm 2 ^a	189 \pm 6 ^c	443 \pm 20 ^c	830 \pm 6 ^c	1183 \pm 75 ^a
	III		100 \pm 11 ^d	134 \pm 14 ^b	255 \pm 16 ^a	363 \pm 7 ^b	1574 \pm 59 ^b
	IV		68 \pm 3 ^c	108 \pm 3 ^a	296 \pm 13 ^b	196 \pm 14 ^a	1439 \pm 132 ^{ab}
Nonanal	I	9 \pm 0.3	199 \pm 3 ^c	217 \pm 21 ^a	210 \pm 18 ^{ab}	200 \pm 15 ^a	826 \pm 59 ^a
	II		92 \pm 2 ^a	224 \pm 21 ^a	428 \pm 13 ^c	314 \pm 32 ^b	801 \pm 61 ^a
	III		136 \pm 11 ^b	202 \pm 11 ^a	226 \pm 10 ^b	220 \pm 8 ^a	852 \pm 31 ^a
	IV		144 \pm 8 ^b	180 \pm 10 ^a	185 \pm 10 ^a	306 \pm 8 ^b	837 \pm 28 ^a

I – air atmosphere, II – air atmosphere + RS extract, III – nitrogen atmosphere, IV – nitrogen atmosphere + RS extract.

a, b... – means in columns with the same letter are not significantly different at $p < 0.05$.

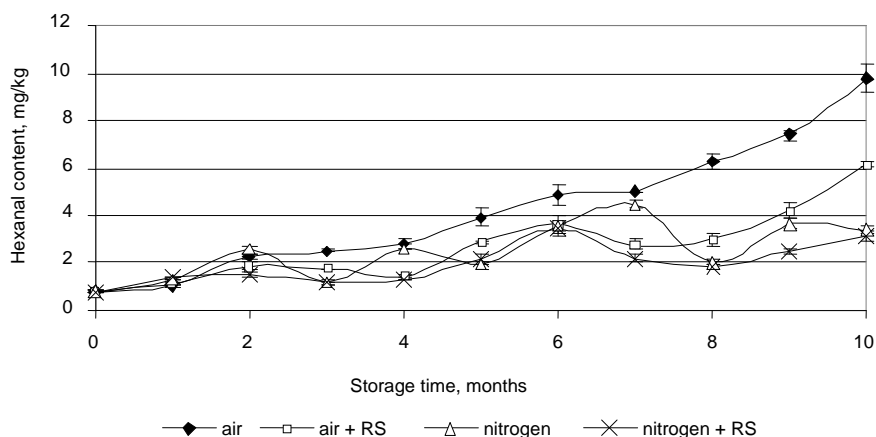


Fig. 2. Development of hexanal in muesli stored at ambient temperature in air and nitrogen atmosphere and with antioxidant addition

bags with nitrogen, lets to limit the hexanal formation. In samples stored only in the nitrogen atmosphere the concentration was estimated at the level of 3.4 mg/kg, whereas in samples with RS extract 3.1 mg/kg. There were no significant differences between ($p < 0.05$) samples stored in the nitrogen atmosphere. It is possible that the strongest influence on the hexanal formation in presented experiment had oxygen.

Jensen et al. [2005] indicate that increased oxygen availability affects the degree of oxidation, additionally exposure of muesli to light resulted in their experiments in increased formation of both radicals as well as hexanal. However, when extruded oats were stored in packages with low O_2 concentration, light had no significant effect [Larsen et al. 2003]. Hazelnuts, which are one of the muesli ingredients, have high concentration of oleic and linoleic acids, and therefore are very susceptible to lipid oxidation. Hexanal and octanal were a major volatile compounds formed during oxidation of hazelnuts, their levels affect the increase in rancidity [Kinderlerer and Johnson 1992]. Authors also indicated that the way to limit oxidative rancidity is to eliminate oxygen, and also light from the package. Furthermore, concentration of other volatile aldehydes changed in the same way as hexanal. The highest increase was observed when samples were stored in air atmosphere. The addition of natural antioxidants as well as modified atmosphere packaging retarded formation of volatile aldehydes, with the exception of nonanal. Although, the nonanal concentration increased during storage from 8.8 to 852 $\mu\text{g}/\text{kg}$, there were no significant differences ($p < 0.05$) between samples stored in modified or air atmosphere, and with or without natural antioxidants. Long-time storage in air also induced formation of unsaturated aldehyde such as *E-2*-octenal, which concentration was 44.7 and 45.5 $\mu\text{g}/\text{kg}$, according to storage conditions. Among the lipid derived volatiles were also alcohols such as 1-pentanol, 1-hexanol and 1-heptanol, but the most abundant was 1-hexanol (Fig. 3). Volatile alcohols were also identified by SPME method by Sides et al. [2001] in oat products. As a result of natural antioxidant addition, formation of 1-hexanol was moderated. Samples stored in air had the highest 1-hexanol concentration estimated at the level 3.4 mg/kg, but when the additives were used concentration was significantly lower 2.5 mg/kg. Similarly to aldehydes formation,

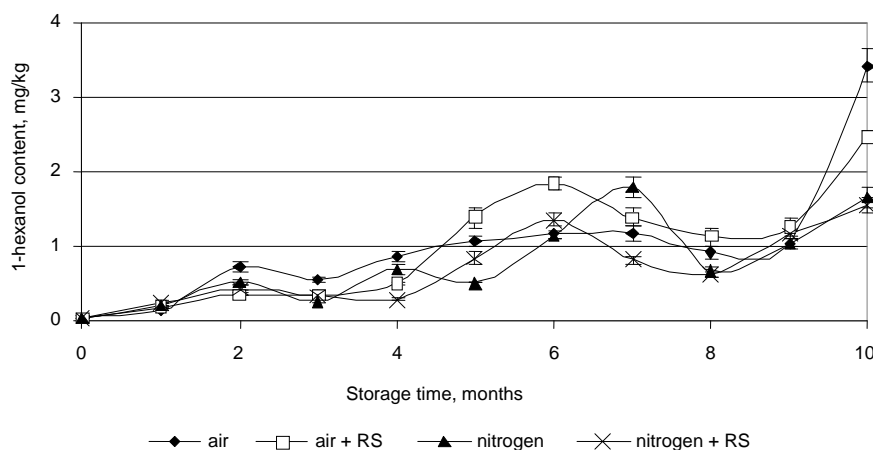


Fig. 3. Development of 1-hexanol in muesli stored at ambient temperature in air and nitrogen atmosphere and with antioxidant addition

the strongest limiting properties had nitrogen atmosphere. After 10 months of storage, formation of 1-octen-3-ol and 1-octanol was observed. 1-octen-3-ol concentration was estimated at the level 35.7 and 46.2 $\mu\text{g}/\text{kg}$, whereas for 1-octanol it was 521 to 647 $\mu\text{g}/\text{kg}$, depending on storage conditions. Although, volatile alcohols could be a result of enzymatic oxidation, according to Heydanek and McGorin [1986] their presence in oat products is connected to autoxidation rather than enzymatic oxidation. Therefore, in muesli which contains 30% of oat flakes, autoxidation is the most probable reason for alcohols formation.

Besides volatile aldehydes and alcohols, 2-heptanone was also present. Its concentration changed similarly to other lipid derived volatile compounds. 2-heptanone was not identified in freshly prepared muesli, however, after only one month of storage its concentration was estimated at the level 12 to 16 $\mu\text{g}/\text{kg}$. At the beginning of storage changes in concentration were slight, however, together with storage time the differences were more significant. After 9 months, the concentration in samples stored in air was 113 and 125 $\mu\text{g}/\text{kg}$, while in samples stored in nitrogen 255 and 222 $\mu\text{g}/\text{kg}$. Additionally, after long time storage 2-nonanone was also present in muesli samples. The highest 2-nonanone concentration was observed in muesli stored in nitrogen 84 $\mu\text{g}/\text{kg}$, whereas the lowest in muesli stored in air with raspberry seeds extract addition 63 $\mu\text{g}/\text{kg}$. Both 2-heptanone and 2-nonanone were an exception, because the most effective storage conditions was air atmosphere, while in other examples it influenced secondary oxidation products formation most.

Comparison of red raspberry and black currant seeds extracts effectiveness towards lipid oxidation inhibition

To compare activities of natural antioxidants, total concentration of secondary oxidation product and PV value was estimated. On the basis of secondary oxidation products concentration after 10 months of storage of black currant seeds extract performed slightly better results in muesli stored in air atmosphere than raspberry seeds extract

(Fig. 4). Control sample stored without any additional substances had the highest volatiles concentration 19.6 mg/kg. Simultaneously, these samples had the highest PV value, estimated at the level 8.7 meq/kg, whereas in freshly prepared muesli it was 3.0 meq/kg, what confirmed undesirable changes in lipids. Both ethanolic extracts significantly moderate oxidative changes in muesli, total concentration of volatiles was estimated at the level of 13.8 and 11.8 mg/kg in samples stored with the addition of raspberry seeds and black currant seeds extract, respectively. For these samples PV values were 6.3 and 5.6 meq/kg. The use of nitrogen atmosphere packaging strongly affected lipid derived volatiles formation. Volatiles concentration in samples stored in modified atmosphere varied from 9.7 to 9.9 mg/kg, but there were no significant differences ($p < 0.05$) between samples with natural additives. Muesli samples stored in nitrogen atmosphere had also the lowest PV values, which differ from 5.0 meq/kg in samples with black currant extract addition to 5.5 meq/kg in muesli without additives. Although, BCS extract contained significantly lower amount of phenolic compounds 340 mg/kg than RS extract 2370 mg/kg d.m., it performed higher antioxidant activity. However, phenolic compounds are probably not the only one responsible for antioxidative activity of extracts from raspberry and black currant. It is known that natural antioxidants can cooperate and perform synergistic inhibition of lipid oxidation [Decker 2002]. Results obtained by Viscidi et al. [2004] showed that natural phenolic compounds addition could give a synergistic effect and protect natural antioxidant present in food. However, Viljanen et al. [2004] indicated that the coexistence of anthocyanins and ellagitannins may not be the best combination for the inhibition of lipid oxidation as raspberry phenolics were among the least potential antioxidant.

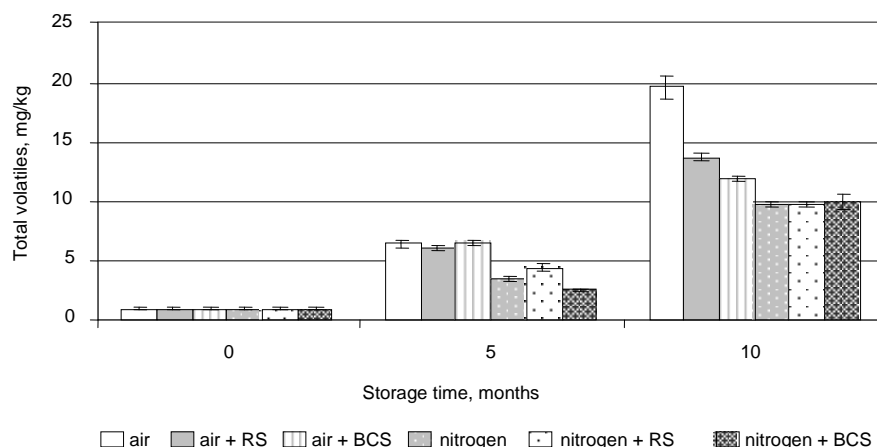


Fig. 4. Effect of raspberry seed extract (RS) and blackcurrant seed extract (BCS) on the total concentration of secondary oxidation products in muesli stored in air and nitrogen atmosphere

On the basis of the obtained results, measurement of volatile compounds concentration, especially hexanal, could be a valuable information about oxidative changes taking place in breakfast cereals such as muesli. The highest increased of hexanal content was observed when samples were stored in air atmosphere, what indicated that oxygen

availability was one of the main factor accelerating lipid oxidation. Air atmosphere was always the least favourable against limiting unsaturated fatty acids oxidation. However, the results showed that undesirable oxidative changes could be retarded by addition of natural antioxidants as well as by packaging in modified atmosphere. The limiting effect was obtained by the addition of 0.3% natural antioxidant before the packaging. Total concentration of volatiles was about 30% lower when natural antioxidants were used. Natural antioxidants protect muesli against lipid oxidation and improve its quality. However, phenolic compounds composition in black currant considerably differ from these in raspberry and therefore their antioxidative effects are different. Moreover, the elimination of air from packages and replacement by nitrogen give the best results in inhibition of lipid oxidation.

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WPLYW ATMOSFERY AZOTU I NATURALNYCH PRZECIWIUTLENIACZY NA OKSYDACJĘ MUESLI W CZASIE DŁUGIEGO PRZECHOWYWANIA

Streszczenie. W pracy badano wpływ naturalnych przeciwutleniaczy z nasion malin i czarnej porzeczki oraz pakowania w modyfikowanej atmosferze na stabilność oksydacyjną muesli, mierzoną poprzez monitorowanie lotnych produktów utleniania lipidów. Efektywność działania przeciwutleniaczy badano w czasie 10-miesięcznego przechowywania w temperaturze pokojowej. Zarówno ekstrakty etanolowe przeciwutleniaczy, jak i atmosfera azotu wpływały na szybkość utleniania lipidów mierzoną zawartością związków lotnych. Związkiem lotnym pochodzącym z oksydacji lipidów obecnym w największych stężeniach był heksanal. Po przechowywaniu jego zawartość wzrosła z 802 $\mu\text{g}/\text{kg}$ do 9,8 mg/kg w muesli przechowywanym w atmosferze powietrza, natomiast w muesli przechowywanym w atmosferze azotu z dodatkiem ekstraktu z nasion malin zawartość heksanolu wzrosła do 3,1 mg/kg . Chociaż naturalne przeciwutleniacze, bogate w związki

lotne, były efektywne w hamowaniu procesu oksydacji lipidów, najefektywniej był on hamowany przez pakowanie w atmosferze modyfikowanej. Dodatek ekstraktów etanolo- wych przeciwutleniaczy nie wzmacniał efektu zastosowania pakowania w atmosferze modyfikowanej. Całkowita zawartość związków lotnych w muesli po 10 miesiącach przechowywania wynosiła 19,6 mg/kg dla prób pakowanych w powietrzu i 13,7 oraz 11,8 mg/kg dla prób z dodatkiem ekstraktów z nasion malin i czarnej porzeczki, natomiast zawartość związków lotnych wynosiła 9,8 mg/kg dla prób przechowywanych w atmosferze azotu bez dodatku przeciwutleniaczy oraz 9,7 i 9,9 mg/kg dla próbek z dodatkiem wymienionych przeciwutleniaczy.

Słowa kluczowe: muesli, ekstrakt z malin, pakowanie w atmosferze azotu, SPME

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