Effect of diet on oxidation and profile of volatile compounds of pork after freezing storage

MARTA BRODOWSKA – DOMINIKA GUZEK – ALEKSANDRA KOŁOTA – DOMINIKA GŁĄBSKA – ELŻBIETA GÓRSKA-HORCZYCZAK – IWONA WOJTASIK-KALINOWSKA – AGNIESZKA WIERZBICKA

Summary

The aim of this study was to evaluate the effect of dietary antioxidants (complex of 100 mg·kg^{-1} vitamin E and 1 mg·kg^{-1} of organic selenium added to fodder) on lipid oxidation and flavour of pork after freezing storage. Meat was derived from animals fed with control fodder, fodder containing linseed oil (rich in polyunsaturated fatty acids) or fodder containing linseed oil and antioxidants. The oxidation process was assessed by analysis of thiobarbituric acid reactive substances (TBARS) and volatile organic compounds, which were analysed using the electronic nose based on the technique of gas chromatography. The level of TBARS was in range from (2.16 ± 0.89) mg·kg^{-1} to (2.94 ± 1.41) mg·kg^{-1} and was associated with the stage of oxidation in all experimental groups. There was no effect of dietary supplementation of antioxidants on lipid oxidation of pork meat after 9 months of freezing storage. In all samples, organic volatile compounds characteristic for oxidation process were identified, but there were differences in the volatile profile between experimental groups and the control group. After a prolonged period of freezing storage, the process of lipid oxidation occurred, regardless from the diet, but the volatile compounds profile varied.

Keywords

thiobarbituric acid reactive substances; volatile compounds; lipids oxidation; pork meat; freezing

One of the major trends creating interest of consumers towards meat products is the need to have the nutritional value of meat increased and to obtain health benefits [1]. The improvement of fatty acids composition of meat may be achieved by increasing the share of polyunsaturated fatty acids (PUFAs), particularly of *n*-3 PUFAs that can reduce the risk of atherosclerosis, risk of diseases of the circulatory system and risk of other diet-related diseases [2]. However, enrichment of meat with *n*-3 PUFAs may lead to an increase of its susceptibility to oxidation. Oxidation process is a major factor causing deterioration of muscle food products. Oxidation is associated with the damage a wide range of products, including nucleic acids, proteins, and lipids, especially phospholipids, while oxidation is initiated. Oxidative stability of meat also depends on antioxidant and prooxidant substances in the muscle [3], as well as duration and conditions of storage [4]. The use of antioxidant substances as a component of animal fodder may be an effective way to prevent lipid oxidation, to minimize the formation of toxic products of oxidation and to maintain composition of fatty acids. The use of antioxidants may also prolong the shelf-life by increasing oxidation defence of muscle food products [5, 6].

Flavour is one of the most important qualities characteristic for pork meat, which indicates freshness. Among various factors that may influence the flavour of meat, diet of the animal has the main role. Especially lipid content and fatty acids profile are the most important attributes, as the precursors of organic volatile compounds determine the aroma of meat [7]. The increased susceptibility of meat to lipid oxidation may have

Marta Brodowska, Dominika Guzek, Elżbieta Górska-Horczyczak, Laboratory of Food Chemistry, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences, Nowoursynowska 159 c, 02-776 Warsaw, Poland.
Aleksandra Kołota, Dominika Głąbska, Department of Dietetics, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences, Nowoursynowska 159 c, 02-776 Warsaw, Poland.
Iwona Wojtasik-Kalinowska, Agnieszka Wierzbicka, Department of Technique and Food Development, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences, Nowoursynowska 159 c, 02-776 Warsaw, Poland.

Correspondence author:
Marta Brodowska, tel.: (+48) 22 59 37 014, e-mail: marta_brodowska@sggw.pl

an impact on the formation of a large number of volatile secondary lipid oxidation products such as aldehydes, ketones, acids or alcohols, and may promote the formation of nitrogen- and sulphur-containing compounds [7]. Prolonged storage may also cause intensification of the lipid oxidation process and generation or conversion of many volatile organic compounds, which contribute to creating the aroma of meat [8, 9].

Freezing is the most popular method of meat preservation after the products are purchased by consumers or in the catering industry [10]. While studies of meat, which was enriched with PUFAs and antioxidants, were confined to characterize the quality of raw and refrigerated meat [4, 5], little information is available on the oxidation of lipids and flavour profile of frozen pork enriched with PUFAs. According to the International Institute of Refrigeration, pork steaks or cuts may be stored in freezing conditions (–18 °C or below) for 10 months [11]. Even though freezing may delay and minimize the processes of lipid oxidation, they cannot be stopped [12]. Additionally, some lipid-soluble radicals may be more stable at low temperatures and as being more mobile, they may promote oxidation [13].

The aim of this study was to evaluate the effect of dietary antioxidants (complex of 100 $mg \cdot kg^{-1}$ vitamin E and 1 $mg \cdot kg^{-1}$ of organic selenium added to fodder) on lipid oxidation and flavour of pork. Muscles were derived from animals fed with linseed oil and antioxidants and were stored for 9 months in freezing conditions. Secondary products of lipid oxidation were assessed on the basis of thiobarbituric acid reactive substances (TBARS) measurement and volatile profile of pork muscle tissue was analysed, depending on the diet of animals.

MATERIALS AND METHODS

Animals and preparation of samples

The study was carried out on crossbreeds of Polish Landrace and Duroc animals. A total of 36 animals were involved in the experiment. Till obtaining the live weight of 60 kg, all animals were fed with a standard fodder. After obtaining the live weight of 60 kg, animals were randomly allotted to 3 feeding groups (12 animals in each group). The control group (C) was fed a standard fodder; the experimental group I (L1) was fed a standard fodder with 3% of linseed oil; the experimental group II (L2) was fed a standard fodder with 3% of linseed oil, 100 $mg \cdot kg^{-1}$ of vitamin E and 1 $mg \cdot kg^{-1}$ of organic selenium. The standard fodder consisted of barley grits (360 $g \cdot kg^{-1}$), wheat middlings (360 $g \cdot kg^{-1}$), maize grits (100 $g \cdot kg^{-1}$), extracted soybean meal (80 $g \cdot kg^{-1}$), extracted rapeseed meal (40 $g \cdot kg^{-1}$) and vitamin-mineral premix (25 $g \cdot kg^{-1}$). The composition and nutrition value of the applied basic fodder was described in the previous paper [14]. In the case of each animal, standard animal management (animals under veterinary care, management typical for animal husbandry in food production according to Polish law regulation) was applied. During the experiment, animals were housed in individual pens until they reached 100 kg ± 5 kg of live weight and then were slaughtered. Slaughter procedure was typical for industrial conditions in Poland. The slaughter procedure and the slaughterhouse were certified as being in accordance with the current EU regulations. For the purpose of the presented analysis, 6 animals from each group were randomly selected. Semimembranosus (SM) muscles were taken into account, because red muscles have been found to have an especially high susceptibility to oxidation and to have a stronger flavour than white muscles. This may be a result of the metabolic type of fibres in red muscles, as they have different activity and a higher content of flavour precursors [15].

The meat cuts were transported to the laboratory. At a time of 48 h post mortem, meat cuts were analysed to measure basic composition (water, fat, protein, connective tissue and ash content) using near-infrared spectrometry (NIR Flex Solids N-500; Büchi Labortechnik, Flawil, Switzerland), pH value (by pH-meter Testo 205, Mera, Warsaw, Poland) and fatty acids profile. Fatty acids were extracted from pork meat by homogenization of samples with the chloroform-methanol solution, according to Folch et al. [16]. The fatty acids composition were determined using gas chromatography (gas chromatograph Shimadzu, Kyoto, Japan). Then, meat cuts were divided across fibres into samples (of approximately 150 g), vacuum-packaged in polyethylene bags (of having low oxygen permeability) and stored at a temperature of –20 °C ± 1 °C for 9 months. After 9 months of freezing storage, TBARS and volatile compounds analysis were performed.

Lipid oxidation analysis

The end-products of lipid oxidation, especially aldehydic products (malondialdehyde and hydroxylnonenal) can be assessed by the analysis of reaction with 2-thiobarbituric acid. The value of TBARS for raw meat was determined according to the method described by Robles-Martinez et al. [17] with some modifications. Pork meat, without

thawing, was cut into samples of 2.5 g and homogenized with 15 ml of trichloroacetic acid solution (200 g·l^{-1} of trichloroacetic acid with 16 ml·l^{-1} phosphoric acid) and 1.25 ml of antioxidant (0.5% propyl gallate and ethylenediaminetetraacetic acid in ethyl alcohol/water, 1 : 1) for about 30 s at 20 Hz (homogenizer Wiggenhauser WT 500; Wiggenhauser, Berlin, Germany). Then it was flushed with 10 ml of trichloroacetic acid extraction solution and filtered. A volume of 5 ml of 2-thiobarbituric acid (0.02 mmol·l^{-1}) was added to 5 ml of filtrate. The samples were heated in a water bath (90 °C) for 40 min to develop the rose-pink colour and then cooled in an ice bath for 10 min. The absorbance of the coloured complex was measured at 530 nm, against a blank, using a UV–VIS spectrophotometer (Shimadzu UV-1800; Shimadzu). The recovery was measured as the assessment of regular samples using addition of standard. A calibration curve was constructed with 1,1,3,3-tetraethoxypropane. The values of TBARS were calculated as milligrams of malondialdehyde (MDA) per kilogram of sample. The analysis was performed for each of 6 animals, for each treatment, in three replicates for each sample.

Volatile compounds analysis

An Alpha MOS Electronic Nose System (Alpha M.O.S., Toulouse, France) was used to measure the volatile compounds of meat. The measurement was conducted using the ultra-fast gas chromatography system with two columns of various polarities (MXT-5 and MXT-1701; Restek, Munich, Germany) with a system of detectors (flame ionization detectors, FID). The headspace technique (HS-100), robotic autosampler and Alpha MOS software (Alpha Soft v. 8.0) for acquisition and processing the data were used.

A amount of 2–3 g of meat in a 20 ml vial was capped with a Teflon-faced silicon rubber cap. Then, vials were placed in the automatic sampler in the headspace system and incubated at 50 °C for 600 s at a centrifugation speed of 8.33 Hz. Analysis of volatile compounds was conducted with two columns simultaneously. A volume of 3000 μl of the gaseous phase was injected in the constant flow on the gas chromatography column with a speed of gas of 125 ml·s^{-1}.

All samples were analysed in five replicates. The method was calibrated using alkanes from *n*-hexane (C_6) to *n*-hexadecane (C_{16}). Volatile compounds were identified on the basis of Kovats' relative retention indices [18] and using AroChemBase database (Alpha M.O.S.) built-in application.

Statistical data analysis

The normality of data distribution was tested using the Shapiro-Wilk test. The statistical differences were tested by Mann-Whitney U-test at a level of significance of $\alpha = 0.05$. Both tests were performed using Statistica Software version 8.0 (StatSoft, Tulsa, Oklahoma, USA). Principal component analysis (PCA) was used to characterize the extracted volatile organic compounds. Alpha Soft software version 8.0 (Alpha Soft, Toulouse, France) built in an Alpha MOS electronic nose system was used for data processing.

RESULTS AND DISCUSSION

Characteristics of meat samples

The pH level was in the range from 5.79 ± 0.08 to 6.26 ± 0.35. The basic composition and fatty acids profiles are presented in Tab. 1 and Tab. 2.

Lipid oxidation

The level of TBARS was in the range from (2.16 ± 0.89) mg·kg^{-1} to (2.94 ± 1.41) mg·kg^{-1} (Tab. 3). There was no effect of the diet on lipid oxidation ($p > 0.05$). The observed values may be interpreted as attributed to the fact that muscles were oxidatively unstable regardless of the applied supplementation.

Vitamin E is a biological antioxidant substance. Its content in meat depends, among other factors, on the diet. The substance acts as a chain-breaking antioxidant, by scavenging lipid peroxyl radicals in cell membranes. The α-tocopherol content in a porcine muscle required to inhibit lipid oxidation is 7–10 mg·kg^{-1} of muscle, which may be achieved

Tab. 1. Basic composition of the semimembranosus muscle.

Fodder group	Moisture [%]	Fat [%]	Protein [%]	Ash [%]	Connective tissue [%]
C	74.3 ± 0.7	3.9 ± 0.8	20.9 ± 0.9	1.2 ± 0.1	1.2 ± 0.2
L1	76.0 ± 0.5	2.3 ± 0.5	21.3 ± 0.4	1.2 ± 0.1	0.8 ± 0.2
L2	72.7 ± 0.9	5.6 ± 0.9	21.0 ± 0.6	1.4 ± 0.1	1.0 ± 0.2

C – standard fodder, L1 – standard fodder with 3% of linseed oil, L2 – standard diet with 3% of linseed oil, 1 mg·kg^{-1} of organic selenium and 100 mg·kg^{-1} of vitamin E.

Tab. 2. Fatty acids composition of semimembranosus muscle.

Fatty acids [g·kg^{-1}]	Fodder group		
	C	L1	L2
C14:0	12.72 ± 0.79	12.81 ± 0.32	12.30 ± 0.71
C16:0	246.92 ± 6.80	243.34 ± 3.20	240.82 ± 6.85
C16:1	27.17 ± 2.44	28.02 ± 2.50	26.92 ± 1.69
C18:0	141.32 ± 5.95	136.19 ± 2.51	140.22 ± 7.10
C18:1 *n*-9	362.40 ± 20.05	386.20 ± 9.34	373.44 ± 16.21
C18:1 *n*-7	34.91 ± 3.60	37.51 ± 2.22	36.24 ± 2.90
C18:2 *n*-6 (linoleic acid)	107.00 ± 8.58	91.13 ± 8.20	97.02 ± 8.38
C18:3 *n*-3 (α-linolenic acid)	16.24 ± 9.90	17.22 ± 7.17	24.12 ± 2.47
C20:4 *n*-6 (arachidonic acid)	6.64 ± 0.78	7.42 ± 0.79	7.41 ± 0.53
C20:5 *n*-3 (eicosapentaenoic acid)	10.88 ± 4.20	8.64 ± 1.20	9.64 ± 2.80
Sum of *n*-6 FA	113.63 ± 8.43	98.6 ± 7.55	104.45 ± 8.11
Sum of *n*-3 FA	28.31 ± 9.45	26.10 ± 6.91	34.02 ± 2.01
Sum of PUFA	141.90 ± 16.16	124.62 ± 13.15	138.46 ± 9.78
Sum of MUFA	424.61± 25.39	451.6 ± 11.42	436.52 ± 18.63
Sum of SFA	402.26 ± 11.64	394.06 ± 2.34	394.34 ± 14.09
Sum of *n*-6 FA / Sum of *n*-3 FA	43.32 ± 11.81	40.08 ± 10.83	30.67 ± 1.63
Sum of PUFA / Sum of SFA	3.53 ± 0.42	3.25 ± 0.31	3.53 ± 0.30

C – standard fodder, L1 – standard fodder with 3% of linseed oil, L2 – standard fodder with 3% of linseed oil, 1 mg·kg^{-1} of organic selenium and 100 mg·kg^{-1} of vitamin E, FA – fatty acids, PUFA – polyunsaturated fatty acids, MUFA – monounsaturated fatty acids, SFA– saturated fatty acids.

by using the supplementation of 100–200 mg·kg^{-1} of fodder [19]. Simultaneously, selenium is an antioxidative trace element, which is involved in activation of the enzyme glutathione peroxidase, and may influence regeneration of vitamin E in biological systems [20].

Vieira et al. [21] concluded that TBARS level of fresh meat was significantly lower than that of meat stored for 90 days at the temperature of –20 °C. Such observations indicated that freezing storage is not sufficient to prevent oxidation and quality changes resulting from this process. Tarladgis et al. [22] suggested that if oxidation is measured as TBARS for conventional raw pork meat, its values should be from 0.5 mg·kg^{-1} to 1.0 mg·kg^{-1}. In frozen meat, TBARS values are in general higher, but they depend on storage time [23, 24]. Antioxidant substances can significantly limit lipid oxidation in meat. It was reported that the dietary vitamin E had an impact on lipid stability of stored meat [25, 26]. Lanari et al. [23] revealed that vitamin E may act protectively on frozen meat for 30 days at a temperature of –20 °C. TBARS values for beef longissimus lumborum muscle, supplemented with vitamin E, were significantly lower than those for controls without supplementation (by about 20%) [23]. However, Hansen et al. [27] determined that the contents of α-tocopherol in pork belly and pork loin had no effect on oxidation decrease during freezing storage. Similar results were demonstrated by other investigators analysing stability of vitamin E in frozen pork chops packed in oxygen-permeable material for 10 months and stored at a temperature of –25 °C [28].

Taking into account the previously mentioned results, it should be stated that the antioxidant po-

Tab. 3. Effect of the dietary treatments on TBARS index of semimembranosus muscle.

Fodder group	TBARS [mg·kg^{-1}]			
	Mean ± *SD*	Median	Minimum	Maximum
C	2.72 ± 1.35	1.96*	1.03	3.47
L1	2.94 ± 1.41	1.78*	1.38	3.76
L2	2.16 ± 0.89	1.72*	1.37	2.88

TBARS – thiobarbituric reactive substances expressed as milligrams of malondialdehyde per kilogram of sample (* – non-parametric distribution). Differences are not statistically significant ($p > 0.05$).
SD – standard deviation, C – standard fodder, L1 – standard fodder with 3% of linseed oil, L2 – standard fodder with 3% of linseed oil, 1 mg·kg^{-1} of organic selenium and 100 mg·kg^{-1} of vitamin E.

tential of meat, including vitamin E content, may be related to fluctuating temperature or time of storage. Explanation for the lack of significant differences of TBARS values in meat samples after fodder supplementation with vitamin E and control could be that α-tocopherol is not able to act as an antioxidant in meat frozen for a long time, due to decreased molecular mobility. An element of the chemical structure of vitamin E is the phytyl side chain, which is responsible for incorporation into and maintaining in cell membranes, however, it may reduce the mobility of the molecule [29]. α-Tocopherol may be immobilized at freezing temperatures, while oxygen, as a smaller molecule, is still mobile. Immobilization of the α-tocopherol molecule usually takes place at temperatures lower than –60 °C [30]. The value of TBARS could quickly increase in meat due to the damage in some cellular structures. In frozen meat, crystals arise which may cause structural changes in the cell membrane. This could be by reason for the release of radicals, oxidative enzymes and other oxidants from destroyed organelles and following promotion of lipid oxidation [30, 31].

Volatile compounds

Raw meat has a gentle odour, which is described as salty, metallic or 'bloody' with a sweet aroma [32]. During the storage, many of the compounds which contribute to aroma are altered and new ones are generated [8, 9]. Meat enriched with PUFAs is especially prone to oxidation of the lipids, which may result in the development of an undesirable odour during prolonged storage [33].

The electronic nose was used for a rapid detection of volatile compounds, depending on the feeding group and resulting oxidation changes, and for discrimination of pork samples. Volatile organic compounds detected in pork meat samples are listed in Tab. 4. Among the observed compounds were amines, aldehydes, esters and alcohols. Some of the mentioned compounds were derived from raw meat, while others were probably the products of lipid oxidation or varied changes resulting from storage. Some volatile compounds which were identified in the frozen and thawed meat were previously also isolated by other authors from raw pork e.g. 3-methyl-1-butanol or propanal [34].

The dietary addition of linseed oil, being rich in PUFAs, and addition of antioxidants (vitamin E and selenium) contributed to differences in the profile of volatile compounds and had an impact on total aroma of meat after 9 months of freezing storage, compared to meat from the control group (Fig. 1). The PCA analysis demonstrated that samples of meat derived from animals fed with a supplemented diet (L1, L2) were characterized by a varying profile of volatiles, in spite of the fact that the identified volatile compounds were mostly the same (Tab. 4). It may indicate that probably the differences of aroma profiles were the result of discrepancy of single compounds or various contents of the same volatile compounds.

The volatile compounds observed in the stored meat may also be formed by alteration and formation processes taking place during lipid peroxidation of *n*-6 or *n*-3 PUFAs. Probably, the level of PUFAs in muscles from various fodder groups

Tab. 4. Tentative identification of volatile compounds in semimembranosus muscle by electronic nose and odour description.

Chemical group of compounds	Volatile compound	Odour description	Fodder groups
Amines	Trimethylamine	ammonical, fishy, pungent	C, L1, L2
Aldehydes	Propanal	ethereal, plastic, pungent	C, L1, L2
	2-Methyl-propanal	burnt, pungent	L2
	Benzenacetaldehyde	floral, grassy, green, hawthorn	C, L1, L2
	Butanal	chocolate, green, malty, pungent	L1, L2
Alkanes	Nonane	alkane, fusel	C, L1, L2
Alcohols	Methanol	alcoholic, pungent	L1
	1-Propanol	alcoholic, fruity, musty, pungent	C, L1, L2
	3-Methyl-1-butanol	alcoholic, balsamic, bitter, burnt, cheese, fermented, fruity, harsh, malty	C, L1, L2
Esters	Methyl butanoate	ester, ethereal, fruity, green, sweet	L1
	Ethyl isobutyrate	nutty	C, L1, L2
	Methyl hexanoate	acetone, fresh, fruity, sweet, thinner	L1, L2

C – standard fodder, L1 – standard fodder with 3% of linseed oil, L2 – standard diet with 3% of linseed oil, 1 mg·kg^{-1} of organic selenium and 100 mg·kg^{-1} of vitamin E.

(Tab. 2) had an impact on changes of odour or even contributed to the appearance of rancid flavours.

Several of the unsaturated fatty acids, such as linoleic and arachidonic acid, are rapidly oxidized to conjugated diene hydroperoxides and, afterwards, degraded to aldehydes or ketones, e.g. 2,4-decadienal, 2-nonenal, 1-octen-3-one, 2,4-nonadienal or 2-octenal. Aldehydes and ketones are the products of early stages of lipid oxidation, that then may be altered to alcohols and esters. The alcohols, such as 1-propanol and 3-methyl-1-butanol, are produced by reduction of the corresponding aldehydes derived from the lipid oxidation process [34]. Esters may be also formed by esterification of some alcohols and organic acids. The volatile esters, such as methyl butanoate and methyl hexanoate, identified in the presented study, may be connected with the oxidation stress taking place in the samples of meat from animals being fed with a fodder supplemented with linseed oil. The mentioned compounds may be responsible for ester, acetone, fresh and even clearly fruity aroma.

The secondary products of lipid oxidation of meat cause rancid, fatty and pungent odour as well as other off-flavours in meat after cold and freezing storage. The short-chain aldehydes tend to be of a sharp and acidic flavour. The development of such flavours was noted in meat by VIEIRA et al. [21] for samples stored for 90 days at –20 °C. In the presented study, propanal was detected, while the sensory description attributed was ethereal, pungent and felt as unpleasant for consumers.

Hexanal and benzenacetaldehyde are considered to be the most important volatile compounds in meat, resulting from the oxidative decomposition of *n*-6 PUFAs, being characterized by rancid flavour that has negative effect on flavour acceptability [12]. TIKK et al. [35] proved that the increase of hexanal content, as a consequence of extended freezing storage, was associated with an increase in TBARS. In the presented study, hexanal was not found, but benzenacetaldehyde was observed in all groups of samples. Other volatile compounds with similar sensory descriptions were also detected. The detection thresholds of many lipid-derived compounds are generally higher than those of water-soluble precursors, e.g. sulphur nitrogen or heterocyclic compounds. Therefore, lipid-derived volatile compounds may overbalance the meat flavour [36].

In the studies of other authors, TBARS values after storage indicated the advancement of the oxidation process in all experimental groups (> 2 mg·kg^{-1} of meat, expressed as MDA) compared with the level in raw meat [22]. According to CHOULIARA et al. [37], when TBARS value was lower than 3 mg·kg^{-1} of meat, it could be perceived as well preserved, in the terms of protection against lipid oxidation and was suitable for consumption, taking into account this aspect. On the other hand, consumers may detect rancidity and off-flavours, which arise during lipid oxidation in meat products, at TBARS values higher than approx. 0.5 mg·kg^{-1}, although the threshold obviously depends on the experience and sensitivity of panellists [38].

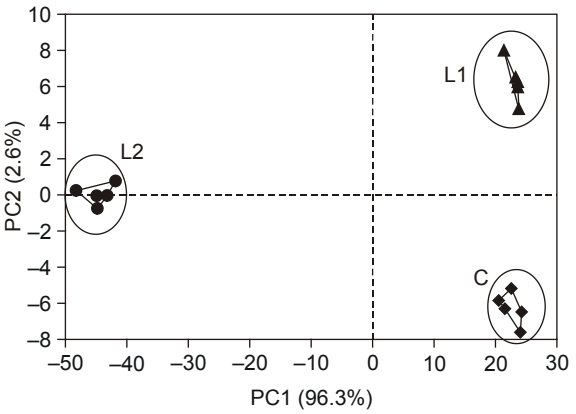

Fig. 1. Principal component analysis of the profiles of volatiles of pork meat after freezing storage.

C – standard fodder, L1 – standard fodder with 3% of linseed oil, L2 – standard diet with 3% of linseed oil, 1 mg·kg^{-1} of organic selenium and 100 mg·kg^{-1} of vitamin E.

CONCLUSIONS

In this study it was revealed that oxidation process is active in frozen pork meat after 9 months of freezing storage. Moreover, lipid oxidation process takes place regardless of animals' diet supplementation. Probably, antioxidants added to forage, were not able to act in frozen meat, due to limitation of mobility or reduction of their activity over time. Supplementation of animals' diet with linseed oil, being a source of PUFAs, and with antioxidants, may have an effect on the profile of volatile compounds of meat after prolonged freezing storage. Oxidative deterioration of lipids of meat in freezing conditions may directly affect occurrence of single volatile compounds and the total aroma of pork, being various depending on the supplementation.

Acknowledgements

Research was realized within a Project BIOFOOD – innovative, functional products of animal ori-

gin No. POIG.01.01.02-014-090/09 co-financed by the European Union from the European Regional Development Fund within the Innovative Economy Operational Programme 2007-2013.

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Received 7 August 2015; 1st revised 17 October 2015; accepted 21 October 2015; published online 1 February 2016.