

Physicochemical properties of meat from rabbits fed rapeseed-oil-enriched diets with different vitamin E levels depending on the packaging and storage method

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The objective of the study was to determine the effect of rapeseed oil (2%) and different levels of an alpha-tocopherol acetate supplement on fatty acid composition and content of vitamin E and thiobarbituric acid reactive substances (TBARS) in the *longissimus dorsi* muscle of rabbits, frozen and stored for a short or long period (14 or 90 days), and to compare the sensory quality of the rabbit meat depending on the packaging and storage method. From 35 to 90 days of age, New Zealand White rabbits (40 animals per group) were fed *ad libitum* complete pelleted diets containing 2% rapeseed oil and 0, 40 or 100 mg/kg alpha-tocopherol acetate as a natural antioxidant. At 90 days, 10 rabbits from each group were slaughtered. The results of the analyses showed that protein content in the *longissimus dorsi* muscle was consistent across groups (19.7-20.4%). No statistically significant differences in water, fat or ash content were observed. After 14 days of frozen storage of the meat, vitamin E levels were lowest in the groups receiving no supplement and increased proportionally to the dietary content of vitamin E in the other groups (3.38-5.24 µg/g). Similar trends persisted after 90 days of frozen storage. Analysis of the effect of PUFA percentage in the rabbit meat on the susceptibility of meat lipids to oxidation revealed a highly significant decrease in TBARS after 90 days of frozen storage in the case of 2% rapeseed oil and 100 mg vitamin E supplement. This is indicative of a slower lipid oxidation rate in the meat. Meat sensory quality scores were found to differ for some traits depending on the storage method (vacuum packing/refrigeration for 14 days or freezing in zip-lock bags for 14 days) and the dietary vitamin E supplement. Vacuum-packed meat had better scores for flavour, aroma, tenderness and juiciness.

KEY WORDS: rabbit meat / rapeseed oil / vitamin E / sensory evaluation / storage method

The dietary value of rabbit meat can be improved by enriching it with nutrients that are beneficial to human health, such as vitamins, microelements or long-chain fatty acids (LC PUFA), mainly of the *n-3* family. These nutrients must be introduced into food because they are often deficient in the human diet. One means of enriching meat with polyunsaturated fatty acids (PUFA) of the *n-3* family is to add vegetable oils to compound feeds [10, 13]. Rapeseed oil has an optimal fatty acid composition, with the lowest content of those that are detrimental to human health and the highest content of essential unsaturated fatty acids (EFA) of the *n-3* family. Moreover, it has an optimal 2:1 ratio of *n-6* to *n-3* fatty acids.

Modification of fatty acid composition to increase the proportion of polyunsaturated fatty acids may have a negative effect on the sensory quality and oxidative stability of meat and its suitability for processing. Due to oxidation of meat lipids numerous compounds are formed which are responsible for a rancid, undesirable odour and flavour, unacceptable to consumers [16]. Oxidation of meat lipids also has a detrimental effect on its colour, texture and nutritional value, due to destruction of EFA and vitamins. Research aimed at prolonging the shelf-life of meat products indicates the need to protect fat from oxidation. This can be achieved with synthetic or natural antioxidants, such as α -tocopherols, ascorbic acid, carotenoids, phenolic acids and certain organic acids [5].

One of the best biological antioxidants is considered to be vitamin E (tocopherol), which neutralizes peroxide free radicals responsible for damage to cell structures and DNA and for lipid oxidation [21].

The rate of oxidation of meat lipids is significantly influenced by external factors, including light, oxygen and temperature. Light energy substantially shortens the induction period of lipid oxidation and is one of the strongest activators of free radical generation. Temperature, like light energy, significantly determines lipid oxidation by stimulating free radical formation. Low positive (4°C) and negative (-10°C) temperatures of fat storage prolong the induction period, but do not eliminate these changes [7, 18].

The aim of the study was to determine the effect of varied α -tocopheryl acetate supplementation (group I – no supplement, group II – 40 mg/kg, group III – 100 mg/kg) of compound feed with 2% rapeseed oil on fatty acid composition, vitamin E content and content of thiobarbituric acid reactive substances in the frozen longissimus dorsi muscle of rabbits following a short or long storage period, and to compare the sensory quality of the meat depending on the packing and storage method.

Material and methods

The study on rabbits was conducted in 2011-2012 on a private rabbit farm in the Podkarpackie Voivodeship. Meat analyses were performed at the Central Laboratory of the National Research Institute of Animal Production.

The material for the experiment consisted of 120 New Zealand White rabbits (60 ♂ and 60 ♀). After weaning from their mothers (35 days), weighing, and individual tattooing, the rabbits were housed in two-storey cages of wire mesh, with four rabbits of each sex in each, in a heated building. Hygienic and technological conditions were in accordance with general principles for this type of production.

From days 35 to 90 of life, the rabbits (40 per group) were fed *ad libitum* pelleted total mixed rations with 2% rapeseed oil, enriched with vitamin E in the form of α -tocopheryl acetate, according to the following experimental design:

- group I – compound feed supplemented with rapeseed oil (2%), without vitamin E
- group II – compound feed supplemented with rapeseed oil (2%) + 40 mg/kg vitamin E
- group III – compound feed supplemented with rapeseed oil (2%) + 100 mg/kg vitamin E

The composition of the compound feed for the rabbits was as follows: dried alfalfa (25%), wheat bran (18.6%), barley meal (22%), maize meal (14%), soybean extraction meal (14%), Pollac milk replacer (2%), fish oil (2%), feed phosphate (1%), NaCl (0.4%) and a vitamin and mineral supplement (1% premix for rabbits) with a coccidiostat (robenidine). The vitamin and mineral supplement, produced for the experiment, contained vitamins A – 1,000,000 IU/kg, D₃ – 150,000 IU/kg, K₃ – 52 mg/kg, B₁ – 50 mg/kg, B₂ – 400 mg/kg, B₃ – 2,000 mg/kg, B₅ – 786 mg/kg, B₆ – 50 mg/kg, B₁₂ – 1,500 mcg/kg, biotin – 10,000 mcg/kg, choline chloride – 12,500 mg/kg, and folic acid – 57 mg/kg; and the minerals Fe – 5,000 mg/kg, Mn – 7,500 mg/kg, Cu – 750 mg/kg, Zn – 5,000 mg/kg, I – 100 mg/kg, Co – 100 mg/kg, Se – 20 mg/kg, and Ca – 33.2%.

The rapeseed oil, obtained from Zakłady Tuszczowe 'Kruszwica' S.A. in Kruszwica, was guaranteed by the producer to contain 27.6% linoleic acid (C18:2 *n-3*) and 10.2% linolenic acid (C18:3 *n-3*).

The compound feeds were balanced according to experimental procedures and the nutrient content was calculated according to 'Dietary recommendations and nutritional value of animal feeds' [27]. The mixtures were balanced in terms of the level of amino acids and minerals according to recommendations by Lebas [12] for this group of animals. Vitamin E content in the samples of prepared feed was as follows: group I – 21.57 mg/kg (natural content of vitamin E from the feed components), group II – 53.99 mg/kg, and group III – 95.24 mg/kg.

After the rearing period (90 days of age), 10 rabbits were randomly selected from each group. The animals were fasted for 24 hours and then slaughtered. Slaughter was carried out according to currently binding methodology, in identical technological conditions for all groups.

After 24-hour chilling (temp. 4°C) both longissimus dorsi muscles were removed from the rabbit carcasses for further analysis after being divided into 5 samples of equal weight.

In the first sample, the quality of the meat was analysed, including the following groups of characteristics: pH at 45 minutes after slaughter (pH_{45}), pH after 24-hour chilling ($\text{pH}_{24\text{h}}$), and proximate chemical composition (content of water, dry matter, protein and fat).

The second muscle sample was deep-frozen for 14 days and the third for 90 days (temp. -20°C) in sealed plastic zipper food storage bags made of HDPE 14/4/32 foil. After the frozen storage period the higher fatty acid composition and the content of vitamin E and thiobarbituric acid reactive substances (TBARS) were determined in the meat lipids.

The fourth muscle sample was deep-frozen for 14 days. The muscles were packed in identical plastic bags of the type described above and then placed in a Mińsk 15M freezer chamber, at -20°C .

The remaining muscles (fifth sample) were vacuum-packed in 200x130 mm PET-PVdC/ CPP laminate shrink bags by PABEX made of polyester film and unoriented polypropylene with a high gas barrier capacity (permeability for $\text{O}_2 = 8.73 \text{ cm}^3/\text{m}^2/24\text{h}/0.1 \text{ MPa}$, for $\text{CO}_2 = 23.89 \text{ cm}^3/\text{m}^2/24\text{h}/0.1 \text{ MPa}$, and for $\text{H}_2\text{O} = 4.25 \text{ g}/\text{m}^2/24\text{h}$). The samples were vacuum-packed in a TEPRO chamber vacuum-packaging machine, model PP-5MG (0.15), and then placed in a Mińsk 15M cooling chamber, in atmospheric air, at a temperature of 4°C maintained automatically by a thermostat for 14 days of storage. The relative humidity of the air in the chamber was 40-50%.

After the cold and frozen storage period, quality analysis of the meat was performed. The frozen meat (the fourth sample) was thawed at $+1^{\circ}\text{C}$ for 24 h.

The pH of the meat was measured in the middle part of the longissimus dorsi muscle with a CyberScan PH 10 PMMV microprocessor pH meter.

Water content was determined according to PN-ISO 1442:2000, content of dry matter by the SOP M.011 gravimetric method (Standard Operation Procedure, M – procedure number at the central laboratory of the National Research Institute of Animal Production), fat by the Soxhlet method according to PN-ISO 1444:2000, and protein by the Kjeldahl method according to PN-75/A-04018.

Lipid content was determined by extraction with a chloroform and methanol solution according to Folch et al. [6]. Methyl esters of fatty acids were prepared according to procedure PN-EN ISO 12966-2:2011. Fatty acids were separated and identified in a VARIAN 3400 gas chromatograph with a flame ionization detector (FID), using an Rtx 2330 capillary column with dimensions of 105 m x 0.32 mm x 0.2 μ . The conditions of the analysis were as follows: column temperature $140\text{-}210^{\circ}\text{C}$, injector temperature 250°C , carrier gas helium (flow rate 3 ml/min), size of injected samples 0.7 μl . Acid standards from Larodan Fine Chemicals AB were used to determine CLA, and standards from Sigma-Aldrich for the remaining acids.

The degree of lipid oxidation (TBARS) was determined by method P 025:2001, according to Pikul [18], as mg malondialdehyde per kg of meat.

Vitamin E was determined by liquid chromatography with a Merck-Hitachi chromatograph on a LiChroCART™ 250-4 Superspher™ 100 RP-18 column (4 microns).

Sensory evaluation was carried out by heating the samples in water containing sodium chloride (0.6%, 1:2 ratio of water to meat) to attain a temperature of 85°C in the centre of the sample. Following heat treatment the samples were cooled to room temperature and then cut into slices (of about 20 g) and placed in plastic boxes. The samples were coded and presented for evaluation in random order. Sensory evaluation was conducted by a panel of five individuals with verified sensory sensitivity, trained according to standard PN-EN ISO 8586-2:1996. The following features were evaluated: aroma, juiciness, tenderness, flavour, and overall assessment. A 5-point scale was used. The evaluation was carried out in a room with a temperature of 20°C, in daylight. Each member of the panel received unsweetened hot tea between samples to neutralize the flavour.

Cooking loss was determined according to the following formula:

$$\text{Cooking loss (\%)} = \frac{\text{sample weight before cooking} - \text{sample weight after cooking} \times 100}{\text{sample weight before cooking}}$$

Statistical computations were performed in the Statistica 9.1 PL statistics package using one-way or two-way analysis of variance with interaction defining the effect of the level of vitamin E in the feed ration and the storage time and means of packaging and storage. Significance of differences between means in groups was estimated by Duncan's multiple range test. Mean values (\bar{x}) for each characteristic are given in the tables.

Results and discussion

The pH₄₅ and pH_{24h} values in the meat of all groups were within the range for normal meat without symptoms of unnatural conversion of muscle to meat (Tab. 1). The pH value, as a measure of meat quality, is an indicator of the progression of post-slaughter changes. In the case of rabbit meat, at 45 minutes after slaughter the mean pH should range from 6.1 to 6.8, and after 24 hours from 5.4 to 5.8 [14, 15]. The rate of the pH decrease depends on the animal's condition at slaughter; it declines faster if the animal was healthy, well-rested and unstressed.

Similar values for initial pH measured 45 minutes after slaughter were noted for this breed of rabbits by Kowalska and Bielański [9] – 6.57, Kowalska et al. [11] – 6.60 and Szkucik and Pysz-Lukasik [25] – 6.21.

Cavani et al. [1] obtained a pH_{24h} value of 5.79 for rabbit meat, while Kowalska and Bielański [9] reported a value of 5.70 and Szkucik and Pysz-Lukasik [24] 5.71. According to

the study by Szkucik and Pyz-Łukasik [24], rabbit muscle tissue becomes fully acidified just 12 hours after slaughter. This process in rabbits is much more rapid than in cattle or pigs, but slower than in broiler chickens [17, 19].

The protein content in the longissimus dorsi muscle was similar in all groups, ranging from 19.7% to 20.4%. The results obtained are lower than those reported by other authors, such as Szkucik and Libelt [23] – 23.91%, Szkucik and Pyz-Łukasik [25] – 23.9%, Kowalska and Bielański [9] – 25.43%, Cygan-Szczegielniak et al. [4] – 23.6%, and Pla et al. [20] – 22.1%. Xiccato [26], on the basis of studies by various authors, states that the protein level in rabbit meat may range from 18.6% to 21.9%. Differences in the protein level depend on the breed and age of the animals, the composition of the feed, the part of the carcass, and preparation for slaughter.

One of the main factors determining the sensory quality of meat is intramuscular fat, which in rabbits contains 47.3% saturated fatty acids (SFA), 35.5% monounsaturated fatty acids (MUFA) and 17.2% polyunsaturated fatty acids [10]. In the present study, as in the case of protein, this component also did not differ significantly between experimental groups, and ranged from 1.90% to 2.11%. Lower intramuscular fat content in the longissimus dorsi muscle was noted by Pla et al. [20] – 1.20%, Łapa [14] – 1.71%, Maj et al. [15] – 1.60%, and Szkucik and Libelt [24] – 1.12%, while higher content was reported only by Kowalska and Bielański [9] – 2.11%.

The water content in the longissimus dorsi muscle did not differ significantly between groups and ranged from 73.4% to 73.7%, which corresponds with results reported by Łapa [14], Szkucik and Libelt [24] and Kowalska [8].

Two-way analysis of variance showed that the vitamin E level and the meat storage time (14 and 90 days) influenced the content of selected fatty acids (Tab. 2). With regard

Table 1
Physicochemical properties of rabbit muscles (*musculus longissimus dorsi*)

Parameter	Group		
	I	II	III
Dry matter (%)	27.3	27.3	27.7
Protein (%)	19.8	19.7	20.4
Water (%)	73.5	73.7	73.4
Fat (%)	2.11	1.96	1.90
pH ₄₅	6.57	6.66	6.62
pH _{24h}	5.73	5.78	5.71

Table 2
Effect of dietary levels of vitamin E and storage time (14 and 90 days) on the content of some fatty acids

Factor	Fatty acids													
	C18:0	C18:1	C18:2	C18:3	C20:4	C20:5	C22:6	SFA	UFA	UFA/ SFA	PUFA n-6	PUFA n-3	PUFA n-6/n-3	
Level of vitamin E														
0 mg/kg	6.31 ^a	27.9 ^a	21.4 ^a	5.05	1.85 ^a	0.08 ^a	0.15 ^a	40.9 ^a	59.1 ^a	1.47 ^a	23.4 ^a	5.28 ^a	4.39 ^{a,b}	
40 mg/kg	6.15 ^a	27.6 ^a	19.5 ^b	5.01	1.08 ^b	0.05 ^a	0.04 ^b	43.9 ^b	56.0 ^b	1.31 ^b	20.6 ^b	5.10 ^a	4.04 ^b	
100 mg/kg	5.66 ^b	23.6 ^b	19.0 ^b	5.15	1.06 ^b	0.15 ^b	0.44 ^c	43.7 ^b	56.3 ^b	1.33 ^b	20.1 ^b	5.74 ^b	3.48 ^b	
Storage time														
14 days	6.39 ^a	27.6	23.8 ^a	5.84 ^a	1.59 ^a	0.08	0.24 ^a	38.2 ^a	61.8 ^a	1.62 ^a	25.5 ^a	6.18 ^a	4.15 ^a	
90 days	5.69 ^b	25.1	16.2 ^b	4.34 ^b	1.06 ^b	0.10	0.17 ^b	47.5 ^b	52.5 ^b	1.11 ^b	17.3 ^b	4.57 ^b	3.79 ^b	
Interaction level of vitamin E and storage time														
	0.07	0.07	0.35	0.25	0.99	0.81	0.24	0.24	0.24	0.53	0.30	0.50	0.79	

Values denoted by different letters within factors differ statistically significantly: A, B, C at $p \leq 0.01$; a, b at $p \leq 0.05$

to vitamin E, significant differences between groups at $p \leq 0.01$ were found for linoleic acid (C18:2), arachidonic acid (C20:4), EPA (C20:5), DHA (C22:6), SFA, UFA, UFA/SFA, PUFA, PUFA *n-6*, PUFA *n-3*, and PUFA *n-6/n-3*, and at $p \leq 0.05$ for stearic acid (C18:0) and oleic acid (C18:1). The meat storage time had a significant ($p \leq 0.01$) effect on the content of stearic (C18:0), linoleic (C18:2), linolenic (C18:3), and arachidonic (C20:4) acids, DHA (C22:6), SFA, UFA, UFA/SFA, PUFA, PUFA *n-6*, PUFA *n-3*, and PUFA *n-6/n-3*. No significant interactions were noted for the characteristics. The significantly ($p \leq 0.01$) lower *n-6/n-3* ratio in the group receiving vitamin E in the amount of 100 mg/kg of feed should be regarded as beneficial to the consumer.

Selim et al. [22], using different levels of vitamins E (0, 40, 80 mg/kg) and C (0, 200, 400 mg/kg) or increased amounts of both vitamins (40 mg/kg E and 200 mg/kg C, 80 mg/kg E and 400 mg/kg C) in feed rations for rabbits, tested the changes taking place during storage of the meat for 10 or 20 days at -20°C , in terms of vitamin loss and the profile of higher fatty acids. They noted a significant ($p \leq 0.01$) effect of vitamin E on the content of PUFA, particularly linoleic and linolenic acids, in the meat kept in frozen storage; the content of these acids after both 10 and 20 days of storage increased as compared to the control (without vitamin supplementation). Corino et al. [3] showed that increased feed supplementation with vitamin E (240 mg/kg) caused a significant ($p \leq 0.01$) increase in oleic acid and thus in total monounsaturated fatty acids, and a lower level of polyunsaturated fatty acids, as compared to the control group receiving 60 mg of vitamin E/kg of feed.

Table 3

TBARS (mg malonaldehyde/kg sample) in rabbit meat after 14 and 90 days of storage and vitamin E level ($\mu\text{g/g}$), and their percentage loss during frozen storage

Parameter	Group		
	I	II	III
TBA-RS 14 days	0.30	0.28	0.27
TBA-RS 90 days	0.72 ^A	0.64 ^a	0.55 ^{Bb}
Vitamin E*	3.38 ^{Aa}	3.99 ^a	5.24 ^B
Vitamin E**	2.77 ^A	3.36 ^B	5.23 ^C
% vitamin E loss	18.0	15.8	0.2

Values denoted by different letters in rows differ statistically significantly: A, B, C at $p \leq 0.01$; a, b at $p \leq 0.05$

*Vitamin E content in muscle tissue after 14 days of frozen storage

**Vitamin E content in muscle tissue after 90 days of frozen storage

Table 4
Effect of dietary levels of vitamin E and the packaging and storage method on the sensory evaluation of rabbit meat

Factor	Sensory evaluation of rabbit meat						
	Cooking loss (%)	Aroma ^{OS}	Flavour ^{OS}	Juiciness ^{OS}	Tenderness ^{OS}	Overall sensory quality ^{OS}	
Level of vitamin E							
0 mg/kg	27.6 ^{Ab}	4.62	4.48 ^{Ab}	4.33 ^A	4.38 ^A	4.46 ^A	
40 mg/kg	26.3 ^B	4.63	4.57 ^b	4.40 ^A	4.54 ^B	4.53 ^B	
100 mg/kg	26.4 ^b	4.56	4.61 ^B	4.51 ^B	4.64 ^C	4.58 ^C	
Packaging and storage method							
Δ	29.1 ^A	4.52 ^A	4.45 ^A	4.22 ^A	4.47 ^A	4.41 ^A	
ΔΔ	24.5 ^B	4.69 ^B	4.66 ^B	4.61 ^B	4.60 ^B	4.64 ^B	
Significance of interaction vitamin E level x packaging and storage method	0.87	0.73	0.02	0.47	0.75	0.57	

Δ – freezing in plastic zip lock bags, ΔΔ – vacuum packaging

OS – sensory scores of meat: 2 points – poor quality, 3 – adequate quality, 4 – good quality, 5 – very good quality

Values denoted by different letters differ within factors statistically significantly A, B, C at p≤0.01; a, b at p≤0.05

The lowest values for the TBARS index after 90 days of frozen storage were noted in the meat from group III (0.55), receiving 100 mg/kg of vitamin E in their feed, and the highest in group I (0.72), which did not receive vitamin E (Tab. 3). The differences noted between groups I and III were significant ($p \leq 0.01$). The low TBARS index after 90 days of storage in group III may be linked to a high level of vitamin E in the meat, as it is a natural antioxidant protecting lipids against oxidation processes.

In the experiment, the increasing addition of vitamin E to the feed increased its content in the meat. After 14 days of storage significant differences were observed in the content of vitamin E between groups I and III at $p \leq 0.01$, and between groups I and II at $p \leq 0.05$. After 90 days the differences between all groups were highly significant.

Two-way analysis of variance showed that the level of vitamin E and the means of meat packaging and storage affected cooking loss and the sensory quality evaluation of the meat (Tab. 4). The varied level of vitamin E in the feed ration had a significant effect ($p \leq 0.01$) on the cooking loss between groups I and II, on flavour between groups I and III, juiciness between groups I and II and group III, and on tenderness and overall sensory quality between group I and groups II and III. Significance at $p \leq 0.05$ was noted for cooking loss between groups I and III and for flavour between groups I and II. The means of packaging and storage had a significant effect, at $p \leq 0.01$, on all characteristics tested. Significant interactions (vitamin E level x means of packaging and storage) at $p \leq 0.05$ were noted in the case of flavour.

Zhang et al. [28] consider the optimal level of supplementation with α -tocopheryl acetate to be 80 mg/kg, as this amount significantly improved the tenderness of meat and delayed lipid oxidation in the meat. Chwastowska-Siwiecka et al. [2] found that muscles vacuum-packed and placed in cold storage for 10 days obtained higher scores for sensory quality characteristics than meat packed in an atmosphere of protective gases and frozen meat.

A significant problem in the case of meat stored in cold and frozen conditions and then subjected to a high temperature is losses in weight due to drip loss (during storage and cooking). In the experiment significantly lower ($p \leq 0.01$) cooking loss was noted in the vacuum-packed meat than in the meat that was frozen. Chwastowska-Siwiecka et al. [2] found that cooking loss was lower in vacuum-packed rabbit meat than in meat packed in an atmosphere of protective gases, irrespective of cold storage time (23.9% and 27.1%, respectively).

The following conclusions were drawn from the results of the study:

- Protein content in the longissimus dorsi muscle was similar in all groups; no differences were noted in water or fat content.
- After 14 days of storage in freezer conditions the lowest vitamin E content was noted in the meat of rabbits that did not receive a vitamin E supplement, while in the others the increase depended on its level in the feed ration; similar tendencies persisted after 90 days of frozen storage.

– When 2% rapeseed oil and 100 mg vitamin E were added to the compound feed, after 90 days of frozen storage there was a highly significant decrease in the TBARS value in the meat, indicating a slower rate of oxidation of meat lipids.

– The sensory quality assessment of the meat differed for some characteristics depending on the amount of vitamin E in the feed and the means of packaging and storage; the vacuum-packed meat received a more favourable evaluation.

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