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The effect of antioxidants on the quality and oxidative stability of animal fats

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Abstract

The aim of the work was to determine the influence of natural and synthetic antioxidants on the quality and stability of animal fats. On the samples, with and without the addition of natural antioxidants (extract of sage, rosemary and ginger) added in the fats at a concentracion of 0.2 % and synthetic antioxidants (PG-propyl galate, BHA-butylhydroxyanisole and BHT-butylhidroxytoluene) added in the fats at a concentracion 0.01 % the values of the peroxide value and the content of free fatty acids were tested. The oxidative stability of fats was determined by the sustainability test at 98 °C during 7 days. Every 24 hours, the peroxide value and free fatty acids were examinedid for all samples of beef, sheep and goat fat. The results of the research show that the applied antioxidants successfully stabilized all animal fats compared to the control sample. Analysis of the composition of fatty acids of base samples (without the addition of natural antioxidants) shows that palmitic, stearic and oleic fatty acids are the most abundant in animal fats. The highest total SFA value was found in sheep fat (66.85 %), and the lowest in goat fat (56.57 %). In beef fat, the total SFA content was 59.26 %. Stearic acid is the most abundant in sheep fat (37.75 %), while beef and goat fat had lower and approximately equal values (27.93 % and 26.91 %). After staeric fat, palmitic fat is the mostabundant in beef fat with 27.78 %, followed by 25.25 % in sheep fat and 24.91 % in goat fat. Of the monounsaturated acids (MUFA), the most abundant in goat fat was oleic acid (38.16 %), while it was somewhat lower in beef (36.61 %), and the lowest in sheep (27.60 %). Total unsaturated fatty acids (UFA) are most abundant in goat fat (43.51 %), followed by beef (40.82 %), while they are significantly less present in sheep's fat (32.95 %). Among polyunsaturated acids (PUFA), the highest content of linoleic acid was found in sheep fat (4.19 %). In beef fat, linoleic acid was 60 % lower compared to sheep fat (0.76 %), and 10% higher compared to goat fat (1.89 %). Based on the content of individual fatty acids and the content of certain groups of fatty acids: SFA, UFA, MUFA, PUFA, the nutritional indices of the tested fats were calculated. It was concluded that the tested samples of beef, sheep, and goat fat without the addition of antioxidants can not be declared as products of high nutritional value. The nutritional value expressed through the PUFA/SFA ratio for beef fat was 0,01, and sheep fat and goat fat 0.06.

Keywords: animal fats, quality, oxidative stability, antioxidants, sustainability test.

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Introduction

Animal fats (beef, sheep and goat tallow) are obtained from adipose tissue usually by dry and wet melting processes. Fats are compounds of saturated and unsaturated fatty acids and glycerol alcohol, which belongs to high molecular weight alcohols (Čorbo, 2008). They contain about 18 % of saturated triglycerides that do not melt in the mouth and create an unpleasant feeling of stickiness (Grompone, 1989). In the diet of the population of Bosnia and Herzegovina, animal fats are traditionally represented, especially goat fat. The properties possessed by beef, sheep and goat fat tissue depend on several factors (breed, age, sex, way of feeding the animal and others (Muradbašić, 2009). Nowadays, animal fats and their derivatives are increasingly used for various industrial purposes, and not only for food preparation (Moslavac et al., 2019). Depending on the presence of a double bond in the aliphatic hydrocarbon chain, we distinguish groups of saturated fatty acids (SFA) and unsaturated (UFA), and within unsaturated, depending on the number of double bonds present in the chain, mono and polyunsaturated fatty acids (MUFA and PUFA) are distinguished. In the diet, one should strive to increase MUFA and PUFA acids. For these reasons, it is necessary to determine the composition of fatty acids in fats in order to assess their nutritional quality, which also affects the nutritional quality of the final product (Grahovac et al., 2022). Animal fats are dominated by palmitic, stearic and oleic acids, which give them a plastic structure, especially beef tallow (Grompone, 1989). The composition of tallow can vary, depending on which part of adipose tissue is used for fat production and on diet (Hauff and Vetter, 2010). Beef tallow, like sheep and goat tallow, is subject to oxidative spoilage and quality changes during production, storage and heat treatment. During the storage of animal adipose tissue and their fat, appropriate autolytic processes occur, which affects the reduction of fat quality. These processes usually go in the direction of oxidation and hydrolytic decomposition. Hydrolysis of fat is a breakdown that results in an increase in the acidity of fat, the proportion of free fatty acids increases. Oxidative spoilage of fat is the most common type of spoilage, and is caused by the action of oxygen from the air on unsaturated fatty acids (Moslavac et al., 2019). Adipose tissue is susceptible to oxidation and microbiological processes due to the presence of the enzyme lipase, which is inactivated at elevated temperatures. For this reason, these fats are poorly sustainable, and after slaughtering the animals, the fatty tissue must be immediately processed or stored at a low temperature (Čorbo, 2008). The stability of fat depends on the type of fat, that is, the composition of fatty acids, as well as the presence of other ingredients that show antioxidant activity (Moslavac et al., 2019). Animal fats are susceptible to spoilage due to the low content of tocopherol, which is a natural antioxidant (Hamilton, 1999). The products resulting from the spoilage of animal fats give an unpleasant smell and taste to the fat, which impairs the sensory properties of the fat (Broadbent and Pike, 2003). Adding antioxidants (substances that inhibit and slow down the process of autoxidation) can improve the fat's resistance to oxidative deterioration. Today, it is known that various natural plant extracts and synthetic antioxidants protect oils and fats from oxidative deterioration. In recent decades, animal fats and their derivatives are increasingly used for various industrial purposes and not only for human consumption (Čorbo, 2008).

Materials and methods

Sampling and preparation of samples for analysis

Kidney beef, sheep and goat adipose tissue or tallow were used in this work. Basic ie. base samples, kidney beef, sheep and goat adipose tissue or tallow (about 2 kg), were taken immediately after the slaughter of animals from slaughterhouses by individual breeders from the area of Sarajevo Canton. Fresh beef, sheep and goat adipose tissue was taken for analysis, from animals of the same breed, approximately the same age and female gender. Samples for analysis were delivered in a fresh, solid and compact state. Natural antioxidants that were used: extract of sage, rosemary and ginger in a concentration of 0.2 %, and synthetic antioxidants: propyl gallate (PG), butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) in a concentration of 0.01 %.

Methods

In order to test the quality of fats and their identification on the basis of unusual fatty acids that are sometimes not characteristic for a certain

type of product, in this case beef, sheep and goat fat, in all tested samples at the beginning, i.e. immediately after fat tissue/tallow melting, the following was determined: the composition of saturated and unsaturated fatty acids, by previous sample preparation, i.e. by saponification and esterification of fatty acids read on GLC, the content of free fatty acids by the standard titration method BAS EN ISO 6858, 2003, the peroxide value by the Wheeler method BAS EN ISO 3960, 2001. To determine the composition of fatty acids gas chromatograph GC/MS-6890 II, Hewlett-Packard with mass selective detector (MSD) 6890 II: FKKT-UL was used (Härtig, 2008). Capillary columns: SP 2560, 100 m x 0,25 mmlD, 0,20 µm; Detector: flame ionization detector FID, 260 °C, separation 100.1; Carrier gas: helium, 20 cm3/sec. After melting, the obtained fat was separated from the cracklings using a hand press. Melted and partially cooled fat in a liquid state is evenly distributed in glass laboratory beakers of 250 ml. The obtained fat does not have a neutral taste. The grease has a firm lubricating consistency and a yellowish-white color. After partial cooling, i.e. before the fat solidifies completely, the process of weighing and adding different antioxidants in different concentrations to each sample was started. Each sample individually, as well as the control samples, are marked with appropriate marks. Fat samples, completely cooled to room temperature, were stored in a refrigerator at +4 to +5 °C until the moment of analysis. From the beginning to the end of the treatment, sustainability was examined, i.e. oxidation stability in all samples of beef, sheep and goat fat, with or without the addition (in the case of control samples) of different antioxidants and in different concentrations, viability test at a constant temperature of 98 °C for a period of 7 days. In the mentioned period, every 24 hours (i.e. after 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours and 168 hours), the changes that occurred using standard titration methods were monitored, namely: peroxide value (PV) and free fatty acids (FFA). The Past 3.15 program was used for statistical data processing (Hammer et al., 2001). In order to determine a statistically significant difference in the values of peroxide value and free fatty acids, under the influence of the type of antioxidant and sampling time, a two-factor analysis of variance was applied, and in the case of statistically significant differences, the Tukey test was used (significance level α=0.05). The aim was to determine whether the type of added antioxidants and the sampling time affect the oxidative stability of beef, sheep and goat tallow samples. For the correlation and presentation of the results multivariate data analysis was used - analysis of the basic components or PCA analysis.

Results and discussion

The results of the examination of the composition of fatty acids are shown in table 1. The total content of saturated acids (SFA) in the tested samples ranged from 56.57 % to 66.85 %. The highest total SFA value was found in sheep fat (66.85 %), and the lowest in goat fat (56.57 %). In beef fat, the total SFA content was 59.26 %. Stearic acid was more abundant in sheep fat (37.75 %), while beef and goat fat had lower and approximately equal values (27.93 % and 26.91 %). After stearic fat, palmitic fat is the most abundant in beef fat with 27.78 %, followed by 25.25 % in sheep fat and 24.91 % in goat fat. Of the monounsaturated acids (MUFA), the most abundant in goat fat was oleic acid (38.16 %), while in beef it was slightly lower (36.61 %) and the lowest in sheep (27.60 %). Total unsaturated fatty acids (UFA) are most abundant in goat fat (43.51 %), followed by beef (40.82 %), while they are much less present in sheep's fat (32.95 %). The highest content of polyunsaturated acids (PUFA) was found in sheep fat (4.19 %). In beef fat, the PUFA content was 60% lower compared to sheep fat (0.76 %), and 10 % higher compared to goat fat (1.89 %). Of the polyunsaturated acids (PUFA), the most abundant in goat and beef fat samples was linoleic acid (1.77 % and 0.74 %), while in the sheep fat sample α-linolenic acid (2.22 %). Moslavac et al., (2019) report a lower value of stearic fatty acid in beef fat, 25.01 %, compared to our results (27.93 %). The same author, according to his research, states a slightly lower value of 35.38 % for oleic acid compared to our research (36.51 %). According to research by Čorbo (2000), the most abundant saturated acids in lamb fat are palmitic acid (from 19.29 % to 24.48 %) and stearic acid (19.65 % to 27.06 %). According to the same author, the most abundant of unsaturated fatty acids is oleic, from 29.76 % to 42.16 %, and other fatty acids are significantly less present. Čorbo (2000) states that in all samples of kidney fat of sheep and rams, the most abundant saturated fatty acids are: stearic acid from 19.05 % to 35.90 % and palmitic acid

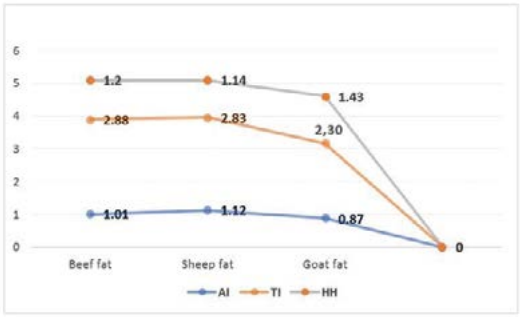

Figure 1 Nutritional indices in the examined fat samples (obtained on the basis of groups and relationships between groups)

from 19.03 % to 22.97 %. The same author states that of the unsaturated fatty acids, oleic acid is the most abundant, at 33.06 % to 42.25 %.

Kegalj et al., (2011) state that the share of certain fatty acids in the intramuscular fat of goat meat was: lauric (29.08 %), myristoleic (23.01 %), arachidonic (20.63 %). For the sake of comparison, the aforementioned authors state in their paper that the proportion of palmitic acid was very low and amounted to 5.55 %, which is a much lower

Table 1 Composition of fatty acids, relationships between specific groups and nutritional indices of the tested fats (without the addition of antioxidants)

Fatty acid (%)	Beef fat	Sheep fat	Goat fat
C 8:0	0.02	0.09	0.09
C12:0	0.04	0.06	0.10
C14:0	3.34	2.66	3.16
C15:0	0.13	0.32	0.49
C16:0	27.78	25.25	24.91
C17:0	0.02	0.72	0.91
C18:0	27.93	37.75	26.91
C14:1	0.51	0.52	0.37
C15:1	0.15	0.20	0.16
C16:1	2.76	0.25	2.44
C17:1	0.09	0.15	0.35
C18:1cis	36.61	27.60	38.16
C18:2 n-6, cis	0.74	1.97	1.77
C18:3 n-3	0.02	2.22	0.12
C20:4 n-6	0.02	0.02	0.02
C20:5n-3	0.02	0.02	0.02
SFA	59.26	66.85	56.57
UFA	40.82	32.95	43.51
MUFA	36.61	27.60	38.16
PUFA	0.76	4.19	1.89
PUFA/SFA	0.01	0.06	0.03
MUFA/SFA	0.67	0.41	0.67
ω6/ω3	37.0	0.89	14.75
AI	1.01	1.12	0.87
TI	2.88	2.83	2.30
HH	1.20	1.14	1.43

Legend: SFA-saturated fatty acids; UFA-unsaturated fatty acids; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids; AI-atherogenic index; TI-thrombogenic index; HH- hypocholesterol/hypercholesterol index

value compared to the proportion of palmitic acid in the goat tallow examined in this paper (24.91 %). Based on the content of individual fatty acids and the content of certain groups of fatty acids: SFA, UFA, MUFA, PUFA, the nutritional indices of the examined fats were calculated and presented graphically (Fig 1). According to the recommendations of the UK Department of Health (1994) and Wood et al. (2004) the values of the ratio of certain groups of fatty acids PUFA/SFA should be greater than 0.4 in order for the food to be declared as a product of high nutritional value. Based on the above, and according to the results from Table 1, it can be concluded that the examined samples of beef, sheep and goat fat without the addition of antioxidants cannot be declared as products of high nutritional value. The nutritional value expressed through the PUFA/SFA ratio for beef fat was 0.01, and for sheep fat 0.06 and goat fat 0.03. AI and TI were calculated based on the formulas proposed by Ulbricht and Southgate (1991), while the HH index was calculated based on the formula proposed by Santos-Silva et al. (2002). Among the tested fats, the AI value was the lowest in goat fat, 0.87, which can be justified by the high content of oleic acid. Sheep fat had the highest AI value (1.12), which is a consequence of the high content of palmitic and stearic acids. TI values for the tested fat samples ranged from 2.30 to 2.88. TI in goat fat was the lowest at 2.30 and the highest in beef, which can be confirmed by the highest presence of oleic, palmitic and stearic acids, which is identical to the AI parameter. The HH index for the tested fat samples ranged from 1.20 to 1.43. The lowest and worst was for sheep fat (1.14) and the best for goat fat (1.43).

Table 2 Initial values of peroxide value and free fatty acids of beef, sheep and goat fat

Parameter	Beef fat	Sheep fat	Goat fat
Peroxide value (mmol O_2/kg)	0.49±0.01	0.49±0.01	0.49±0.00
FFA (%)	0.72±0.00	0.11±0.00	0.17±0.00

The initial values of peroxide value (PV) and free fatty acid content (FFA) in beef, sheep and goat fat are shown in table 2. The obtained results were compared and are in accordance with the Rulebook on minced meat, semi-finished products and meat products (Official Gazette BiH No. 82/13), as well as the research results of other authors mentioned in this paper. According to the aforementioned Rulebook (Official Gazette of Bosnia and Herzegovina No. 82/13), in ruminant fats, the value of the peroxide value must not exceed 4 mmol O_2/kg, and the content of free fatty acids must not exceed 0.75 %. From table 2, it can be seen that the initial values of the peroxide value for all three animal fats were 0.49 mmol O_2/kg, and that they are in accordance with the values of the mentioned Rulebook, which also indicates their good and appropriate quality. Moslavac et al. (2019) in their research state the initial value of the peroxide value in beef fat as 0.00 mmol O_2/kg. The content of free fatty acids in all three tested fats ranged from a minimum of 0.11 % (sheep fat) to a maximum of 0.72 % (beef fat). The specified values listed in Table 2 for beef, sheep and goat fat indicate that the fats are of good quality and that they are in accordance with the aforementioned Rulebook. The initial values of the content of free fatty acids in beef, sheep and goat fat are different and amount to 0.72 % for beef, 0.11 % for sheep and 0.17 % for goat fat.

From the results shown in Table 3, it can be seen that there was a change in the oxidation stability of beef fat without the addition of antioxidants (control sample) during 168 hours of carrying out the viability test at a temperature of 98 °C. The obtained results show that during the test there was a statistically significant increase in the value of the peroxide value, and after 168 hours of the test the determined value was 19.64 mmol O_2/kg. With the addition of natural antioxidant ginger extract (0.2 %), greater efficiency of protection was achieved, greater stability, i.e. resistance of beef fat to oxidative spoilage. After 168 hours of the viability test, the value of the peroxide value obtained was the lowest compared to the samples with the addition of other natural antioxidants (11.77 mmol O_2/kg). Propyl gallate showed the highest protection efficiency in beef fat against oxidation. After 168 hours of the viability test at 98 °C, the peroxide value was the lowest (0.96 mmol O_2/kg). The influence of natural and synthetic additives on the oxidative properties of beef fat is reported by

Table 3 Average values of the peroxide value of beef fat ± SD sampled at different time intervals without and with the addition of synthetic and natural antioxidants

Sample	Concentration (%)	Peroxide value (mmol O_2/kg)							
		0 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Control	-	0.49^{Aa}±0.01	1.49^{Ab}±0.00	3.33^{Ac}±0.00	4.66^{Ad}±0.41	6.93^{Ae}±0.10	6.93^{Ae}±0.00	13.53^{Af}±0.09	19.64^{Ag}±0.06
PG	0.01	0.49^{Aa}±0.01	0.49^{Ba}±0.00	0.49^{Ba}±0.01	0.49^{Ba}±0.01	0.49^{Ba}±0.01	0.49^{Ba}±0.00	0.74^{Ba}±0.35	0.96^{Ba}±0.03
BHT	0.01	0.49^{Aa}±0.01	0.49^{Ba}±0.01	0.96^{Ba}±0.01	0.96^{Ca}±0.01	0.98^{BC}a±0.03	0.99^{BCa}±0.01	1.24^{BCa}±0.31	1.90^{Cb}±0.00
BHA	0.01	0.49^{Aa}±0.01	0.49^{Ba}±0.00	0.98^{Ba}±0.01	2.19^{Db}±0.27	3.16^{Dc}±0.34	3.24^{Dcd}±0.13	5.39^{De}±0.12	9.38^{Df}±0.05
Extract of sage	0.20	0.49^{Aa}±0.01	1.00^{ABa}±0.01	1.98^{Cb}±0.00	3.17^{Ec}±0.37	5.21^{Ed}±0.03	5.31^{Ede}±0.04	13.24^{Af}±0.30	16.02^{Eg}±0.22
Extract of rosemary	0.20	0.49^{Aa}±0.01	1.99^{Ab}±0.01	3.14^{Ac}±0.32	5.85^{Fd}±0.12	6.77^{Ae}±0.23	6.86^{Aef}±0.10	14.40^{Eg}±0.43	16.83^{Fh}±0.00
Extract of ginger	0.20	0.49^{Aa}±0.01	1.49^{Ab}±0.02	3.14^{Ac}±0.36	4.03^{Ad}±0.25	4.90^{EFe}±0.27	4.95^{EFef}±0.00	11.36^{Fg}±0.15	11.77^{Fgh}±0.16

Legend: A-B Different capital letters in columns indicate statistically significant differences in peroxide number values for antioxidants added to beef fat
a-b Different lowercase letters in rows indicate statistically significant differences in peroxide number values with respect to sampling time interval (0-168 h

Moslavac et al. (2019). Research results show that applied antioxidants successfully stabilize beef fat. Of the natural antioxidants, rosemary extract type Oxy'Less CS had the highest antioxidant activity in beef fat. It achieved a higher efficiency of protecting beef fat from oxidation, compared to other tested natural antioxidants. Synthetic antioxidants propyl gallate and butylhydroxyanisole successfully increased the stability of beef fat, and propyl gallate showed greater antioxidant activity. After 120 hours of testing, the authors determined that the peroxide value of beef fat with the addition of propyl gallate was 0.00 mmol O_2/kg. According to the mentioned authors, the weakest protection of beef fat compared to the control sample is shown by alphatocopherol (15.84 mmol O_2/kg after 120 hours), while in this paper the weakest protection is shown by rosemary extract (16.83 mmol O_2/kg after 168 hours).

Table 4 shows the change in the oxidation stability of sheep fat without the addition of antioxidants (control sample) during 168 hours of the viability test at a temperature of 98°C. The obtained results show that during the test there was

Table 4 Average values of peroxide number ± SD of sheep fat sampled at different time intervals without and with the addition of synthetic and natural antioxidants

Sample	Concentration (%)	Peroxide value (mmol O_2/kg)							
		0 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Control	-	0.49^{Aa}±0.01	2.25^{Ab}±0.35	2.46^{Abc}±0.02	7.11^{Ad}±0.45	8.33^{Ae}±0.12	11.52^{Af}±0.51	15.02^{Ag}±0.03	20.06^{Ah}±0.20
PG	0.01	0.49^{Aa}±0.01	0.49^{Ba}±0.01	0.49^{Ba}±0.00	0.49^{Ba}±0.01	0.49^{Ba}±0.01	0.49^{Ba}±0.00	1.74^{Bb}±0.37	2.46^{Bc}±0.02
BHT	0.01	0.49^{Aa}±0.01	0.99^{BCa}±0.01	0.99^{BCa}±0.01	2.44^{Cb}±0.02	2.99^{Cbc}±0.02	4.95^{Cd}±0.07	7.46^{Ce}±0.05	14.71^{Cf}±0.00
BHA	0.01	0.49^{Aa}±0.01	1.50^{Acb}±0.00	1.50^{CDb}±0.00	2.65^{CDc}±0.38	2.97^{Ccd}±0.04	2.97^{Dcd}±0.00	5.34^{Dce}±0.15	5.39^{Dce}±0.00
Extract of sage	0.20	0.49^{Aa}±0.01	1.49^{ACb}±0.01	3.07^{Ac}±0.33	3.62^{Ecd}±0.32	6.37^{De}±0.60	6.90^{Eef}±0.05	11.39^{Eg}±0.00	15.55^{Ch}±0.64
Extract of rosemary	0.20	0.49^{Aa}±0.01	1.49^{ACb}±0.00	3.48^{AEc}±0.02	5.17^{Fd}±0.31	6.41^{De}±0.13	8.91^{Ff}±0.00	11.69^{Eg}±0.27	14.09^{Ch}±0.28
Extract of ginger	0.20	0.49^{Aa}±0.01	1.49^{ACb}±0.02	2.43^{Ac}±0.07	3.66^{EGd}±0.37	6.37^{De}±0.09	6.90^{Eef}±0.05	11.39^{Eg}±0.16	13.15^{Eh}±0.26

Legend: A-B Different capital letters in columns indicate statistically significant differences in peroxide number values for antioxidants added to sheep fat
a-b Different lowercase letters in rows indicate statistically significant differences in peroxide number values with respect to sampling time interval (0-168 h)

a statistically significant increase in the value of the peroxide value (PV), and after 168 hours of the test, the determined value of the peroxide value was 20.06 mmol O_2/kg. Positive results were obtained with the application of tested natural antioxidants for the purpose of stabilizing sheep fat, and the fat was effectively protected from oxidative deterioration. With the addition of natural antioxidant ginger extract (0.2 %), greater efficiency of protection was achieved, greater stability, i.e. resistance of sheep fat to oxidative spoilage. After 168 hours of carrying out the sustainability test, the peroxide value obtained was the lowest compared to other investigated natural antioxidants and amounted to 13.15 mmol O_2/kg. The highest efficiency of sheep fat protection against oxidation, compared to the tested antioxidants, was obtained with the use of propyl gallate. After 168 hours of the sustainability test, the lowest value of PV (2.46 mmol O_2/kg) was determined, which indicates that the value determined after 168 hours of the test was in accordance with the Ordinance. According to the research of Muradbašić (2009), the value of the peroxide value was determined in the tested samples of sheep fat, which were 1.99 mmol O_2/kg for the Pramenka male sample, and 0.99 mmol O_2/kg for the Pramenka female sample. The determined values were related to the value of the peroxide value in the first month of the test (30 days). After 3 months of storage in the refrigerator, the tests were repeated on both samples. The value of peroxide value for sheep fat (Pramenka male) was 4.50 mmol O_2/kg, and for the fat sample (Pramenka female) the determined value was 4.47 mmol O_2/kg. According to Ünsal et al. (1995), the peroxide value for Marakaman sheep was 0.50 mmol O_2/kg, which is a lower value compared to the results of the aforementioned author, and almost the same initial value of the peroxide value of sheep fat determined in this work (0.49 mmol O_2/kg). The value of peroxide value for adipose tissue of sheep was determined by Nour-El-Din et al. (1984) which is 1.48 mmol O_2/kg. Čorbo (2000) states that after 24 hours in a thermostat at a temperature of 98°C, lamb fat had a very high peroxide value, and due to its lower viability, only the fat of the female throats of the piglets stood out (26.58 mmol O_2/kg), while the other three basic samples had similar values of peroxide value (about 21 mmol O_2/kg). According to research by Čorbo (2000), sheep fat showed an extremely low peroxide value for sample U4, and an extremely high one for samples U1 and U3. The basic samples deteriorated more strongly in sheep (lambs 18.01 mmol O_2/kg and crossbreds 21.96 mmol O_2/kg) than rams (lambs 12.63 mmol O_2/kg and crossbreds 8.57 mmol O_2/kg).

Table 5 shows the change in the oxidation stability of goat fat without the addition of antioxidants (control sample) during 168 hours of the viability test at a temperature of 98 °C. The obtained results show that during the test there was an increase in the value of the peroxide value (PV), and after 168 hours of the test, the peroxide value was determined to be 79.21 mmol O_2/kg. Goat fat

Table 5 Average values of the peroxide value of goat fat ±SD sampled at different time intervals without and with the addition of synthetic and natural antioxidants

Sample	Concentration (%)	Peroxide value (mmol O_2/kg)							
		0 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h
Control	-	0.49^{Aa}±0.00	2.24^{Ab}±0.37	6.47^{Ac}±0.66	13.37^{Ad}±0.89	22.58^{Ae}±0.65	27.34^{Af}±0.16	44.39^{Ag}±0.31	79.21^{Ah}±0.41
PG	0.01	0.49^{Aa}±0.00	0.49^{Ba}±0.00	0.49^{Ba}±0.01	0.49^{Ba}±0.00	0.49^{Ba}±0.00	0.99^{Ba}±0.01	0.99^{Ba}±0.01	2.40^{Bb}±0.11
BHT	0.01	0.49^{Aa}±0.00	0.99^{Ba}±0.01	0.99^{BCa}±0.00	0.99^{BCa}±0.01	0.99^{BCa}±0.01	0.99^{BCa}±0.01	2.45^{Cb}±0.00	2.45^{Bb}±0.00
BHA	0.01	0.49^{Aa}±0.00	0.99^{Ba}±0.01	0.99^{BCa}0.01	0.99^{BCa}±0.01	0.99^{BCa}±0.01	0.99^{BCa}±0.01	2.45^{Cb}±0.00	2.45^{Bb}±0.00
Extract of sage	0.20	0.49^{Aa}±0.00	1.21^{Ba}±0.29	1.44^{BDa}0.02	3.45^{Db}±0.07	5.19^{Dc}±0.07	8.45^{Dd}±0.28	15.46^{De}±0.11	20.51^{Cf}±1.10
Extract of rosemary	0.20	0.49^{Aa}±0.00	1.72^{Bb}±0.34	3.40^{Ec}±0.09	6.98^{Ed}±0.53	12.69^{Ee}±0.26	17.41^{Ef}±0.59	30.73^{Eg}±0.21	58.42^{Dh}±0.45
Extract of ginger	0.20	0.49^{Aa}±0.00	0.98^{Ba}±0.01	3.35^{Ec}±0.07	6.44^{Ed}±0.00	9.18^{Fe}±0.19	9.36^{DFe}±0.20	18.56^{Ff}±0.35	23.68^{Eg}±0.49

Legend: A-B Different capital letters in columns indicate statistically significant differences in peroxide number values with respect to antioxidants added to goat fat
a-b Different lowercase letters in rows indicate statistically significant differences in peroxide number values with respect to sampling time interval (0-168 h)

shows the greatest instability during the application of the viability test at a temperature of 98 °C. With the addition of the natural antioxidant of sage extract (0.2 %), greater protection efficiency, greater stability, i.e. resistance of goat fat to oxidative deterioration. After 168 hours of the sustainability test, the determined value of PV is the lowest and is 20.51 mmol O_2/kg in comparison to the other tested natural antioxidants. The highest efficiency of protection of goat fat from oxidation, in relation to the tested antioxidants, was obtained with the use of propyl gallate. After 168 hours of the viability test, the peroxide value was the lowest (2.40 mmol O_2/kg). According to Ganić et al. (2022), peroxide values of Herzegovinian dry goat meat ranged from 1.53 mmol O_2/kg to 4.65 mmol O_2/kg, depending on

Figure 2 Influence of the type of added antioxidant and the time interval on the content of free fatty acids in beef fat

the part of the carcass used for testing.

On Fig. 2, it can be seen that the content of free fatty acids gradually increases, especially after 144 hours in the control sample, and in other fat samples after 168 hours of treatment at a temperature of 98°C. Using natural and synthetic antioxidants according to the results from graph 2, it can be concluded that their presence in beef fat has positive effects on the content of free fatty acids, comparing the obtained results with the control sample. According to Muradbašić (2009), the value of the content of free fatty acids in beef fat for male Simmental was 1.10 %, and for female Simmental the estimated value was 1.40 %. Also, the same author states that the value of the content of free fatty acids after three months of storage in the refrigerator was lower in both tested samples of beef fat, and amounted to 1.00% for male Simmental and 0.70 % for female Simmental. From Fig. 3, it can be seen that the content of free fatty acids increased or stagnated after the 24th hour of testing, and a slightly higher increase could only be observed after 144 hours in the control sample, and in the other sheep fat samples after 168 hours. treatment at a temperature of 98°C.

Using natural and synthetic antioxidants

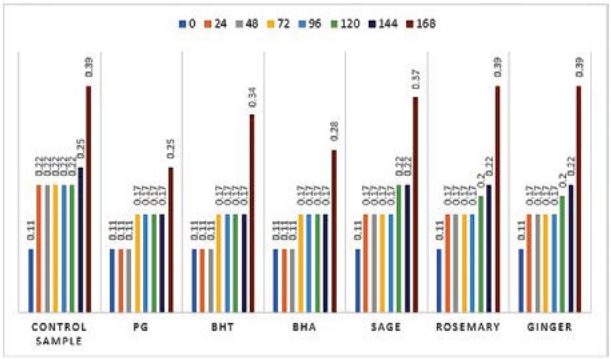

Figure 3 Influence of the type of added antioxidant and the time interval on the content of free fatty acids in sheep fat

according to the results from Fig. 3, it can be concluded that their presence in sheep fat has positive effects on the content of free fatty acids. According to research by Čorbo (2000), the content of free fatty acids in the kidney fat of Pramenka lambs was 1.12 % (0.60 %) and for Križana lambs 0.69 % (0.54 %). However, the author states that the lowest value was the adult heads of Pramenka (sheep and rams), whose value was 0.43 % on average. Female necks predominantly had a slightly higher content of free fatty acids compared to male necks in all groups, and a higher value was especially expressed in adult crossbred sheep (1.41 %). Muradbašić (2009) states in his research that the value of the content of free fatty acids in the first month of testing in the male Pramenka sheep fat sample was 0.50 %, and for the female Pramenka sheep fat sample, the determined value was 0.60 %. The same author states that after storing the fat in the refrigerator for 90 days, there was an increase in the content of free fatty acids in both samples of sheep fat. The value of the content of free fatty acids for the Pramenka male sheep fat sample was 1.40 %, and for the Pramenka female sheep fat sample 0.70 %. The values of the content of free fatty acids in sheep fat samples were higher with the length of

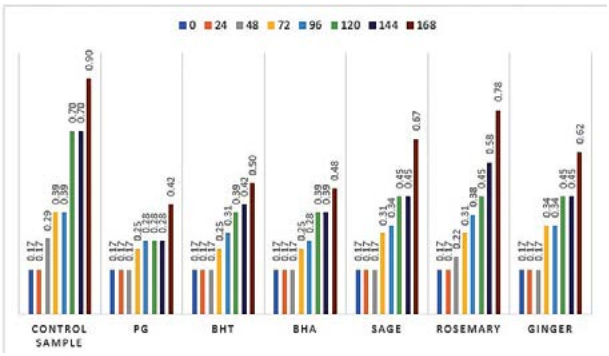

Figure 4 Influence of the type of added antioxidant and the time interval on the content of free fatty acids in goat fat

treatment (168 hours) and under the influence of high temperature.

From Fig 4, it can be observed that after 24 hours of treatment there was no increase in FFA in any sample of goat fat. After 48 hours, it can be seen that the value of the control sample and the sample of goat fat with the addition of rosemary extract increased, while the value of FFA stagnated in the other samples (0.17 %). After 72 hours of treatment, an increase in the content of free fatty acids can be observed in all samples of goat fat. The increase in the value of FFA content continues until the very end of the test, where the best result of fat preservation was found in the fat sample with the addition of propyl gallate (PG), and the weakest in the fat sample with the addition of rosemary extract. The analysis of the main components was carried out on the basis of a correlation matrix that included 24 parameters for three groups of animal fat samples (beef, sheep and goat fat). From Fig 5, it can be seen that a high positive correlation was achieved between the content of FFA, C16:0, C14:0 and the ratio of ω-6/ω-3 fatty acids, while the mentioned parameters were negatively correlated with the content of C15:0, C8:0, C17:0 and C18:2 n-6, cis. Also, a high positive correlation was found between the content of SFA, C18:0, C18:3 n-3, C15:1 and the value of the AI index, which had a negative correlation with the content of C17:1, C12:0, C18:1 cis, MUFA and HH index values.

From Fig 5, it can be seen that the values of the peroxide value, the contents of heptadecene, lauric, oleic acid, monounsaturated fatty acids and the values of the HH index were positioned in relation to the sample of goat fat that were characteristic of it. The values of the arytrogen index, the contents of SFA, PUFA, C18:0, C18:3 n-3, C15:1, the ratio of PUFA/SFA fatty acids on the graph (Fig. 5) were positioned on the right side of the plot in relation to the sample of sheep fat for which they were characteristic. The values of the ratio of ω-6/ω-3 fatty acids, the content of free fatty acids, C14:0 and C16:0 acids were positioned on the lower left side of the plot in relation to the sample of beef fat that had the characteristic values of the mentioned parameters.

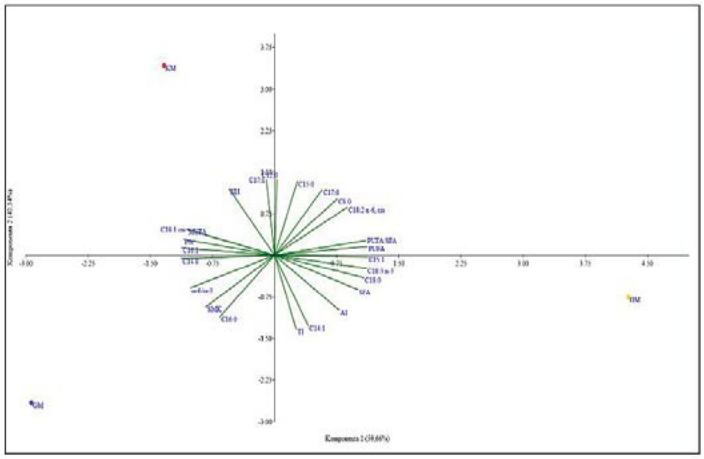

Figure 5 Principal component analysis (PCA) – graphical display of animal fat samples according to fatty acid composition and their sustainability parameters

Conclusion

The highest total value of SFA was found in sheep fat (66.85 %). Of the monounsaturated acids (MUFA), the most abundant in goat fat was oleic acid (38.16 %). Total unsaturated fatty acids (UFA) are most abundant in goat fat (43.51 %). The highest content of polyunsaturated acids (PUFA) was found in sheep fat (4.19 %). The nutritional indices of the examined fat samples, obtained on the basis of the content of individual and certain groups of fatty acids SFA, UFA, MUFA, PUFA, show that goat fat has the best composition of fatty acids and ratio of fatty acids within the compared groups. Atherogenic (AI) and thrombogenic indices (TI) are the lowest in goat and hypocholesterol index (HH) in sheep fat. The atherogenic index (AI) is the highest in sheep and the hypocholesterol index (HH) is the lowest in sheep and the highest in goat fat. The initial values of the peroxide value for all three animal fats were 0.49 mmol O_2/kg, and initial values of the content of free fatty acids in all three tested fats ranged from a minimum of 0.11 % to a maximum of 0.72 %. The values of the peroxide number and the content of free fatty acids, after 168 hours of treatment at a temperature of 98 °C and with the addition of different antioxidants in different concentrations, in beef, sheep and goat fat showed good stability and increased resistance to oxidation. Of the synthetic antioxidants, propyl gallate had the best antioxidant properties in all three tested animal fats, which was determined based on the obtained results of determining the peroxide value and the content of free fatty acids. The results of the peroxide value show that of the natural antioxidants, the addition of ginger extract showed the best antioxidant properties in beef and sheep fat (11,77 mmol O_2/kg for beef and 13,15 mmol O_2/kg for sheep fat) while in goat fat, sage extract gave the best sustainability (20,51 mmol O_2/kg). Beef and goat fat had better sustainability with the addition of ginger extract (1,06 % for beef and 0,62 % for goat fat) and sheep with the addition of sage extract (0,37 %), which was shown by the results of the content of free fatty acids compared to the control sample after 168 hours of treatment. All the obtained results are in accordance with the values prescribed by the applicable Rulebook for this type of fat. Natural and synthetic antioxidants have a beneficial effect and at the same time improve the stability of beef, sheep and goat fat, despite exposure to high temperature and prolonged storage. Analyzes performed on all samples showed that it is possible to effectively protect beef, sheep and goat fat from oxidative deterioration.

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Utjecaj antioksidansa na kvalitetu i oksidativnu stabilnost animalnih masti

Sažetak

Cilj ovog istraživanja bio je utvrditi utjecaj prirodnih i sintetskih antioksidansa na kvalitetu i stabilnost animalnih masti. Na uzorcima, sa i bez dodatka prirodnih antioksidansa (ekstrakt kadulje, ružmarina i đumbira) dodanih u masti u koncentraciji od 0,2 % i sintetskih antioksidansa (PG-propil galat, BHA-butilhidroksianizol i BHT-butilhidroksitoluen) dodanih u masti u koncentraciji 0,01 % ispitivane su vrijednosti peroksidnog broja i sadržaja slobodnih masnih kiselina. Oksidativna stabilnost masti određena je testom održivosti na 98 °C tokom 7 dana. Svaka 24 sata ispitivane su peroksidna vrijednost i slobodne masne kiseline za sve uzorke govođe, ovčje i kozje masti. Rezultati istraživanja pokazuju da su primijenjeni antioksidansi uspješno stabilizirali sve animalne masti u odnosu na kontrolni uzorak. Analiza sastava masnih kiselina baznih uzoraka (bez dodataka prirodnih antioksidansa) pokazuje da su palmitinska, stearinska i oleinska masna kiselina najzastupljenije u animalnim mastima. Najveća ukupna vrijednost SFA utvrđena je u ovčjoj masti (66,85 %), a najniža u kozjoj masti (56,57 %). U govođoj masti ukupan sadržaj SFA iznosio

je 59,26 %. Stearinska kiselina je najzastupljenija u ovčjoj masti (37,75 %), dok su goveđa i kozja mast imale niže i približno jednake vrijednosti (27,93% i 26,91%). Nakon staerinske, palmitinska kiselina je najzastupljenija u goveđoj masti s 27,78 %, zatim 25,25% u ovčjoj i 24,91% u kozjoj masti. Od mononezasićenih kiselina (MUFA) u kozjoj masti najviše je zastupljena oleinska kiselina (38,16%), dok je nešto niža u goveđoj (36,61 %), a najmanje u ovčjoj (27,60 %). Ukupne nezasićene masne kiseline (UFA) najviše su zastupljene u kozjoj masti (43,51 %), zatim u goveđoj (40,82 %), dok su značajno manje zastupljene u ovčjoj masti (32,95 %). Među polinezasićenim kiselinama (PUFA), najveći sadržaj linolne kiseline je u ovčjoj masti (4,19 %). U goveđoj masti linolna kiselina je bila 60 % niža u odnosu na ovčju (0,76 %), a 10 % viša u odnosu na kozju (1,89 %). Na osnovu sadržaja pojedinačnih masnih kiselina i sadržaja pojedinih grupa masnih kiselina: SFA, UFA, MUFA, PUFA, izračunati su nutritivni indeksi ispitivanih masti. Zaključeno je da se ispitani uzorci goveđe, ovčje i kozje masti bez dodatka antioksidansa ne mogu deklarirati kao proizvodi visoke nutritivne vrijednosti. Nutritivna vrijednost izražena kroz omjer PUFA/SFA za goveđu mast bila je 0,01, a ovčja i kozja mast 0,06.

Ključne riječi: animalne masti, kvaliteta, oksidativna stabilnost, antioksidansi, test održivosti.

Die Wirkung von Antioxidantien auf die Qualität und oxidative Stabilität von tierischen Fetten

Zusammenfassung

Ziel der Arbeit war es, den Einfluss von natürlichen und synthetischen Antioxidantien auf die Qualität und Stabilität von tierischen Fetten zu bestimmen. An den Proben mit und ohne Zusatz von natürlichen Antioxidantien (Salbei-, Rosmarin- und Ingwerextrakt), die den Fetten in einer Konzentration von 0,2 % zugesetzt wurden, und synthetischen Antioxidantien (PG-Propylgalat, BHA-Butylhydroxyanisol und BHT-Butylhidroxytoluol), die den Fetten in einer Konzentration von 0,01 % zugesetzt wurden, wurden die Werte der Peroxidzahl und der Gehalt an freien Fettsäuren geprüft. Die oxidative Stabilität der Fette wurde durch den Nachhaltigkeitstest bei 98 °C während 7 Tagen bestimmt. Alle 24 Stunden wurden der Peroxidwert und die freien Fettsäuren bei allen Proben von Rinder-, Schaf- und Ziegenfett untersucht. Die Forschungsergebnisse zeigen, dass die verwendeten Antioxidantien alle tierischen Fette im Vergleich zur Kontrollprobe erfolgreich stabilisiert haben. Die Analyse der Fettsäurezusammensetzung der Basisproben (ohne Zusatz natürlicher Antioxidantien) zeigt, dass Palmitin-, Stearin- und Ölfettsäuren in den tierischen Fetten am häufigsten vorkommen. Der höchste Gesamtgehalt an SFA wurde in Schafsfett (66,85 %) festgestellt, der niedrigste in Ziegenfett (56,57 %). In Rinderfett lag der Gesamtgehalt an SFA bei 59,26 %. Stearinsäure ist am häufigsten in Schafsfett enthalten (37,75 %), während Rinder- und Ziegenfett niedrigere und annähernd gleiche Werte aufwiesen (27,93 % und 26,91 %). Palmitinsäure ist mit 27,78 % am häufigsten in Rinderfett enthalten, gefolgt von 25,25 % in Schafsfett und 24,91 % in Ziegenfett. Von den einfach ungesättigten Fettsäuren (MUFA) war die Ölsäure im Ziegenfett am häufigsten (38,16 %), im Rinderfett etwas seltener (36,61 %) und im Schaffett am wenigsten (27,60 %). Ungesättigte Fettsäuren (UFA) sind am häufigsten in Ziegenfett enthalten (43,51 %), gefolgt von Rindfleisch (40,82 %), während sie in Schafsfett deutlich weniger vorhanden sind (32,95 %). Von den mehrfach ungesättigten Säuren (PUFA) wurde der höchste Gehalt an Linolsäure in Schafsfett festgestellt (4,19 %). In Rinderfett war der Linolsäuregehalt um 60 % niedriger als in Schaffett (0,76 %) und um 10 % höher als in Ziegenfett (1,89 %). Auf der Grundlage des Gehalts an einzelnen Fettsäuren und des Gehalts an bestimmten Fettsäuregruppen (SFA, UFA, MUFA, PUFA) wurden die Nährwertindizes der untersuchten Fette berechnet. Es wurde festgestellt, dass die getesteten Proben von Rinder-, Schaf- und Ziegenfett ohne Zusatz von Antioxidantien nicht als Produkte mit hohem Nährwert deklariert werden können. Der Nährwert, ausgedrückt durch das PUFA/SFA-Verhältnis, betrug bei Rinderfett 0,01, bei Schaf- und Ziegenfett 0,06.

Schlüsselwörter: Tierische Fette, Qualität, oxidative Stabilität, Antioxidantien, Nachhaltigkeitstest

El efecto de los antioxidantes en la calidad y estabilidad oxidativa de las grasas animales

Resumen

El objetivo de esta investigación fue determinar la influencia de los antioxidantes naturales y sintéticos en la calidad y estabilidad de las grasas animales. En las muestras, con y sin adición de antioxidantes naturales (extracto de salvia, romero y jengibre) añadidos a la grasa a una concentración del 0,2% y antioxidantes sintéticos (PG-propilgalato, BHA-butilhidroxianisol y BHT-butilhidroxitolueno) añadidos a se ensayó la grasa a una concentración de 0,01% se ensayaron los valores del índice de peróxidos y el contenido de ácidos grasos libres. La estabilidad oxidativa de la grasa se determinó mediante una prueba de viabilidad a 98 °C durante 7 días. Cada 24 horas, se analizó el índice de peróxido y los ácidos grasos libres para todas las muestras de grasa de res, oveja y cabra. Los resultados de la investigación muestran que los antioxidantes aplicados estabilizaron con éxito todas las grasas animales en comparación con la muestra de control. El análisis de la composición de ácidos grasos de las muestras base (sin la adición de antioxidantes naturales) muestra que los ácidos grasos palmítico, esteárico y oleico son los más abundantes en las grasas animales. El valor total más alto de SFA se encontró en la grasa de oveja (66,85 %) y el más bajo en la grasa de cabra (56,57 %). El contenido total de SFA en la grasa de res fue de 59,26%. El ácido esteárico es el más abundante en la grasa de ovino (37,75 %), mientras que la grasa de vacuno y caprino presenta valores inferiores y aproximadamente iguales (27,93 % y 26,91 %). Después de la grasa esteárica, la grasa palmítica es la más abundante en la grasa bovina con un 27,78 %, seguida del 25,25 % en la grasa ovina y el 24,91 % en la grasa caprina. De los ácidos monoinsaturados (MUFA), el ácido oleico es el más abundante en la grasa de cabra (38,16%), mientras que es algo menor en la de vacuno (36,61%) y el que menos en la de ovino (27,60%). Los ácidos grasos insaturados totales (UFA) son los más representados en la grasa de cabra (43,51 %), seguidos de la carne de vacuno (40,82 %), mientras que están significativamente menos representados en la grasa de oveja (32,95 %). Entre los ácidos poliinsaturados (PUFA), el mayor contenido de ácido linoleico se encuentra en la grasa de oveja (4,19%). En la grasa de res, el ácido linoleico fue un 60 % más bajo en comparación con la grasa de oveja (0,76 %) y un 10 % más alto en comparación con la grasa de cabra (1,89 %). Sobre la base del contenido de ácidos grasos individuales y el contenido de grupos individuales de ácidos grasos: SFA, UFA, MUFA, PUFA, se calcularon los índices nutricionales de las grasas analizadas. Se concluyó que las muestras analizadas de grasa bovina, ovina y caprina sin la adición de antioxidantes no pueden ser declaradas como productos de alto valor nutritivo. El valor nutricional expresado a través de la relación PUFA/SFA para la grasa de res fue de 0,01 y para la grasa de oveja y cabra de 0,06.

Palabras claves: grasas animales, calidad, estabilidad oxidativa, antioxidantes, prueba de sustentabilidad

Impatto degli antiossidanti sulla qualità e la stabilità ossidativa dei grassi animali

Riassunto

L'obiettivo di questa ricerca era determinare l'impatto degli antiossidanti naturali e sintetici sulla qualità e la stabilità dei grassi animali. Sui campioni considerati, con e senza aggiunta di antiossidanti naturali (estratto di salvia, rosmarino e zenzero), aggiunti al grasso in concentrazione dello 0,2 %, e di antiossidanti sintetici (PG-propil gallato, BHA-butilidrossianisolo e BHT-butilidrossitoluene), aggiunti al grasso in concentrazione dello 0,01 %, sono stati rilevati il numero di perossidi e il contenuto di acidi grassi liberi. La stabilità ossidativa del grasso è stata determinata mediante un test di sostenibilità a temperatura costante (98 °C) per 7 giorni. Si è proceduto, così, al rilevamento ogni 24 ore del numero di perossidi e del contenuto di acidi grassi liberi per tutti i campioni di grasso di manzo, pecora e capra considerati. I

risultati della ricerca hanno mostrato che gli antiossidanti impiegati hanno stabilizzato con successo tutti i grassi animali rispetto al campione di controllo. L'analisi della composizione in acidi grassi dei campioni di base (senza l'aggiunta di antiossidanti naturali) ha mostrato che gli acidi grassi palmitico, stearico e oleico sono più abbondanti nei grassi animali. Il valore totale più alto di SFA è stato riscontrato nel grasso di pecora (66,85 %), il più basso nel grasso di capra (56,57 %). Nel grasso di manzo, invece, è stato riscontrato un contenuto totale di SFA del 59,26 %. L'acido stearico è risultato maggiormente presente nel grasso di pecora (37,75 %), mentre nel grasso di manzo e di capra sono stati riscontrati valori inferiori e approssimativamente uguali tra loro (27,93% e 26,91 %). Dopo quello stearico, l'acido più abbondante presente nel grasso di manzo (27,78 %) è quello palmitico, presente nel grasso di pecora con una percentuale del 25,25 % e nel grasso di capra con una percentuale del 24,91 %. Tra gli acidi monoinsaturi (MUFA), l'acido oleico risulta maggiormente presente nel grasso di capra (38,16%), mentre è leggermente inferiore nella carne bovina (36,61 %) e notevolmente inferiore nel grasso di pecora (27,60 %). Gli acidi grassi insaturi totali (UFA) sono i più rappresentati nel grasso di capra (43,51 %), seguito da quello di manzo (40,82 %), mentre sono significativamente meno rappresentati nel grasso di pecora (32,95 %). Tra gli acidi polinsaturi (PUFA), il più alto contenuto di acido linoleico è presente nel grasso di pecora (4,19 %). Nel grasso di manzo, l'acido linoleico è risultato inferiore del 60 % rispetto al grasso di pecora (0,76 %) e superiore del 10% rispetto al grasso di capra (1,89 %). È stato poi possibile calcolare gli indici nutrizionali dei grassi testati sulla base del contenuto dei singoli acidi grassi e del contenuto dei singoli gruppi di acidi grassi (SFA, UFA, MUFA e PUFA). Si è concluso che i campioni testati di grasso bovino, ovino e caprino senza l'aggiunta di antiossidanti non possono essere dichiarati prodotti ad alto valore nutrizionale. Il valore nutrizionale espresso attraverso il rapporto PUFA/SFA per il grasso bovino è risultato pari a 0,01, mentre per il grasso ovino e caprino è risultato pari a 0,06.

Parole chiave: grassi animali, qualità, stabilità ossidativa, antiossidanti, test di sostenibilità