

OXIDATIVE STABILITY EFFECT OF BASIL, GARLIC
AND MUSCAT BLOSSOM EXTRACTS ON LIPIDS
AND MICROBIOLOGY OF MINCED MEAT

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Abstract

In this paper the results of tests of the antioxidant activity, the fatty acids and microbiological composition of the three different extracts (basil, garlic and muscat blossom) and their effect on the 1st, 3rd and 5th day of stored pork minced meat at -1°C are presented. The changes on the secondary products of lipid oxidation, acid level, peroxide number, the total number of bacteria and the presence of *Proteus*, *Clostridii*, *Escherichia coli*, *Salmonela*, *Listeria monocitogenes* are studied, as well as the sensory qualities of colour surface section colour and odour. The minced meat onto which 0.3 g/kg garlic extract was applied in comparison with the groups to which is added an extract of basil had better microbiological status and lower oxidative changes. The saturated fatty acids in all tested groups of minced meat range from 38.011% to 43.009%, the polyunsaturated fatty acids from 7.375% to 14.775% and the monounsaturated fatty acids from 46.589% to 50.054% of the total amount of fatty acids. In terms of sensory characteristic the odour Group IV (onto which 0.3 g/kg extract basil was applied) score slightly better in terms of Group IV (to which 0.3 g/kg garlic extract was applied).

Key words: basil, garlic, muscat blossom extracts

Introduction. The minced meat as well as the other meat products belong to the high-risk products and are subjects of putrescence. The surface contamination and the temperature range of storage are of greatest impact over the keeping of the minced meat. In such cases it comes to decrease or worsening of the quality of minced meat due to which chemical and microbiological changes take place which result in the putrescence of the meat. During the oxidative changes various chemical compositions are formed (hydroperoxides, epoxides, aldehydes, ketones) and contribute to the worsening of the quality, the colour, the taste, the structure and the security of the product [1-2]. The mincing of the meat allows for good

conditions for development of micro-organisms. The reaching part of the meat increases, the fascia and aponeuroses are broken (linking tissue surface on the muscle fibers) and the biological barrier for entering of the microorganisms deep into the meat is broken. The damaged muscle fibers create a sap which increases the wetness of the meat. Due to this, the minced meat is a subject to a fast bacterial degradation [3]. The most common cause for the putrescence of the minced meat are the bacteria *Pseudomonas species*, *Bronchotrigh thermosphacta*, *Serratia*, *Enterobacter*, *Proteus*, *Clostridium* [4].

In recent years the research for the usage of natural antioxidants has increased. The increased interest is linked with the fact that they improve the absorption of proteins sugar and fat and have stronger anti-oxidative and antimicrobial characteristics than the synthetic antioxidants. The high antioxidant effect of spices and extracts derived there from is closely connected with the presence of chemical compounds mainly phenols which participate in the inhibiting of the oxidative processes. The extracts obtained from plant materials are rich in phenols and are a good alternative to synthetic antioxidants [5].

The extracts of basil, garlic and muscat blossom contain biologically active compounds with antioxidant and antimicrobial effect. In the basil and basil extract the main ingredients are the carvacrol linalol and eugenol. In garlic and garlic extract the major bioactive components are sulphur compounds allin, diallyl disulphide, allyl disulphide and allicine. The main bioactive components in the muscat blossom and muscat blossom and its extract are muristicine saphrole, elimicine, eugenol and isoeugenol [6-8]. Because in the literature there is scarce data on the impact of extracts of basil, the muscat blossom and garlic on the quality and durability of minced meat, the purpose of our study was to investigate the influence of different concentrations of the given extracts on oxidative changes of minced pork.

Material and methods. As a material in the study a minced meat of second category is used (without table salt) which was adopted according to the rules for minced meat, preparations of minced meat and meat products (Official Gazette of Republic of Macedonia No 63 from 29.04.2013). The minced meat was produced according to the sanitary and veterinary rules applied for the Republic of Macedonia. First, the groups A with basil extract were prepared, then the groups B and C with garlic extract, that is, the Muscat flower:

Group A Basil	Group B Garlic	Group C Muscat blossom
Group 1 a controlled test without the addition of basil extract.	Group 1 a controlled test without the addition of garlic extract.	Group 1 a controlled test without the addition of arillus myristicae extract.
Group 2 a controlled test with the addition of 0.1 g/kg of basil extract.	Group 2 a controlled test with the addition of 0.1 g/kg of garlic extract.	Group 2 a controlled test with the addition of 0.1 g/kg of arillus myristicae extract.

Group A Basil	Group B Garlic	Group C Muscat blossom
Group 3 a controlled test with the addition of 0.2 g/kg of basil extract.	Group 3 a controlled test with the addition of 0.2 g/kg of garlic extract.	Group 3 a controlled test with the addition of 0.2 g/kg of arillus myristicae extract.
Group 4 a controlled test with the addition of 0.3 g/kg of basil extract.	Group 4 a controlled test with the addition of 0.3 g/kg of garlic extract.	Group 4 a controlled test with the addition of 0.3 g/kg of arillus myristicae extract.

The experiment was conducted three times. The extracts (basil, garlic and Muscat blossom) were products of the company ECOM Food Industry Corporation Canada. The extracts are microbiologically clean 100%, i. e. their microbiological image is perfect. The extracts were applied after the mincing of the meat. After the mincing, the meat was packed in plastic plates with dimensions 240 mm × 130 mm and depth of 50 mm. The so filled in meat into the plates was vacuumed by a multi-functional vacuum machine MULTIVAC (German production). After vacuuming the pork meat was stored in fridge on the temperature of -1°C . On the first, the third and the fifth day of storage the acid level, peroxide number and the microbiological status of the pork minced meat samples was studied. At the end (on the fifth day) a sensor research was made on the groups of minced meat and the qualities of free fatty acids in the minced meat.

Examining the acid value (AV)/or free fatty acids FFA and the peroxide value (PV). The acid level was studied according to the method ISO 660 – (2000) while the peroxide number according to the method ISO 3960 – (2001).

Microbiological examination. For the microbiological study 20 g of each sample was taken which was homogenized with 180 ml distilled water before seeding, while some dilutions were made. The number of bacteria is presented as log.CFU/g. The total number of bacteria was studied in the *Proteus*, *Clostridia*, *E. coli*, *Salmonella*, *Listeria monocitogenes*. *Proteus* (brilliant green $37^{\circ}\text{C}/24-48$ h) *E. Coli* (brilliant green bile lactose broth $42^{\circ}\text{C}/24-48$ h), *Clostridia* (blood agar $37^{\circ}\text{C}/48$ h), *Salmonella* (bismuth sulphite agar $37^{\circ}\text{C}/24-48$ h), *Listeria monocitogenes* (Frazer broth base Palcam agar Oxoid ISO 11290/2010, total number of bacteria (nutrient agar $37^{\circ}\text{C}/24-48$ h) ISO 4833/2008).

Fatty acids composition. The fatty acid composition of the minced meat was determined by gas chromatography. The method of work on the sample is AOAC 996.06 GC-FID-7890 A with appliance of the tool Gas chromatograph with Flame Ionized Detector. The fat from the analyzed samples of minced meat are extracted by hydrolysis methods (acid hydrolysis). The pirogal acid is added so as to avoid liberation of the fatty acids. The methylation of fatty acids was performed according to AOAC GC - FID – 7890. The obtained methyl esters of fatty acid (FAMES) were analyzed using the appliance of Gas chromatograph with Flame Ionized Detector and a capillary column (SP 2560 100 mx 0.25 mm

to 0.25 μm). Operating conditions: injector temperature 225 °C detector 285 °C. Initial temperature of 100 °C kept for 4 minutes increased by 3 °C every minute to a final temperature of 240, hold 15 minutes. The holder gas was helium with a flow rate of 0.75 ml/min. Certain FAMES from the analyzed groups of minced meat were identified separately on the basis of comparison with the respective retention time (which is characteristic of their molecular mass as identification parameter) with retention time standards FAMES (which include *cis* and *trans* isomers of fatty acids) standard mix. The analyzed content of each fatty acid is expressed in percentage (%).

Sensory evaluation. The sensor research was made by 7 experienced professionals which studied the colour of the surface, colour of cutting and smell of the research groups of minced pork meat. The evaluation was made on a nine-degree scale determined by VNIIMP Moscow.

Statistical analysis. The obtained results were statistically elaborated with the computer program Microsoft Excel 97/2003. The results are presented with a mean value \pm Sd.

Results and discussion. The results of the hydrolytic and oxidative changes at the groups of studied samples with basil, garlic and Muscat flower extract are given in Table 1.

The acid level in all tested samples of minced meat during storage increases, where the increase at the control samples is higher. The quantity of oxidation products of fatty acids that is expressed through the peroxide value was 0.052 mmol/kg in the control group and 0.72 mmol/kg 0.05 in the group with addition of 0.3 g/kg extract of basil. In the groups with addition of garlic extract, the peroxide value was initially 0.048 mmol/kg in the control and 0.063 mmol/kg in the group with addition of 0.3 g/kg extract of garlic. In the groups with addition of extract of muscat blossom the peroxide value at the beginning was 0.058 mmol/kg in the control group and 0.075 mmol/kg in the group with addition of 0.3 g/kg extract of muscat blossom. During the storage at a temperature of -1 °C in the groups with added extract of garlic and basil and muscat blossom, the peroxide value increases whereas the highest values were obtained in the control group (3.37–3.80 mmol/kg) and lowest among groups with added 0.3 g/kg extract (1.69–1.98 mmol/kg). The provisions of the Regulation on requirements in terms of quality of minced meat, meat preparations and meat products (Official Gazette No 63 from 29.04.2013) do not indicate allowed values for peroxide value and the acid level in meat products and therefore the interpretation on the obtained results is not possible. According to OSTRICH-MATIJAŠEVIČ [9], sensory changes of meat products are visible even when the peroxide value is greater than 5 mmol/kg. It can be concluded that the process of oxidation in the tested samples is not expressed.

PIČURIČ et al. [10] investigated the anti-oxidative activity of basil and basil extract and found that the extract of basil shows relatively little antioxidant

Table 1

Results from the oxidative changes in the examined groups of vacuumed pork minced meat stored at temperature -1°C

Extract of basil				
Days	Group I Control Mean \pm SD	Group II Mean \pm SD	Group III Mean \pm SD	Group IV Mean \pm SD
	AV POV	AV POV	AV POV	AV POV
1	3.18 \pm 0.08 0.052 \pm 0.08	2.42 \pm 0.4 0.28 \pm 0.5	2.20 \pm 0.08 0.55 \pm 0.05	1.98 \pm 0.5 0.72 \pm 0.05
3	3.78 \pm 0.02 3.51 \pm 0.07	2.56 \pm 0.8 1.42 \pm 0.4	2.33 \pm 0.7 1.63 \pm 0.4	4.09 \pm 0.8 1.89 \pm 0.02
5	4.09 \pm 0.05 3.70 \pm 0.04	3.52 \pm 0.05 1.72 \pm 0.7	3.59 \pm 0.2 1.82 \pm 0.04	4.81 \pm 0.2 1.78 \pm 0.05
Garlic extract				
	AV POV	AV POV	AV POV	AV POV
1	2.58 \pm 0.02 0.048 \pm 0.08	2.23 \pm 0.2 0.063 \pm 0.8	2.35 \pm 0.06 0.057 \pm 0.02	2.33 \pm 0.3 0.063 \pm 0.2
3	3.73 \pm 0.7 3.21 \pm 0.02	3.80 \pm 0.4 2.17 \pm 0.6	3.69 \pm 0.05 0.13 \pm 0.7	3.28 \pm 0.05 1.86 \pm 0.3
5	4.08 \pm 0.5 3.37 \pm 0.7	3.97 \pm 0.02 1.62 \pm 0.3	3.97 \pm 0.03 1.61 \pm 0.06	3.01 \pm 0.2 1.69 \pm 0.04
Extract of Muscat blossom				
	AV POV	AV POV	AV POV	AV POV
1	2.54 \pm 0.05 0.058 \pm 0.05	2.40 \pm 0.7 0.063 \pm 0.8	2.32 \pm 0.06 0.067 \pm 0.04	2.20 \pm 0.4 0.075 \pm 0.2
3	3.85 \pm 0.07 2.28 \pm 0.07	3.70 \pm 0.8 2.17 \pm 0.6	3.55 \pm 0.02 0.13 \pm 0.7	3.20 \pm 0.04 1.86 \pm 0.3
5	4.15 \pm 0.5 3.80 \pm 0.3	3.92 \pm 0.04 1.62 \pm 0.3	3.80 \pm 0.04 1.75 \pm 0.05	3.75 \pm 0.7 1.98 \pm 0.07

Legend: AV-acid value POV-peroxide number

Group I – without an extract addition

Group II – with the addition of 0.1 g/kg extract

Group III – with the addition of 0.2 g/kg extract

Group IV – with the addition of 0.3 g/kg extract

effect. HAMID et al. [11] investigated the antioxidant activity of fresh garlic, onion powder and garlic extract of minced meat from a camel and they established that the best results are obtained using the garlic extract. MUHISLIN et al. [12] examined the impact of a mixture of extract of rosemary, sodium acetate and calcium acetate in burgers kept at a temperature of 5°C and discovered that at the hamburgers with adding of this mixture there were fewer bacteria and less oxidative changes compared with samples in which it was not added.

The results related to the microbiological research on all samples of minced meat are given in Table 2. At none of the examined sample groups of minced meat the presence of *Proteus*, *Clostridii*, *Escherichia coli*, *Salmonela*, *Listeria monocitogenes* is found. Only the total number of bacteria that are mostly bacilli are identified. The table shows that during the vacuum storage of minced pork meat the highest values of the total number of bacteria was found in the control groups and the lowest total number of bacteria in Group IV. The highest total number of bacteria in Group IV was in the group with addition of ex-

tract of basil (3.25 log CFU/g) compared to the group with addition of garlic extract (2.96 log CFU/g) and muscat blossom (3.32 log CFU/g) on the fifth day of storage. During the entire period of testing the total number of bacteria did not cross the value of 3.92 log CFU/g. NGUEFACK et al. [13] investigated the antibacterial activity of extracts derived from various plants (*Cymbopogon citratus*, *Ocimum basilicum*, *Ocimum gratissimum*, *Thymus vulgaris* and *Zingiber officinale*) or their antibacterial activity against *Staphylococcus aureus*, *Listeria monocitogenes*, and found that these extracts showed antibacterial activity in different degrees. However, the lowest antibacterial activity was shown at extracts of *Zingiber officinale* and *Ocimumum Basilicum* and strongest at extracts *O. Gratissimum C. Citratus*. YIN and CHENG [14] reported that the onion extract reduces the development of *Bacillus cereus*, *Clostridium Botulinium type A*, *E. coli*, *Lactobacillus plantarum*, *Staphylococcus aureus* and fungi and Salmonela volatile compounds of garlic reduces the development of *Candida albicans* and *Pencilium*. The results that we obtained are consistent with the results which were found by the above-named authors. The results show that garlic extract in comparison with the extract of basil has a stronger antioxidant and antimicrobial activity. Besides the extracts of decreased oxidation the microbiological status also have an impact and vacuum on the packaging of minced pork meat.

T a b l e 2

A microbiological status of the examined groups of vacuumed pork minced meat stored at temperature of -1°C

Extract of basil (log CFU/g)				
Days	Control group I Mean \pm SD	Group II Mean \pm SD	Group III Mean \pm SD	Group IV Mean \pm SD
1	3.20 \pm 0.22	3.04 \pm 0.25	3.07 \pm 0.82	2.84 \pm 0.55
3	3.34 \pm 0.48	3.25 \pm 0.52	3.20 \pm 0.88	3.14 \pm 0.52
5	3.39 \pm 0.28	3.32 \pm 0.42	3.29 \pm 0.48	3.25 \pm 0.58
Garlic extract (log CFU/g)				
Days	Control group I	Group II	Group III	Group IV
1.	3.53 \pm 0.25	3.51 \pm 0.42	3.41 \pm 0.82	3.32 \pm 0.20
3.	3.60 \pm 0.28	3.36 \pm 0.48	3.30 \pm 0.85	3.20 \pm 58
5	3.70 \pm 0.42	3.17 \pm 0.52	3.30 \pm 0.88	2.96 \pm 0.22
Muscat blssom extracts (log CFU/g)				
Days	Control group I	Group II	Group III	Group IV
1.	3.72 \pm 0.28	3.60 \pm 0.40	3.52 \pm 0.18	3.45 \pm 0.25
3.	3.80 \pm 0.22	3.52 \pm 0.42	3.48 \pm 0.80	3.40 \pm 50
5	3.92 \pm 0.42	3.25 \pm 0.28	3.35 \pm 0.55	3.32 \pm 0.20

The fatty acid composition of the tested groups of minced pork meat is given in Table 3. The composition of the fatty acids include monounsaturated,

T a b l e 3

The fatty acid content of the minced meat with basil, garlic and muscat blossom extract

Basil extract				
Fatty acid %	Group I Control	Group II (0.1 g/kg)	Group III (0.2 g/kg)	Group IV (0.3 g/kg)
C 14: 0	1.874	1.864	1.835	1.1915
C 14 :1	0.479	0.463	0.470	0.453
C 16:0	24.564	24.354	24.079	24.446
C 16:1	3.547	3.473	3.453	3.508
C 17:0	0.334	0.334	0.328	0.342
C 17:1	0.408	0.400	0.390	0.403
C 18:0	11.971	11.846	11.799	11.828
C 18:1 n 9 t	/	/	/	/
C 18:1 n 9 c	42.155	42.481	43.240	42.569
C 18:2 n 6 c	14.149	14.274	13.898	14.010
C 18: 3n6	0.517	0.501	0.506	0.523
Garlic Extract				
C 14:0	2.186	2.114	2.143	2.161
C 14:1	0.598	0.664	0.614	0.659
C 16:0	27.488	27.513	27.293	27.240
C 16:1	3.981	4.221	4.148	4.405
C 17:0	0.370	0.401	0.392	0.400
C 17:1	0.472	0.483	0.481	0.510
C 18:0	12.965	12.884	12.913	12.879
C 18:1 n 9 t	/	/	/	/
C 18:1 n 9 c	44.479	44.218	44.401	44.396
C 18:2 n 6 c	6.894	6.861	6.885	6.823
C 18:3 n 6	0.567	0.571	0.566 7	0.552
Muscat blossom extract				
C 14:0	2.148	2.151	2.134	2.120
C 14:1	0.471	0.446	0.483	0.494
C 16:0	25.291	25.301	25.215	25.187
C 16:1	3.815	3.768	3.884	3.753
C 17:0	0.348	0.351	0.348	0.356
C 17:1	0.442	0.430	0.436	0.437
C 18:0	12.394	12.527	12.551	12.514
C 18:1 n 9 t	0.493	0.461	0.482	0.470
C 18:1 n 9 c	45.261	45.507	45.251	45.551
C 18:2 n 6 c	7.921	7.684	7.808	7.789
C 18:3 n 6	1.408	1.332	1.394	13.123

polyunsaturated and saturated fatty acids. The content of fatty acids is expressed as % of total identified fatty acids. Table 3 shows that there is no major difference in the composition of fatty acids in all groups.

Saturated fatty acids (C14:0, C16:0, C17:0, C18:0) in all tested groups of minced meat range from 38.011% to 43.009%. The polyunsaturated fatty acids (C18: 2n6c, C18: 3n6) in the groups of tested minced meat are represented from 7.375% to 14.775%. The monounsaturated fatty acids (C 14: 1, C 16 :1, C 17:1, C 18:1n9, C 18: 1n9c) are represented from 46.589% to 50.054%. In the muscle fat tissue in general the most common are fatty acids: 18 1; C16: 0; C 18: 0. [15]. According to WHETSELL et al. [16] these acids in beef meat make up 80% of total fatty acids. GANDEMEER [17] RAES et al. [18] state that in intramuscular fat tissue the highest percentage of fatty acids goes to the saturated and monounsaturated fatty acids. ŠEVIČ et al. [19] examined the fatty acid composition of meat breeds Mangulica and Landrace and found that saturated fatty acids in meat from these two breeds of pigs was 33.31 and 38.74%, polyunsaturated fatty acids 11.55 and 12.33% and monounsaturated fatty acids 41.83 and 50.25%. Our results are consistent with the results which are obtained by the above given authors. The compound of fatty acids is a result of extracellular fat tissue second category meat for minced meat used in analyses.

The results of sensory testing on the groups of minced meat are given in Table 4. The table shows that the poorest results in terms of sensory properties are seen in the control groups and the best in Groups IV. In terms of sensory characteristic odour Group IV (with 0.3 g/kg basil extract added) score slightly

T a b l e 4
Sensory analysis of the pork minced meat groups

Extract of basil				
Sensory properties	Control group I Mean ± SD	Group II Mean ± SD	Group III Mean ± SD	Group IV Mean ± SD
colour of surface	5.55 ± 0.18	7.22 ± 0.25	6.48 ± 0.28	7.22 ± 0.12
colour section	5.58 ± 0.12	6.92 ± 0.40	6.52 ± 45	7.25±0.10
Smell	5.55 ± 0.10	6.72 ± 0.25	6.45 ± 0.45	7.40 ± 052
Garlic extract				
colour of surface	5.50 ± 0.22	7 ± 0.15	6.20 ± 0.25	7.28 ± 0.20
colour section	5.5 ± 0.2	7 ± 0.20	6.60 ± 0.45	7.480 ± 0.12
Smell	6.0 ± 0.18	6 ± 0.20	5.56 ± 0.28	7.00 ± 0.48
Muscat blossom extract				
colour of surface	5.42 ± 0.25	7.12 ± 0.18	6.12 ± 0.22	7.12 ± 0.20
colour section	5.35 ± 0.12	6.78 ± 0.15	6.20 ± 0.25	7.18 ± 0.18
Smell	5.285 ± 0.18	6.20 ± 0.25	5.25 ± 0.28	7.0 ± 0.22

better in terms of Group IV (with 0.3 g/kg garlic extract and muscat blossom added) probably as a result of the strong and penetrating odour of garlic extract in comparison with extracts of basil. AWAN et al. [8] examined the impact of sensory properties of garlic extract applied in the products of chicken meatloaf and found that garlic extract may to some extent improve the taste of the product.

Conclusions. The tests and the obtained results bring us to conclusion that the garlic extract added in an amount of 0.3 g/kg have better antioxidant and antimicrobial activity compared with basil extract added in an amount of 0.3 g/kg. In sensory characteristic the odour is slightly better in the group where 0.3 g/kg of basil extract was added in comparison with the group where 0.3 g/kg was added. Probably due to the strong and penetrating odour of the garlic extract in comparison to the basil extract. The extracts of garlic, basil and muscat blossom do not have an impact on the fatty acid composition of the groups of minced meat.

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