JAP&T TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2020, *26* (2), 74-81

Journal of Agroalimentary Processes and Technologies

Influence of growing and processing factors on the fatty acid profile of poultry lipids

Cosmina Andrea Chirilă[1], Raymond Nandy Szakal[1*], Daniel Ioan Hădărugă[2], Nicoleta Gabriela Hădărugă[1]

[1]*Department of Food Science, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timişoara, Calea Aradului 119, 300645-Timişoara, Romania*
[2] *Department of Applied Chemistry, Organic and Natural Compounds Engineering, Polytechnic University of Timişoara, Carol Telbisz 6, 300001-Timişoara, Romania*

Abstract

The main goal of the study was to evaluate the fatty acid (FA) profile of lipids separated from chicken meat by various methods, as well as the evaluation of the influence of growing/processing conditions on this profile. Non-thermal / mechanical separation and moderate temperature and pressure extraction techniques have been used for the separation of lipid fractions from breast and thigh chicken meat. Meat samples were collected from farm and household poultry. The FA profile was determined by gas chromatography-mass spectrometry (GC-MS) of the derivatized lipid fractions to the corresponding fatty acid methyl esters. It was emphasized that monounsaturated fatty acids are the most important in chicken lipid fractions, oleic acid (as methyl ester) being in a relative concentration of 19.7-33.2%. Thermal processing affects the composition of the lipid profile of poultry meat, both on the main fatty acids and degradation compounds. Hexanal and some *trans* isomers of the essential fatty acids have been identified in processed poultry meat, but at relatively low content.

Keywords: fatty acid (FA), poultry lipids, chicken, chromatography-mass spectrometry (GC-MS)

1. Introduction

Poultry production for meat and/or eggs has continuously increased in the European Union, from 13,000 thousand tons in 2011 to 16,200 thousand tons in 2019, according to data from the European Commission [1]. The data are for chicken, turkey and duck, but the highest increase was observed for duck (over 15% in 2018, compared to 2017). During the same period, chicken production for meat increased by 2.4%. On the other hand, the production price has decreased in the last year at EU level by 1.2%, reaching ~189 Euro/100 kg chicken carcass [1]. In comparison, the average price in the United States was 178.5 Euro/100 kg during this period, and in Brazil, a major exporter in this market, only 110.5 Euro/100 kg.

In Romania, chicken production increased progressively between 2017 and 2019 [1]. If in 2017 the productivity was 398 thousand tons, in 2018 and 2019 it increased to 428 thousand, respectively 445 thousand tons, which represents an increase of 7.5% for 2018/2017 and 4.0% for 2019/2018 [1]. The highest productivity in the EU was observed in Poland (2508 thousand, 2582 thousand and 2700 thousand tons in the period 2017-2019, with an increase of 4.6% over 2019/2018). On the other hand, the most important increase for two consecutive years was observed for Hungary (11.2% for 2018/2017), respectively Hungary and Ireland, with an increase for 2019/2018 of 6.4% and 5.5%, respectively [1]. A decrease in productivity for 2018/2017 was found only in Italy (-3.0%) and Sweden (-1.6%), and in 2019/2018 only for Slovenia (-0.8%). Meanwhile, meat production across the EU had a similar increase, this being 2.1% for 2019/2018, with the highest increase for Estonia (13.6%), respectively Malta, Slovakia and Denmark with 11.0% each. Romania ranked sixth in this respect, with an increase in chicken productivity

Corresponding authors: rszakal@smithfield.ro

of 9.1% [1]. A significant decrease was observed for Belgium (-6.5%) and France (-5.5%). Imports into the EU were mainly from Thailand, Brazil and Ukraine, in proportions of 38.3, 37.2 and 15.2% in 2018, respectively 38.2, 36.7 and 14.6% in 2019. Regarding EU chicken exports to other countries, the largest in the last two years being to the Philippines, Ghana, Ukraine and South Africa (9.5/10%, 9.3/9.8% and 10/8.6% for 2018/2019). However, exports from the EU exceeded imports, by a significant percentage of over 100% (EU exports in 2018 of 1779 thousand tons, respectively imports of only 813 thousand tons in the same period) [1].

Poultry lipids are mixtures of various hydrophobic compounds from the class of fatty acids, mainly triglycerides of these fatty acids, but also mono- and diglycerides, respectively free fatty acids [2-4]. In addition, lipid fractions may contain other hydrophobic compounds, but in much lower concentrations (*e.g.*, tocopherols, sterols, etc.). Separation of the lipid fraction from poultry can be done by several methods, each with its advantages and disadvantages. The unprocessed lipid fraction can be separated manually or mechanically from the portion in which it is found in larger quantities, but the separation yield is relatively low. On the other hand, the separation of the lipid fraction in this way does not affect its chemical composition (lipid profile), and the degradation of the compounds of interest can be minimized [5, 6].

Methods of efficiently obtaining the lipid fraction from poultry meat involve separation by extractive methods. One can use the less expensive method of separation by heating in water-pressing, which does not last long and which also allows the selection of the extraction temperature [7-9]. The lipid fraction can be separated with very good yields, but there is the disadvantage of higher temperature and degradation caused by the presence of water [10-17]. If repeated solid-liquid extraction methods are required that needs hydrophobic solvents (*e.g.*, hexane, petroleum ether), efficient extraction can be performed but the disadvantages are several: high extraction temperature (extraction at the boiling points of the solvents), the use of relatively toxic organic solvents, which must be completely removed by distillation and requires another step at high temperature, and the cost of the process is much higher [18, 19]. However, the extraction methods are appropriate for an accurate determination of lipids in meat samples.

The aim of the present study was to evaluate the lipid profile of processed chicken meat by various methods, as well as to identify possible degradation compounds that may occur in these processing conditions.

2. Materials and method

2.1. Materials

The poultry samples required for the separation of the lipid fraction, used in the present study, were selected from the western part of the country, for poultry raised on farms or in private households. The samples were coded as follows: "*K-F*" – chickens raised in intensive regime (46°9′28″ N, 23°38′2″ E), *Gallus gallus domesticus* L., "*K-H*" – chickens raised in the household (46°22′46″ N, 23°16′47″ E), *Gallus gallus domesticus* L., "*Bst_UnPr/Pr*" – intensively raised chicken breast (46°35'56" N, 26°54'23" E), sample unprocessed or thermally processed, *Gallus gallus domesticus* L. and "*Thg_UnPr/Pr*" – intensively raised chicken thighs (46°35'56" N, 26°54'23" E), thermally processed sample, *Gallus gallus domesticus* L.

2.2. Separation of the lipid fraction

The lipid fractions from the chicken samples were mechanically separated (unprocessed) or obtained by the heat pressing method (processed) using a 6L volume aluminum pressure vessel (Tefal Classic 6L, Rumilly, Haute-Savoie, France). The samples were cut into medium pieces of 50-150 cm (with skin and bones) and mixed with distilled water in a ratio of 1:4. Samples of 0.37-1.3 kg were used for lipid extraction. The extraction time was one hour at ~0.15 MPa at boiling temperature. The cooled liquid part was decanted and the boiled meat was pressed by hand to remove all the liquid. The liquid layer was centrifuged at ~3200 rpm and room temperature for 15 minutes using a Heraeus AG centrifuge (Hanau, Germany). The layers were cooled to 4 °C to solidify the lipid layer, which was separated and stored in the refrigerator until further analysis.

2.3. Transesterification of glycerides to the fatty acid methyl esters

Transesterification (derivatization) of glycerides to the fatty acid methyl esters (FAME) was performed in a 100 mL round-bottomed flask with reflux condenser, in which ~100 mg of sample, 5 mL of methanol · BF_3 solution (20% BF_3, Lewis acid), refluxed in a water bath for 30 minutes, then 5 mL

of hexane were introduced and reflux was continued for another 30 minutes. The mixture was treated with 15 mL of saturated NaCl solution, stirred vigorously for 15 seconds, then the flask was made up with the same sodium chloride solution until the organic layer separated in the neck of the flask, from where it was separated and dried over anhydrous Na_2SO_4.

2.4. Gas chromatography – mass spectrometry (GC-MS)

The determination of the fatty acid profile of the chicken lipid fractions was performed using a Hewlett Packard 6890 Series GC, coupled with the Hewlett Packard 5973 Mass Selective Detector. The analysis conditions for gas chromatograph were: Zebron 5-MS capillary column (length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm), temperature program 50 °C - 300 °C, heating rate 6 °C/min, injector and detector temperature 300 °C, carrier gas (mobile phase): Helium, injection volume 2 μL, solvent delay 4 min. For the mass spectrometer the conditions were: energy EI 70 eV, temperature source 150 °C, scanning range 50-300 amu, scanning speed 1 s^{-1}. The MS identification was performed by comparison the experimental MS with the spectra from the 2011 NIST database, and the relative percentage concentrations resulted from the ratio between the area of the GC peak for a given compound and the sum of the areas of all the peaks of the separated compounds. The analyzes were performed in duplicate and average values have been discussed (*RSD* < 5%).

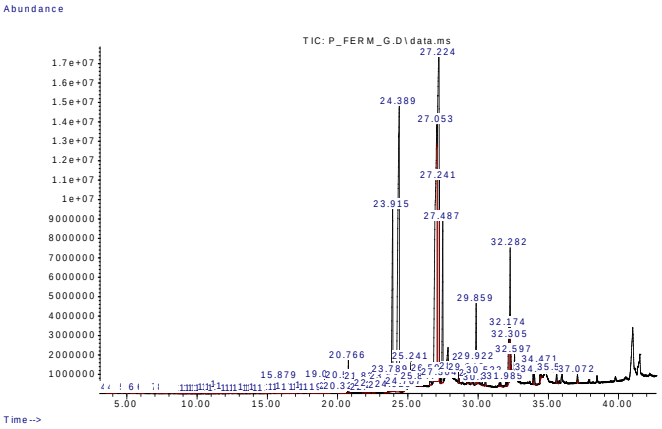
3. Results and discussion

In the studies performed, the method that offers the most advantages was used, namely the separation of the lipid fraction by heating in water-pressing, followed by the separation of the lipid layer at low temperature and drying (removal of water traces) on absorbent material. The degradation of labile compounds in the class of fatty acid glycerides, especially polyunsaturated ones, was thus minimized. For comparison, non-processing mechanical separation of the lipid fraction (unprocessed samples) was also used, which were evaluated in terms of lipid profile.

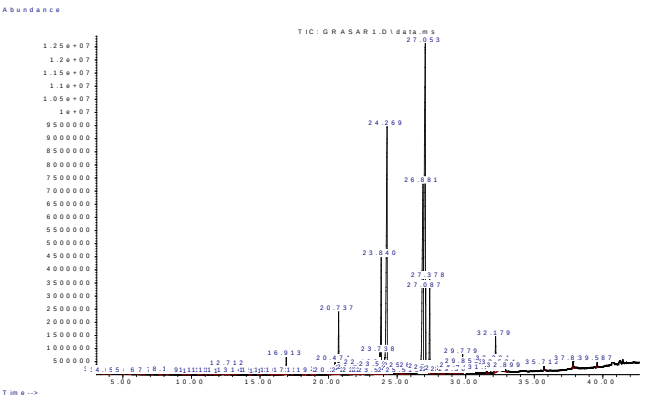
The separation yields of the lipid fraction from chicken meat generally varied in the range of 1.61-5.13%, without a highlight depending on the mode of growth or processing.

3.1. Lipid profile of meat separated from farm and household poultry

For the lipid fraction separated from the "*K-F*" samples, the values for the most concentrated acids were close (25.1% for linoleic acid and 24.9% for oleic acid), while palmitic acid was identified at a concentration of 19.9% (Figure 1a and Table 1). A fairly high concentration was also observed for palmitoleic acid (7.1%), while stearic acid showed a concentration of only 5%. The degradation compounds were in this case at much lower concentration values, the most important being the hexanal (0.04%). The percentage of monounsaturated fatty acids was highest (35%), while saturated and polyunsaturated fatty acids were identified at relatively close concentrations (26.3% and 27%, respectively). Of these, omega-6 and omega-9 fatty acids were the most important (Table 1).



(a)



(b)

Figure 1. Gas chromatograms from GC-MS analysis for derivatized lipid fraction, separated from intensively reared chicken, code "*K-F*" (a), and chicken reared in the household, code "*K-H*" (b)

Similar results were obtained in the case of samples of separate lipid fractions from chicken raised in private households (code "*K-H*"). GC-MS analysis for derivatized samples of these lipid fractions (Figure 1b and Table 1) indicated high concentrations of oleic acid (33.23%).

The next, in order of concentration, was palmitic acid (21.44%) and only the third in this list was identified linolenic acid (16.87%). In addition, palmitoleic acid, a monounsaturated acid, was much more concentrated in these samples (6.32%). Stearic acid was identified in concentrations of ~4.7%, and myristic acid at 3.09%. Other saturated acids identified at concentrations below 1% were caprylic, capric, lauric, pentadecanoic and margaric acids. Among the mono- and polyunsaturated acids, also in concentrations below 1.9%, myristoleic, vaccenic, arachidonic and 11-eicosenoic acids were identified (Table 1). Also, some degradation compounds of the aldehyde class were identified, but at very low concentrations (hexanal or malon-dialdehyde, especially in the "*K-F*" samples).

Table 1. Lipid profile of the derivatized sample of the farm and household chicken meat (codes "*K-F*" and "*K-H*"), as well as unprocessed and processed breast and thigh chicken meat (codes "*Bst_UnPr*", "*Thg_UnPr*", "*Bst_Pr*" and "*Thg_Pr*"). MS identification, retention indices (*RI*) and the relative concentration of the corresponding fatty acid methyl esters (*RSD* < 5% for duplicate analysis) have been presented. *SFA*, *MUFA* and *PUFA* stand for saturated, monounsaturated and polyunsaturated fatty acid, respectively. *Omega-3*, *-6*, and *-9* stand for the corresponding omega-FAs

MS Identification	***RI***	***K-F***	***K-H***	***Bst_UnPr***	***Thg_UnPr***	***Bst_Pr***	***Thg_Pr***
Hexanal, dimethyl acetal	975	0.04	0.02	-	0.01	0.03	0.02
Malonaldehyde, bis(dimethyl acetal)	1025	0.03	-	-	0.01	-	0.01
Caprylic acid, methyl ester	1125	0.02	0.05	0.02	-	0.03	0.02
Nonanal, dimethyl acetal	1278	0.02	-	0.01	0.01	0.01	0.01
Capric acid, methyl ester	1326	0.03	0.32	0.03	0.03	0.04	0.03
Lauric acid, methyl ester	1525	0.05	0.8	0.05	0.04	0.06	0.05
Azelaic acid, dimethyl ester	1546	0.01	-	0.01	-	-	-
Myristoleic acid, methyl ester	1714	0.31	0.58	0.29	0.25	0.33	0.32
Myristic acid, methyl ester	1728	0.82	3.09	0.77	0.69	0.98	0.84
Pentadecanoic acid, methyl ester	1830	0.11	0.44	0.08	0.07	0.12	0.1
7,10-Hexadecadienoic acid, methyl ester	1895	0.28	0.34	0.23	0.17	0.3	0.27
Palmitoleic acid, methyl ester	1912	7.09	6.32	5.66	4.96	6.88	6.73
Palmitic acid, methyl ester	1940	19.9	21.44	17.81	17.62	20.99	19.2
9,12-Hexadecadienoic acid, methyl ester	1992	1.39	0.08	2.37	0.3	0.08	-
Margaric acid, methyl ester	2029	0.13	0.26	-	-	0.16	0.16
Linoleic acid, methyl ester	2103	25.14	16.87	16.26	14.41	24.16	19.39
Oleic acid, methyl ester	2113	24.91	33.23	21.45	19.66	21.93	23.09
trans-Vaccenic acid, methyl ester / Elaidic acid, methyl ester	2114	1.61	1.86	1.26	1.26	1.01	1.4
Stearic acid, methyl ester	2129	5.03	4.7	3.99	4.41	5.28	4.22
Arachidonic acid, methyl ester	2236	0.25	0.12	0.33	0.79	0.43	0.61
cis-11-Eicosenoic acid, methyl ester	2276	0.61	0.42	0.54	-	0.36	0.52
Other compounds		*12.22*	*9.12*	*28.84*	*35.31*	*16.82*	*23.01*
SFA		26.34	31.19	23.09	23.65	28.09	25.24
MUFA		35	43.89	29.59	26.39	30.83	32.41
PUFA		26.99	17.78	16.69	14.58	24.65	19.84
omega-3		0.04	0.04	0.03	0.03	0.03	0.03
omega-6		25.48	17.46	16.67	14.58	24.63	19.83
omega-9		27.13	35.49	23.25	20.92	23.3	25.01

3.2. Lipid profile of unprocessed and processed poultry meat

In order to evaluate the influence of the conditions of separation of lipid fractions, samples of lipid fractions from chicken meat (chest - "breast" and pulp - "thigh") were studied, for which the separation was done mechanically, without any thermal processing (code "*UnPr*"), respectively by the pressing-heating method (code "*Pr*").

Analysis of the fatty acid profile for the lipid fractions separated from the same source, but by the different methods mentioned above, indicated both differences in fatty acid composition, but especially for the presence and concentration of degradation compounds (Figures 2a-2d, 3a-3h and Table 1). Thus, for the samples not subjected to thermal processing (but derivatized by the transesterification method), the most concentrated acid was oleic acid (21.5% in the chicken breast sample, respectively 19.7% for the chicken pulp sample). The concentrations of palmitic and linoleic acids were close, in the case of the "*Bst_UnPr*" chicken breast sample they were 17.8% and 16.3%, and in the case of the "*Thg_UnPr*" chicken leg sample of 17.6% and 14.4% (Table 1). Palmitoleic acid (5.7% and 5%, respectively), as well as myristoleic, myristic, vaccenic/elaidic, arachidonic and 11-eicosenoic acids were also identified by GC-MS, at concentrations below 5.7%. It is worth noting the almost total lack of aldehydes characteristic of the degradation of fatty acid glycerides, namely hexanal and malon-dialdehyde. In the case of the "*Bst_UnPr*" sample, hexanal was not identified (only nonanal, but at a very low concentration of 0.01%), while in the "*Thg_UnPr*" sample, hexanal and malon-dialdehyde were identified in concentrations below 0.01% (Table 1).

The fatty acid profile is changed after processing. A change in relative concentrations was observed for both the fatty acids of interest, but especially for the degradation aldehydes (Figures 2a and 2b, Table 1). Thus, hexanal was identified at concentrations up to 0.03% (Figures 3c-3d), but malon-dialdehyde, heptanal, octanal, nonanal and decanal were also identified. However, it is not possible to make a clear demarcation between unprocessed and processed samples, the variations in composition being important even for samples of the same species, raised and processed in the same way.

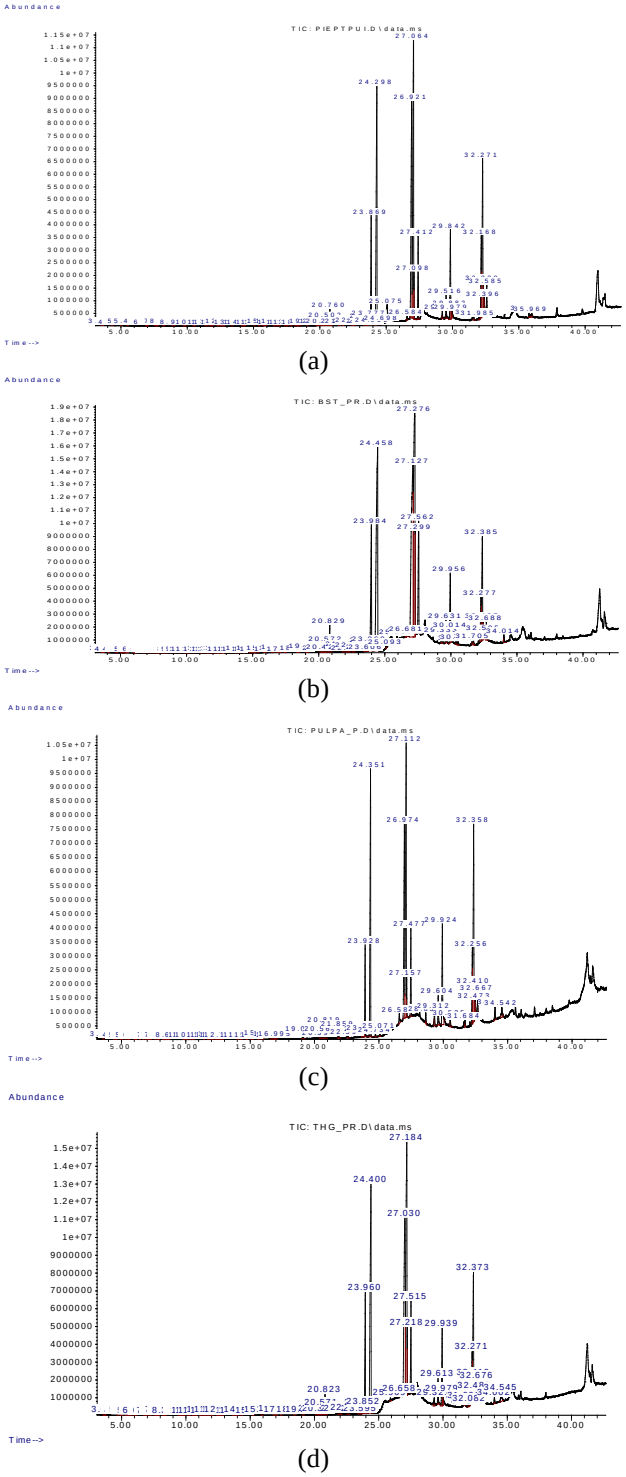


Figure 2. Gas chromatograms from GC-MS analysis for the derivatized lipid fraction, separated from unprocessed chicken breast, code "*Bst_UnPr*" (a), from thermally processed chicken breast, code "*Bst_Pr*" (b), from unprocessed chicken thigh, code "*Thg_UnPr*" (c), and from thermally processed chicken thigh, code "*Thg_Pr*" (d)

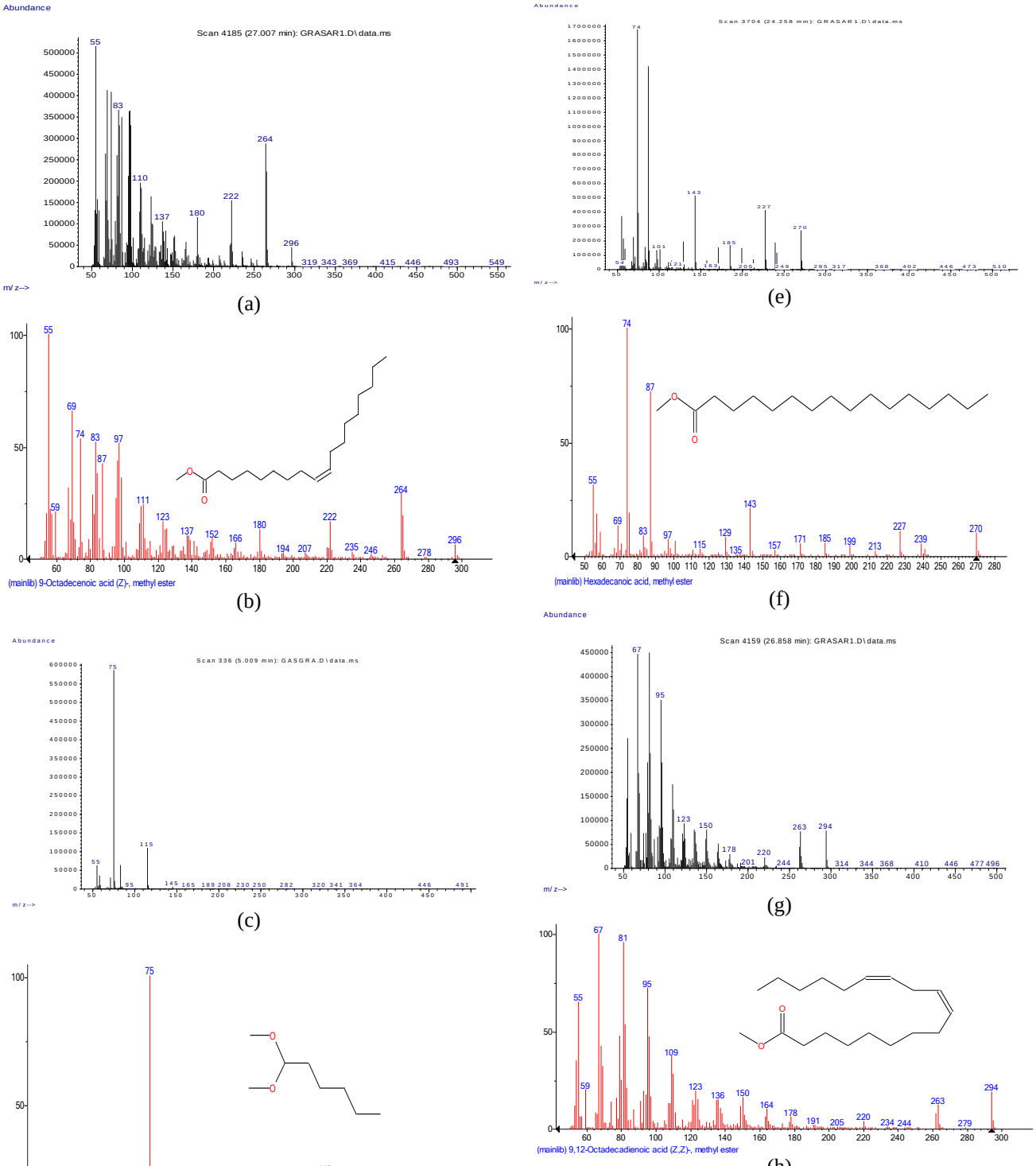


Figure 3. Mass spectra from GC-MS analysis for the derivatized poultry lipid fraction, corresponding to oleic acid, methyl ester, (a) – experimental and (b) – from the NIST database, for the main degradation compound, hexanal (as dimethyl acetal), (c) – experimental and (d) – from the NIST database, as well as for one saturated fatty acid, palmitic acid, methyl ester, (e) – experimental and (f) – from the NIST database, and for an polyunsaturated fatty acid, linoleic acid, methyl ester, (g) – experimental and (h) – from the NIST database

4. Conclusion

Following the studies performed on the lipid profile of poultry meat in correlation with the growth or processing environmental factors, the following main conclusions can be drawn: (1) various samples of poultry meat, selected from various areas of Romania, were selected, grown in intensive system or in private households, for which the lipid profile was evaluated by gas chromatography-mass spectrometry (GC-MS) methods; (2) the lipid profile of the separated fractions of chicken meat indicated a relatively high concentration of mono- and polyunsaturated fatty acids (as methyl esters), of which the most important were oleic and linoleic acids (19.7-33.2% and 14.4-25.1%, respectively); (3) lipids separated from chicken meat had important concentrations of saturated acids, representative being palmitic acid (17.6-21.4%), but also stearic acid (4-5.3%); (4) the analysis of the lipid profile of the chicken samples also indicated some degradation compounds, but at relatively low concentrations, especially in the case of thermally processed samples.

This is the case for hexanal (such as dimethyl acetal) or *trans* isomers of unsaturated acids (*e.g.*, elaidic acid).

Acknowledgements: Authors wants to thank to PNCDI III 2015-2020 – ID 368 institutional development project "Ensuring excellence in R&D within USAMVBT", from the institutional performance subprogram 1.2, development of the R&D national system program 1. This work was supported by proving the equipment's of the Faculty of Food Engineering Timişoara – "*Food Science*"- Research Center

References

1. ***. EU Market Situation for Poultry. Bruxelles: Committee for the Common Organisation of the Agricultural Markets; 2019 20 June 2019.
2. ***. Fats and fatty acids in poultry nutrition and health. Cherian, G.; Poureslami, R., editors. Nottingham: Context Products Ltd.; 2012.
3. Chirilă, C.A. Chimia şi biochimia cărnii – materie primă (porc/vită/pasăre/oaie/capră). Universitatea de Ştiinţe Agricole şi Medicină Veterinară a Banatului "Regele Mihai I al României" din Timişoara (IOSUD), Şcoala Doctorală "Ingineria Resurselor Vegetale și Animale". 2019;Raport de cercetare ştiinţifică R1.
4. Dalziel, C.J.; Kliem, K.E.; Givens, D.I. Fat and fatty acid composition of cooked meat from UK retail chickens labelled as from organic and non-organic production systems. Food Chemistry. 2015;179:103-108. http://dx.doi.org/10.1016/j.foodchem.2015.01.118.
5. Padilla, S. quality characteristics of poultry products. Handbook of poultry science and technology Primary Processing. Hoboken, New Jersey: John Wiley & Sons, Inc.; 2010. p. 453-465.
6. Soriano-Santos, J. Chemical composition and nutritional content of raw poultry meat. Handbook of poultry science and technology Primary Processing. Hoboken, New Jersey: John Wiley & Sons, Inc.; 2010. p. 467-489.
7. ***. Handbook of poultry science and technology. Primary Processing. Hoboken, New Jersey: John Wiley & Sons, Inc.; 2010.
8. ***. Handbook of poultry science and technology. Secondary Processing. Hoboken, New Jersey: John Wiley & Sons, Inc.; 2010.
9. Chirilă, C.A. Metode de separare/purificare/derivatizare a fracţiunilor lipidice. Universitatea de Ştiinţe Agricole şi Medicină Veterinară a Banatului "Regele Mihai I al României" din Timişoara (IOSUD), Şcoala Doctorală "Ingineria Resurselor Vegetale și Animale". 2019;Raport de cercetare ştiinţifică R2.
10. Chirilă, C.A. Procese de izomerizare/degradare a fracţiunilor lipidice din carne (materie primă). Universitatea de Ştiinţe Agricole şi Medicină Veterinară a Banatului "Regele Mihai I al României" din Timişoara (IOSUD), Şcoala Doctorală "Ingineria Resurselor Vegetale și Animale". 2019;Raport de cercetare ştiinţifică R3.
11. David, I.; Orboi, M.D.; Simandi, M.D.; Chirilă, C.A.; Megyesi, C.I.; Rădulescu, L.; Lukinich-Gruia, A.T.; Muntean, C.; Hădărugă, D.I.; Hădărugă, N.G. Fatty acid profile of Romanian's common bean (*Phaseolus vulgaris* L.) lipid fractions and their complexation ability by β-cyclodextrin. PLoS ONE. 2019;14(11):e0225474. https://doi.org/10.1371/journal.pone.0225474.
12. Hădărugă, D.I.; Birău-(Mitroi), C.L.; Gruia, A.T.; Păunescu, V.; Bandur, G.N.; Hădărugă, N.G. Moisture evaluation of β-cyclodextrin/fish oils complexes by thermal analyses: A data review on common barbel (*Barbus barbus* L.), Pontic shad (*Alosa immaculata* Bennett), European wels catfish (*Silurus glanis* L.), and common bleak (*Alburnus alburnus* L.) living in Danube river. Food Chemistry. 2017;236:49-58. http://dx.doi.org/10.1016/j.foodchem.2017.03.093.
13. Hădărugă, D.I.; Hădărugă, N.G.; Mureşan, S.; Bandur, G.; Lupea, A.X.; Păunescu, V.; Riviş, A.; Tatu, C. Fatty Acid/β-Cyclodextrin Nanoparticles: Thermal Analyses and Molecular Modeling Studies. Journal of Agroalimentary Processes and Technologies. 2008;14(1):43-49.
14. Hădărugă, D.I.; Ünlüsayin, M.; Gruia, A.T.; Birău-Mitroi, C.; Rusu, G.; Hădărugă, N.G. Thermal and oxidative stability of Atlantic salmon oil (*Salmo salar*

L.) and complexation with β-cyclodextrin. Beilstein Journal of Organic Chemistry. 2016;12:179-191. http://dx.doi.org/10.3762/bjoc.12.20.

15. Hădărugă, N.G.; Hădărugă, D.I.; Păunescu, V.; Tatu, C.; Ordodi, V.L.; Bandur, G.; Lupea, A.X. Thermal stability of the linoleic acid/alpha- and beta-cyclodextrin complexes. Food Chemistry. 2006;99:500-508. http://dx.doi.org/10.1016/j.foodchem.2005.08.012.
16. Hădărugă, N.G.; Szakal, R.N.; Chirilă, C.A.; Lukinich-Gruia, A.T.; Păunescu, V.; Muntean, C.; Rusu, G.; Bujancă, G.; Hădărugă, D.I. Complexation of Danube common nase (*Chondrostoma nasus* L.) oil by β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin. Food Chemistry. 2020;303:Art. 125419. https://doi.org/10.1016/j.foodchem.2019.125419.
17. Ünlüsayin, M.; Hădărugă, N.G.; Rusu, G.; Gruia, A.T.; Păunescu, V.; Hădărugă, D.I. Nano-encapsulation competitiveness of omega-3 fatty acids and correlations of thermal analysis and Karl Fischer water titration for European anchovy (*Engraulis encrasicolus* L.) oil/beta-cyclodextrin complexes. LWT - Food Science and Technology. 2016;68:135-144. http://dx.doi.org/10.1016/j.lwt.2015.12.017.
18. Holser, R.A. Oxidative stability of fatty acids. In: Cherian, G.; Poureslami, R., editors. Nottingham: Context Products Ltd.; 2012. p. 85-98.
19. Belitz, H.-D.; Grosch, W.; Schieberle, P. Food chemistry. Berlin Heidelberg: Springer-Verlag; 2009. http://dx.doi.org/10.1007/978-3-540-69934-7.