JAP&T TPA-Timişoara

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

Journal of Agroalimentary Processes and Technologies 2020, *26* (2), 60-73

Journal of Agroalimentary Processes and Technologies

Classical *versus* modern analysis of poultry lipids – a review

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Abstract

The review presents a comparison literature survey on the classical and modern analysis of poultry lipids. The history and classification of poultry meat, as well as the chemical composition, stability and degradation of lipids, and analysis methods involved for the determination of poultry lipid profile have been emphasized. The main methods described in this review and their applications are classical physical chemical techniques (such as refractive index, iodine value, polar compounds, or thibarbituric acid test) as well as gas chromatography coupled with various detectors that includes mass spectrometry or flame ionization detectors, derivatization methods, Fourier transform infrared spectroscopy for *trans* fatty acid determination, or high resolution thin layer chromatography.

Keywords: poultry lipids, poultry meat, gas chromatography

1. History and applications of the poultry meat products

Poultry are raised mainly for meat, but also for eggs, the most important being broilers and laying hens (*Gallus gallus domesticus* Linnaeus, 1758), respectively the turkey for meat (*Meleagris gallopavo* Linnaeus, 1758). Historically, poultry were raised in households or on small, individual farms, either for family consumption of meat and eggs or for local sale. In the interwar period began the more intense production of poultry and eggs, especially for sale in stores, also during this period selecting chicken breeds for meat and eggs, respectively, and farms specialize in certain directions of production - meat or eggs. Chickens are now appearing, as a specific breed for mass production. In the following period, the chicken processing sector developed, modernized industrial facilities appeared and the effective marketing of chicken meat in the form of carcasses began. It is also beginning to separate the food preparation sectors, livestock farms and processing facilities, each with different locations, which will prevent the spread of potential diseases [1-3].

After the Second World War, in 1949, the process of evaluating the quality of chicken carcasses began, through the US Department of Agriculture, which set standards that producers must meet. During this period, egg production begins by raising laying hens in cages with lattice for easier collection of eggs and prevention of the spread of diseases and pests [1-3]. Also during this period, there is a decrease in egg consumption due to cholesterol controversies, based on scientific studies.

During the 1960s there is the so-called "vertical integration" in the meat industry, much more efficient and profitable, increased profitability and the emergence of new methods of advertising, due to technological development in the field of communications [1-3]. In the following period, a continuous development of this sector is observed, with the appearance of various control and inspection bodies for maintaining/improving the quality of products, safety and health of consumers. There is a strong modernization of chicken production, process automation, research in innovative products, eradication of diseases and pests, genetic improvements and new technologies.

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The variety of marketed products, based on chicken, is also developing, with a significant increase in consumer preference for semi-prepared or "ready-to-eat" products. There is an explosion of outlets and fast food restaurants, whose menu is mainly based on chicken, in the US there is a consumption that has exceeded that of beef products. Technologies have been developed in which products have a high content of antioxidants (*e.g.* vitamin E) or omega-3 fatty acids, in particular by improving feed for laying hens and chickens [1-7, 8 , 9, 10]. Abbasi and colleagues succeeded in increasing the fat content of omega-3 fatty acids by adding flaxseed oil to the diet of broilers. They obtained nanoemulsions based on flaxseed oil and whey protein concentrate, which were introduced into the diet of broilers. The fatty acid profile showed an 80% increase in omega-3 fatty acid content in chicken meat and 98% in chicken breast, respectively, when supplementing the diet with flaxseed oil nanoemulsion and vitamin E [11]. Similar studies, supplementing the diet with α-linolenic acid from various plant sources, showed an increase in the concentration of omega-3 fatty acids in the meat of chicken breast and leg by 4-9 times, compared to control cases. The most significant increases were observed for long-chain omega-3 acids, such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) [12, 13].

Exports and imports, especially carcasses, chicken meat and chicken fat, have grown significantly in the last two decades. Exports from the USA increase in the direction of Asia and South America, over 20% of the total production being exported during this period [1, 14]. The HACCP system ("Hazard Analysis and Critical Control Points") appears, which presents hygiene norms and rules in the production and processing companies, generally in the field of public alimentation, which was implemented in USA at the beginning of 1998, and in the EU by Regulation Nº 852/2004 [15-17]. For laying hens, minimum standards have been established in the case of rearing in cage batteries, by Directive 1999/74/EC, respectively the introduction of the free rearing system (without cages) [18-20]. On the other hand, broilers are reared in accordance with Directive 2007/43/EC on minimum rules for the protection of reared broilers [21, 22].

In recent years, emphasis has been placed on how to raise poultry, especially those intended for egg production, respectively organic production [11, 12, 14, 23-26]. In the EU they are established by regulations and standards on the breeding, transport and slaughter of poultry, by the Regulation of the European Commission EC Nº 889/2008 [25, 27-31].

2. Classification of poultry products

As for broilers, they are processed from 30-35 days to a maximum of 55-60 days of growth, being clearly established the times of day and the temperature at which they are collected. Special containers must be used for transport to processing locations [2].

The classification of poultry raised for processing is shown below [32]:

- Young chickens (5-6 weeks), weighing up to 0.9 kg
- Chicken for carcass, intended for frying
- Chickens, usually under 13 weeks of age, regardless of gender, with soft, foldable skin, smooth texture and flexible chest cartilage
- Baked chickens, usually take 3-5 months, regardless of gender, with soft, foldable skin, smooth texture, but less flexible cartilage of the chest
- Young rooster, usually under 8 months
- Chicken for baking or other preparations, usually over 10 months, with less tender flesh and less flexible cartilage of the chest
- Mature cock, with coarse, hard skin and dark flesh, respectively the hard chest.
- Young turkey for cooking (under 16 weeks), regardless of gender, with soft, foldable and smooth texture, respectively flexible chest cartilage
- Young turkey, less than 8 months old, with soft, foldable skin, but less flexible cartilage of the chest
- Mature turkey, under 15 months, with relatively smooth skin
- Mature turkey over 15 months, with hard skin and less tender flesh
- Young ducklings (usually under 8 weeks old), regardless of gender, with skin, flesh and soft trachea
- Young duck, less than 16 weeks old, regardless of gender, for which the meat is not fully cured
- Mature duck, usually less than 6 months old, regardless of gender, with hardened meat

- Young goose with smooth flesh and less developed wings
- Mature goose, regardless of gender, with hardened meat
- Young or immature pigeon
- Mature pigeon, with hardened skin and flesh.
- Other birds such as pheasants, quails, etc.

As for the part or area that is used for processing, the most traded are boneless breast, boned breast, drumstick, chicken halves, front or back halves, bone thighs, whole thighs, chicken quarters, quarters of the chest with or without wing, upper thigh, leg with back, fillet muscles, whole wings or portions of wings, neck and legs, etc. [32]. The classification of the marketed poultry parts is also made according to the complete or partial presence, respectively the absence of bones or skin, depending on the destination or preparation method, weight, as well as depending on the marketing method (freshly chilled, frozen in ice, but unpackaged, packaged under carbon dioxide, packaged and frozen) or growing method (traditional production, grown outdoors or in "green" conditions, grown organically or in an antibiotic-free system) [2, 3, 32].

3. Chemical composition of the poultry meat products

In addition to protein intake, poultry meat has a significant fat content. The most important compounds in the lipid fraction of poultry meat are fatty acid triglycerides, but there are also mono- and diglycerides, respectively free fatty acids, as well as hydrophobic antioxidant compounds (tocopherols, carotenoids) in much lower concentrations [23, 33]. It was established that an adult has a distribution of energy taken from the diet according to the class of macronutrients with a significant percentage for carbohydrates (45-65% of the total energy provided through the daily diet), fats and proteins (20-35%, respectively 10-35%), of which omega-6 and omega-3 polyunsaturated fatty acids (expressed as α-linolenic acid) represent 5-10%, respectively 0.6-1.2% [33, 34]. For a daily energy intake of 2000 kcal, the average distribution of nutrient classes is as follows [34]: protein 50 g, fat 65 g, saturated fat 20 g, monounsaturated fat 10 g, carbohydrates 300 g, fiber 25 g, sugar 50 g. On the other hand micronutrients and other components necessary for the body should be taken from the diet in the following average amounts: cholesterol 300 mg, calcium 1000 mg, iron 18 mg, magnesium 400 mg, phosphorus 1000 mg, potassium 3500 mg, sodium 2400 mg, zinc 15 mg, copper 2 mg, vitamin C 60 mg, thiamine 1.5 mg, riboflavin 1.7 mg, niacin 20 mg, vitamin B_6 2 mg, folate 400 µg, vitamin B12 6 µg, vitamin A 5000 IU (international units), vitamin E 20 mg, pantothenic acid 10 mg and vitamin D 400 IU [34].

Regarding the distribution of these components in meat from poultry marketed for food, an approximate composition is shown in Table 1 [34]. On the other hand, the amino acid profile of the proteins present in chicken meat indicates that all 20 essential amino acids are present in such food raw materials, with variations and a maximum for glutamic acid, in the range 113.0 - 150.7 mg / g protein (Table 2).

Poultry lipids are very important both nutritionally and in terms of quality and stability/shelf-life of these products, because the glycerides of polyunsaturated fatty acids present in this fraction are easily degradable, with the formation of derivatives that reduce organoleptic and nutritional characteristics, but also with negative effects on human health (as is the case of free radicals resulting from their oxidation/self-oxidation). The profile of fatty acids (existing mainly as triglycerides) differs depending on many factors, such as the conditions of growth and diet, age and species, the poultry part considered and even the conditions of preparation of samples for analysis [33, 34]. The average fatty acid composition for young and adult chicken meat, respectively skin, is shown in Table 3.

Table 1.Approximate composition of poultry meat (g/100 g) [34]

Component	Chicken meat	Turkey meat	Duck meat	Quail meat
Proteins	12.1	13.7	12.8	13.1
Lipids	11.1	11.9	13.8	11.1
Fibers	0.0	0.0	0.0	0.0
Carbohydrates	1.2	1.1	1.4	1.4
Ash	1.0	0.8	1.2	1.1
Water	74.6	72.5	70.8	74.3

Table 2. Amino acid profile of chicken meat, determined by Kjeldahl method ($N \times 6.25$, expressed as mg/100 g protein) [34]

Nº	Amino acid	Concentration range (mg/g protein)	Nº	Amino acid	Concentration range (mg/g protein)
1	Alanine	34.0 – 60.6	11	Cysteine	< 7.3
2	Valine	38.0 – 50.0	12	Phenylalanine	30.7 – 40.2
3	Glycine	38.8 – 70.1	13	Glutamic acid	113.0 – 150.7
4	Leucine	63.7 – 80.7	14	Histidine	14.6 – 40.0
5	Isoleucine	33.0 – 55.0	15	Tyrosine	23.0 – 39.8
6	Proline	41.0 – 53.0	16	Lysine	63.2 – 85.0
7	Threonine	31.5 – 48.8	17	Arginine	56.0 – 68.3
8	Serine	32.8 – 51.4	18	Hydroxyproline	< 7.1
9	Aspartic acid	72.0 – 97.2	19	Cystine	9.2 – 13.0
10	Methionine	14.7 – 32.5	20	Tryptophan	10.7 – 11.7

Table 3. Fatty acid composition of chicken meat and skin (g/100 g) [33, 34]

Fatty acid	Young meat	Adult meat	Skin
Saturated fatty acids			
C14:0	0.6	0.9	1.1
C16:0	20.1	19.5	22.3
C18:0	8.3	7.1	5.6
Monounsaturated fatty acids			
C14:1	1.1	1.1	0.0
C16:1	2.6	4.3	5.9
C18:1	22.0	27.9	33.1
Polyunsaturated fatty acids			
C18:2	15.8	19.5	23.8
C18:3	0.6	0.9	1.6
C20:4	4.5	2.9	0.6
C20:6	3.0	0.9	0.0
Other fatty acids	2.0	1.5	0.0

Cholesterol is a controversial compound in the lipid fraction of these products. It is indispensable in the stabilization and functionality of cell membranes and central nervous system tissues, but can have negative effects on accumulation due to excessive consumption of products with a high content of this component, such as atherosclerosis. On the other hand, cholesterol is biosynthesized in the body by the liver, at a level about three times higher than that taken from the diet. In chicken and turkey, the cholesterol content varies between 53-83 mg/100 g, lower in the latter case [33, 34]. Other components of chicken meat are trace elements (Fe, Zn, Se), fat-soluble vitamins (A, D, E and K) and water-soluble vitamins (C, B_1, B_2, B_6, B_{12}, niacin, pantothenic acid and biotin), the average content of which are presented above [34, 35].

4. Stability and degradation of poultry lipids

Poultry production involves several stages such as rearing under various conditions (on farms, outdoors or in cages, in households), transport and stunning, slaughter - evaluation/sorting, processing, packaging as raw material, preservation (cooling or freezing). Poultry meat as a raw material can be marketed or subsequently subjected to other processes such as: heating, drying, irradiation, addition of food additives, etc. [2, 3]. The handling and processing of poultry inevitably leads to degradation and chemical transformation of various components such as lipid oxidation and protein denaturation [36, 37]. In the first case, degradation involves a series of radical reactions with stages of initiation (1), propagation (2) and termination (3) of the reaction chain:

(1) $R^{\cdot} + O_2 \rightarrow RO_2^{\cdot}$

$RO_2^{\cdot} + R\text{-}H \rightarrow ROOH + R^{\cdot}$

$RO^{\cdot} + R\text{-}H \rightarrow R\text{-}OH + R^{\cdot}$

(2) $ROOH \rightarrow RO^{\cdot} + {}^{\cdot}OH$

$2ROOH \rightarrow RO_2^{\cdot} + RO^{\cdot} + H_2O$

(3) $2\ R^{\cdot} \rightarrow R\text{-}R$ (stable compounds)

$R^{\cdot} + RO_2^{\cdot} \rightarrow$ stable compounds

$2\ RO^{\cdot} \rightarrow$ stable compounds

The most susceptible to oxidation are mono- and especially polyunsaturated compounds such as oleic, linoleic, linolenic acids (and glycerides) in the methylene groups of the residues -CH=CH-CH_2-CH_2- and -CH=CH-CH_2-CH=CH- [38-44]. The action of free radicals above forms free radicals derived from fatty acids (or glycerides), peroxy radicals, hydroperoxides, oxy radicals and, further, results in aldehydes or formylated acids, which lead to decreased product quality (Figure 1) [37].

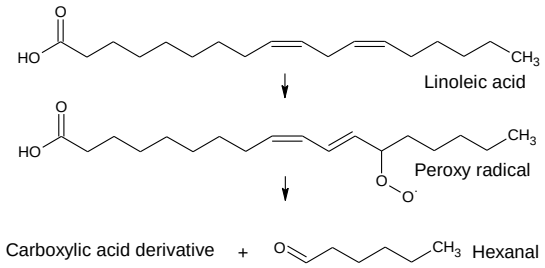

Figure 1. Schematic degradation of linoleic acid to the off-flavored aldehydes (hexanal)

The lipid fraction in poultry meat contains three major classes of glycerides of fatty acids: saturated fatty acid derivatives (SFA), monounsaturated fatty acid derivatives (MUFA) and polyunsaturated fatty acid derivatives (PUFA). The most important fatty acids in poultry are mono- and polyunsaturated acids - oleic and linoleic acids, in the form of glycerides (for example, olein or linolein, Figure 2). The lipid profile represents the distribution of these fatty acids in the lipid fraction, without determining their number and position on the glyceride structure, which is very difficult to achieve. However, the lipid profile can be evaluated by spectroscopic methods (semi-quantitative, by infrared spectroscopy) or by gas chromatography coupled with mass spectrometry, after a prior derivatization of the glycerides to the methyl esters of fatty acids, much more volatile.

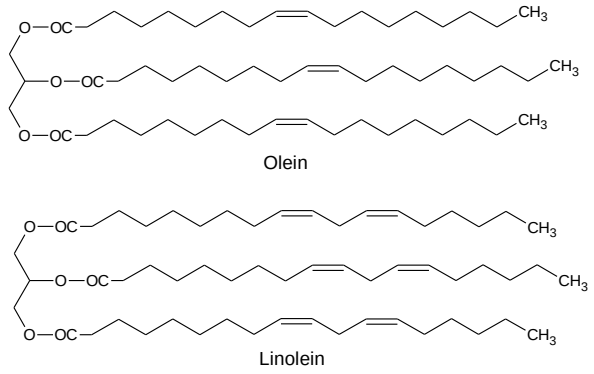

Figure 2. Exemplification of structures from the lipid fraction of poultry meat

5. Methods of analysis and evaluation of poultry lipid profile

Lipid analysis, in general, involves both classical physical chemical determinations, as well as modern determinations, which allow the identification and quantification of components and even degradation compounds. Preliminary processing of lipid samples is also very important in order to reduce determination errors and interferences.

Among the ***classical physical chemical methods*** are [45]:

- *Refractive index* - is determined using a refractometer at 20 °C, 25 °C or 40 °C, in the latter case for grease. For beef lard, the refractive index at 40 °C is in the range of 1.454-1.458, for rapeseed oil of 1.465-1.467, and for sunflower oil of 1.467-1.469.
- *Melting range* - is determined in various ways and with various equipment, with values up to 48 °C, for example in the case of beef fat.
- *Ignition, self-ignition and smoke points* - are useful from an application point of view, as frying and refined oils have smoke points above 200-300 °C.
- *Cooling test* - measures the resistance of an oil to crystallization.
- *Color test* - performed by the Lovibond method (based on yellow standards), respectively spectrophotometric; the absorbance of oils and fats is measured at 460, 550, 620 and 670 nm, and the color index is determined by the following equation:

Color index = 1.29·A460 + 69.7·A550 + 41.2·A620 - 56.4·A670

- *Iodine index* - measures the degree of unsaturation of an oil or fat and is based on the addition of iodine to the double bonds in the structure of unsaturated fatty acids. Iodine is used in excess, and the unreacted fraction is titrated with sodium thiosulfate in the presence of starch as an indicator. This value can also be calculated from the composition of the oil or fat in fatty acids according to the equation below. It is useful in characterizing oils in hydrogenation processes and as an indicator for lipid oxidation.

Iodine index (triglycerides) = (% hexadecenoic acid×0.950) + (% octadecenoic acid×0.860) + (% octadecadienoic acid×1.732) + (% octadecatrienoic acid×2.616) + (% eicosenoic acid×0.785) + (% acid docosenoic×0.723)

- *Saponification index* - the amount of potassium hydroxide needed to saponify a given amount of oil or fat. Excess KOH is worked on, and after saponification, unreacted KOH is determined by titration with a solution of mineral acid of a given concentration and known factor.
- *Acidity index* - allows to determine the content of free fatty acids in oil and fat samples. The index is useful in evaluating the refining processes of oils and fats.
- *Solid fat index and solid fat content* - measured by dilatometry, by evaluating the change in volume to change in temperature. Modern methods for determining solid fat content use pulsed MRI.
- *Consistency*.
- *Polar compounds in frying fats* - quantification of polar compounds, conjugated dienoic acids and fatty acid composition. Separation of polar compounds involves chromatographic methods. A content of more than 27% polar components makes fat or oil unusable for subsequent frying.
- *Measurement of lipid oxidation level* - self-oxidation (which causes rancidity and the appearance of odor-degrading odor compounds):
  - *Peroxide index* (determine the number of milliequivalents of peroxide per kg of fat; excess potassium iodide reacts with peroxides, and the iodine formed in the reaction is titrated with standard sodium thiosulphate solution in the presence of starch as an indicator)
  - *Anisidine index* (the amount of aldehydes in the oil and fat samples is determined by reaction with *p*-anisidine and spectrophotometric measurement of the reaction product)
  - *Determination of hexanal* (by gas chromatography coupled with "headspace" - gas analysis above the sample)
  - *Thiobarbituric acid test (TBA)* - allows the measurement of the secondary compound resulting in the oxidation processes of lipids, malondialdehyde. A colored compound is formed in the reaction which is measured spectrophotometrically (Figure 3):

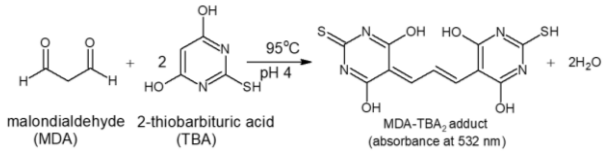

Figure 3. Reaction of malondialdehyde with 2-thiobarbituric acid

  - *Fluorescence microscopy* - to locate more advanced oxidations (for example, in the presence of "Nile Blue" dye).
- *Determination of lipid oxidation stability* by the Schaal method - maintaining a sample of a known quantity in a high temperature oven (usually 60 °C) and determining the rancidity level by a known method (sensory evaluation or peroxide index).
- *Stability index* of the oil or *active oxygen method* - involves determining the period of induction of oxidation by bubbling purified air through the oil or fat sample, maintained at high temperature (110-130 °C), then capturing the volatile compounds generated (especially of formic acid) in deionized water and conductivity measurement (Rancimat method and "Oxidative Stability" - Omnion), respectively the method of active oxygen, which involves determinations of the peroxide index.

Modern methods involve the use of sophisticated equipment, with increased accuracy and sensitivity and used in modern research either singularly or in combination with the classical methods presented previously [45-52]. The concentrations of the specific compounds of the lipid fractions, such as free fatty acids, mono-, di- and triacylglycerols (triglycerides), phospholipids, sterols (including cholesterol), pigments and fat-soluble vitamins, are mainly determined. Fractions containing saturated, mono- or polyunsaturated fatty acids, omega-3 and omega-6 acids, or their ratio, *trans* acids and conjugated polyunsaturated acids, respectively sucrose polyesters, medium or short chain fatty acid triglycerides are also differentiated. The most used techniques are gas chromatography (GC), high performance/pressure liquid chromatography (HPLC) and more recently high resolution thin layer chromatography (HR-TLC).

- *Gas chromatography (GC)* - can be combined with *mass spectrometry* detection (*GC-MS*) or *hydrogen flame ionization* (*GC-FID*), being the most used modern technique for assessing the profile of fatty acids in oils and fats, after prior derivatization, usually to fatty acid methyl esters (FAME) (Figure 4). The method is presented in detail below.

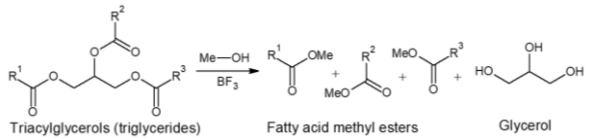

Figure 4. Transesterification reaction by boron-trifluoride - methanol of triglycerides to the corresponding fatty acid methyl esters

- *Determination of cis, cis polyunsaturated fatty acids* - these diastereoisomers cannot be very well differentiated from other isomers by GC, so the liposidase method is used. This involves the conversion of *cis,cis*-1,4-methylene dienes (-CH=CH-CH_2-CH=CH-) to conjugated dienes (-CH=CH-CH=CH-CH_2-), which are measured spectrophotometrically at 234 nm.
- *Determination of trans fatty acids* - is performed by infrared spectroscopy, based on the absorption band from 966 cm^{-1}, and as an internal standard is used methyl elaidate. Modern methods use the ATR-FTIR technique ("attenuated total reflectance - Fourier transform infrared spectroscopy").
- *Determination of cholesterol* - there are several methods of determination, but that involves saponification of oils or fats and extraction of cholesterol (non-saponifiable compound), followed by derivatization to trimethyl-silyl ether with trimethyl-silyl-imidazole (TMSI, Figure 5), hexamethyl-disilazane (HMDS) or trimethylchlorosilane (TMCS) and quantification by GC is the most suitable method [47, 50, 51, 52 , 53].

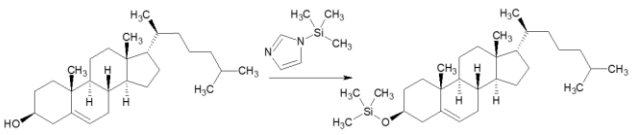

Figure 5. Derivatization of cholesterol to the corresponding trimethylsilyl-ether

- *Separation of the lipid fraction by thin layer chromatography (TLC)* - silica gel G is used as adsorbent and mixture of solvents hexane:diethyl ether:formic acid in a volume ratio of 80:20:2 as elution system, and the development is performed with 2',7'-dichlorofluorescein under UV light. It is separated in ascending order of retention rates: phospholipids, monoglycerides, diglycerides, cholesterol, free fatty acids, triglycerides and close to the front line of cholesterol esters.

6. Applications of gas chromatographic methods for the analysis of lipids from food products

The analysis of fat and its fatty acid profile in various foods follows several common steps, regardless of the product analyzed [49, 51, 52]. One method involves the prior hydrolysis of the glycerides present in the fat, followed by esterification to methyl esters (FAME) with a solution of BF_3 in methanol and their extraction into hexane or heptane. Dry FAME solutions on anhydrous sodium sulfate are analyzed by GC-MS/FID, which allow both identification based on MS spectra and quantification with high accuracy by GC-FID.

In addition to the method presented above, there are several methods and techniques for derivatization to FAME of glycerides present in the lipid fractions of raw materials and foods, the most important being presented below [49]:

- Methanolysis in acid catalysis (hydrochloric acid in methanol or sulfuric acid in methanol)
- Diazomethanolysis
- Transesterification with sodium methoxide in methanol
- Transesterification with boron trifluoride in methanol
- Transesterification with tetramethylammonium hydroxide in basic catalysis.

In addition to classical analysis of the fatty acid profile of lipids, derivatization and GC technique allow specific analyzes, such as analysis of erucic acid content of *Brasicaceae* species, using internal standard, analysis of butanoic acid content of milk fat, butter or food contain these ingredients from the dairy industry [49].

In the latter case, the methyl ester of pentanoic acid is used as an internal standard, respectively a polar capillary column of the cyanopropyl-silicone or polyethylene glycol type. Another example is the differentiation between *cis* or *trans* unsaturated fatty acids, which are identified and quantified as methyl esters, after derivatization by saponification with methanolic NaOH, followed by esterification with BF_3 in methanol, in heptane solution. An interesting study is the determination of bound fatty acids in position 2 of triglycerides, by selective saponification of fatty acid residues in positions 1 and 3 of triglycerides using lipase from the pancreas, separation of monoglycerides by liquid chromatography on silica gel, followed by saponification and methylation to methyl esters of fatty acids at position 2 of triglycerides. The technique is useful for determining the distribution of fatty acids in triglycerides, which allows the detection of counterfeits [49].

The GC technique and the derivatization method are also very important for identifying the presence of conjugated linoleic acid in meat lipids of various origins. Conjugated linoleic acid (C18:2 c9t11, abbreviated CLA – "conjugated linoleic acid") occurs mainly in the lipid fractions of ruminants, but is more difficult to analyze due to the possibility of structural changes in the double bonds (position and isomers) during the derivatization process. Aldai and colleagues have established that the best method of derivatization is by using trimethylsilyl diazomethane (TMS-DM), with the formation of methyl ester with advantages in terms of conversion and degradation processes (Figure 6). The method involves saponifying lipids with a strong base (5M KOH in 1:1 methanol-water, at 60 °C for 60 min, diluting with 0.5% NaCl and adding petroleum ether to remove hydrophobic non-saponifiable matter such as cholesterol or some acyl-lipids), acidification with glacial acetic acid, addition of 2-methoxypropane, removal of the solvent under nitrogen flow at 40 °C, re-dissolution of the residue in 1:2 toluene-methanol and addition of TMS-DM in hexane. Esterification is performed at 40 °C for 10 min. Excess of diazomethane is removed in a stream of nitrogen at 40 °C, the residue is dissolved in hexane stabilized with butyl-hydroxytoluene (BHT) as an antioxidant, centrifuged, and the supernatant is analyzed by GC. Tricosanoic acid methyl ester (C23:0) is used as an internal standard [47].

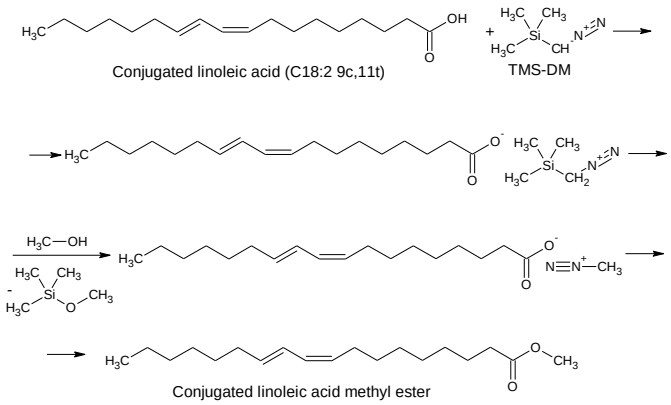

Figure 6. Derivatization reaction of the conjugated linoleic acid (C18:2 9c,11t) to the corresponding methyl ester using trimethylsilyl-diazomethane (TMS-DM)

7. Applications of gas chromatographic methods for the analysis of poultry lipids

Regarding the profile of fatty acids in the lipid fractions of poultry, most recent studies use the GC-MS technique, which allows the identification of methyl esters of fatty acids resulting from the derivatization of the lipid fraction, both based on MS spectra, by comparison with comprehensive MS databases, as well as with the help of FAME mixtures, based on retention indices. Givens and colleagues evaluated the fatty acid composition of lipid fractions in birds raised for marketing in the UK, in an intensive or open space regime. The broilers were analyzed in whole or in part by liquid-solid extraction of the lipid fraction with petroleum ether, which were transesterified with sodium methoxide in anhydrous toluene. Lipid profile analysis was performed by capillary column GC-MS, and identification was performed using a standard FAME mixture and based on MS spectra. The quantification of the components was performed by the internal standard method, with docosan as reference substance [23, 54]. The lipid fractions in chickens raised in intensive and open space showed the highest concentrations in *cis*-monounsaturated acids, with a maximum value in the latter case (5441 mg/100 g). The most important in this class was oleic acid (C18:1 c9, with values of 3132 mg/100 g and 4349 mg/100 g for chickens raised in intensive and open space, respectively). In terms of omega-3 fatty acid content, EPA, DPA and DHA showed similar total values, ranging from 30-35.5 mg/100 g.

On the other hand, saturated fatty acids were more concentrated in the samples from chicken raised in the open diet, with a value of approximately 3688 mg/100 g, the most important in this class being palmitic acid (C16:0, 2910 mg/100 g). On the other hand, the lipid fractions in various parts of the samples showed similar distributions. Thus, monounsaturated fatty acids were almost three times higher in the lipid fraction separated from skinless chicken breast, compared to the chicken breast with skin (2277 mg/100 g for the chicken breast with skin and 868 mg/100 g for the skinless sample), while this distribution was less than double in the skin pulp sample (4536 mg/100 g for skin meat and 2581 mg/100 g for skinless meat). The omega-3 acids mentioned above showed rather close values for skin or skinless samples (EPA + DPA + DHA 18.3-22.9 mg/100 g, respectively 28.4-29.2 mg/100 g) [54]. Similar studies conducted by the same research team revealed that chicken breast and pulp from organically reared ("bio") birds did not have a favorable profile in terms of quality and composition compared to products obtained from farmed birds, in the classical, conventional regime [55]. Thus, there were only small differences between the composition in saturated, mono- and polyunsaturated fatty acids in the samples of breast meat and pulp intended for "fast food" type products, but in the products prepared for consumption the concentrations of palmitoleic, oleic, elaidic acids (*trans*) and α-linolenic acid (omega-3) were higher. Also, long hydrocarbon chain fatty acids showed lower concentrations in processed products [55].

There are significant differences in the fatty acid profile of the dietary lipid fractions of poultry. Experiments were performed on the percentage of fat by varying the content in dextrose, corn flour, soybean (with 48% protein), soy protein isolate, poultry by-products, casein, coconut oil, chicken, lime powder, dicalcium phosphate, sodium chloride, magnesium sulphate, potassium sulphate, methionine, glycine, choline, potassium chloride, vitamin mixture (vitamin A, 5500 IU as *trans*-retinyl acetate, colecalciferol 1100 IU, vitamin E, 11 IU as *rac*-α-tocopheryl acetate, riboflavin 4.4 mg/kg, Ca pantothenate 12 mg/kg, nicotinic acid 44 mg/kg, choline 220 mg/kg, vitamin B_{12} 6.6 µg/kg , vitamin B_6 2.2 mg/kg, menadione 1.1 mg/kg, folic acid 0.55 mg/kg, biotin 0.11 mg/kg, thiamine (as mononitrate) 1.1 mg/kg, ethoxyquin 125 mg/kg), mineral mixture (Mn 60 mg/kg, Zn 50 mg/kg, Fe 30 mg/kg, Cu 5 mg/kg, I 1.5 mg/kg), mixtures for protection against diseases and parasites [56]. Lipid fractions were evaluated under dietary supplementation with ingredients high in polyunsaturated fatty acids (soy and fish oil, coconut or corn oil). It has been found that a dietary supplement with soy and fish oil with a content of only 0.18% linoleic acid leads to a good development of birds, compared to the use of supplements based on corn and soy oils with a higher content of linoleic acid (2.20%). In addition, no significant changes in the fat content of liver or chicken breast (4.18-5.45% and 1.20-1.56%, respectively) were observed when supplementing the diet with soy and casein or soy with fish [56]. A significant increase in lipid content in the liver was observed when supplementing the diet with 1-2% corn oil (6.58-7.56%, compared to 4.91% in the case of control samples) [56].

Another study involved the analysis of the lipid fraction and fatty acid profile for farm-raised chicken breast and leg meat under normal conditions, with commercially available food, based mainly on wheat and soy, but also different proportions of oats, sources of phosphorus, calcium, sodium, choline, amino acids, vitamins, trace elements and enzymes. The total lipid content of the chicken breast and pulp was 3.85(±1.16)% and a range of 1.32-6.78% and 8.21(±2.40)% and a range of 4.33-15.29%. On the other hand, the saturated fatty acid content for the breast and pulp of farmed chicken was 33.17(±4.91)% and a range of 28.58-49.24%, and 30.85(±1.89)% and a range of 27.09-34.83%, respectively, for monounsaturated fatty acids. These values were 42.861(±1.97)% and a range of 38.06-47.95%, as well as 43.58(±1.62)% and a range of 39.21-48.05% for monounsaturated fatty acids, respectively. Finally, for polyunsaturated acids the values were 23.57(±4.98)% and a range of 10.94-29.75%, 25.93(±2.69)% and a range of 21.49-31.97%, respectively [57].

If the fat content of the diet was increased, for example from 2.52% for control to 9.41% for high-fat diets, and the content of monounsaturated fatty acids in these diets decreased, with the increase of polyunsaturated ones, especially omega-3 acids (α-linoleic acids - ALA, EPA, DPA and DHA), the lipid content of chicken breast and pulp did not change significantly, but the composition of lipid fractions showed a significant increase in omega-3 fatty acids, quantified by GC-MS after

derivatization to FAMEs [12]. Chicken breast increased from 0.7% in control samples to 15.4% for the diet high in polyunsaturated acids in the case of ALA, from 0.3% to 2.3% for EPA, from 0.5% to 4.3% for DPA and from 0.5% to 2.4% for DHA, with an increase in the relative concentration of omega-3 fatty acids from 2.1% to 25.5%. In the case of chicken leg, this increase was from 1.8% to 25.9% [12].

Flaxseed oil, which has a high content of α-linolenic acid (ALA, 40-60%), was used to increase the content of omega-3 fatty acids in chicken lipids. The oil was added to the diet of chickens for meat in nanoencapsulated form in matrices based on whey protein concentrate, by obtaining nanoemulsions by ultrasound. The basic diet consisted of corn, flour and soybean oil, mono- and dicalcium phosphate, mixtures of vitamins and minerals, methionine and salt, with an energy value of 2950-3050 kcal/kg, protein content 19.06-21.22%, calcium 0.86-0.92%, phosphorus 0.33-0.41%, sodium 0.14-0.18%, lysine 1.00-1.15%, methionine and cysteine 0.69 -0.83% and threonine 0.72-0.81% [11]. The flaxseed oil diet was obtained from the basic diet, to which was added non-encapsulated or nanoencapsulated flaxseed oil (1 mL nanoemulsion - equivalent to 0.1 g flaxseed oil/kg body weight/day), without or with vitamin E additions (200 mg/kg). In the diets used, the relative concentrations of linoleic acid (omega-6, LA) and ALA (omega-3) were compared, both as concentrations and as ratios, taking into account that in the basic diet the concentrations of LA and ALA were 58.04% and 5.80%, and in flaxseed oil 21.49% and 40.37%, respectively. The ALA content in chicken pulp lipids increased to 1.92% and 2.10% for flaxseed diets without or with the addition of vitamin E, and for 2.53% and 2.80% for diets with chicken pulp in the case of flaxseed oil nanocapsules, compared to the 1.66% content in the basic diet. On the other hand, DPA and DHA increased from 0.11% and 0.13% for the basic diet to 0.13-0.36% and 0.14-0.19% for flaxseed diets, respectively. Maximum were observed for supplementation with nanoencapsulated flaxseed oil in whey protein concentrate and vitamin E [11]. Similar studies were conducted by Kalakuntla et al., who added to the diet of chickens various oils more or less rich in omega-3 fatty acids, such as sunflower oil, soybean oil, mustard seed oil, olive oil, flaxseeds oil and fish oil. The lipids in chicken meat thus raised showed in all cases high concentrations of omega-3 fatty acids, significant being the case of chickens fed diets based on flaxseed oil or fish oil. The relative concentration of omega-3 fatty acids increased from 0.82-1.18% to 10.49-10.61% and 12.21-13.34%, respectively, with the highest values in the case of lipids from chicken breast [58]. The distribution of omega-3 fatty acids was quite high, EPA and DHA being in concentrations of 2.37-2.72% and 5.27-5.76% in lipids in chicken breast and pulp raised with dietary supplementation with oil. α-Linolenic acid was the most concentrated omega-3 acid in chicken lipids raised on flaxseed oil diets (5.02% for chicken breast lipids and 5.68% for from chicken leg raised with flaxseed oil diets) [58].

8. Applications of some spectroscopic methods for the analysis of poultry and other lipids

Infrared (IR) spectroscopy is a particularly useful technique for assessing the importance of fatty acid classes in birds' diets and their lipids [59]. IR is also useful in detecting the level of degradation of unsaturated fatty acids or counterfeiting, for example by evaluating the stretching band characteristic to the *trans* ethylene bond [60-62].

A widely used technique is Fourier transform infrared spectroscopy (FTIR) and especially coupled with total reflection attenuation system (ATR-FTIR). Such techniques, combined with methods of multivariate data analysis such as principal component analysis - PCA or projection in latent structures/partial least squares method - PLS, but also other chemometric methods such as PCR (principal component regression), LDA (linear discriminant analysis), MLR (multiple linear regression), or SIMCA (soft independent modeling of class analogies), have allowed the classification of various oils and fats. Thus, the classification of olive oils according to the olive harvesting region was performed by FTIR-CA and FTIR-PLS-DA techniques, which involve cluster analysis (CA) or PLS-discriminant analysis (PLS-DA), but also PCA. Thus, FTIR "fingerprints" specific to olive oils from various regions were identified [53]. In addition, studies have been performed to identify other vegetable oils (soybean, sunflower, rapeseed or corn) in olive oil based on FTIR and Raman spectroscopy data. The PLS-DA technique allowed to discriminate with a 100% percentage of pure olive oils compared to mixtures, especially based on FTIR "fingerprints" [63]. The characteristics of olive oil in terms of ethyl ester contents of fatty acids, waxes, diacylglycerols and colored pigments

have been successfully predicted by FTIR and UV-Vis coupled with PLS techniques [64]. On the other hand, by using self-organizing maps (SOM) in the analysis of FTIR data, it was possible to discriminate between samples of various edible oils [65], which was also done for a number of vegetable oils based on SB-ATR-FTIR ("single bounce") data on the various classes of fatty acids and the processing of these data by PLS [59]. FTIR techniques were combined with high-resolution ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (^{1}H- and ^{13}C-NMR) to differentiate between light and heavy hydrocarbon-based non-edible oils [66], and FTIR data, FT-NIR spectroscopy (Fourier transform - near infrared spectroscopy) and FT-Raman allowed discrimination between edible oils and fats by LDA and CVA (canonical varied analysis), especially based on vibration C=C bond, the techniques being useful for identifying counterfeits of such products [67]. The identification of the falsification with lard of the fat of lamb, beef or chicken could also be done by FTIR-PLS or FTIR-DA based on fingerprints in the range 1500-900 cm^{-1} [68]. Counterfeiting could also be identified for sesame oil mixed with hazelnut, canola or sunflower oils by ATR-FTIR-PLS [61], kukui nut oil (*Aleurites moluccanus*) with sunflower oil, soybean or corn oils [62], respectively soybean oil in corn oil-based mixtures by the same FTIR-PLS technique [69].

The chemical class fatty acid composition for microencapsulated fish oil supplements was predicted based on ATR-FTIR data [70]. Classification by ATR-FTIR-PLS showed high accuracy, especially for total oil, total omega-3 fatty acids, respectively EPA and DHA acids.

The combined SB-FTIR-PLS method has been successfully used to determine omega-6 and omega-3 fatty acids, respectively, the ratios between them, in lipids in poultry feed [71], and supplements on marine animal oils with a high content of omega-3 fatty acids were classified by FTIR-PLS and FT-NIR-PLS [72].

An interesting study is the authentication of liquid egg compositions based on the ATR-FTIR-PCA and FT-NIR-PCA techniques. Samples of whole egg, egg white or egg yolk and eggs with added egg white or water were analyzed. The last two types of samples, falsified, were separated by 100%, especially when using the FTIR-NIR technique [73].

Determination and prediction of fat and protein content in chicken carcass and breast was performed by FT-NIR and regression analysis, with values of the determination coefficient r^2 of over 0.91 [74]. The multilinear correlation equations used as independent variables the logarithm of the reflectance inverse at 2336-2348 cm^{-1}, 2270 cm^{-1} and 1734 cm^{-1} for the carcass, respectively at 2270 cm^{-1}, 1778 cm^{-1} and 1445 cm^{-1} for the chicken breast:

$$\%\ Fat_{(carcass)} = b_{00} + 221 \cdot \log[1/R(2336\text{-}2348)] - 281 \cdot \log[1/R(2270)] + 103 \cdot \log[1/R(1734)]$$

$$\%\ Fat_{(breast)} = b_{00} - 168 \cdot \log[1/R(2270)] + 740 \cdot \log[1/R(1778)] - 520 \cdot \log[1/R(1445)]$$

where b00 is the intersection with the ordinate [74].

The fat content of chicken breast or various foods based on chicken breast (such as nuggets) was predicted with great accuracy by FT-NIR and FT-NIT techniques (Fourier transform - near infrared reflectance / transmittance). This required the use of lipid databases and the obtaining of lipid models with errors below 1.6% [75].

9. Conclusion

An updated literature review on the classical and modern analysis of poultry lipids have been performed. Among classical physical chemical techniques, modern gas chromatography - mass spectrometry, including derivatization methods, as well as Fourier transform infrared spectroscopy coupled with multivariate statistical analysis techniques have been emphasized for the evaluation lipid profile and classifications or identifying the adulteration of various oils and fats, especially for poultry lipids.

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 Timișoara – "*Food Science*"- Research Center

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