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PhD Thesis Summary

Studies on biologically active and toxic compounds by spectroscopic methods

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CONTENT

Key words:

- Mass Spectrometry (MS), Gas Chromatography (GC), GC-MS Coupling, quantitative analysis methods
- Amino acids, fatty acids, quantitative calculation, inbred lines
- FAME, PUFA, EFA, SFA analysis
- Vegetable oils, seeds, trout meat and plasma, nutritional quality, heavy metal content
- ICP-MS, trace elements, Omega 3, Omega 6

INTRODUCTION

Detailed knowledge of chemical processes taking place in the structure of living organism, both vegetal or animal, only became possible with the development of modern instrumental analysis techniques. These techniques allow today to obtain a large number of data, while reducing the time required for the collection, identification and interpretation stages.

Gas Chromatography (GC) is currently one of the most important analytical methods when it comes to determining individual substances in complex mixtures, in organic chemical analyses. Mass spectrometry (MS), used as a detection method, offers remarkable possibilities for qualitative and quantitative determination of compounds, going up to the order the order in which the groups are bounded in the molecule. Among the most important features of mass spectrometry we can list: high sensitivity, upper detection limits, reduced time required and applicability of the method in a wide variety of fields: organic chemistry, food and environmental pollution control, forensic investigations, process monitoring, atom physics, reaction kinetics, inorganic chemistry analyses, etc.

The applications of analytical methods in the food industry are multiple and particularly important. Current studies aim at developing and optimizing methods capable of determining the composition of widely used food (chromatographic / metabolomic fingerprint), identifying certain essential compounds for optimal metabolism or human immune function, protein synthesis, maintaining mineral balance, etc.

Other studies aim at increasing the productivity of cereals / vegetables or fruits, developing species of hybrids resistant to various environmental conditions, with superior nutritional value and high yield.

Amino acids are organic substances of physiological importance, the basic components of proteins, indispensable elements in human nutrition. They can be considered to be the most important nutrients in the human body, contributing to the synthesis and efficient use of proteins. Of the many amino acids known today in nature (over 700), 20 amino acids, used in the synthesis of over 50,000 unique proteins and 20,000 required enzymes. Of these 20 amino acids, nine essential amino acids are distinguished that the body cannot synthetize them by itself and which are obtained by eating various foods. The human body does not store the surplus of essential amino acids, which are used to create new proteins on a regular basis. For this reason, the continued supply of these amino acids is necessary to maintain health.

The other type of amino acids, called non-essential amino acids, can be synthesized by the body, so regular consumption is not required.

Fatty acids are important components of lipids present in plants, animals and micro-organisms. Typically, these are carboxyl groups with a long aliphatic chain, most of them having an even number of carbon atoms. If the carbon-carbon bonds are all unique, the acid is saturated; if any of these bonds are double or triple, the acid is unsaturated, showing increased reactivity. The fatty acids are not present in free state in nature, but mixed with glycerol to form triglycerides. A number of fatty acids are considered essential because they cannot be synthesized by the human or animal body. Such acids, such as omega-3 and omega-6, are

required in cellular processes, but also in the synthesis of some other fatty acids. Omega-3 and omega-6 fatty acids are derived from linoleic acid and alpha-linoleic acid, respectively. The intake of omega-3 and omega-6 acids is therefore particularly important for maintaining optimal health.

The evolution of food habits over time has led to a change in fatty acid consumption, characterized by an increase in the intake of foods rich in Omega-6 fatty acids and an alarming reduction in Omega-3 intake, which has led to an important imbalance in the Omega- 6 / Omega-3, with serious repercussions on human metabolism and on health.

Essential Omega-3 fatty acids are found in vegetal sources: grain / vegetable seeds, oils extracted from them, or fish meat (mackerel, herring, trout). Omega-6 is found in seeds and nuts, but also in oils extracted from them, especially in refined vegetable oils. Unlike Omega-3, Omega-6 is produced in varying amounts in the human body, having as the main cause the hormonal imbalance.

Determination of minerals and trace elements is also of great interest. Some metals (Fe, Mg, Zn and Cu) are considered as essential micronutrients for the body. For this reason, the presence of metals in the diet (in particular in vegetable oils) is an important factor in assessing biochemical and nutritional properties, given their ability to influence the oxidation rate. Other metals, also known as heavy metals, play a harmfull role on contaminated food. Food contamination can be caused by water, air, soil, the contact with machinery, plant or technical equipment, etc. The toxicity of these metals is the result of their binding to the important enzymatic systems present in the animal cell or to the parts of the cell membranes. The toxic effect is manifested when thresholds values are exceeded; under these values, some metals (Cu, Co, Fe, Ni, Zn) may even have a beneficial role, essential for the proteins involved in certain metabolic pathways.

When exceeding the maximum admissible limits, some heavy metals (As, Hg, Pb, Cd) can cause kidney tubular necrosis, impaired renal function, while chronic exposure to Pb, Hg, Cd leads to nephrotoxic effects.

The nutritional quality of the metal content in the studies carried out during the PhD stage, was tested using the mass spectrometry with inductively coupled plasma method (ICP-MS), using the Perkin Elmer ICP-MS instrument.

Some minerals (Ca, Na, Mg, K, Zn) were determined from the seed samples by flame atomic absorption spectrometry, using an Analytik Jena, ContrAA 700 equipment, in an acetylene-air flame.

The aim of the thesis is to develop analytical methods and to optimize GC-MS systems (gas chromatograph coupled with mass spectrometer) for measuring fatty acids and amino acids in seed extracts, various vegetable oils, plasma and trout, to develop methods for analysis and comparison of nutrient composition, as well as to measure heavy metal content by inductively coupled plasma mass spectrometry (ICP-MS).

Structure of the thesis

The PhD thesis is structured in 8 chapters, an introduction chapter, a chapter presenting Conclusions and Personal Contributions, respectively a section of references consulted in writing the paper.

The first chapter contains a description of mass spectrometry as one of the most important methods and techniques used in quantitative analysis, applicable in a wide range of fields: atomic physics, physics and kinetics of chemical reactions, most of the analytical domains. Theoretical notions on operating principles, main sample insertion systems, ion sources, respectively types of detectors used in mass spectrometry are presented, depending on field of analysis and required performance.

Chapter II is a theoretical approach of the most common chromatographic analysis method, gas chromatography, a method widely used today for quantitative and qualitative analyses of mixtures, for compound purification, gas flow analysis, oxygen, nitrogen, dioxide and nitrogen monoxide percent measurements, in medicine (blood tests), measurement and analysis of essential oils or aromas and nutrients in food industry. Main types of chromatographic columns, as well as detection systems used, with their specific applications are also presented.

Chapter III briefly discusses two modern applications of mass spectrometry, namely gas chromatograph coupled with mass spectrometer (GC-MS) and the inductively coupled plasma mass spectrometer tandem (ICP-MS).

GC-MS is a very useful analytical technique when it is applied to confirm and identify volatile and semi-volatile elements in a complex mixture, in determining the molecular weight and elemental structure of unknown volatiles / semi-volatiles, etc.

The ICP-MS method is based on the combination of inductively coupled plasma as ionization method with mass spectrometry as a method of ion separation and detection. In addition to classical soil, water, food samples, currently a wide range of biological samples, both solid and liquid, can be analysed by ICP-MS: blood, urine, plasma, serum, interstitial fluids, internal organs, hair, bones and even cells.

Chapter IV presents the process of validating analytical methods, that is, the approach by which it is established through laboratory studies whether a method fulfils the criteria for the analytical applications for which it was selected. Validation consists of a compliance / accreditation methodology that takes into account the purpose, characteristics, performance and acceptability limits, elements characterized by a series of parameters, also presented in this chapter.

The first four chapters lay the theoretical basis for analyses performed in the second part of the doctoral thesis.

Chapter V presents a qualitative and quantitative analysis using gas chromatography coupled with mass spectrometry to identify amino acids and fatty acids from 25 maize seed samples. The study was conducted to increase productivity of maize production and to develop hybrids resistant to different environmental conditions with superior nutritional value and high yield.

Chapter VI contains high-precision analyses to compare the content of fatty acids in plasma and trout (Oncorhynchus mykiss). In this regard, a simple and reliable method was developed by which fatty acids were separated and identified by a Trace GC gas chromatograph equipped with a RTX-5MS capillary column coupled to a Trace DSQ quadrupole mass spectrometer.

Chapter VII presents a method for measuring and comparing the composition of nutrients (fatty acids and metal ions) present in various vegetable oils extracted from flax, poppy, grapes, hemp, nuts, soy, pumpkin, sesame seeds and comparison of results obtained with those found in trout meat. Detection and measurements of fatty acid concentrations were performed by GC-MS, and the ICP-MS technique (mass spectrometry inductively coupled with plasma) was used to characterize the metal ion content (Na, Mg, K, Mn, Cd, Cu, Cr).

Chapter VIII presents precision analyses for measuring the content of fatty acids, minerals and heavy metals from seed extracts: flax, poppy, grape, hemp, nuts, pumpkin, sesame, watermelon and chia. The analytical methods used in these measurements were also the GC-MS respectively ICP-MS couplings.

Chapter IX concludes the thesis by presenting final conclusions as well as personal contributions brought by this paper. The following possible directions of research are outlined, using the experimental results found through this doctoral thesis.

1. GC-MS analysis of aminoacids and fatty acids from maize seeds

Aim of study

Within this study we aimed at developing a qualitative and quantitative analysis method of amino acids and fatty acids from 25 maize seed samples. The study was conducted to increase productivity of maize production and to develop hybrids resistant to different environmental conditions with superior nutritional value and high yield.

We have analysed several inbred lines of maize seed in order to help improve culture [1], [2]. Inbreeding is always achieved through artificial insemination, making it virtually impossible to make inbred lines by another method.

The amounts of protein and amino acids are influenced by genetic and environmental factors such as climatic conditions, soil type, amount of fertilizer used, etc. Research to improve nutritional quality is necessary in order to get adequate amino acid content in feed, as maize usually contains small amounts of essential amino acids such as methionine, lysine, threonine or others that can limit farm animal growth [3].

Gas chromatography coupled with mass spectrometry (GC-MS) is an excellent technique for quantitatively identifying and quantifying amino acids and fatty acids [4], [5] which is the reason for choosing this technique for qualitative and quantitative analysis of free amino acids in maize samples, within this study.

Materials and methods - experimental stage

For qualitative and quantitative analysis of amino acids and fatty acids, 25 samples consisting of maize seeds were used. The studied inbred lines represent the nucleus of 5 maize

grain varieties: TC 209 (1.1), TC 316 (2.1), TC 243 (3.1), TB 367 (4.1), D 105 (5.1), experimented with 5 types of cytoplasm: original, (CIT T 248), (CIT TB 329), (CIT TC 177) and (CIT TC 221). Inbred maize lines were marked: 1.2-1.5; 2.2-2.5; 3.2-3.5; 4.2-4.5; 5.2-5.5.

An amount of 400 mg of maize flour was extracted with 5 ml of 6% trichloroacetic acid ($C_2HCl_3O_2$) in an ultrasound bath for 5 minutes, the extraction process being repeated twice. The samples were centrifuged for 5 min at 6000 rpm, collecting the supernatant. 0.5 ml of this supernatant and 10 µl of [^{15}N] methionine used as internal standard were passed through a 4x40 mm column Dowex 50W-W8 ion changer resin. The collected solution was dried in a stream of nitrogen at 60 °C or using a vacuum centrifuge at 60 °C [2].

Derivatization of amino acids

Compound analysis by GC/MS applies to volatile and semi-volatile compounds. However, there are a large number of compounds that aren't volatile enough to be analysed by gas chromatography and mass spectrometry. By using chemical processes, interest components can be changed to become more volatile.

Analysis of amino acids by gas chromatography-mass spectrometry often involves multiple derivatizations.

In the case of the amino acid experimental study, the derivatization included an esterification of the carboxyl function by adding 200 µl 3M butanol / HCl for 1 hour at 110°C followed by acetylation of the amine function using 100 µl of trifluoroacetic anhydride for 20 min at 60°C. The trifluoroacetylation reaction serves to reduce polarity of the amino group. Excess solvents were removed with a stream of nitrogen, and the derivatized dried components were dissolved in 500 µl of ethyl acetate and injected into the GC-MS.

Derivatization of fatty acids

Fatty acid samples were converted to corresponding methyl esters (FAME) for GC analysis. Methanol: acetyl chloride 4: 1 (v: v) was used as esterification agent. The esterification process took 20 minutes at 80 °C. Excess reagent was removed by evaporation in a stream of nitrogen at 60 °C, the acquired methyl esters being then dissolved in 500 µl of dichloromethane. After adding the internal standard (10 µg C11: 1), 1 µl of sample was automatically injected into the gas chromatograph.

Experimental equipment

For separation and identification of amino acids and fatty acids, a Trace GC gas chromatograph coupled with a Trace DSQ Thermo Finnigan quadrupole mass spectrometer analyser (Figure 1.1) were used. Separation was performed on a Rtx-5MS, 30 m long, 0.25 mm in diameter, 0.25 µm solid phase thickness capillary column.

Figure. 1.1. *Thermo Finnigan Trace GC-Thermo DSQ system*

The temperature program for amino acid separation started at 70 °C for 2 min, then increased at a rate of 5 °C / min to 100 °C, then by 10 °C / min to 260 °C and then by 17 °C / min to 290 °C, this temperature being maintained for 5 min.

For separation of fatty acids, the following temperature program was used: 50°C, 1 min, successive increment of 8 °C/min to 300 °C. The helium flow (carrier gas) was 1 ml/min.

Mass spectrometer operating conditions were the same for both types of analysed compounds: transfer line temperature: 250 °C, injector temperature: 200 °C, ion source temperature: 250 °C. Electron energy was 70 eV and the emission current was 100 mA. As internal standard ^{15}N-methionine was used for quantitative measurement of amino acids, respectively undecaenoic acid (C11: 1), for quantitative measurement of fatty acids.

Quantitative calculation of amino acids

For detection and quantification of amino acids, the isotopic dilution method is used. An isotopically labelled internal standard, analogue of the amino acid of interest, typically ^{15}N - methionine, is added to each sample of supernatant resulting from extraction. The fractional isotopic abundances of methionine and its ^{15}N -labelled isotopomer were calculated from the experimentally determined isotopic ratios.

Isotopic contributions can be described by means of a set of simultaneous linear equations with the general form:

$$I_x = \sum_{x=i,j} A_i X_j \qquad (1)$$

where I_x represents the relative isotopic abundance of ion x, A_i represents the contribution of each isotopic species to each mass in the recorded domain, and X_j is the unknown fractional abundance.

If we express the set of simultaneous equations in matrix notation, the relative abundance of each isotopic species (A_i) is calculated for two ions, m/z 171 and m/z 172:

$$I = AX \qquad (2)$$

where: A - the abundance matrix (m x n), containing the contribution of each isotopic species, I - the measured intensity; X - the amount of compound.

The equations set's solution is obtained by the least square method, as follows:

$$X = (A^T A)^{-1} A^T I \quad (3)$$

where: A^T – transpose of matrix A.

The pseudo-inverse matrix, A^{-1}, is calculated as follows:

$$A^{-1} = \frac{1}{\det A} A^* \quad (4)$$

where A is the determinant matrix and A^* is the adjoint of matrix A.

The response factors for each amino acid (the non-dimensional coefficients used to correct the detector's response) are calculated by:

$$F_i = \frac{A_i/A_j}{m_i/m_j} \quad (5)$$

where m_i is the amount corresponding to compound *i*, m_j is the amount corresponding to compound *j*, and A_i and A_j are the areas of compounds *i* and *j*.

Amino acids in the maize seed samples were calculated using the formula:

$$C_i(\%masă) = \frac{\frac{m_j * A_i}{F_i * A_j}}{\sum_{i=1}^{n}\left(\frac{A_i}{F_i}\right)} * 100 \quad (6)$$

where C_i is the amount of compound *i*, m_j is the amount of internal standard, A_i and A_j are the areas of peaks *i* and *j*, and F_i is the response factor corresponding to compound *i*.

Quantitative calculation of fatty acids

For quantitative measurement of fatty acids, we used 10 mg of undecenoic acid (C11: 1) as internal standard, after derivatization.

The quantitative calculation of fatty acids was performed using the following formula:

$$F_i = \frac{A_i/A_j}{m_i/m_j} \quad (7)$$

$$m_i(\mu g) = m_j(\mu g) \frac{A_i}{F_i \cdot A_j} \quad (8)$$

where: F_i - response factors calculated using fatty acid standards,

m_i, m_j - the amounts corresponding to compounds *i* respectively *j*;

Ai, *Aj* - areas of compounds *i*, respectively *j*.

Results

Measuring amino acids in maize seed samples

Detection of amino acids in the studied inbred lines of maize seed extracts was performed using the NIST Spectrum Library. Thus, 17 (seventeen) amino acids were identified, according to Table 1.1.

Table 1.1. *Amino acids identified in the inbred lines of maize seed extracts*

No.	Identified constituent	Chromatographic retention time (min)
1	Alanine (Ala)	10.56
2	Glycine (Gly)	11.28
3	Threonine (Thr)	11.92
4	Serine (Ser)	12.38
5	Valine (Val)	12.72
6	Leucine (Leu)	13.94
7	Isoleucine (Ile)	14.09
8	Gamma-aminobutyric acid (GABA)	15.27
9	Proline (Pro)	16.01
10	Methionine (Met)	17.48
11	Aspartic acid (Asp)	18.60
12	Phenylalanine (Phe)	18.70
13	Ornithine (Orn)	18.75
14	Tyrosine (Tyr)	19.75
15	Lysine (Lys)	19.98
16	Glutamic acid (Glu)	20.03
17	Histidine (His)	22.35

Free amino acids separation chromatograms from two varieties of inbred lines of maize seeds, 3.1 and 5.4.: (Ala: 10.56 min, Gly: 11.28 min, Ser: 12.38 min, Val: 12.72 min, GABA: 15.27 min, Pro: 16.06 min, Met: 17.48 min, Asp: 18.6 min, Lys: 19.98 min, Glu: 20.03 min, His: 23.35 min) are shown in Figure 1.2.

Comparison of the total free amino acids is shown in fig. 1.3. The bars represent the total free amino acid results for the maize seeds TC 209 (1.1), TC 316 (2.1), TC 243 (3.1), TB 367 (4.1), D 105 (5.1), and the total amino acid results for maize seeds inbred lines 1.2, 1.3, 1.4, 1.5, obtained from the experiments TC 209 (1,1), with cytoplasmic types: 2 (CIT T 248), 3 (CIT TB 329), 4 (CIT TC 177) and 5 (CIT TC 221) and so on; 1.1 is compared to seeds 1.2, 1.3, 1.4 and 1.5; 2.1 with 2.2-2.5; 3.1 with 3.2-3.5; 4.1 with 4.2-4.5; 5.1 is compared to seeds 5.2-5.5.

Highest values for the total number of free amino acids were obtained for the inbred line 3 followed by 2, 4, 1 and 5.

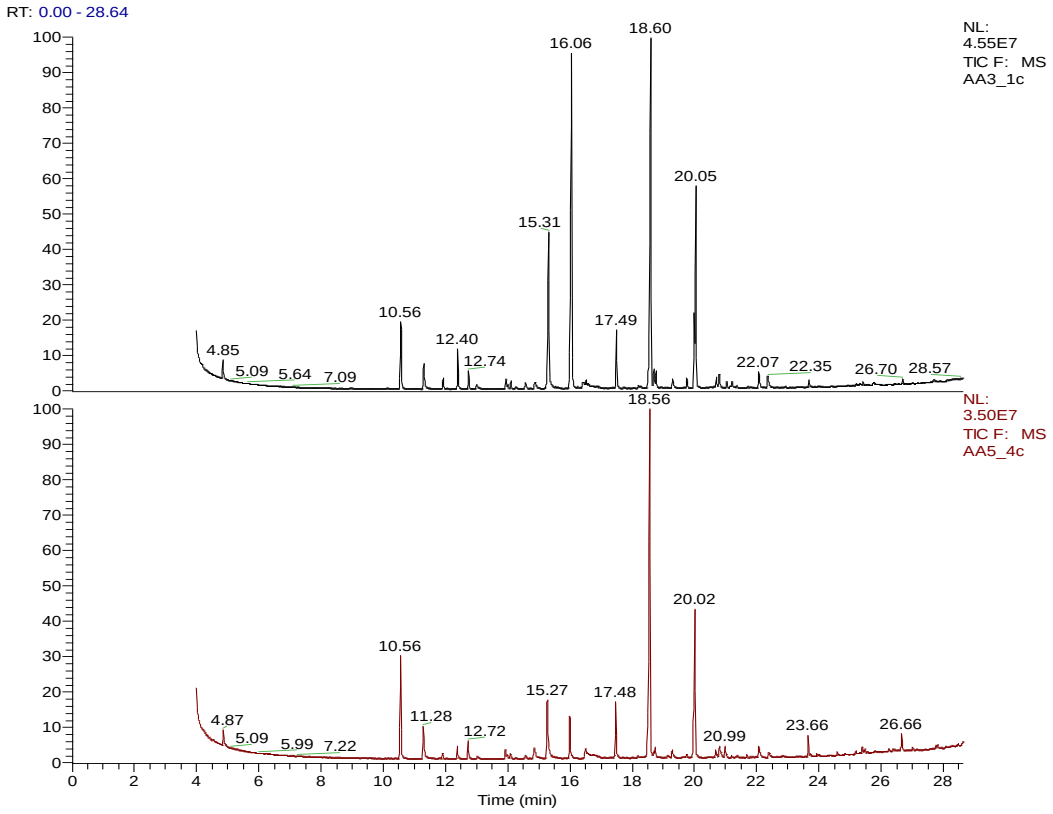

Figure. 1.2. *Comparison of free amino acid variation from two types of maize seed (inbred lines), 3.1 and 5.4*

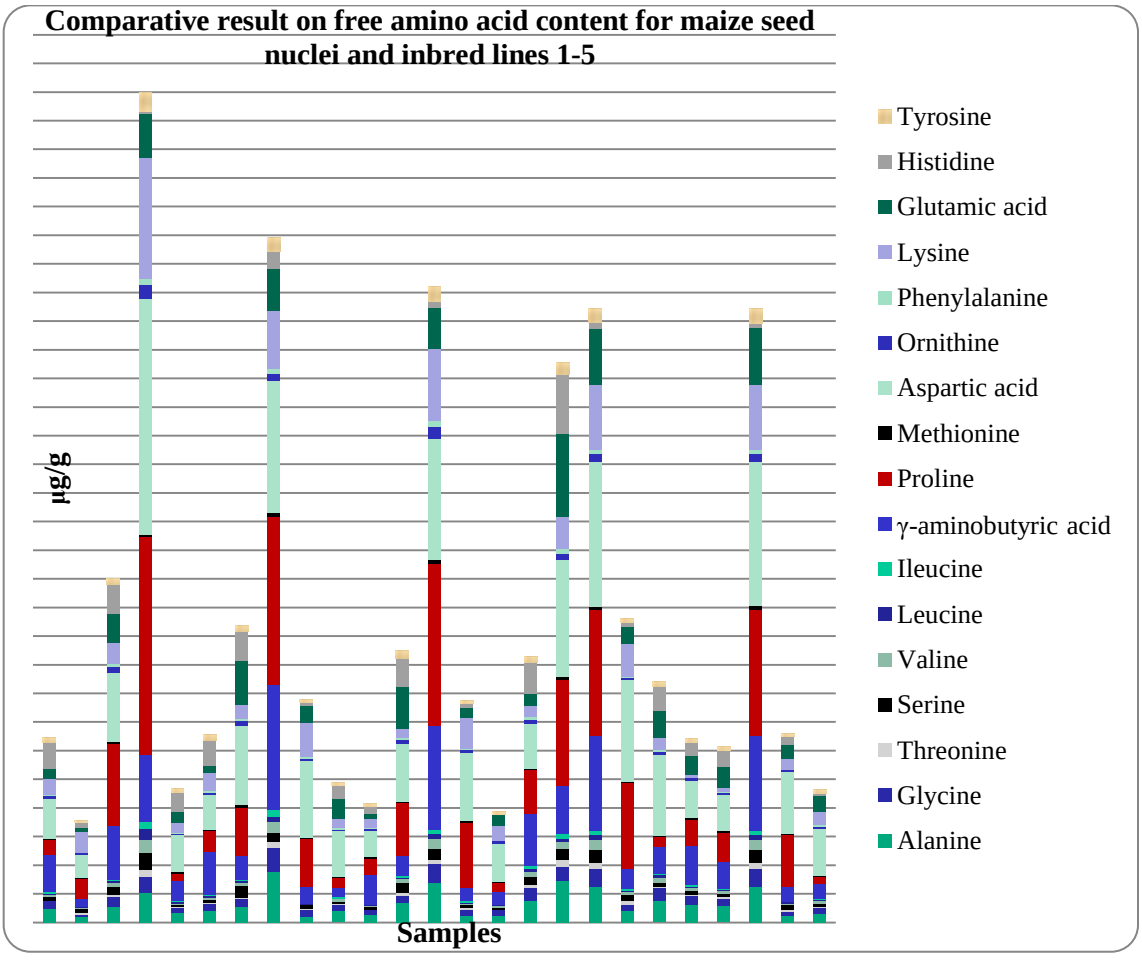

Figure. 1.3. *Comparison of total free amino acids in maize inbred lines 1-5*

Results of amino acids values in inbred maize seed lines, in μg.g-1, for seed nuclei are shown in Table 1.2 and Fig. 1.4. for inbred seed lines.

Table 1.2. *Amino acids values, in μg.g-1, for seed nuclei*

μg.g·[1]	1.1	2.1	3.1	4.1	5.1
Ala	94.36±18.16	40.23±8.30	112.17±16.51	207.05±27.04	64.66±9.88
Gly	53.56±3.34	17.15±073	64.09±10.88	110.56±10.85	38.53±6.56
Thr[1]	6.55±0.24	8.40±2.07	17.06±4.02	53.39±7.21	3.85±0.21
Ser	23.62±8.55	31.47±6.31	51.75±14.90	113.23±5.55	12.35±3.19
Val[1]	19.37±2.05	7.45±1.29	27.85±3.47	96.21±8.59	13.23±0.27
Leu[1]	7.53±0.93	3.29±0.73	12.86±2.04	72.80±14.28	9.10±0.12
Ile[1]	7,54±1.08	3.65±0.72	13.85±0.54	48.14±1.04	6.75±0.81
Pro	100.84±12.57	140.50±24.25	569.22±93.61	1522.20±165.37	50.22±2.38
Met[1]	10.25±0.92	11.72±0.11	16.63±0.15	16.94±1.05	9.89±1.88
Asp	280.32±39.11	155.14±17.66	483.75±127.60	1642.84±260.53	261.27±73.59
Orn[1]	15.47±2.84	14.88±3.19	40.46±1.63	102.62±13.95	8.05±2.51
Phe[1]	6.96±2.12	3.48±0.81	18.60±2.12	39.14±9.46	7.01±0.36
Lys[1]	113.10±1.53	142.06±30.20	149.33±44.05	845.47±87.43	64.77±7.48
Glu	72.47±4.29	30.55±1.59	202.87±56.70	305.95±63.99	75.74±21.68
Tyr	40.21±1.95	14.05±2.99	44.10±10.08	136.09±12.08	25.34±4.07
His[1]	177.78±80.68	37.96±17.01	204.04±83.11	15.03±5.13	139.49±10.65
Total	1293.29	713.44	2404.68	5798.44	933.87
EAA[1]	458.91	273.12	612.83	1496.88	326.84

Results point out that, for example, in the experiment between seed nucleus (3.1) with cytoplasmic types 2,3,4 and 5, the largest amino acids are proline 885.12 - 1179.54 μg·g^{-1} and aspartic acid 840.07 - 1006.52 μg·g^{-1}. The highest values for total free amino acids were obtained for inbred lines 3.4, resulting from the Type 4 cytoplasm experiment.

Other major amino acids from maize seeds were gamma-aminobutyric acid, glutamic acid, lysine, alanine and histidine in amounts of 65.59 - 871.96 μg·g^{-1}, 41.75 - 577.24 μg·g^{-1}, 23.89 - 507.42 μg·g^{-1}, 36.72 - 352.11 μg·g^{-1} and 6.02 - 211.43 μg·g^{-1}. Seed nuclei values for proline, aspartic acid and lysine were much higher in sample 4.1 as shown in Table 2., respectively 1522.20 μg·g^{-1}, 1642.84 μg·g^{-1} and 845.47 μg·g^{-1}. Small amounts of glycine 31.34 - 125.03 μg·g^{-1}, serine 11.85 - 89.81 μg·g^{-1}, valine 6.30 - 76.21 μg·g^{-1}, ornithine 9.62 - 89.18 μg·g^{-1} were determined from inbred maize lines.

Tyrosine levels of 108.99 μg·g^{-1} were higher than isoleucine (≤ 45.67 μg·g^{-1}), methionine (≤ 32.99 μg·g^{-1}), threonine (≤ 42.40 μg·g^{-1}) and leucine (≤ 36.73 μg·g^{-1}).

In the results achieved, Asp and Glu values represent the sum of aspartic acid and asparagine, respectively glutamic acid and glutamine, because the derivatization method changes glutamine and asparagine in glutamic acid and respectively aspartic acid.

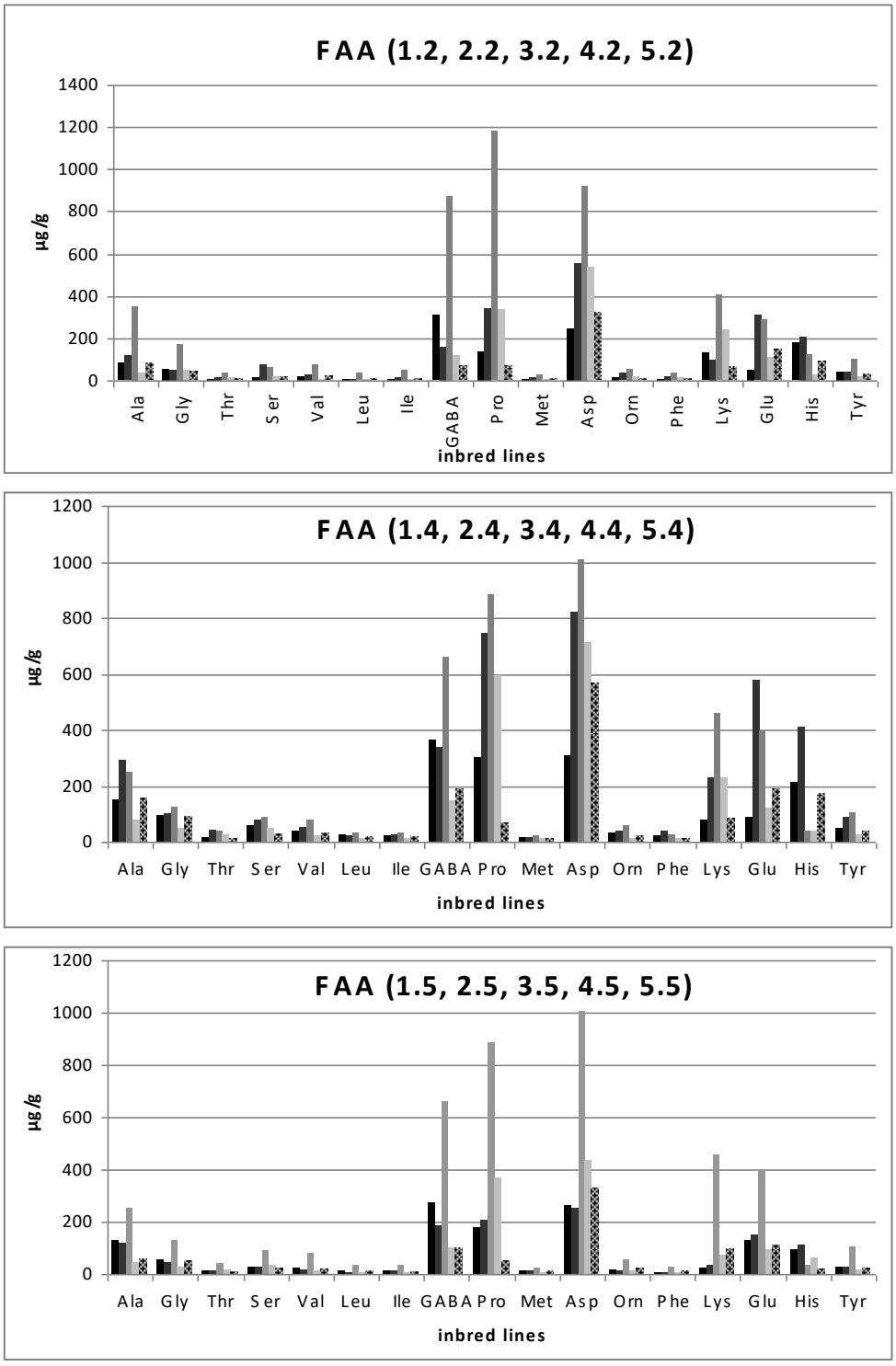

Figure. 1.4. *Free amino acids from maize inbred lines*

Figure 1.5 shows a comparison of total essential amino acids measured in maize samples (Ala, Thr, Val, Leu, Ile, Se, Met, Orn, Phe, Lys, His). Compared to nuclei seeds (1.1, 2.1, 3.1,

4.1, 5.1) with high levels of essential FAA (in seeds 4.1), inbred lines 3.2, 3.3, 2.4, 3.4 and 3.5 showed the highest concentration.

Results are useful for choosing inbred lines with good traits on nutritional and functional qualities for development of elite sprout plasma selection processes.

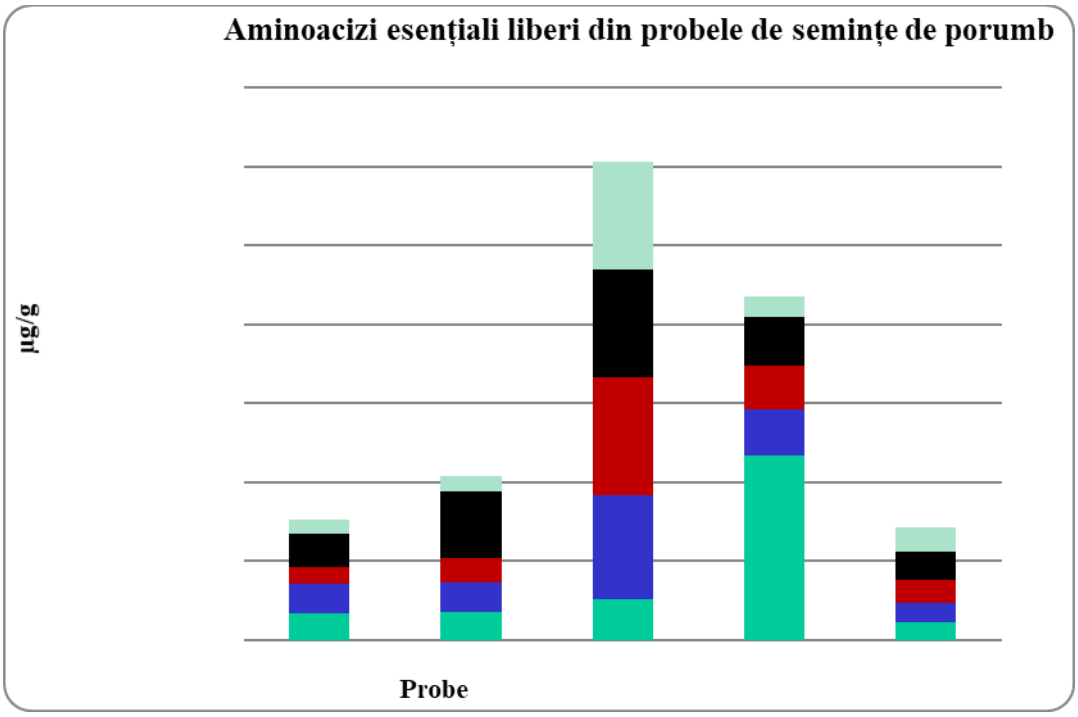

***Fig. 5**. Essential free amino acids from maize seed samples. Sample rating, bottom-up:1: 1.1, 1.2, 1.3, 1.4, 1.5; 2: 2.1, 2.2, 2.3, 2.4, 2.5; 3: 3.1, 3.2, 3.3, 3.4, 3.5; 4: 4.1, 4.2, 4.3, 4.4, 4.5 si 5: 5.1, 5.2, 5.3, 5.4, 5.5; the first sample (colour green) of each group represents the nucleus of the maize seed and the other 4 samples represent the lines*

Fatty acids measurement

Following the analysis of the 25 seed samples of inbred maize lines, the predominant fatty acids found were: C18: 2, C18: 1, C16: 0, C18: 0.

The method was validated by injecting a standard fatty acid solution, which underwent the same derivatization procedure. Fatty acids elution order is shown in Table 1.3.

***Table 1.3.** Fatty acids found in the inbred lines of maize seed extracts*

No.	Constituent	Rt(min)
1	C11:1 (hendecenoic acid=SI)	16.09
2	C16:1 (palmitoleic acid)	23.13
3	C16:0 (palmitic acid)	23.4
4	C18:2 (linoleic acid)	25.55
5	C18:1 (oleic acid)	25.6
6	C18:0 (stearic acid)	25.79
7	C20:1 (11-eicosenoic acid)	27.75

8	C20:0 (eicosanoic acid, arachidic acid)	27.99
9	C22:0 (benenic acid) M=354	30.04
10	C24:0 (lignoceric acid) M=382	31.87

The dominant saturates acids were: palmitic acid, 15.83-20.81%, respectively stearic acid, 3.2-3.7%. The most common fatty acid in maize seed samples was linoleic acid, an Omega-6 polyunsaturated fatty acid, with a percentage of 45.06% -62.34%, followed by oleic acid, a mono- unsaturated (MUFA) fatty acid, with values ranging from 15.03% to 23.59%. Figure 1.6 shows the saturated fatty acids and Figure 1.7 illustrates the unsaturated fatty acids identified in the inbred maize lines 1-5.

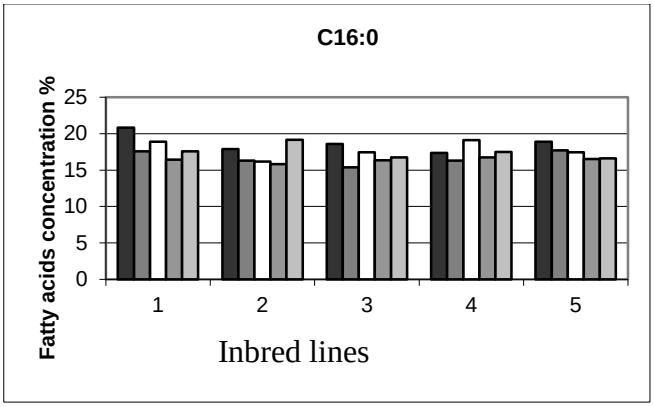

Figure 1.6. *Saturated fatty acids from maize seeds inbred lines1-5*

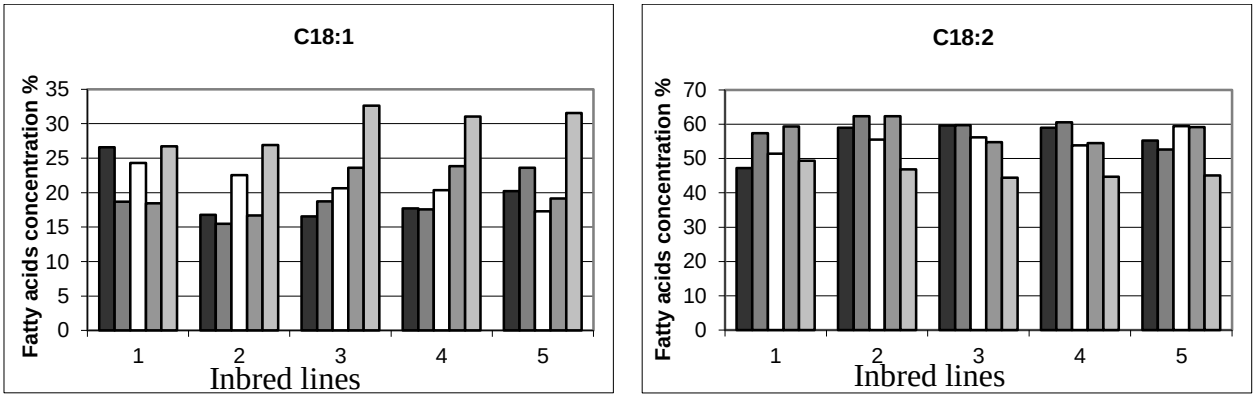

Figure 1.7. *Unsaturated fatty acids from maize seeds inbred lines1-5*

The black bars in above graphs represent results for maize seed nuclei TC 209 (1.1), TC 316 (2.1), TC 243 (3.1), TB 367 (4.1), D 105 (5.1) and the other four bars are the results for inbred lines of maize seeds 1.2, 1.3, 1.4, 1.5, obtained in TC 209 (1.1) experiments, with the following cytoplasmic types: 2 (CIT T 248), 3 (CIT TB 329) and 5 (CIT TC 221), and so on; 1.1 is compared to 1.2, 1.3, 1.4 and 1.5; type 2.1 with 2.2-2.5, 3.1 with 3.2-3.5, 4.1 with 4.2-4.5, and 5.1 with 5.2-5.5.

Method validation

Within this study, we performed method validation by using amino acid standards. The precision obtained for R.S.D showed values less than 23% and the accuracy was less than 20% for amino acid standards containing 60 respectively 80 µg/ml. L.O.D. și L.O.Q. of analytical procedures ranged from 10^{-3} to 10^{-2} µg $µl^{-1}$.

Conclusions

The extraction method and GC-MS technique proved to be suitable for measuring free amino acids (FAA) in maize inbred lines. Highest values of free amino acids measured in maize seeds were for aspartic acid and proline, followed by lysine, alanine, glutamic acid, histidine and gamma-aminobutyric acid. FAA measured and showed important differences between studied maize inbred lines.

Dominant saturates acids were palmitic acid and stearic acid. Most common fatty acid in maize seed samples was linoleic acid, an Omega-6 polyunsaturated fatty acid, followed by oleic acid, a mono-unsaturated fatty acid (MUFA). The concentration of oleic acid is strongly negatively correlated with that of linoleic acid, suggesting that genotype selection can improve functional and nutritional qualities of these inbred lines.

By choosing lines with the best traits, nutritional and functional quality of maize seeds can be improved.

2. Determination of fatty acids in plasma and trout meat by GC-MS

Aim of the study was to develop a simple and reliable method to compare fatty acids in trout plasma and meat [6].

Materials and methods - experimental stage

Fatty acids (FA) were determined from plasma and trout samples. FA were extracted from 0.5 ml of plasma by adding 0.5 ml of chloroform: methanol 2: 1 (v: v). The solution was stirred vigorously for 30 seconds at room temperature. 1 g of trout meat was crushed with 1 g of quartz sand in a ceramic pot and homogenized with 5 ml of distilled water. After a 5 minute centrifugation, the supernatant was collected and the fat acids were extracted using the same solvent and the plasma extraction conditions. The samples were centrifuged for 5 min (5800 rpm) and the higher methanol - the aqueous phase was removed. The lower chloroform phase containing the extracted fat acids is dried in a stream of nitrogen at 60 °C. The lipids were converted to the appropriate FAME (methyl esters of fatty acids) by esterification of the carboxylic functions with 200 µl of methanol: acetyl chloride 4: 1 (v: v), 20 min, 80 °C. The derivatives were evaporated to dryness with a stream of nitrogen at 60 °C, and then dissolved in 500 µl of dichloromethane. 10 µg of C11: 1 were added to each sample for quantitative determination by GC-MS.

Experimental apparatus

The fatty acids were separated and identified using a Trace GC gas chromatograph equipped with a RTX-5MS capillary column (30m x 0.25 mm ID, 0.25 µm film thickness) and coupled to a Trace DSQ quadripolar mass spectrometer (Thermo Finnigan). The temperature program for separating FAME was: 50 °C for 2 min, increasing at 8 °C/min at 310 °C (8 minutes). The helium was used as a carrier gas at a flow rate of 1 ml / min. 1 µl of each sample was injected into GC-MS using the splitting mode (10: 1) with the TriPlus autosampler. The electron impact ion source (EI) worked at 70 eV. The following conditions were assured: the transfer line temperature was set at 250 °C, the injector temperature at 200 °C and the ion source

temperature at 250 °C. The emission current was 100 μA. Qualitative analysis was performed in the 50 - 500 a.m.u.

Results and discussions

The fatty acid profile of freshwater fish is unique in variety and degree of unsaturation [8-14]. Their nutritional role is recognized, the two main omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, with many beneficial effects on the body in both growing children and adult. Thus, administration of these omega-3 fatty acids can help lower cholesterol levels, regulate blood pressure, normalize heart rate, reduce the risk of breast cancer [15], [16]. The seasonal variation of these nutrients is studied extensively [17-19]. Therefore, it is essential to have a simple and rapid method for the qualitative and quantitative characterization of uncommon fatty acids (long chain polyunsaturated fatty acids). The sensitivity and selectivity of the method make GC-MS a particularly useful method for FAME analysis [7], [20]. Figure 2.1 shows the total ionic chromatogram of a mixture of trout plasma fatty acids. FAME were identified using the NIST Spectrum Library.

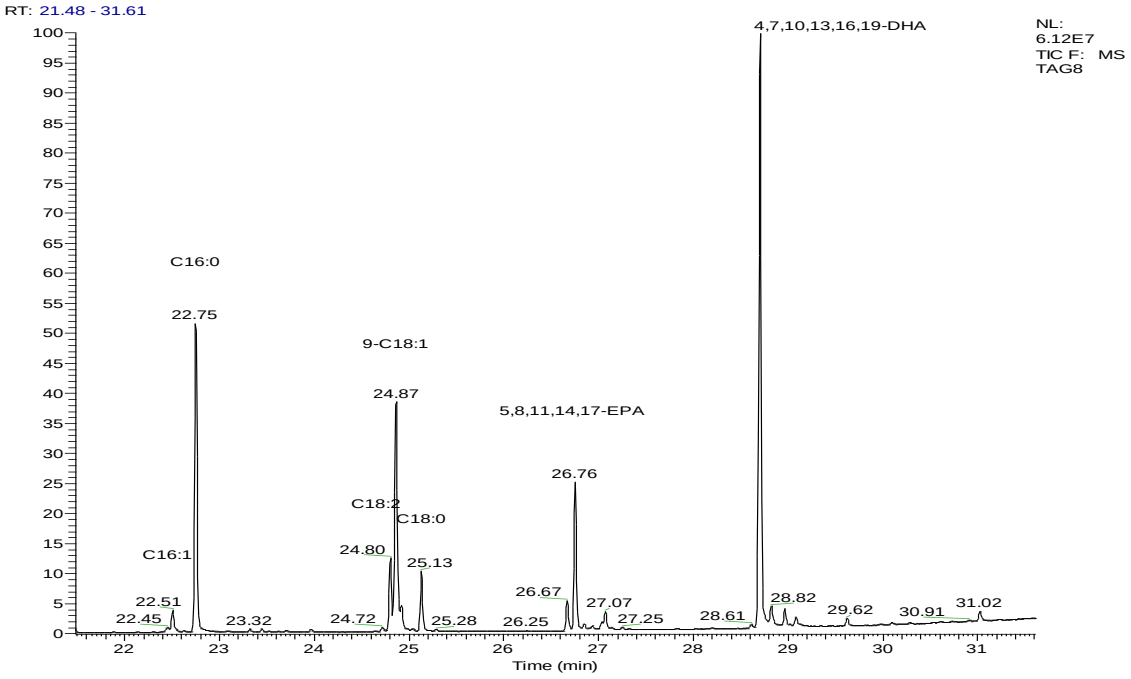

Figure 2.1. *ICT Chromatogram for the separation of fatty acids from trout plasma by GC-MS*

Table 2.1. shows the composition of fatty acids (in% by weight of total fatty acids) of plasma and trout. Saturated fatty acids represent only 25.61% (in plasma) and 31.04% (in meat) of total fatty acids, palmitic acid (C16: 0) having the highest concentration. Stearic acid (C18: 0) is present in relatively small proportions (5.42% and 6.27%). Unsaturated fatty acids (UFA) represent more than half of the total fatty acids found in plasma and meat samples (74.39% and 68.95%, respectively). Most monounsaturated acids (MUFA) were C16: 1, C18: 1n-7, C18: 1 n-9, oleic acid (C18: 1n-9) being the most abundant. Linoleic acid (C18: 2 ω-6) represents 19% (in plasma) and 11.3% (in meat) of the total UFA. PUFA ω-3 (sum of EPA and DHA) represents approx. 26% and 36.6%, respectively, of the total of fatty acids found in trout and meat plasma.

Table 2.1. *Concentrations of fatty acids (%) in plasma and trout, n = 5*

	R_t (min)	Fatty acids (%) Plasma	Meat
hexadecenoic acid (C16:1)	22.51	2.86	2.19
hexadecanoic acid (C16:0)	22.75	20.18	24.78
9,12 octadecadienoic acid (C18:2)	24.80	14.13	7.81
9-octadecenoic acid (C18:1)	24.87	27.79	18.91
octadecenoic acid (C18:1)	24.93	3.62	3.43
octadecanoic acid (C18:0)	25.13	5.42	6.27
5,8,11,14,17 eicosapentaenoic acid (C20:5)(EPA)	26.76	5.41	8.51
4,7,10,13,16,19 docosahexaenoic acid(C22:6)(DHA)	28.71	20.59	28.11
SFA		25.61	31.04
UFA		74.39	68.95
EPA		5.41	8.51
DHA		20.59	28.11

(Rt - retention time; SFA - saturated fatty acids, UFA - unsaturated fatty acids;

SFA = C16: 0 + C18: 0; UFA = C16: 1 + C18: 2 + 9-C18: 1 + C18: 1 + EPA + DHA)

Figure 2.2. shows the considerable proportion of unsaturated fatty acids (UFA) compared to unsaturated (SFA) in plasma and meat. It should be noted that ω-3 PUFA accounts for 53% of the total UFA.

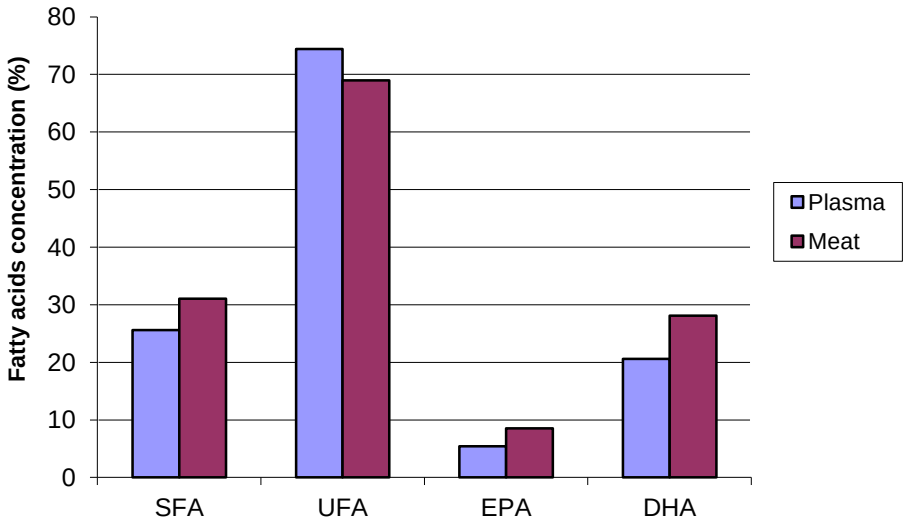

Figure 2.2. *Content of fatty acids in trout plasma and meat samples.*

In plasma, the ω-3/ω-6 ratio was 1.84, while in the meat, 4.68. The DHA (22: 6ω3)/EPA (20: 5ω3) ratio was 3.8 in plasma and 3.3 in the meat. This result can be explained by the fact that EPA is not found in large amounts in tissues, being used rapidly in DHA biosynthesis and eicosanoids.

Conclusions

The GC-MS method developed here for determining fatty acids in plasma and trout samples is simple and appropriate for the intended purpose [6], [21]. Validation parameters obtained were good.

The concentration of PUFA in plasma and trout was higher than that of other fatty acids in the following order: PUFA> MUFA> SFA. Our results prove that trout is a valuable source of essential fatty acids.

3. Comparison of the nutrient composition of some vegetable oils

Aim of the study

The purpose of the study was to evaluate and compare the content of fatty acids and metal ions present in various vegetable oils. The values of the obtained fatty acids were compared with the trout meat. In this respect, a method of extraction, chromatographic separation with the identification of the specific spectra of vegetable oils from different types of seeds was developed and validated. Quantitative analysis of fatty acids derivatized as methyl esters (FAME - Fatty Acids Methyl Esters) was performed by GC/MS technique.

The study also proposes a simple method of determining the metal ion content (Na, Mg, K, Mn, Cd, Cu and Cr) using the ICP-MS inductive coupled plasma mass spectrometer.

Materials and methods - experimental stage

Analysis of total fatty acids in some vegetable oils was performed by mass spectrometry technique coupled with gas chromatography (GC/MS). The fatty acids were derivatized as methyl ester, separated by GC and identified by MS techniques. Some vegetable oils used as food supplements, purchased from the organic market in Romania and coming from different European countries, including Romania, were compared in terms of composition in fatty acids and metal ions. The oil samples studied were flaxseed, poppy, grape, hemp, nut, soy, pumpkin, sesame and olives.

To evaluate their fatty acid content a Trace DSQ Thermo Finnigan quadrupole mass spectrometer coupled with a Trace GC gas chromatograph was used. Oil samples derivatized as fatty acid methyl esters (FAME) were separated on a RTX-5MS capillary column, 30 m x 0.25 mm, film thickness 0.25 μm. The temperature program used was: 50 °C, 2 min, 8 °C / min at 310 °C (10 min); the carrier gas was helium 6.0 at a flow rate of 1 ml / min. The following conditions were followed: transfer line, temperature 250 °C, injector temperature 250 °C; ion source temperature 250 ^{o}C; Splitter: 10: 1; the electron energy was 70 eV and the emission current, 100 μA.

Derivatization and separation consisted of the following steps [22]: Vegetable oil samples (20 μl) were transformed into FAME for GC analysis by esterification with 200 μl of methanol: acetyl chloride (4: 1 v / v) for 20 minutes at 80 ^{o}C. The derivatives were evaporated to dryness in a stream of nitrogen at 60 °C and then dissolved in 500 μl of dichloromethane. 1μl was injected into the GC automated with the autosampler. The identification of fatty acids was obtained by comparing methyl esters of fatty acids from the mass spectra recorded in the m / z range 35 - 500 using the mass spectra of the FAME standards of the NIST library.

Elementary analysis of the metal content was performed by plasma inductively coupled mass spectrometry, (ICP-MS). The following operating conditions were applied: nebulization gas flows of 0.86 L min^{-1}; auxiliary gas flow of 1.2 L min^{-1}; gas and plasma flow of 15 L min^{-1}; Lens voltage of 7.25 V; power 1100 W ICP RF; CeO / Ce = 0.027; Ba^{++} / Ba^{+} ratio = 0.025.

Larger unsaturated fatty acids (UFA), ranging from 71.49% (olive oil) to 89.51% (linseed oil), have been found in the investigated vegetable oils. The sequence of fatty acids determined in oils was as follows: olives (71.49%) < sesame (76.94%) < pumpkin (78.16%) < soybean (82.92%) < hemp (86.19%) < grapes (87.61%) < poppy (88.93%) < flax (89.51%). Values are significantly higher than those of unsaturated fatty acids derived from trout, where the lowest determined value is 68.96%. (Table 3.1 and 3.2).

Table 3.1. *Fatty acid methyl esters identified in vegetable oils*

FAME	Flax	Poppy	Grape	Hemp	Nuts
C16:1	0.06	0.39	0.15	-	0.27
C16:0	7.15	9.06	9.08	10.05	12.09
C18:2	18.34	71.93	63.10	58.35	59.32
C18:1	68.34	14.83	24.40	11.78	10.83
C18:3	-	-	-	16.06	9.46
C18:0	3.35	2.00	3.32	3.75	3.11
EPA	1.77	1.78	-	-	3.85
C20:1	1	-	-	-	-
-C20:0	-	-	-	-	-
DHA	-	-	-	-	-
C22:0	-	-	-	-	-

Table 3.2. *The fatty acid methyl esters found in vegetable oils and trout*

FAME	Soy	Pumpkin	Sesame	Olives	Trout
C16:1	-	0.52	0.61	2.09	2.19
C16:0	12.40	17.18	15.62	22.70	24.80
C18:2	54.31	57.42	42.23	3.32	7.81
C18:1	27.98	20.22	32.54	65.20	22.30
C18:3	-	-	-	-	-
C18:0	3.89	4.65	5.48	4.43	6.27
EPA	-	-	0.65	-	8.51
C20:1	-	-	0.91	0.87	-

C20:0	0.38	-	0.89	1.35	-
DHA	0.63	-	-	-	28.10
C22:0	0.42	-	1.08	-	-

UFA / SFA ratios ranged from 2.51 (olives) to 8.52 (linseed) and recorded similar variations as UFA. For trout, the ratio was 2.22 (Figure 3.1).

Concentrations of polyunsaturated fatty acids (PUFAs) determined in the studied vegetable oils varied in the following order: olives (3.32%) < linseed (20.11%) < sesame (42.88%) < soybean (54, 94%) < pumpkin (57.42%) < grapes (63.11%) < nuts (72.6%) < poppy (73.71%) < hemp (74.41%).

The PUFA value of trout meat was 44.43%. With the exception of linseed oil, these levels are comparable to those of rich PUFA vegetable oils such as grape seed (65.40%), sunflower (66.00%), pepper (67.80% Perilla (69.90%), flax seeds (71.80%), black currant seeds (75.30%), safflower (77.30%) and hemp seeds (79.10%).

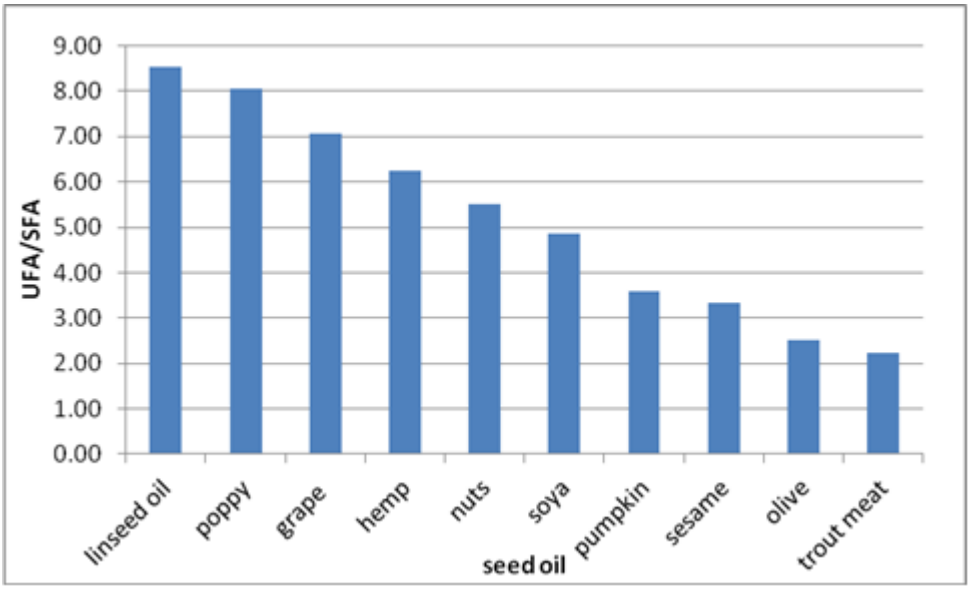

Figure 3.1. *Comparison of the UFA / SFA ratio in the studied samples*

Lenoleic acid (C18: 2) had the highest value in all studied vegetable oils, except for flax and olive oil, and oleic acid (C18: 1) was the most important.

Linolenic acid (C18: 3) was present only in hemp (16.06%) and nuts (9.46%).

DHA fatty acids are the largest in trout and are not found in vegetable oils, but 8.51% EPA in fish meat showed a low value in vegetable oils, of 3.85% in nuts, 1.78% in poppy and flax seed and 0.65% in sesame oil.

PUFA / SFA ratios ranged from 0.12 (olives) to 6.66 (poppy seeds), and were found in the following order: olives < sesame < pumpkin seeds < soybeans < nuts < grapes < hemp < poppy.

Method validation

The method was validated using standard fatty acids. Good validation parameters were obtained. Correlation coefficients for individual compounds calculated for linearity were in the range of 0.982 - 0.983. The accuracy for relative standard deviation (RSD) shows values smaller than 10% for the important fatty acids, in the 2.2 - 8.9% range. The analytical results reported for the concentrations of the fatty acids analyzed are the mean of two distinct measurements.

Conclusions

The GC-MS method developed for the determination of fatty acids from various vegetable oils extracted from seeds has proved to be well-chosen. The validation parameters of the quantitative analysis method were good. The concentration of polyunsaturated fatty acids (PUFA) determined in vegetable oils was higher than that of saturated fatty acids (SFA), in the order: PUFA> MUFA> SFA. Our results have shown that vegetable oils extracted from the seeds analyzed are a valuable source of essential fatty acids.

The comparative determination of FAME in the studied vegetable oils by the GC-MS method showed interesting results: the UFA levels determined from vegetable oils exceeded UFA values in trout, in addition, the PUFA values determined from most vegetable oils were higher compared with that determined from trout (except olive, linseed and sesame oil).

The method used was found to be appropriate for the analysis of small quantities of food samples, and the results could be used for various purposes: food quality control, food processing control, nutrition classification, diet control and metabolic studies.

4. Comparative analysis of the content of fatty acids and metals in seed extracts

Aim of the study

The purpose of this study was to identify and analyze the fatty acid content, as well as to determine minerals and metals from seed samples from a variety of plants. Thus, for qualitative and quantitative determination of fatty acids, selected seeds from a variety of plant species, purchased from the Romanian market, were taken into account. Gas chromatography coupled with mass spectrometry (GC-MS) was used, a modern technique that proved to be particularly useful to achieve the established goal.

Since the determination of mineral nutrients in the diet is of great interest, some metals such as iron, magnesium, zinc and copper are considered essential micronutrients, we have developed a method of qualitative and quantitative analysis based on the inductively coupled plasma mass spectrometry technique (ICP-MS). Some minerals (Ca, Na, Mg, K, Zn) were determined from the seed samples by flame atomic absorption spectrometry using an Analytik Jena, ContrAA 700, in an acetylene-air flame.

Materials and methods - experimental stage

The studied samples were: flaxseed, poppy, grape, hemp, walnut, pumpkin, sesame, watermelon and chia.

The analysis of the fatty acids in the analyzed seeds was carried out by mass spectrometry technique coupled with gas chromatography (GC / MS). The fatty acids were derivatized as methyl ester, separated by GC and identified by MS techniques [23-24].

200 mg of each sample type of plant seed referred to above were crushed and then extracted using 0.6 ml of NaCl and 0.8 ml of methanol, passed through an ultrasonic bath for 1 minute then mixed with 0.8 ml of chloroform for 2 minutes, the solution obtained being centrifuged for 3 minutes at 5800 rpm. The lower layer was collected and the extraction was repeated with 0.4 mL of chloroform. The extract obtained was dried, the fatty acids being converted into methyl esters (FAME) by esterification of the carboxylic functions, and finally adding 10 μl of C11: 1 (undecaenoic acid) as an internal standard.

To assess the content of seed samples in fatty acids a Trace DSQ ThermoFinnigan quadrupole mass spectrometer coupled with a Trace GC gas chromatograph was used. Separation of fatty acid methyl esters was performed using a RTX-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 μm). Each sample was extracted in duplicate.

To determine the content of minerals (metals and trace elements), the inductively coupled plasma mass spectrometry technique (ICP-MS) was used. The seed samples were dried, homogenized and then passed through a 20 mesh screen to obtain extremely fine particles. The microwave digestion method has been optimized using a Mars 5 Microwave system. Sample digestion was performed following a program presented in Table 4.1. These solutions were then analyzed by the ICP-MS technique after appropriate dilution using an external standard calibration.

In order to trace the calibration curve for the quantitative analysis, a high purity ICP Multi Element Standard Solution XXI CertiPUR solution, manufactured by Merck Germany, was used. Some minerals (Ca, Na, Mg, K, Zn) were determined from the seed samples by flame atomic absorption spectrometry using an Analytik Jena, ContrAA 700, in an acetylene-air flame. Standard solutions with a concentration of 1000 mg · l-1 for each mineral (Ca, Na, Mg, K, Zn) were obtained by dilution with 1% (v / v) nitric acid solution.

Table 4.1. *The program used to digest the samples for extraction.*

Phase	Power		Time range	Pressure	Temperature	Scheduled time (min)
	Level	%	(min)	(psi)	(°C)	
1.	800	100	15	800	200	20
2.	15 min-cooling					

Results and discussions

The chromatogram of separation of methyl esters of fatty acids identified in linseed is shown in Figure 4.1. FAME were identified using the NIST Spectrum Library.

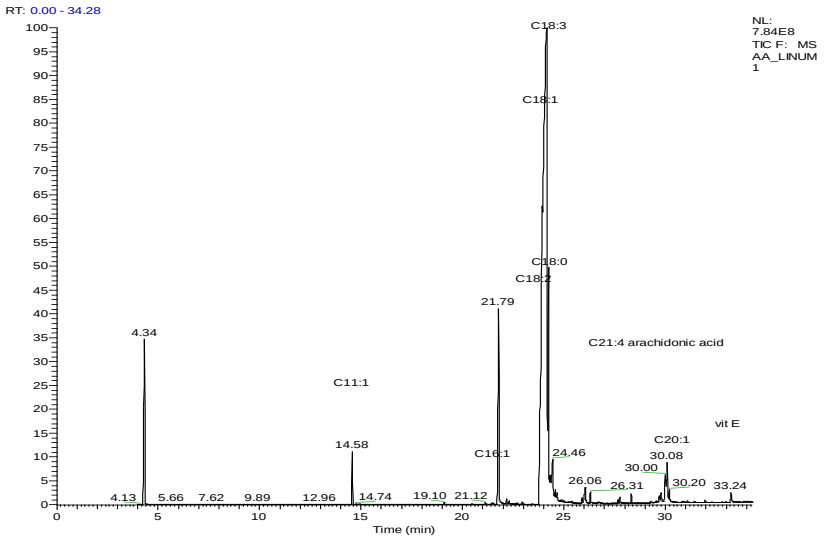

Figure 4.1. *Separation chromatogram in FAME seed*

The applied method allowed the identification and evaluation of the concentrations of a significant number of fatty acids present in the analyzed seed extracts as follows: unsaturated fatty acids (C16: 1 palmitoleic acid, C18:1 oleic acid, C18:2 linoleic acid, α-linoleic acid C18:3) and saturated fatty acids (C16:0 palmitic acid, C18:0 stearic acid, C20:0 arachidic - eicosanoic acid, C22:0 behenic acid, C24:0 lignococcal acid). Analysis of their composition revealed the presence on average of 14% of monounsaturated fatty acids (MUFA), 16% of saturated fatty acids (SFA), and an average of 70% for polyunsaturated fatty acids (PUFAs).

From the polyunsaturated fatty acid (PUFA) category, the linoleic acid (C18:2 - LA), omega-6 and α-linolenic acid (C18:3 - ALA) , omega-3 are considered as essential fatty acids.

The concentration of total saturated fatty acid content varied between 11.13% (grape and pumpkin seeds) and 28.22% (watermelon seeds).

The ratio of unsaturated fatty acids / saturated fatty acids (UFA / SFA) for the analyzed seeds showed the following values: 7.99% for grape and pumpkin seeds, 6.92% for nut, 5.81% for chia, 57% for poppy seeds, 5.21% for hemp, followed by (4.54%), sesame (4.02%) and watermelon (2.64%).

Alpha-linolenic acid (ALA), the main component of Omega-3 acid, plays an important role in the prevention of cardiovascular disease. ALA contributes to protecting blood vessels from destruction caused by inflammatory processes. In the case of the consumption of flax seeds, for example, the concentration of two other fatty acids in the blood stream was increased: eicosapentaenoic acid (ETA) and docosapentaenoic acid (DPA), acids whose presence also contributes to anti-inflammatory protection [12] [25-26].

The content in Omega-6 (C18:2 linoleic acid - LA) shows the following concentrations in descending order of their value: poppy, grape, nuts, hemp, pumpkin, watermelon, sesame, and chia.

Regarding Omega-3 (C18:3 alpha-linoleic acid - ALA), the values determined from the seed analyzed in descending order are: chia, flax, grape, hemp, walnut, pumpkin, watermelon, poppy, completely absent in the case of sesame.

The values determined for fatty acids in mg / g range from 169.7 for chia seed to 133.4 for poppy seed 98.6 (sesame), 53.95 (nut), 43.98 for pumpkin), 27.41 (hemp), 19.0 (grapes) and 9.02 in the case of watermellon.

Using the method developed from the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique, we aimed to determine the content of trace elements (substances with a simple structure in the biological processes of the organisms) for cobalt (Co), chromium (Cr) , nickel (Ni), arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), selenium (Se) and copper (Cu), in extracts from analyzed seed samples (fig. 4.2).

The heavy metals identified with the highest concentration in the seed samples analyzed are: Cu, Ni, Cr and Se. Lead did not exceed the recommended maximum values, whereas cadmium exceeded these values only in poppy seeds.

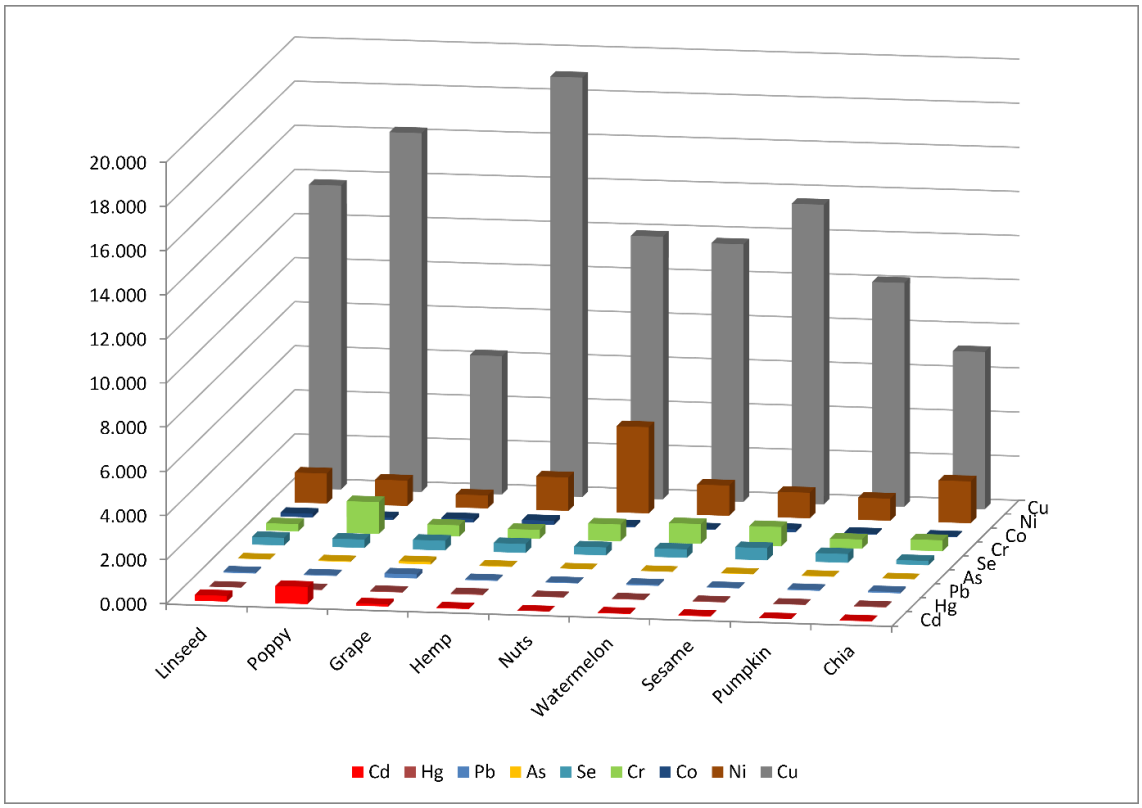

Figure 4.2. *Concentrations of the metals determined in the seed samples analyzed, (µg / g)*

All minerals identified in seed samples were present in high quantities (Figure 4.3), the highest concentration being Ca, determined in grape and poppy seeds, while in sesame and chia it is present in low concentrations . Magnesium showed high values for all analyzed seeds, with the highest concentration being recorded in hemp seeds [24].

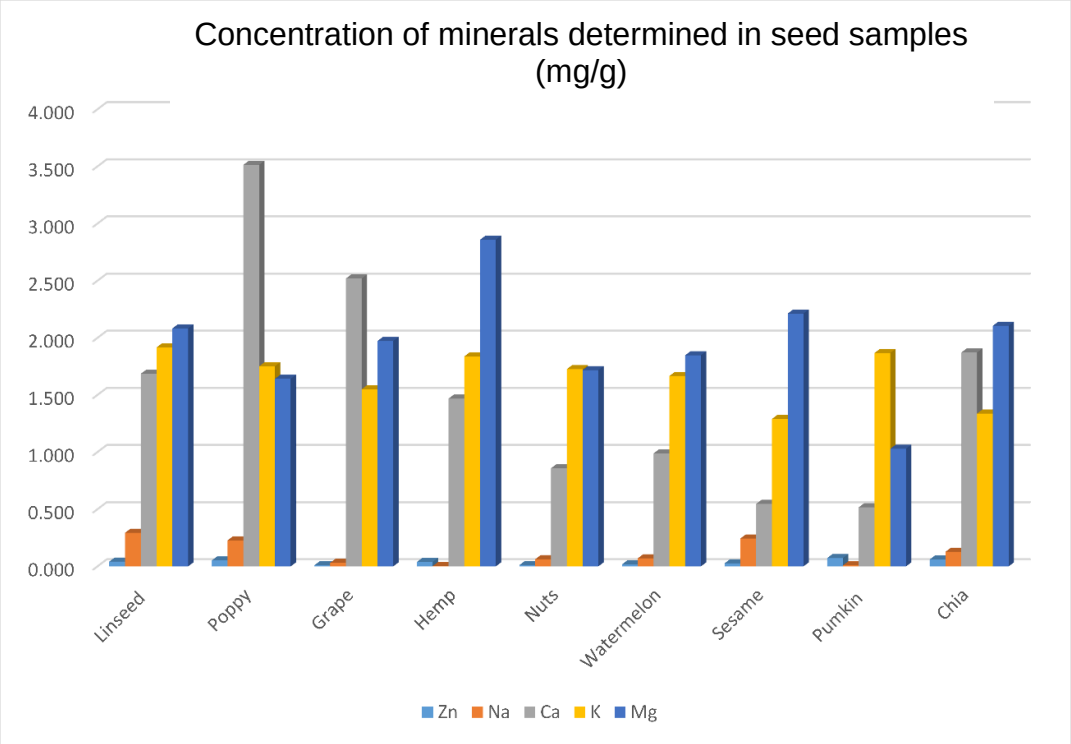

Figure 4.3. Concentration values of the main minerals identified in seed samples analysed

Results validation

Fatty acid standards have been used to validate the assay method. The applied GC-MS method presented superior validation parameters. The accuracy of the method is characterized by Relative Standard Deviation (R.S.D.) of less than 10% for the important fatty acids determined. The value for the recovery parameter was 83.3%.

Accuracy (expressed as bias - measurement error) and accuracy (expressed as relative standard deviation) for the ICP-MS techniques used were determined by analyzing CRM-NCS ZC85006 Tomato certified reference materials (China National Analysis Center for Iron and Steel, Beijing, China). The accuracy was obtained by comparing the measured concentration with the values of the certified material and expressed as a percentage of the recovery yield R. The obtained results indicated the placement of ICP-MS determined ICP-MS concentrations in the confidence interval of 95%, the relative standard deviation being less than 10% for all determined metals.

Conclusions

The method of analysis using gas chromatograph coupling - mass spectrometer has proved to be a simple, rapid and efficient method. Concentrations of polyunsaturated fatty acids (PUFAs) determined in the seed analyzed were higher than saturated fatty acids, confirming their beneficial nutritional value for health.

The analyzed seeds showed high values for identified minerals, calcium (Ca) found in the highest concentration in poppy seeds and grapes.

Concentrations of heavy metals have met the recommended values, except for cadmium (Cd), for poppy seeds, demonstrating the importance of monitoring and controlling them in the diet.

Chia and flaxseeds, with high concentrations of Omega-3 linoleic acid, have shown an important nutritional value.

GENERAL CONCLUSIONS

Based on the experimental results obtained by the studies conducted during the PhD, we can draw the following conclusions:

- ✓ At present, there is a growing interest in the nutritional qualities of foodstuffs due to their influence on human health. One of the main causes of cardiovascular disease and cancer is the content of our diet, the incorrect diet choices. For this reason, scientific research on the optimization of micronutrient content in food and the growing demand for healthy food make it necessary to identify simple and effective analytical methods.
- ✓ Gas chromatography is a technique widely used for the identification and analysis of fatty acids as a result of their derivatization in fatty acid methyl esters (FAME), with multiple applications in the analysis of the nutritional qualities of vegetable oils, various plant extracts and seeds, etc.
- ✓ In the research, we have developed a method of qualitative and quantitative analysis of the content of amino acids and fatty acids from 25 seeds of corn seeds. In this regard, we analyzed several inbred lines of corn seed to increase the productivity of production and development of hybrid species resistant to different environmental conditions with high nutritional value and increased yield. Separation and identification of amino acids and fatty acids were made by GC-MS with isotopic dilution. In order to validate the method, standards amino acid were used. The precision obtained was good, RSD showing values less than 23% and the accuracy was less than 20% for amino acid standards containing 60 and 80 μg / ml respectively).
- ✓ The extraction method and GC-MS technique have proven to be suitable for the determination of free amino acids (FAAs) in corn inbred lines. The highest values of free amino acids determined in corn seeds were for aspartic acid and proline, followed by lysine, alanine, glutamic acid, histidine and gamma-aminobutyric acid. The FAA determined and showed important differences between the corn inbred lines studied. By choosing the lines with the best traits, the nutritional and functional quality of corn seeds can be improved.
- ✓ I have developed a method of qualitative and quantitative determination of the fatty acids content of plasma and trout. The method of extraction, chromatographic separation and spectrum identification of fatty acids from trout plasma has been optimized. The fatty acids were separated and identified by GC-MS. The method was validated using standard fatty acids of 20 μg / ml and 60 μg / ml. The detection limit (LOD) was 1ng and the accuracy gave values less than 20%. Saturated fatty acids identified were only 25.61% (in plasma) and 31.04% (in meat) of the total fatty acids, palmitic acid (C16: 0) having the highest concentration. Unsaturated fatty acids (UFA) accounted for more than half of all fatty acids found in plasma and meat samples (74.39% and 68.95%, respectively).

- ✓ PUFA concentration in plasma and trout was higher than that of other fatty acids in the following order: PUFA> MUFA> SFA. The high MUFA levels in fish plasma demonstrate the high nutritional qualities of this type of meat.
- ✓ Methods of extraction, chromatographic separation, identification and quantitative analysis of fatty acid from vegetable oils (obtained from flax, poppy, grapes, hemp, nuts, soybeans, pumpkin, sesame and olives) have been developed and the comparative analysis with the values of methylated fatty acids determined from fish plasma (FAME) and other nutrients. Vegetable oils used as food supplements, purchased from the organic market in Romania, have been compared in terms of composition in fatty acids and metal ions. Elemental analysis of the metal content was performed by plasma inductively coupled mass spectrometry, (ICP -MS). The method was validated using standard fatty acid samples. The concentration of polyunsaturated fatty acids (PUFA) determined in vegetable oils was higher than that of saturated fatty acids (SFA), in the order: PUFA> MUFA> SFA. Our results have shown that vegetable oils are a valuable source of essential fatty acids. The comparative determination of FAME in the studied vegetable oils showed interesting results: the UFA levels determined from vegetable oils exceeded UFA values in trout, in addition, the PUFA values determined from most vegetable oils were higher compared with that determined from trout (except olive, linseed and sesame oil).
- ✓ A method has been developed to determine the content of fatty acids, namely minerals and trace elements, present in samples of seeds belonging to different species (flax, poppy, grape, hemp, walnut, pumpkin, sesame, watermelon and chia). The analysis of the fatty acids in the analyzed seeds was carried out by mass spectrometry technique coupled with gas chromatography (GC / MS). The fatty acids were derivatized as methyl ester, separated by GC and identified by MS techniques. To determine the content of metals, minerals and trace elements, the inductively coupled plasma mass spectrometry technique (ICP-MS) was used. Some minerals (Ca, Na, Mg, K, Zn) were determined from the seed samples by flame atomic absorption spectrometry using an Analytik Jena, ContrAA 700, in an acetylene-air flame.
- ✓ The analysis method using the gas chromatograph coupling - mass spectrometer has proved to be a simple, fast and efficient method. Concentrations of polyunsaturated fatty acids (PUFAs) determined in the seed analyzed were higher than saturated fatty acids, confirming their beneficial nutritional value for health.
- ✓ The analyzed seeds showed high values for identified minerals, calcium (Ca) found in the highest concentration in poppy and grape seeds. Concentrations of heavy metals have met the recommended values, except for cadmium (Cd), for poppy seeds, demonstrating the importance of monitoring and controlling them in the diet. Chia and flaxseeds, with high concentrations of Omega-3 linolenic acid, have shown an important nutritional value.
- ✓ ICP-MS and GC / MS techniques are physical high-performance quantitative analysis techniques with high precision traceability, with many interdisciplinary applicative possibilities.

The research results were disseminated in 9 scientific papers, published in specialized magazines indexed by ISI and / or BDI, respectively held at national and international scientific sessions and manifestations, both in the country and abroad (22 participations).

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