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Fatty acids distribution in different pastries

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Abstract

Fats are one of the three major categories of foods. They provide our body with a very concentrated source of energy. Fats contain many of the essential fatty acids for health. The intake of fatty acids in food is essential for a healthy diet. An optimal ratio of saturated and unsaturated fatty acids is 0.8, and between omega 3 and omega 6 acids are 1.2. Hypercholesterolemia occurs above these values , and the products are easily oxidizable. The fatty acid content of twelve pastries is analyzed by the gas chromatographic method. The distribution of eight fatty acids present in various pastries was studied, the ratio of saturated and unsaturated fatty acids. These characteristics are essential for nutritional information and have shown significant differences.

Keywords: fatty acids, pastries, analysis

1. Introduction

Fatty acids are found either in the free state or in the form of carbonates. Among the natural derivatives of fatty acids, lipids occupy a predominant place. Except for non-saponifiable lipids, all others contain at least one fatty acid residue. Therefore, the group of fatty acids will be much more detailed compared to other classes. The main food sources of fatty acids are products of animal origin (meat and dairy). Consumption of saturated fatty acids increases LDL-cholesterol levels [10]. Fatty acids differ in the number of carbon atoms and the number of double bonds in the carbon atom chain. Fatty acids are with acids without double bonds (saturated) and acids with double bonds (unsaturated). For the reaction of unsaturated fatty acids in the body, the position of the double bonds in the carbon chain is essential. Depending on the position and number of double bonds, fatty acids are divided into three important families: n = 3, n = 6, and n = 9. They have as properties: a large number of carbon atoms, even number of carbon atoms, linear chain, without ramifications, are monocarboxylic [3]. Large amounts of unsaturated fatty acids are found in vegetable oils (olive oil, corn oil, peanut oil, sunflower oil), and some vegetable fats, including coconut, seed palm, and palm oil, contain large amounts of saturated fatty acids, also found in large quantities in animal fats, but excluding marine fats [2].

1.1 The structure of fatty acids

The substances under the name of the fatty acids are composed only of carbon (C), hydrogen (H), and oxygen (O), bearing, as the organic acid, the carboxyl group.

The carboxylic functional group, also called carboxyl (-COOH), is significant for all organic acids. Its ionic form is monovalent, negative, acidic (COO-).

The introduction into a molecule of the carboxylic group takes place due to a carboxylation reaction, and the loss of this group by a compound takes place as a result of a decarboxylation reaction. The number of carbon atoms is always even.

For example, we present below the chemical structure of butyric acid and its symbol: CH3-CH2-CH2-COOH (symbol: 4: 0) [18].

1.2. Fatty acids and classification of fatty acids

Carboxylic acids having in the molecule a linear chain with an even number of carbon atoms (n> = 4) are called fatty acids [17].

1.2.1. Classification of fatty acids

Fatty acids are classified according to two criteria:
By chain length:
• Short-chain fatty acids (4-6 carbon atoms)

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• Medium-chain fatty acids (8-12 carbon atoms).
• Long-chain fatty acids (14 or more carbon atoms) [1].

The two digits of the symbol represent the number of carbon atoms, respectively, the number of double bonds (=). Thus the acid is composed of 4 carbon atoms and has no double bond [8].

După gradul de nesaturare:

• Saturated fatty acids (when there are no double bonds) [1].

They predominate in animal fats:
- *Luric acid*, CH3- (CH2) 10-COOH, (n = 12) predominates in butter obtained from coconut milk;
- *Butanoic acid (butyric acid)*, CH3-CH2-CH2-COOH, is the first in the series of fatty acids because it has four carbon atoms and is found in butter made from cow's milk;
- *Palmitic acid*, CH3- (CH2) 14-COOH (n = 16) and stearic acid, CH3- (CH2) 16-COOH, (n18) are the main constituents of fat in animal bodies;
- *Capronic, caprylic, and carinic acid* (n = 6, 8, respectively 10 C atoms), found in the fat of goat's milk [14].

• Unsaturated fatty acids (when a double bond is formed) [5]

They are part of the oils that are extracted from the seeds or fruits of some plants:
- *Linolenic acid* C18: 3 double bonds C = C) is part of the esters found in soybean oils, corn.
- *Olric acid* (18 C atoms and a double bond) is the main constituent of fat in cocoa butter and some oils;

• Monounsaturated fatty acids (when there is a single, double bond)

- *Erucic acid*, which can be found in rapeseed, coniferous, mustard oil;
- *Oleic acid*, found in pumpkin seeds, olive oil and in small quantities, almost in all oilseeds, in lard;
- *Laurinoleic acid*, found in goat's milk [16].

• Polyunsaturated fatty acids (when there are two or more double bonds)
- *Linolenic acid* (omega 3) is found in fish and flax
- *Gamma-linolenic acid* (omega 6) which is found in poppy seeds, peanuts, breast milk, grape seeds, and blackcurrants;
- *Arachidonic acid* is found in peanuts, animal fats, pork liver [5].

2.Materials and Method

The analyzed material consisted of 12 pastry samples for which the fatty acid profile was determined by chromatographic analysis determining the ratio between saturated and unsaturated fatty acids and between omega fatty acids 3,6 and 9.

2.1 Methods of chromatographic analysis of fatty acids

Currently, mainly chromatographic methods are used: thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFC) as well as combined techniques: GC / MS, SFC / MS, LC / MS, GC / IR, GC / FTIR [6].

2.1.1 Preparation of biological material for chromatographic analysis

To ensure the viability of the qualitative and quantitative analysis of fatty acids, immediately after collecting the biological material undergoes some preliminary protection for the acid at a low temperature, mainly due to the oxygen effect against oxidation or UV-VIS [11].

Sensitive tissues (serum or plasma) are immediately frozen at -80 degrees Celsius, while solid tissues are frozen in liquid nitrogen.

Tissues are stored at -80 degrees Celsius until analytical procedures. The key step in the preparation of biological materials for fatty acid analysis involves the separation of lipids from the soluble component remaining in water by their efficient and selective extraction [17].

Prior to extraction, the liquid tissue becomes frozen while the solid tissue undergoes liquid nitrogen dust into the atmosphere [12].

Extraction is performed using an organic solvent. The analysis of extruded garage acids involves their classification into those classes, usually by thin-layer chromatography (TLC) or solid-phase extracts (SPE) [13].

Thin-layer chromatography (TLC) is the oldest and largest technique for isolating those classes of lipids, allowing the separation of a complex mixture on silica gel.

TLC does not require any expensive and sophisticated cost for equipment or numerous solvent. However, it cannot prevent the oxidation of polyunsaturated fatty acids during the process of long-term exposure to oxygen in the air; this is the main disadvantage [15].

2.1.2. Chromatographic analysis of fatty acids

Volatile carboxylic acid derivatives are most commonly obtained by derivatizing carboxyl or hydroxyl groups or multiple bonds into unsaturated fatty acids.

Volatile fatty acid derivatives are usually produced by single-step acidic or alkaline transesterification. Also, a carboxyl group of fatty acids is derived by esterification of the products obtained by hydrolysis in an alkaline medium. For acidic esterification, hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) in methanol (MeOH) or boron trifluoride (BF_3) in methanol is used, which is the most commonly used. Boron trifluoride in methanol causes the transesterification of existing fatty acid esters present in all lipid classes [7].

The time required for transesterification is different: free fatty acids (2 min), then phosphoglycerides (10 min), triacylglycerols (30 min), and sphingomyelin (90 min). The fastest transesterification has been shown to be at 100 degrees Celsius. Transesterification is performed in the presence of butylated hydroxytoluene (BHT) as an antioxidant to prevent oxidation of unsaturated fatty acids. The methyl esters obtained are extracted with n-hexane (n-pentane) and washed with water. The efficiency of esterification performed in this way in the biological material is about 97-99%, but the short durability of boron trifluoride is a disadvantage of this method [8].

2.1.3. High-performance liquid chromatography (HPLC) in fatty acid analysis

To perform the analysis of fatty acids at room temperature, we are using HPLC. The primary difficulty in analyzing fatty acids using HPLC is the lack of chromophore groups in their molecules, which prevents direct detection using a UV detector or a fluorescence (FL) detector. Although most lipids show the absorption of UV radiation at a wavelength of about 100 nm, this wavelength is not recommended because organic solvents have a maximum absorption in the same range. Therefore, determining compounds are converted to derivatives before being placed on the column (pre-column) or after separation on the reactor column located directly in the chromatograph, usually between the column and the detector (post-column). At the same time, the determination of fatty acids in the form of derivatives improves the sensitivity and selectivity of the determination. Most analyzes of fatty acids and their derivatives are performed in the reverse phase using columns filled with modified silica gel modified with octadecylsilyl (ODSC18), octolsilyl (C8) [9].

In the analyzed samples, the derivatization was performed directly on the finely pulverized and dried material (0.1 grams) with 20% methanolic boron trifluoride (2 ml), for one hour, at 80 °C, on the ultrasonic bath. The separation of the organic phase was performed by adding 2.5 ml of 10% sodium chloride solution and extracting the methyl esters of the fatty acids in 2 ml of hexane. The hexane solution of methyl esters of 1 µ fatty acids was injected into the gas chromatograph coupled with Shimadzu QP2010 plus mass spectrometry at 250 ºC. Injection temperature 250 °C. Separation of esters was performed on an ATwax column with L = 30m, d = 0.25mm, granulation = thickness 1µm.

Initial temperature program in column T = 110 ºC, followed by increasing the temperature to 250 ºC, at a rate of 7º / min, a temperature that was maintained for 10 min. Total separation time 22 min. He carrier gas with column flow rate 2 ml / min, total flow rate 24 ml / min, linear velocity = 37.8 cm / s. Pressure 278 kPa, Splitting rate 1/10.

Detection of compounds separated by mass spectrometry, electronic ionization T ion source = 210 ºC, T interface = 255 ºC.

Identifying the separated and detected compounds were performed based on the NIST 05 spectrum library's software [18].

3. Results and discussions

The variation of the gas-chromatographic analysis of the fatty acids from the extracted fat of the 12 analyzed samples is presented in tables 1 and 2. The concentration of the eight analyzed fatty acids from the 12 samples is presented.

The 12 samples analyzed are:
S1- corn with cheese
S2-Cheese donuts
S3-Jam donuts
S4-chocolate donuts
S5-Salts

S6-Wholemeal pretzels
S7- simple pretzels
S8-Sesame pretzels
S9-Poppy pretzels
S10-simple biscuits
S11-whole digestive biscuits
S12-digestive biscuits

The ratio of saturated fatty acids: unsaturated fatty acids for sample 1 (corn with cheese), is 1.45, being two times higher than the optimum of 0.8, a value over which is considered in nutrition - biochemical pathology and which has a hypercholesterolemic action.

Saturated fatty acid ratio: unsaturated fatty acids for sample 2 (corn with cheese), is 0.62, close to optimum, with a high percentage of ω6 acid but low of ω3, ω6 / ω3 ratio of 16.2.

Saturated fatty acid ratio: unsaturated fatty acids for sample 3 (Jam donuts), is 0.64, close to optimal, with a high percentage of acid ω6, but low of ω3, the ratio ω6 / ω3 of 22.5.

Saturated fatty acid ratio: unsaturated fatty acids for sample 4 (chocolate donuts), is 1.17, higher than the optimum, with a high percentage of ω6 acid, but low of ω3, the ω6 / ω3 ratio of 22.06.

Saturated fatty acid ratio: unsaturated fatty acids for sample 5 (salts), is 0.24, much lower than the optimal value, with a low percentage of acid ω6, but high of ω3, the ratio ω6 / ω3 of 48.01.

Saturated fatty acid ratio: unsaturated fatty acids for sample 6 (Wholemeal pretzels), is 0.71, very close to the optimal value, with a high percentage of acid ω6, but low of ω3, the ratio ω6 / ω3 of 61.58

Figure 1, shows the concentration of fatty acids in the samples (1-6).

Table 1. Variation of fatty acids in the analyzed samples (samples 1-6)

Nr. Crt.	Fatty acid	S1 (%)	S2 (%)	S3 (%)	S4 (%)	S5 (%)	S6 (%)
1	Caprine acid C10: 0	0.341	0.273	0.16	0.135	0.047	0.062
2	Lauric acid C12: 0	0.614	0.551	0.398	9.216	0.082	0.685
3	Myristic acid C14: 0	22.830	0.833	0.933	13.624	0.282	0.871
4	Palmitic acid C16: 0	6.484	29.913	30.848	17.788	13.561	29.954
5	Stearic acid C18: 0	29.546	9.045	5.914	21.600	5.464	10.114
6	Oleic acid C18: 1	37.290	30.078	34.108	21.966	29.979	29.277
7	Linoleic acid C18: 2	2.766	27.599	26.467	22.775	49.552	28.574
8	Linolenic acid C18: 3	0.131	0.273	1.172	1.172	1.032	0.464
	SFA saturated fatty acids	59.815	40.615	38.253	54.087	19.437	41.685
	MUFA	37.290	30.078	34.108	21.966	29.979	29.277
	PUFA	2.895	29.307	27.639	23.947	50.584	29.038

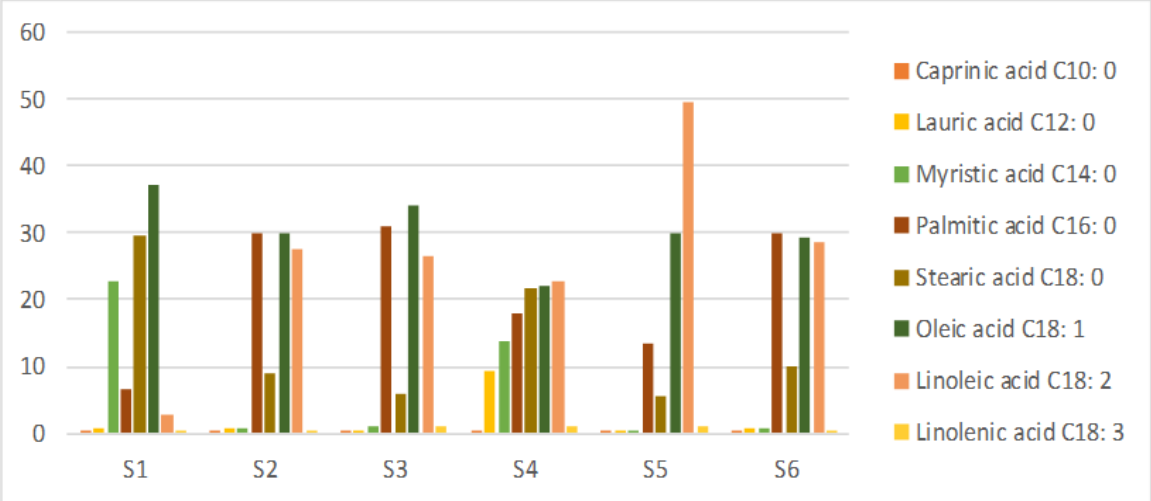

Figure 1. The concentration of fatty acids in samples (1-6)

Table 2. ANOVA comparison test results of the effect of storage days

Nr. Crt.	Fatty acid	S7 (%)	S8 (%)	S9 (%)	S10(%)	S11(%)	S12(%)
1	Caprine acid C10: 0	0.094	0.029	0.085	0.010	0.10	0.096
2	Lauric acid C12: 0	1.099	0.231	1.013	0.041	0.75	0.458
3	Myristic acid C14: 0	1.289	0.304	1.161	0.199	1.12	0.693
4	Palmitic acid C16: 0	34.863	11.983	36.043	11.912	32.86	27.881
5	Stearic acid C18: 0	11.596	4.656	11.348	5.114	5.01	5.007
6	Oleic acid C18: 1	32.526	30.414	32.297	32.008	37.33	43.356
7	Linoleic acid C18: 2	18.201	52.042	17.771	50.45	21.30	19.908
8	Linolenic acid C18: 3	0.333	0.340	0.281	0.266	1.49	2.601
	SFA saturated fatty acids	48,94	17.104	49.651	17.286	39.88	34.145
	MUFA	32.526	30.414	32.297	32.008	37.33	43.356
	PUFA	18.534	52.382	18.052	50.716	22.79	22.509

Table 3. Statistici descriptive

Lot	N	Media	Deviation std	Std error. of the average	95% confidence interval of the average		Minimum	Maximum
					Inferior limit	Upper limit		
1	12	0,11933	0,098175	0,028341	0,05696	0,18171	0,010	0,341
2	12	1.26150	2,526483	0,729333	-0,34375	2,86675	0,041	9,216
3	12	3.67825	7,082287	2,044480	-0,82162	8,17812	0,199	22,830
4	12	23.67417	10,520694	3,037063	16,98964	30,35870	6,484	36,043
5	12	10.36783	7,744817	2,235736	5,44701	15,28865	4,656	29,546
6	12	32.55242	5,264704	1,519789	29,20738	35,89745	21,966	43,356
7	12	28.11708	15,148548	4,373009	18,49216	37,74201	2,766	52,042
8	12	0.91583	0,754285	0,217743	0,43658	1,39508	0,131	2,601
Total	96	12.58580	14,703430	1,500663	9,60661	15,56499	0,010	52,042

The legend:
1-Caprinic acid C10: 0
2-lauric acid C12: 0
3-Myristic acid C14: 0
4-palmitic acid C16: 0
5-Stearic acid C18: 0
6-oleic acid C18: 1
7-linoleic acid C18: 2
8-linolenic acid C18: 3

Saturated fatty acid ratio, unsaturated fatty acids for sample 7 (simple pretzels), is 0.95, slightly higher than the optimum value, with a high percentage of ω6 acid, but low of ω3, ω6 / ω3 ratio of 54.65.

The ratio of saturated fatty acids: unsaturated fatty acids for sample 8 (sesame pretzels), is 0.20, much lower than the optimal value, with a high percentage of acid ω6, but low of ω3, the ratio ω6 / ω3 of 153, 06.

Saturated fatty acid ratio: unsaturated fatty acids for sample 9 (poppy pretzels), is 0.98, slightly higher than the optimal value, with a high percentage of acid ω6, but low of ω3, the ratio ω6 / ω3 of 63.24.

Saturated fatty acid ratio: unsaturated fatty acids for sample 10 (simple biscuits) is 0.20, much lower than the optimum value, with a high percentage of ω6 acid but low of ω3, ω6/ ω3 ratio of 189.6.

Saturated fatty acid ratio, unsaturated fatty acids for sample 11 (whole digestive biscuits), is 0.66, very close to the optimal value, with a high percentage of ω6 acid, but low of ω3, ω6 / ω3 ratio of 43.46.

Saturated fatty acid ratio: unsaturated fatty acids for sample 12 (digestive biscuits), is 0.51, very close to the optimum value, with a high percentage of ω6 acid, but low of ω3, ω6 / ω3 ratio of 7.65.

Figure 2, shows the concentration of fatty acids in the samples (7-12)

Figure 3 shows the concentration of saturated acids, MUFA, PUFA from the 12 samples analyzed.

Table 3, shows descriptive statistics of the fatty acids analyzed from the 12 samples.

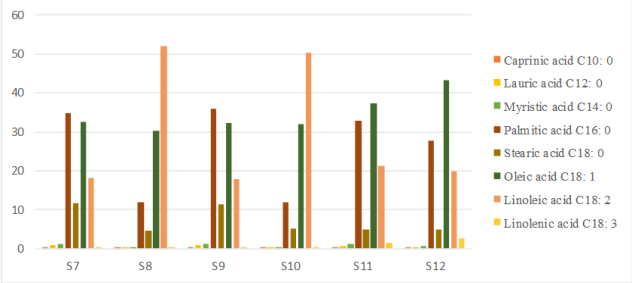

Figure 2. The concentration of the fatty acids of the samples (7-12)

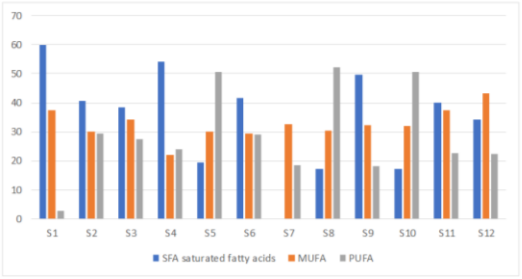

Figure 3. The concentration of saturated acids, MUFA, PUFA from the 12 analyzed samples

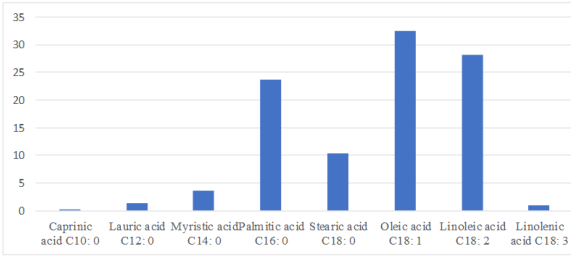

Figure 4. Media representation values for all eight lots

Figure 4 represents the averages of the values from the eight analyzed lots. Samples with a high content of saturated fatty acids have a longer shelf life but are nutritionally shorter because saturated fatty acids are precursors of metabolites responsible for inflammatory processes.

Saturated fatty acids with a long chain (palmitic, stearic) are challenging to digest and absorb and can create various digestive problems.

"Of the total lipids needed by the human body, it is recommended that over 66% come from glycerides formed from unsaturated acids". Of the samples analyzed, only samples 8, 9, and 10 correspond to this recommendation.

4.Conclusion

The role of constitutive lipids as phospholipids, glycolipids, and sphingolipids is an essential component of cell membranes. Each of the 130 trillion cells in the human body is bounded by a membrane, which plays a crucial role. The type of fatty acids influences the properties of these membranes in phospholipids.

The presence of double bonds in fatty acid chains determines the geometry of fatty acid molecules, making them take up more space than saturated fatty acids. In this way, unsaturated fatty acids take up more space in the cell membrane, increasing the membrane's fluidity.

Polyunsaturated fatty acids in cheap oils are incorporated into cell membranes, altering their physical and functional properties and diminishing cells' ability that provides immunity to fight cancer cells.

The analysis of the samples revealed a ratio of saturated fatty acids / unsaturated fatty acids.

The intervals of the eight lots were highly significant differences (ANOVA test, $p < 0.001$).

As the body cannot assimilate ω6 and ω3 fatty acids after studying bakery samples, we noticed that the highest acid ratio ω6 and ω3 have simple biscuits (sample 10) and sesame pretzels (sample 8).

In the eight analyzed samples, the presence of trans acids was not noticed.

The highest concentration is represented by oleic acid and the lowest by caprine acid, but it is found in the bakery products studied.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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References

1. Christie W. W., *Lipid analysis isolation, separation, identification, and structural analysis of lipids*, Editura Pergamon Press, Oxford, 1982
2. Dan D. Bedeleanu, *Biochimie Medicina & Farmaceutica* , editura Dacia, Cluj-Napoca, 1985
3. Drochioiu, G., Duţă, I., *Biochimie Generală*, Casa Editorială Demiurg, Iaşi, 2003
4. Gârban, Z., *Nutriţie Umană, Volumul I*, Editura Didactică şi pedagogic, Bucureşti, 2000
5. Gârban,Z., *Biochimie, Tratat comprehensive*, Volumul I, Editura Didactică şi pedagogic, Bucureşti, 2000
6. Cosman, G., Badea, M., Bîgiu, N., *Biochimie clinic*, Editura Universității Transilvania dn Brașov, 2002
7. Goina T., Constantinescu E., Ciulei I., Râcz G., Grigorescu E., *Farmacognozie, Editura Didactică și pedagogic*, București, 1967
8. Gottschalk T.E., Bailey J.E., *International Cosmetic Ingredient Dictionary and Handbook*, Editura Washington: CTFA, 2008
9. Ianculov I., Filimon, M., *Analiza biochimică*, Editura Orizonturi Universitare, Timișoara, 2003
10. Istudor V., *Farmacognozie.Fitochimie.Fitoterapie, vol. I*, Editura Medicală, București, 1998
11. Liebig C. C., *Il bagno dispersione oleosa*, Editura Novalis, Milano, 2007
12. Ștefănescu I., *Lubrifiere și materiale de ungere*, Editura AIUS, Craiova, 2005
13. Valnet J., *Aromaterapia.Guarire con le piante*, Editura Giunti, Milano, 2006
14. Arellano, M., Jomard, P., El Kaddouri, S., Roques, C., Nepveu, F. & Couderc, F. Routine analysis of short-chain fatty acids for anaerobic bacteria identification using capillary electrophoresis and indirect ultraviolet detection. *Journal of Chromatography B: Biomedical Sciences and Applications*, **2000**, *741*(1), pp. 89-100., retrieved from address https://www.ncbi.nlm.nih.gov/pubmed/10839136, Accesat în 20.04.2021
15. Bergman, E.N., Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews*, **1990**, *70*(2), pp. 567- 590., retrieved from addresshttps://pdfs.semanticscholar.org/5ab8/f360949164551ac957c2aa9e30011cdc8c31.pdf, access in 20.04.2021
16. Chen, H.M. & Lifschitz, C.H., Preparation of fecal samples for assay of volatile fatty acids by gas-liquid chromatography and high-performance liquid chromatography. *Clinical Chemistry*, **1989**, *35*(1), pp. 74-6., retrieved from address https://stud.epsilon.slu.se/9845/1/ewald_n_161130.pdf, access in 25.04.2021
17. McOrist, A.L., Abell, G.C.J., Cooke, C. & Nyland, K., Bacterial population dynamics and fecal short-chain fatty acid (SCFA) concentrations in healthy humans. *British Journal of Nutrition*, **2008**, *100*(1), pp. 138-146 https://www.ncbi.nlm.nih.gov/pubmed/18205991, access in 25.04.2021
18. Bielawska, K., & Dziakowska, I & Roszkowska-Jakimiec, W.. Chromatographic determination of fatty acids in biological material. *Toxicology mechanisms and methods*, **2010**, *20*, 526-37. 10.3109/15376516.2010.515081.https://www.researchgate.net/profile/Katarzyna_Bielawska/publication/46303595_Chromatographic_determination_of_fatty_acids_in_biological_material/links/554b16450cf21ed213590540/Chromatographic-determination-of-fatty-acids-in-biological-material.pdf, access in 24.04.2021