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Extraction of biologically active substances from peach kernels and use as an additive in the food industry

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Annotation - In the next stage of our research, we studied the fatty acid content of proteins, vitamins, minerals and fats from biologically active substances isolated from peach seeds grown in the climatic conditions of Uzbekistan. To do this, we synthesized methyl esters of fatty acids from the studied oils under alkaline conditions.

Keywords – Peach seeds, pressing, extraction, Soxlet, substances, drying, ceramic mortar, gasoline, refrigerator, desiccator, chromatography, injector, detector, column, thermostat.

In the vegetable oil industry, there are two main methods used in the world to extract oil from oilseeds:
- Technical method of oil extraction - pressing;
- Extraction of oil from a solution of volatile organic solvents - extraction.

The pressing method does not allow to completely separate the oil from the seeds. The particles coming out of the press are always covered with a thin layer of oil, which is held in place by gravitational forces that are several times higher than the pressure created in modern presses. The oil content of the presses is 4 ... 7%, and the oil content of the presses is 15 ... 17%.

The total amount of oil in the oil raw material can be extracted only by extraction.

The oil content of peach seeds was determined by Soxhlet apparatus. The essence of the method is based on the extraction of oil from crushed peach seeds into an organic solvent, dissolving the solvent, drying and extracting the separated oil.

50 grams of peach seeds grown in the country are cleaned of impurities and dried in an oven at 100-105 ° C for one hour. After drying the seeds should have a moisture content of 3-5.5%. After determining the moisture content of the dried seeds, grind them in a ceramic mortar, weigh 8-10 grams of the crushed sample and put it in a cartridge bag made of filter paper. The cartridge is placed in the desiccator of the soxlet apparatus. Hexane is used as a solvent in extraction.

The net weight of the Soxhlet tube is weighed and recorded in a workbook, and hexane is added to 1/3 of the volume of the container. The tube is then connected to a desiccator and placed on an electric heater. We pour gasoline into the desiccator until the cartridge with crushed seeds sinks and connect it to the refrigerator and connect the refrigerator to the tap.

Once all parts of the appliance are securely connected, connect the heater to the mains. During heating, the gasoline in the flask should boil uniformly over the entire volume of the flask. Gasoline boils and its vapors rise from the desiccator's steam pipe to the refrigerator, where they turn into a liquid under the influence of water and drop onto the cartridge.

The falling gasoline dissolves the oil in the seeds. If the level of gasoline in the desiccator rises above the level of the siphon loop pipe of the desiccator, then the gasoline is poured from the desiccator into the flask. Then the process continues and the second time the gasoline is poured into the flask, the extraction time is calculated. The extraction process takes 4-6 hours. To make sure that the extraction process is complete, add a drop of the solution from the extractor to the flask on the filter paper and wait for the solvent to evaporate. If no yellow oil stain remains on the filter paper after the solvent has evaporated, this indicates that the extraction process is complete. At the end of the extraction process, disconnect the electric

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heater from the power supply, and after 20 minutes, turn off the water supply to the refrigerator. At the end of the extraction process, separate the flask from the flask, assemble the flask, and place the solvent flask in it to separate the solvent by evaporation. Continue the evaporation process until the solvent in the tube is used up. Then separate the flask from the apparatus, cool it, and after it has cooled, weigh it with the oil, record the result in a workbook, and calculate the amount of fat in the seed from the following expression:

$$\times = \frac{a - b}{p} * 100$$

Here,

a is the weight of the flask with oil, grams; b is the weight of the empty flask, grams; p is the weight of the seed sample taken for extraction, grams.

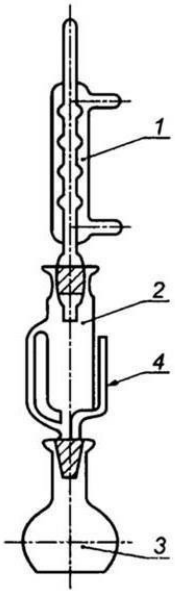

Figure 1. The structure of the soxlet apparatus. 1 refrigerator, 2 extractor, 3 extraction tube, 4 siphon tube.

Weighing from 100g, 0.500 l bottoms were placed in flat flasks and extracted by adding 1: 3 extraction gasoline to each. Each stage of extraction processes was carried out for 1.5-2 hours. During the extraction, the oils dissolved in the extraction gasoline were filtered in a Teflon funnel. This process was performed 3 times each. The gasoline extracts in each tube were placed in round tubes with a bottom of 1 liter, and each tube was blown out of gasoline in a rotor evaporator, and the remaining oil at the bottom of the tube was removed from the tubes using a dispenser and weighed. The amount of fat obtained is given in the table.

1– Table. Peach seed fat

Peaches	Fat content,%
Seed	41±0.17

In the next stage of our research, we studied the fatty acid content of oils extracted from peach seeds grown in the climatic conditions of Uzbekistan. To do this, we synthesized methyl esters of fatty acids from the studied oils under alkaline conditions. The full text of the method is given in Chapter 2. The obtained ethers were analyzed on the GS-MS device under the following conditions:

Chromatograph: HP 9010. 50m capillary column PEG-based stationary phase. Chromatography conditions: injector: 180℃; Detector - 250°C; program for column thermostat: initially at 100℃ for 0.5 min, increasing from 10℃ to 230℃ per minute. Hold at 230℃ for 5 min. The velocity of the gas-carrying gel is 1.7 ml / min.

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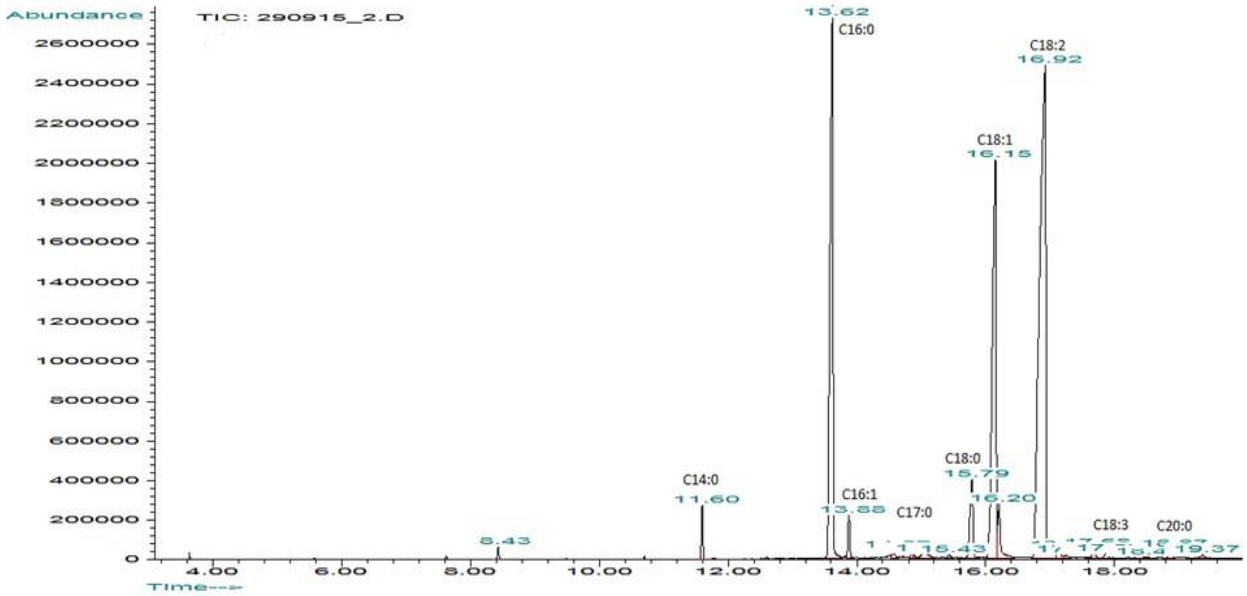

Figure 2. GX is a fatty acid chromatogram of peach oil.

Mass - parameters of the selective detector (mode scan., 40 to 1000 Amu); the electron pulse of ionization is 70 eV. Mass - Spectrum Library Database / W9N11.L and Database / RTLPEST3.L

The lipid component composition of different types of peaches varies from country to country. The results of the research vary. The quality and quantity of oil content of peach oil depends on the type and climatic conditions of the peach and the method of extraction and solvent. Using the GX-MS method, 6 fatty acids of peach oil were obtained by extraction of hexane.

Linoleic and linolenic acids are important for medicinal use. These acids are converted to arachidonic acid in the body, converted into prostaglandins under the action of cyclooxygenases, and thromboxanines, and under the action of lipooxygenases - leukotrienes, which improve vascular permeability, improve viscosity of internal organs and skin.

Table 2.

Ingredients of Peach Seed Oil.

Fatty acids	**Quantity**	**Amount,%**
Myristic acid 14:0	0,02	0,02
Palmitin C16:0	10,68	10,34
Stearin 18:0	0,27	0,28
Araxidon C20:0	0,002	0,0071
Linol C18:2	3,01	3,21
Olein 18:1	12,57	12,39

The amount of water-soluble vitamins in defatted peach seeds and fruits.

The amount of water-soluble vitamins was determined by high-efficiency liquid chromatography. To do this, weigh 5-10 g of the sample taken from the dried fruit and defatted seeds at room temperature. The bottom of the sample is placed in a flat 300 ml flask and 50 ml of 40% ethanol solution. The mixture was boiled with intense stirring for 1 h, equipped with a magnetic stirrer, reverse coolant, and then stirred for 2 h

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Date of Publication: 17-04-2022

at room temperature for 2 h. The mixture is precipitated and filtered. The remaining 25 ml of 40% ethanol was re-extracted twice.

The filtrates were combined and placed in a 100 ml volumetric flask to the line and filled with 40% ethanol (5-10%). The resulting solution was centrifuged at 7,000 rpm for 10 min. The resulting solution was removed from the top for analysis.

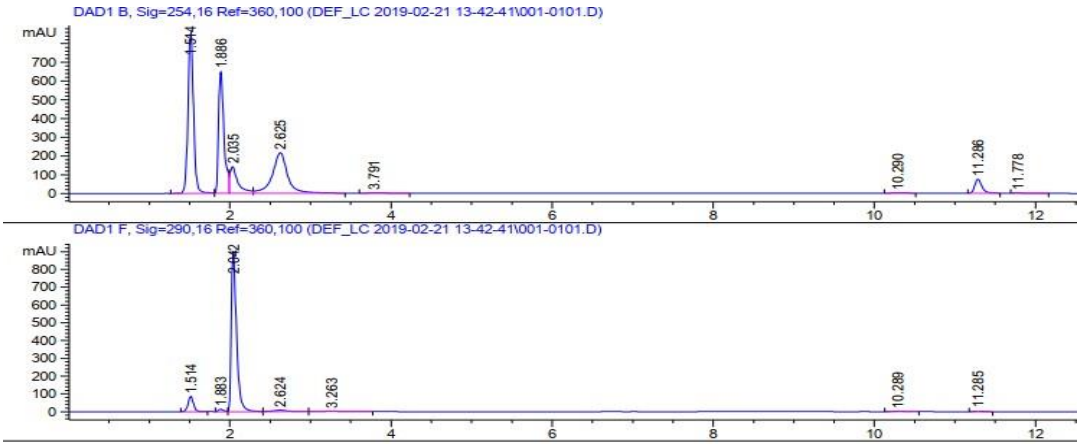

Figure 3. Chromatogram of standard water-soluble vitamins.

Working solutions of water-soluble vitamins at a concentration of 1 mg / ml were prepared. To do this, 50.0 mg of clear weight was weighed on an analytical balance from each vitamin standard and filled to the point where it was dissolved in 40% ethanol in a 50 ml volumetric flask.

In the literature, phosphorus, acetate buffer systems and acetonitrile were used as eluent in the detection of water-soluble vitamins by high-efficiency liquid chromatography. We decided to use acetonitrile with an acetate buffer system.

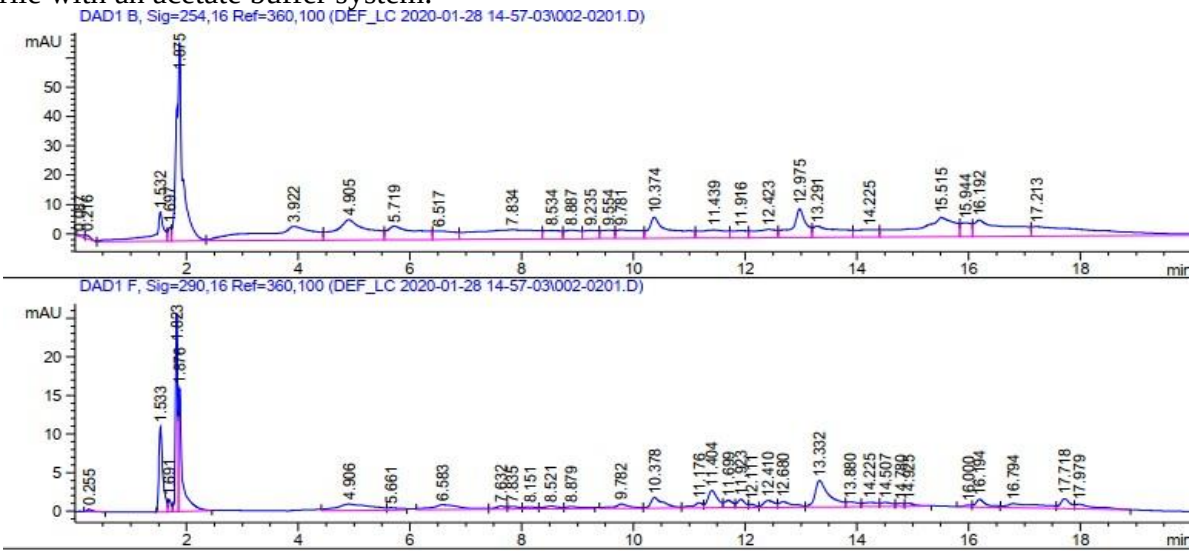

Figure 4. Peach fruit water-soluble vitamins chromatogram Chromatography conditions:

-Chromatograph Agilent-1200 (equipped with autodaster)
-Kolonka Exlipse XDB C 18 (obratshenno-fazniy), 5 µm, 4,6 x150mm
-Diode matrix detector (dad), 204 nm, 254 nm, 290 nm identified.
-Flow rate 1ml / min
- Elyuent acetate buffer: acetonitrile:
0-5 min 96: 4,
6-8 min 90:10,
9-15 min 80:20, 15-17 min 96: 4,
thermostat temperature 250C0, -5 µl input quantity (vkol)

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The chromatograph was initially filled with standard working solutions, followed by prepared working solutions. Figure 4.

Chromatographic conditions: -chromatograph Agilent-1200 (equipped with auto-dispenser) -column Exlipse XDB C18 (obrashenno-phase), 5mkm, 4.6 × 150 mm. -diode matrix detector (DAD), 254nm, 290nm identification, -flow rate 1 ml / min, -eluent acetate buffer: acetonitrile: 0-5 min 96: 46-8 min 90-109-15 min 80-20 15-17 min 96: 4 -thermostat temperature 250ºC -5 µl input volume (vkol)

Based on the peaks on the chromatogram, the vitamins were determined on the basis of standard vitamins. Vitamins are identified by standard vitamins issued by a certified company.

Table 3. The amount of water-soluble vitamins in the samples obtained

Peaches	V1	V3	PP	V9	V6	C
	QUANTITY (mg / g)					
Fruit	0,42± 0,02	0,35± 0,04	0	0,085± 0,02	0,19± 0,03	8,06± 0,01
Degreased seeds	0,32± 0,03	0,33± 0,13	0	0,065± 0,01	0,12± 0,02	2,23± 0,01

We found that water-soluble vitamins are good in fruits and seeds. For example, it was found that the amount of vitamin V1 was 0.42 mg / g, while vitamin C was 8.06 mg / g. Such a difference is also observed in the amounts of vitamins V3, V9, V6, V5.

The total and soluble protein content of defatted peach seeds

The total protein content of peach seeds was determined by the Keldal method. In this method, all the proteins in the sample are determined relative to the amino group in the amino acid, ie nitrogen. In this case, the protein is generally hydrolyzed mainly in sulfuric acid. Soluble proteins were determined by the Louri method. In this method, samples are detected on a spectrophotometer based on a color reaction based on folic reagent.

Table 4. Peach seeds contain total and soluble protein

Peaches	Keldal	Louri
	QUANTITY %	
Seeds	17,33±0.5	7,26±0.2

Gel filtration of desalinated samples was carried out on a sorbent TSK-55f placed in a column 2.6x100 cm. 0.01 M Tris-HCl with a pH of 7.5 was used as a buffer for elution. The flow rate was 1 ml / min. Elucidated proteins and peptides were detected at 280 nm. The chromatography of the results is shown in Figure 9.

The number of proteins in the samples and their molecular weights were determined using DDS electrophoresis methods (Figure 10).

TSK-55f sorbent was used to separate and purify the separated protein sum, and as a result, 3 fractions were isolated. When the molecular masses of the isolated fractions were studied using DDS-electrophoresis, it was found that protein was present in fraction 1 at 48, 35 and 17 kda, in fraction 2 at 65, 34 and 15 kda, and in fraction 3 at only 16 kda.

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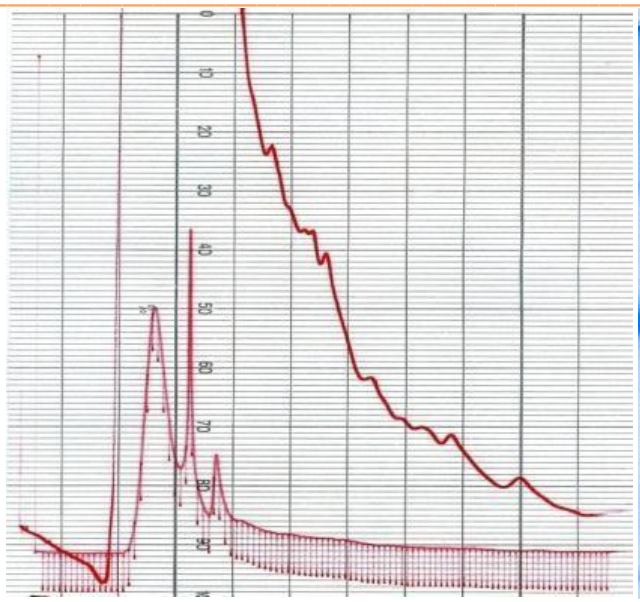

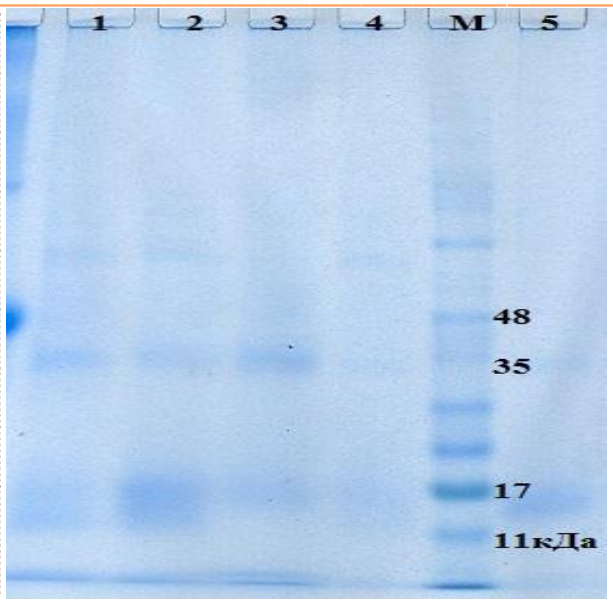

Figure 5. Peach Seed Figure 6. Gel chromatogram of the total fraction of peach seeds and total protein separated from the fruit, the protein and peptides and their (column 78 / 2.6 gel TSK-55, wave fraction gel-electrophoresis results length 280 nm, flow rate 1 ml / min) . (Examples: 1-protein-peptides isolated from seed; 2-protein-peptides isolated from fruit; 1-fraction 3-TSK-55 gel-1; 4-fraction-2; 5-fraction-3; M-protein marker)

After determining the protein content of defatted peach seeds, work was carried out to determine the amount of free amino acids in it.

In addition, the amount of free amino acids in the sample was determined by chromatography. The degreased sample was extracted for one hour in an ultrasonic water bath. The extract was centrifuged at 15,000 rpm for 15 min and 10% acetic acid (THUK) was added in a 1: 1 ratio to precipitate the protein peptides, and re-centrifuged. 0.1 ml of it was synthesized with FITC and FTG (phenylthiocarbomoil) was obtained. The results are presented in Table 5.

Table 5. **The amount of free amino acids in the seed flour**

Amino acid	Ekstrakt mg/g	Amino acid	Eksrtakt mg/g	Amino acid	Eksrtakt mg/g
Asparagin	0,74	Prolin	4,40	Treonin	2,87
Glyutamin	0,70	Tirozin	0,82	Argenin	0,60
Serin	0,11	Valin	1,19	Alanin	3,36
Glitsin	0,26	Metionin	1,36	Triptofan	0,78
Asparagin	0,26	Izoleysin	1,11	Fenilalanin	0,71
Glyutamin	1,65	Leysin	0,74	Lizin	0,07
Sistein	0,88	Gistedin	0		

The samples examined revealed that the free amino acids contained 20 Lamino Acids. It should be noted that almost no changes were observed in the composition of essential amino acids. The amounts of non-exchangeable amino acids are given in the table above.

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Date of Publication: 17-04-2022

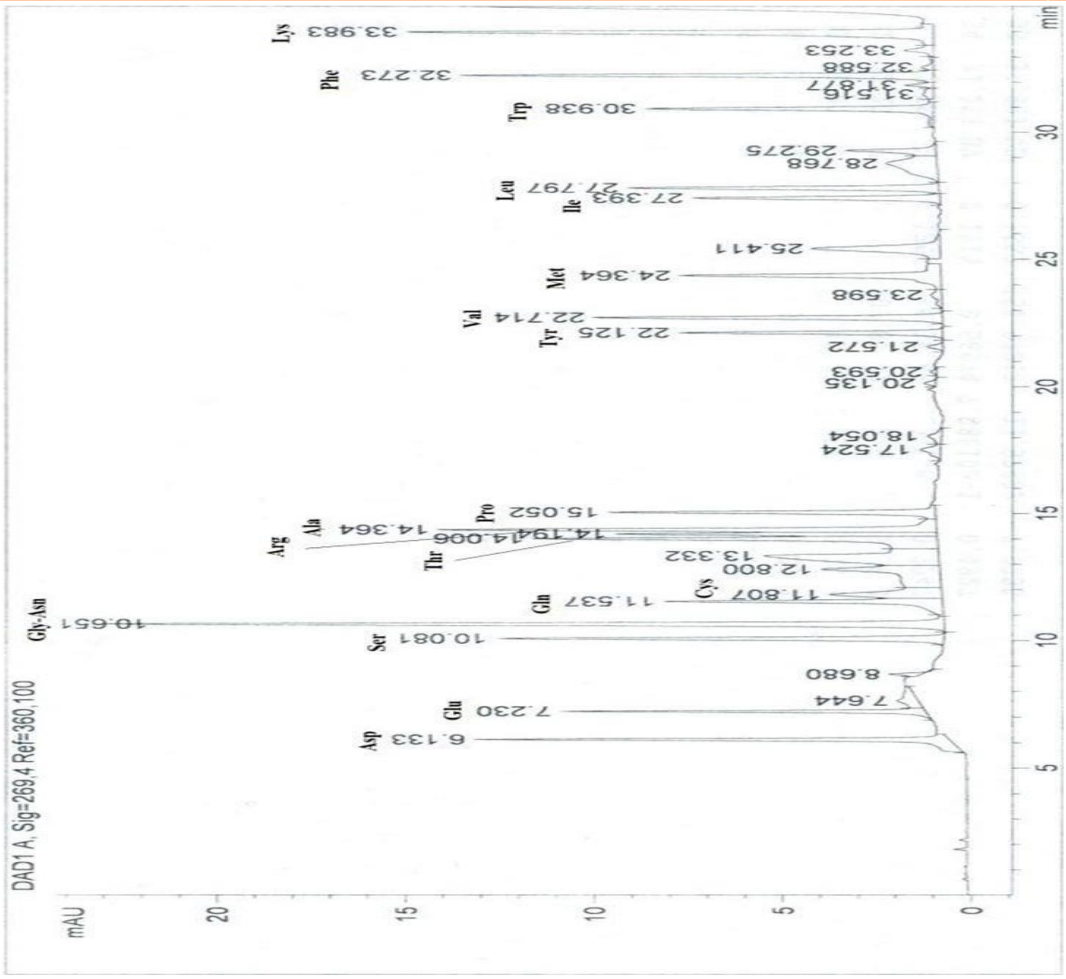

Figure 6. Chromatogram of standard amino acids.

Detection of FTG amino acids was performed on Agilent Technologies 1200 chromatograph. Solution A: 0.14 M CH3COO Na + 0.05%, pH 6.4, B: Acetonitrile. Flow rate 1.2 ml / min, 269 nm. Gradient% B / min: 1-6% / 0-2. 5 min; 6-30% / 2.51-40 min; 30-60% / 40.1-45 min; 60-60% / 45.1-50 min; 60-0% / 50.1-55 min.

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