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Amino Acid Profile and Content in Crude Herbal Drugs (Fruits) of *Rosaceae* Species

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ABSTRACT

Objective: Free and combined amino acids are important constituents of medicinal plants. The objective of the study was to investigate the influence of the conservation method on amino acid composition and content of free and combined amino acids in *Rosaceae* fruits.

Materials and methods: Fresh, dried, and frozen fruits of rowan tree (*Sorbus aucuparia* L.), Siberian hawthorn (*Crataegus sanguinea* Pall.), and cinnamon rose (*Rosa cinnamomea* L.) were stored during fruit-bearing season in Botanical garden of Sechenov University. Amino acids were analysed by RP-HPLC-UV after pre-column derivatization with o-phthalaldehyde and 3-mercaptopropionic acid (OPA) (derivatization of primary amines), or with fluorenylmethyloxycarbonyl (FMOC) (secondary amines).

Results: the composition and the content of free and combined amino acids of *Rosaceae* fruits conserved by a few methods were studied. The effect of drying and freezing on the composition and content of amino acids in the rowan tree, Siberian hawthorn and cinnamon rose fruits was researched to determine the most appropriate way to preserve them.

Conclusions: Drying can be recommended as the most appropriate conservation method to preserve amino acids content and composition in the rowan tree, Siberian hawthorn and cinnamon rose fruits.

Keywords: *Sorbus aucuparia* L., *Crataegus sanguinea* Pall., *Rosa cinnamomea* L., Rosaceae, fruits, amino acids, conservation method, HPLC method.

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INTRODUCTION

Fruits of *Rosaceae* species (*Sorbus aucuparia* L., *Crataegus sanguinea* Pall., *Rosa cinnamomea* L.) contain different groups of biologically active substances (BAS): simple sugars, flavonoids (anthocyanins and flavonols), polysaccharides, tannins, hydroxycinnamic and organic acids and amino acids [1-4]. The last group of BAS is currently under-studied in this type of raw material. Biochemical studies demonstrate an important role for amino acids in different organs and organ systems function. Amino acids as protein constituents take part in all vital processes alongside with nucleic acids, carbohydrates, and lipids. They are being in dynamic balance in numerous metabolic reactions. Tyrosine and phenylalanine are the primary metabolites that serve as precursors for flavonoids. Glutamic and aspartic acids lie at the core of nitrogen fixation, transport and elimination of biologically active forms of maintaining nitrogen balance. Methionine can improve damaged heart function. Glycine and his derivatives act as hypolipidemic agents; some amino acids stimulate insulin secretion by pancreatic b-cells [5-11].

Rowan tree fruits contain a considerable amount of amino acids such as cystine, cysteine, lysine, histidine, arginine, aspartic acid, glycine, alanine, tyrosine. It is well-known that indispensable amino acids are essential nutrients and their deficiency leads to metabolic disorders, growth impairment, and developmental difficulties. High levels of indispensable amino acids such as lysine, threonine, valine, methionine, isoleucine, phenylalanine were found in hawthorn and cinnamon rose fruits [7, 11, 12].

Fresh fruits contain free and combined amino acids. Due to limited shelf life (according to the instruction on storage, the shelf-life of fresh soft fruits is not greater than 3 days), using the freshly harvested material is expensive and time consuming for the pharmaceutical industry. In order to preserve biologically active substances and increase production cost-effectiveness, the conservation is using. Drying is the most frequently used conservation method in pharmacy, while freezing is more common in the food industry. It should be noted that it's impossible to fully maintain amino acids content using those methods, but examining their influence on composition and content is allowing to improve those methods and avoid changes in the pharmacological properties of herbal drugs when possible [13, 14]. So this data is very important for the development of monographs for pharmacopoeia [15].

The research aims to determine the composition and content of free and combined amino acids in *Rosaceae* fruits by HPLC-UV.

MATERIALS AND METHODS

2.1. Plant Material

The objects of our study were samples of fresh, dried, and frozen fruits of the rowan tree, Siberian hawthorn, and cinnamon rose. Fruits were collected during the fruit-bearing season in the Botanical garden of Sechenov University. Storage was conducted in accordance with the instruction on collecting and drying drug plants. Freezing was carried out in a freezer at -18-20° C, drying was made in an oven at 60-80°C.

2.2. HPLC-UV conditions

Amino acid content and composition were analyzed by RP-HPLC after pre-column derivatization with o-phthalaldehyde and 3-mercaptopropionic acid (OPA) (derivatization of primary amines), or with fluorenylmethyloxycarbonyl (FMOC) (secondary amines) [7,12,16-19].

HPLC procedures are widely used in the analysis of crude herbal drugs [20-24].

Chromatograph Agilent 1260 (solvent supplying system and degassing system for 4 solvents; diode array detector,

column thermostat; autosampler). Software – ChemStation (ver. A.09.03). Chromatographic column – XBridge BEH300 C18 Sugar-Pak (WATERS, USA), column length – 250 mm, internal diameter – 4.6 mm, pore size – 5 μm. Standard amino acids solution 79248-5X2MI (USA) was purchased from Sigma-Aldrich. Autosampler was programmed to perform precolumn derivatization: 5 μl of borate buffer, 1 μl of OPA reagent were loaded into sample loop, mixed, then 1 μl of FMOC reagent was loaded, mixed again, 8 μl were injected. Eluent flow-rate – 0.8 ml/min. Detection by UV at 338 nm (reference wavelength – 380 nm).

2.3. Preparation reagent solutions

2.3.1. Preparation of OPA reagent

50 mg of o-phthalaldehyde were dissolved in 1 ml of methanol, 40 ml of 3-mercaptopropionic acid (CAS 107-96-0, 63768 Sigma (>99,0%)) were then added, made up to the mark of 10 ml with 0.4 N sodium borate aqueous solution (pH = 10.4).

2.3.2. Preparation of FMOC reagent

50 mg of fluorenylmethyloxycarbonyl (CAS 28920-43-6, Sigma 23186 (>99.0%)) were dissolved in 10 ml of acetonitrile.

2.3.3. Preparation of borate buffer

76 g of sodium tetraborate decahydrate were dissolved in 1000 ml of distilled water, then 1 M sodium hydroxide was added to pH = 10.4. The resulting solution was filtered using fluoroplastic membrane filter with 0.45 μm pore.

2.3.4. Preparation of mobile phase A

1.36 g of sodium acetate trihydrate was put into 1000 ml measuring cup, then 500 ml of distilled water and 90 μl of triethylamine were added, then mixed. 1-2% acetic acid was added to pH = 7.2, then 1.5 ml of tetrahydrofurane was added, then mixed. The resulting solution was filtered with a fluoroplastic membrane filter of 0.45 μm pore diameter.

2.3.5. Preparation of mobile phase B

1.36 g of sodium acetate trihydrate was put into 500 ml measuring cup, then 100 ml of distilled water was added, then mixed until complete dissolution. 1-2% acetic acid was added to pH = 7.2, then 200 ml of methanol and 200 ml of acetonitrile were added, then mixed. The resulting solution was filtered with a fluoroplastic membrane filter of 0.45 μm pore diameter.

2.4. HPLC-UV analysis of amino acids

Precisely weighed amount (about 10.0 g) of dried, fragmented fruits of cinnamon rose, rowan tree, and hawthorn, and homogenized suspension of fresh, frozen fruits were put into 250 ml round-bottomed flask, then 250 ml of water were added, and the extraction conducted for 1 hour in the boiling water bath using reflux condenser. The extract was filtered with glass filter (POR-40), the filtrate was boiled to dryness. The dry extract was dissolved in 10 ml of water. 50 μl was subjected to amino acid analysis (free amino acids). Hydrolysis of samples was performed according to Manual on Quality and Safety Control of Biological Food Additives R 4.1.1672-03 "Amino acids composition analysis. Testing" with 6M hydrochloric acid [12]. Hydrolysates were filtered. Dry residue on the filter was washed with hot water. Filtrates were evaporated on a water bath. 20 ml of water and 50 μl of resulting solutions were added to residues and then chromatographed (combined amino acids). The resulting hydrolysate was used for analyzing. Amino acids were identified by retention time. The mixture containing 19 amino acids was used as an internal standard. Each peak area was calculated automatically and the quantitative content was determined.

RESULTS AND DISCUSSION

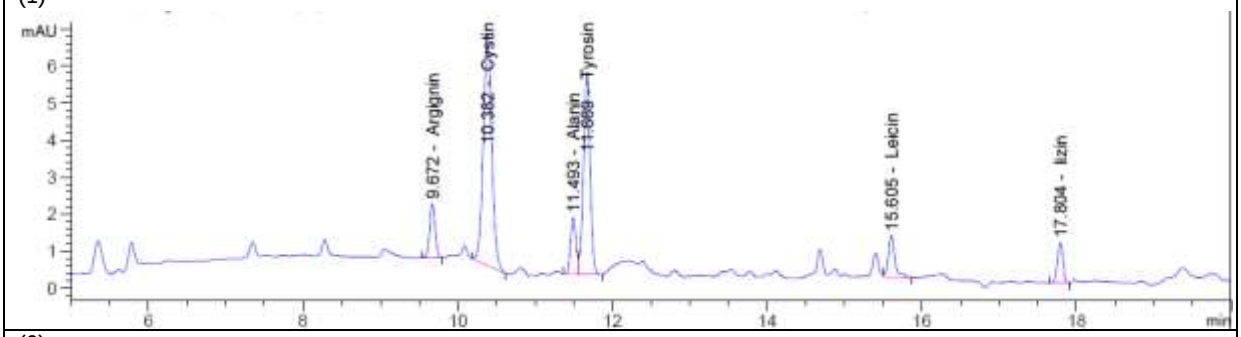
3.1. Amino acids profile and content

Chromatography data are shown in Figures 1-9. Results of qualitative analysis of detected amino acids are presented in Tables 1-3.

3.1. Siberian hawthorn fruits

In fresh fruits of Siberian hawthorne were identified 6 free and 5 combined amino acids. Total amount in fresh fruits was 1.86±0.05 mg% and 0.88±0.03 mg% respectively; frozen – 2 free and 3 combined amino acids (0.46±0.02 mg% and 0.21±0.01 mg% respectively); in dried – 1 free and 5 combined amino acids (1.20±0.04 mg% and 6.72±0.15 mg% respectively). Proline content is over 80% of the identified amino acids total content.

(1)



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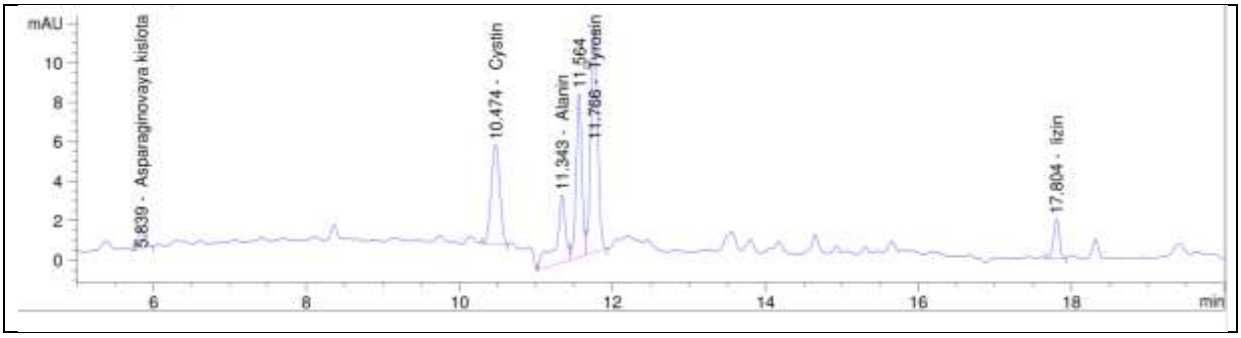


Figure 1: Chromatogram of amino acids profile of fresh hawthorn fruits before (1) and after (2) hydrolysis

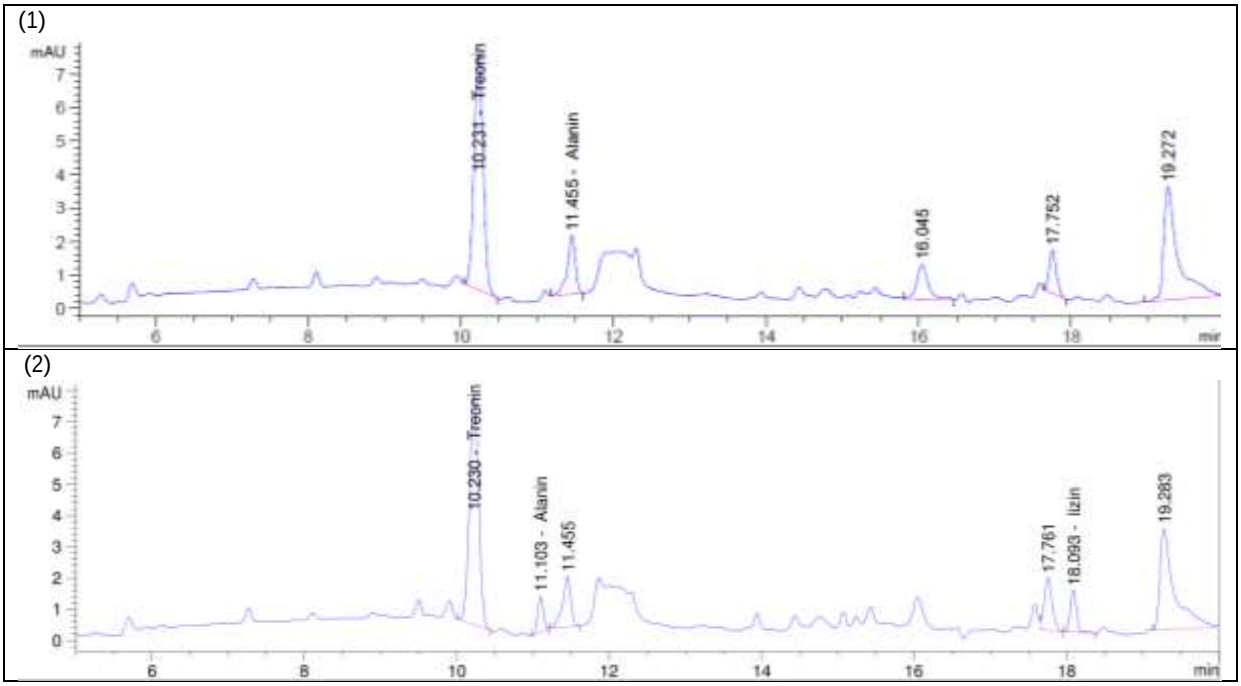


Figure 2: Chromatogram of amino acids profile of frozen hawthorn fruits before (1) and after (2) hydrolysis

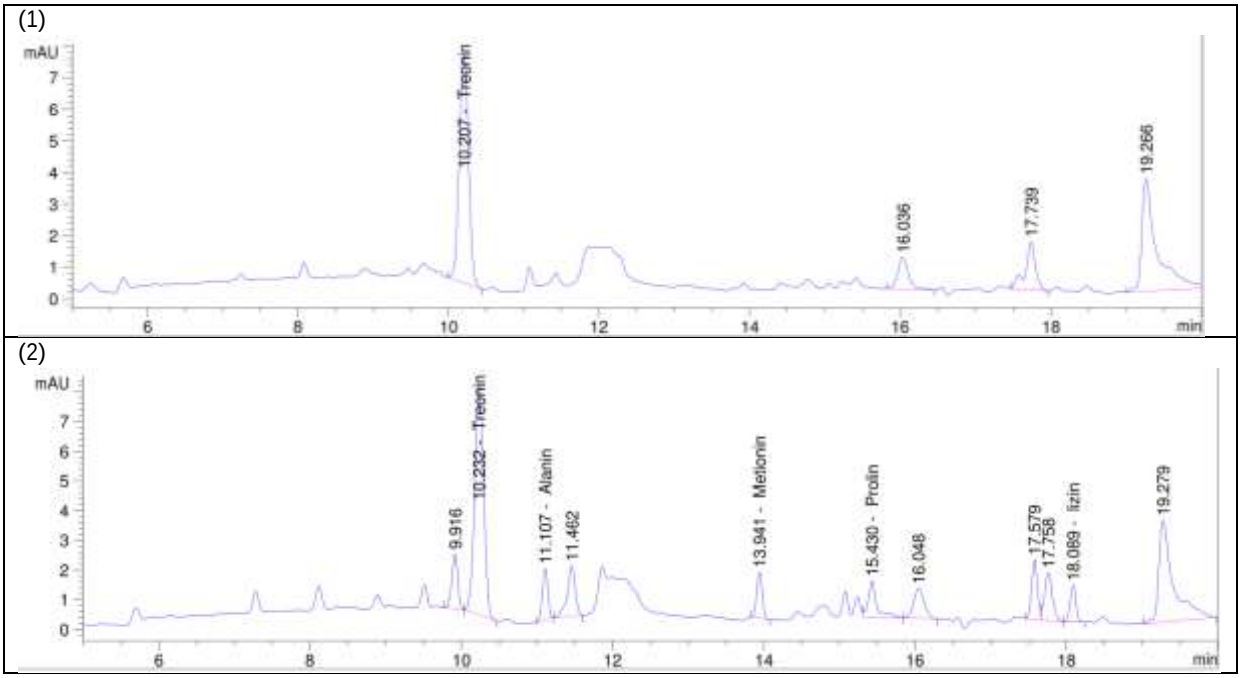


Figure 3: Chromatogram of amino acids profile of dried hawthorn fruits before (1) and after (2) hydrolysis

Table 1: Amino acids content (mg %) in fresh, frozen, and dried hawthorn fruits

Amino acids	Siberian hawthorn fruits					
	Before hydrolysis			After hydrolysis		
	Fresh	Frozen	Dried	Fresh	Frozen	Dried
Aspartic acid	-	-	-	0.01	-	-
Arginine	0.06	-	-	-	-	-
Threonine	-	0.38	1.20		0.17	0.51
Cystine	1.41	-	-	0.49	-	-
Alanine	0.04	0.08		0.07	0.02	1.08
Thyrosine	0.28	-	-	0.27	-	-
Methionine	-	-	-	-	-	0.06
Proline	-	-	-	-	-	5.02
Isoleucine	-	-	-	-	-	-
Leucine	0.05	-	-	-	-	-
Lysine	0.02	-	-	0.02	0.02	0.05
Total content	1.86±0.05	0.46±0.02	1.20±0.04	0.88±0.03	0.21±0.01	6.72±0.15

3.2. Rowan tree fruits

Rowan tree fruits' amino acid content analysis showed the presence of 9 free amino acids in fresh fruits, 6 – in frozen, and 2 – in dried. The total amount of free amino acids is 4.04±0.12. 1.81±0.06 and 1.9±0.04 mg% respectively. After acidic hydrolysis there were found 16 amino acids in fresh fruits (3.18±0.08mg%), 7 – in frozen (0.56±0.02mg%), and 9 – in dried (7.41±0.18mg%).

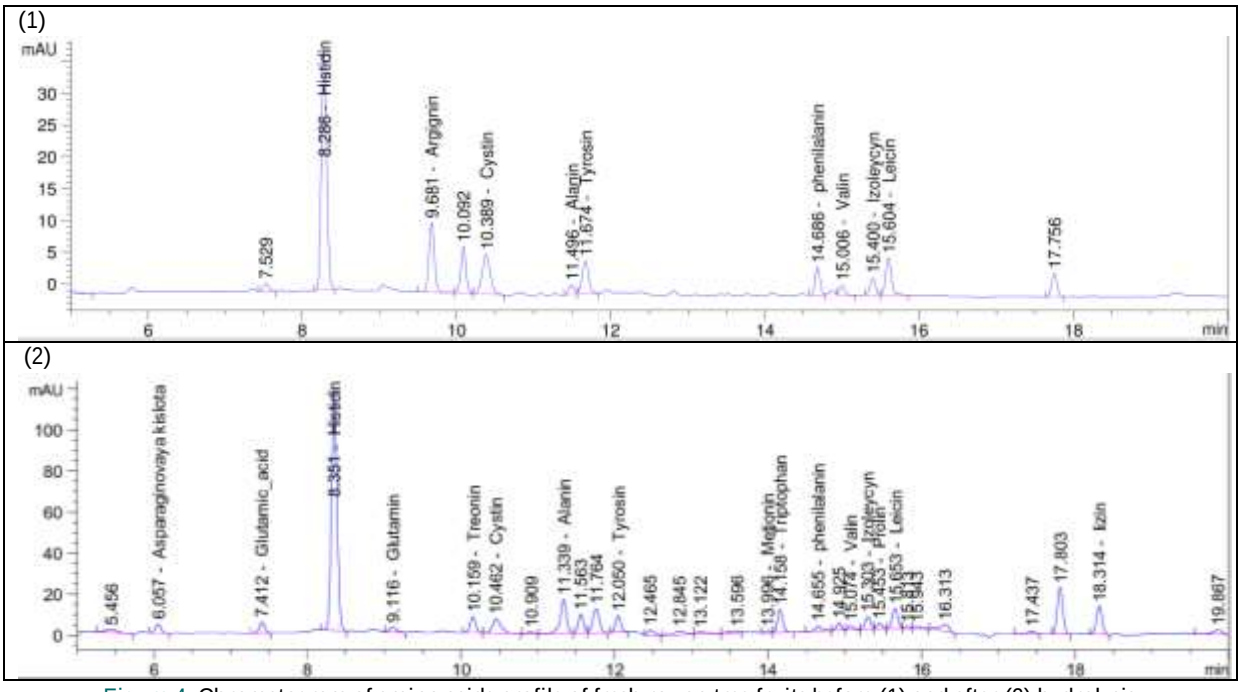


Figure 4: Chromatogram of amino acids profile of fresh rowan tree fruits before (1) and after (2) hydrolysis

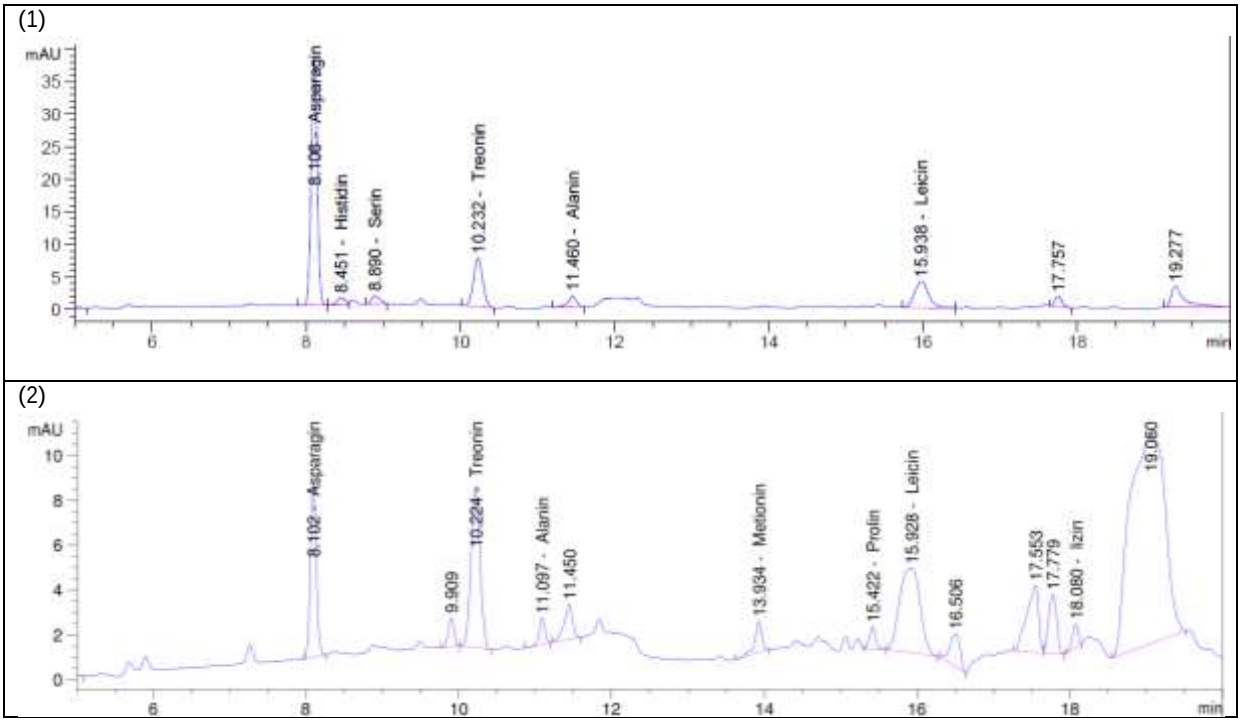


Figure 5: Chromatogram of amino acids profile of frozen rowan tree fruits before (1) and after (2) hydrolysis

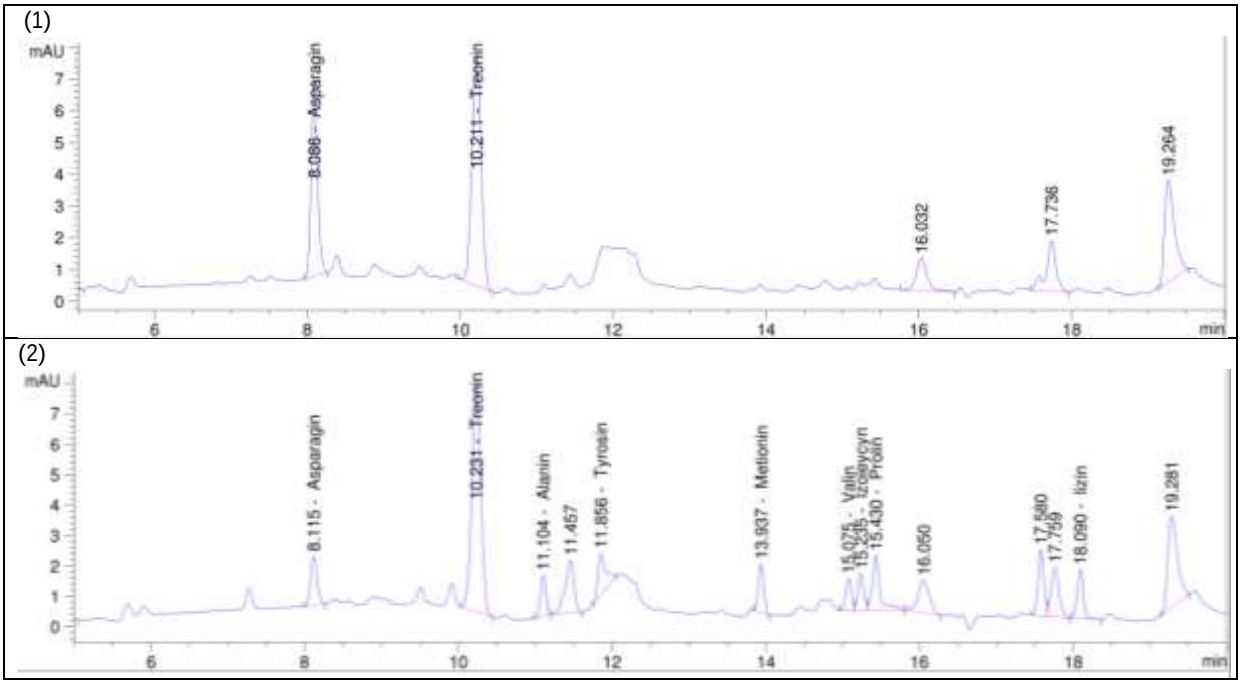


Figure 6: Chromatogram of amino acids profile of dried rowan tree fruits before (1) and after (2) hydrolysis

Table 2: Amino acids content (mg %) in fresh, frozen, and dried rowan tree fruits

Amino acids	Rowan tree fruits					
	Before hydrolysis			After hydrolysis		
	Fresh	Frozen	Dried	Fresh	Frozen	Dried
Glutamic acid	-	-	-	0.06	-	-
Asparagine	-	1.01	0.69	-	0.03	0.04
Aspartic acid	-	-	-	0.22	-	-
Histidine	1.43	0.06	-	1.00	-	-
Serine	-	0.05	-	-	-	-

Glutamine	-	-	-	0.03	-	-
Arginine	0.41	-	-	-	-	-
Threonine	-	0.37	1.21	0.06	0.06	0.54
Cysteine	1.29	-	-	0.30	-	-
Alanine	0.03	0.07	-	0.11	0.01	0.05
Thyrosine	0.25	-	-	0.07	-	0.11
Methionine	-	-	-	0.008	0.009	0.06
Triptophane	-	-	-	0.10	-	-
Phenylalanine	0.37	-	-	0.07	-	-
Valine	0.04	-	-	0.01	-	0.004
Proline	-	-	-	0.97	0.38	6.50
Isoleucine	0.05	-	-	0.03	-	0.04
Leucine	0.17	0.25	-	0.06	0.07	-
Lysine	-	-	-	0.08	0.005	0.07
Total content	4.04±0.12	1.81±0.06	1.9±0.04	3.18±0.08	0.56±0.02	7.41±0.18

3.3. Cinnamon rose fruits

As it is seen from tables and chromatograms, 5 free and 15 combined amino acids were identified in fresh cinnamon rose fruits (total content was 2.25±0.04 mg% and 2.38±0.05 mg% respectively); 4 free and 2 combined amino acids in frozen fruits (0.71±0.03 mg% and 0.10±0.02 mg% respectively); 5 free and 8 combined amino acids in dried fruits (2.11±0.06 mg% and 2.99±0.08 mg% respectively).

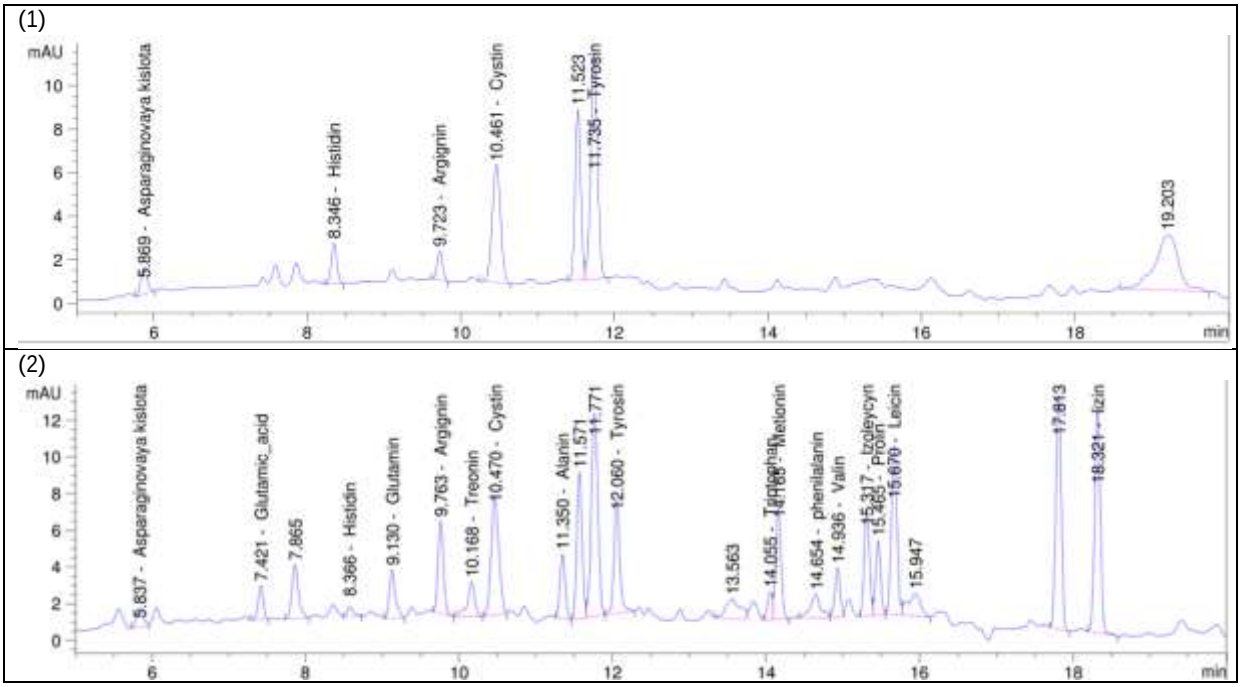
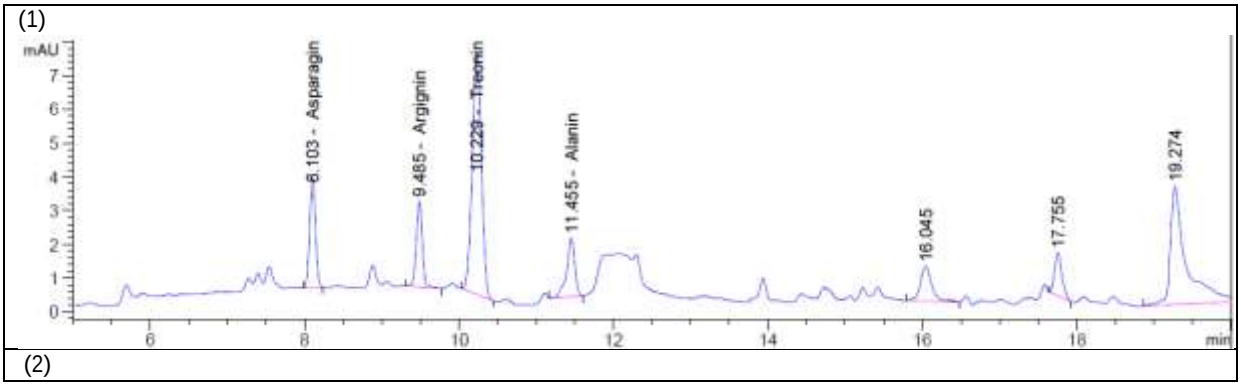


Figure 7: Chromatogram of amino acids profile of fresh cinnamon rose fruits before (1) and after (2) hydrolysis



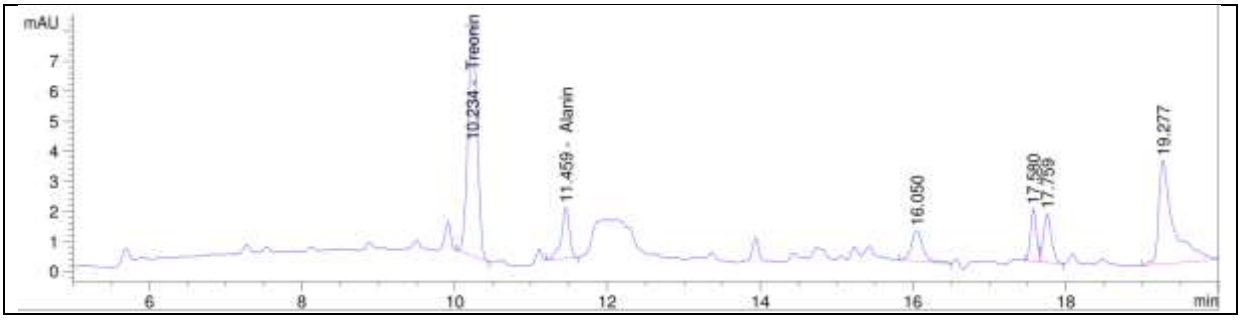


Figure 8: Chromatogram of amino acids profile of frozen cinnamon rose fruits before (1) and after (2) hydrolysis

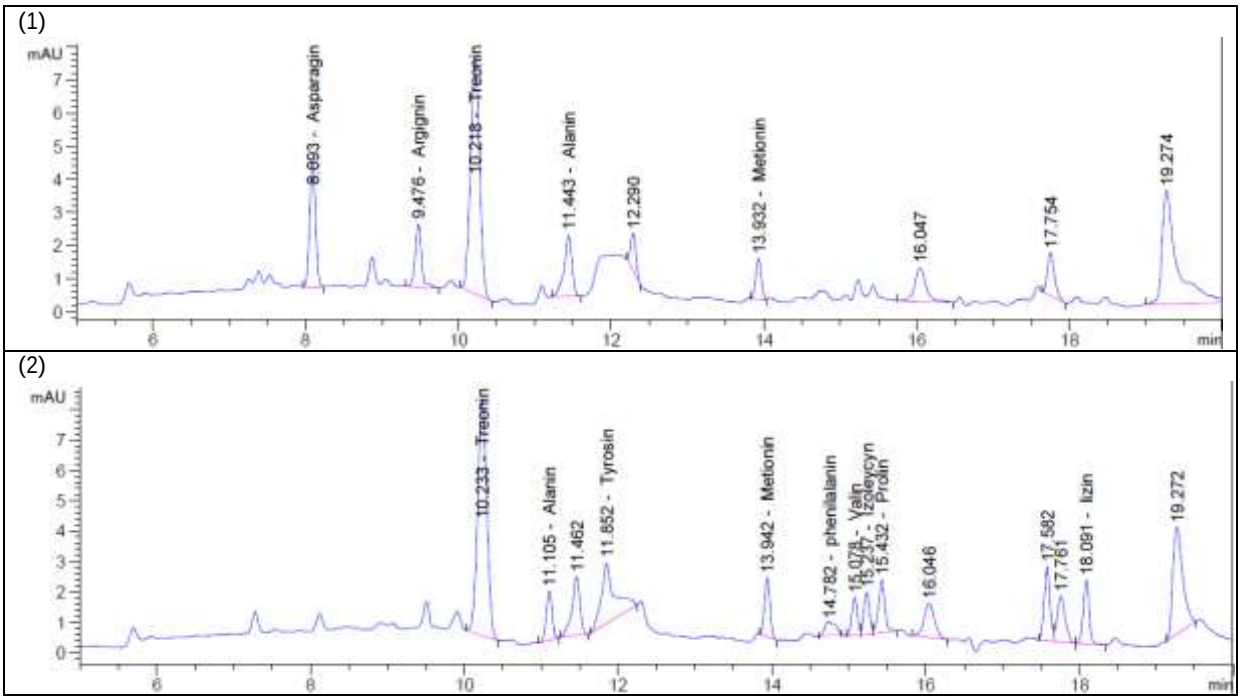


Figure 9: Chromatogram of amino acids profile of dried cinnamon rose fruits before (1) and after (2) hydrolysis

Table 3: Amino acids content (mg %) in fresh, frozen, and dried cinnamon rose fruits

Amino acids	Cinnamon rose fruits					
	Before hydrolysis			After hydrolysis		
	Fresh	Frozen	Dried	Fresh	Frozen	Dried
Asparagine	-	0.09	0.33	-	-	-
Aspartic acid	0.30	-	-	0.009	-	-
Histidine	0.08	-	-	0.02	-	-
Glutamine	-	-	-	0.03	-	-
Arginine	0.05	0.12	0.26	0.05	-	-
Threonine	-	0.42	1.16	0.02	0.10	0.27
Cystine	1.24	-	-	0.30	-	-
Alanine	-	0.08	0.24	0.002	0.003	0.03
Thyrosine	0.58	-	-	0.06	-	0.01
Methionine	-	-	0.11	0.04	-	0.03
Phenylalanine	-	-	-	0.005	-	0.02
Valine	-	-	-	0.002	-	-
Proline	-	-	-	1.69	-	2.57
Isoleucine	-	-	-	0.003	-	0.02
Leucine	-	-	-	0.07	-	-
Lysine	-	-	-	0.08	-	0.04

Total content	2.25±0.04	0.71±0.03	2.11±0.06	2.38±0.05	0.10±0.02	2.99±0.08

CONCLUSION

Amongst our study objects cinnamon rose and rowan tree fruits can be considered as sources of highly digestible amino acids, other biologically active substances and microelements in medical therapy. Given these results, it can be said that drying is the most reasonable conservation method to preserve amino acids composition and content in cinnamon rose, Siberian hawthorn, and rowan tree fruits.

ACKNOWLEDGMENT

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CONFLICTS OF INTEREST

None.

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