Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 05 May 2026
Revised 11 June 2026
Accepted 24 June 2026

Natural Resources for Human Health 2026, Vol. 6 (5): 1176–1189

KEYWORDS:
Rubus idaeus; Rubus caesius; amino acid profiling; foodomics; high-performance liquid chromatography [HPLC]; functional foods; bioactive compounds; plant metabolites; nutritional composition; stems and roots; free amino acids; nutraceutical potential.

Comparative Foodomic Profiling of Free Amino Acids in Stem and Root of Rubus idaeus L. and Rubus caesius L. Using HPLC

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ABSTRACT:

OBJECTIVE: The present work was to comparatively determine the free amino acid content in stem and root of Rubus idaeus L and Rubus caesius L using high-performance liquid chromatography [HPLC] and the application of this material in terms of biologically active components of functional and nutraceutical foods.

MATERIALS AND METHODS: The stem and root organs [above-ground and underground] of plants Rubus idaeus L and Rubus caesius L, which grow in the Fergana region of Uzbekistan, were collected, cleaned, dried in shade and ground. Free amino acids in proteins were precipitated by 20% trichloroacetic acid [TCA], and the extracted amino acids were derivatized with phenylisocyanate [to phenylthiocarbamyl derivatives, PTC] then analyzed by high-performance liquid chromatography on the diode-array detection system with the column Discovery HS C18. Amino acids were identified and quantified using retention time [RT] based on the established chromatographic conditions using a standard mixture of amino acids. The quantitative estimation of amino acids was carried out by comparison of peak areas using standard amino acid mixtures under similar conditions. Results are expressed as the average ± standard deviation [SD] [n = 3].

RESULTS: Based on the conducted HPLC analysis, 20 free amino acids were identified in plants Rubus idaeus L. And Rubus caesius L. In Rubus idaeus L., highest concentration of amino acids detected were cysteine [2.397 mg/g DW], threonine [0.931 mg/g DW], proline [0.910 mg/g DW], glutamine [0.816 mg/g DW] and arginine [0.659 mg/g DW], while in Rubus caesius L. They were serine [1.525 mg/g DW], valine [1.068 mg/g DW], asparagine [0.971 mg/g DW], glutamine [1.125 mg/g DW], and aspartic acid [0.775 mg/g DW]. The total amount of amino acids was higher in Rubus caesius [9.342 mg/g DW] than in Rubus idaeus [8.15 mg/g DW]. Amino acids, including essential ones like threonine, valine, methionine, leucine, isoleucine, tryptophan, phenylalanine and lysine, have been found in both plants.

CONCLUSION: The investigation has revealed that plants Rubus idaeus L. And Rubus caesius L. Possess diverse array of amino acid profile which may have nutritive and biological importance. These amino acids present in stem and root of plants demonstrate good potential to be employed as unexploited resources for functional foods and nutraceuticals. This investigation supports the general trends of the application of foodomics techniques to profile the nutritional and bioactive constituents of unexploited plant sources.

1. INTRODUCTION

Rubus [Rosaceae] represents a genus consisting of over 250 species throughout the temperate world which are noted for both their nutritional and medicinal significance. Within this genus the consumption of Rubus idaeus L. [red raspberry] and Rubus caesius L. [European dewberry] is widespread owing to their rich contents of vitamin, polyphenols and antioxidants. Whilst the fruit of these two species have been extensively investigated the rest of the plants, specifically the stem and root, have generally received far less attention as possible sources of beneficial molecules [1]. Foodomics, a novel interdisciplinary approach which has become more popular in recent years, uses analytical chemistry, omics science and nutritional science to give an overview of the composition of food and its biological activity. These techniques have been used extensively to give detailed analyses of individual biomolecules present in food products; one particular biomolecule, the amino acid, is vital to many physiological processes including protein synthesis, metabolism, defence mechanisms and immunity. Free amino acids are considered to be important indicators of the quality and metabolic activity of plants and the individual composition of free amino acids within the plant tissues of different species, parts of the plant or different environments vary widely and can be used to characterise food products and their functions. However, in recent years even though many foodomic technologies have been employed a comprehensive analysis of the free amino acid composition of non-edible or under-utilised parts of plants, specifically Rubus stem and roots has not been completed [2]. Thus, the purpose of this project was to analyse and compare the free amino acid composition of Rubus idaeus L. And Rubus caesius L. Stem and root by use of HPLC in order to further characterise these two Rubus species through foodomics and examine potential as other sources of health-giving biomolecules.

Rubus is a genus of plants in the Rosaceae family, which includes about 700 species. These are shrubs with bright green leaves and small white or pink flowers. Some species of rubus bear berries, such as Rubusidaeus L. [raspberry] and Rubuscaesius L. [gray blackberry]. Representatives of the genus are famous for their aromatic berries, which humanity has been eating since ancient times. The fruits of wild species are tasty and widely available, so cultivated varieties began to be selected at the end of the 19th century. Today, there are dozens of varieties of Rubusidaeus L. and Rubuscaesius L., as well as various interspecific hybrids of these plants [3].

A Raspberry is the common name of a highly disseminated and economically significant species of Rubus, belonging to the family Rosaceae. Rubusidaeus L., or simply, the raspberry plant is originally from Europe and North Asia and has spread its popularity and cultivation throughout the world. Raspberry is appreciated worldwide for its tasty and refreshing flavor and nutritional values. Its usages have varied over the centuries; as food, medicine and for cosmetic purposes, reflecting the significance it holds across sectors.

The objective of this referat is to present Rubusidaeus L. In depth, covering its botany, cultivation, uses and beneficial health properties. Through the presentation, it is hoped that the reader will appreciate why this fruit is not only desirable but also beneficial for human health. Raspberry is a deciduous shrub, characteristic of temperate regions, distinguished by specific morphological and biological growth characteristics from the other representatives of its family Rosaceae [4].

It is a perennial, somewhat sprawling shrub, typically of moderate growth, reaching heights ranging between 1 to 2m, although some cultivated forms grow taller than this. The raspberry hasarching, thorny stems, called canes, that are woody by their second year of growth, but they are relatively pliable and have a green to reddish-brown color during their first year. Small, stiff prickles grow on these canes and render them a bit difficult to harvest without injury.

Leaves The raspberry plant has alternate, compound leaves that generally are composed of 3 to 5 leaflets. The leaflets are oval or lanceolate, have toothed or serrated edges and are hairy underneath but not above; they are dark green above and paler below [5]. In addition to leaves, the plant also bears a delicate flower that is generally white or tinged with pink.

It grows on spikes at the tips of short branches in the leaf axils; and can be found alone or in clusters that can have a broad panicle. The raspberry flower is perfect and pollinates on self but more frequently with insects. Fruit Raspberry produces an aggregate fruit composed of 3-20 clusters, or drupelets that contain one seed and are united into a fleshy structure [5, 6]. As the raspberry ripens the seed within each of these drupelets enlarge, expanding outwards and giving the raspberry fruit its characteristic shape [7]. Raspberries are the fifth-highest value crop for production value in Michigan, United States; raspberry cultivation is practiced around the globe, the total world production of the fruit amounted to millions metric tons annually. Raspberries are popular for both fresh consumption and for the preparation of jams, juices, desserts and as a fruit for the production of alcoholic drinks such as wines and liqueurs.

It also has a market as a cash crop in several countries; USA, Canada, Poland, Mexico and China are the leading raspberry producing countries worldwide. Raspberries are an extremely nutritious food source. In addition to being enjoyed fresh, it can be utilized in a variety of prepared dishes, such as pies, tarts and cobblers, sauces and jams [8].

The nutritional worth of raspberry makes it a valuable food, both in fresh form and used as an ingredient in processed food items and beverages. The high vitamin, mineral, and antioxidant content of raspberries gives it the ability to benefit your health in a myriad of ways. Here are some of the benefits of raspberries to your health:

[9-11].

Nutrient-dense: Raspberries contain a significant amount of nutrients; these include the vitamin C, the manganese and the folate. These particular vitamins and minerals are essential to a person's immune function and skin healing and formation. In addition to vitamin C, manganese contributes to the proper formation of bone tissue, antioxidant defense and metabolism. There are also small amounts of vitamins A, E and some B vitamins found within this berry.

Antioxidants: These are potent health-promoting compounds, raspberries are packed full of it and especially antioxidants in the form of anthocyanins and ellagic acid. The health benefits of antioxidants have long been associated with reducing the effects of oxidation in our cells and protecting against various chronic diseases such as cancer, cardiovascular diseases and neurodegenerative diseases. Laboratory studies have also found that ellagic acid has promising potential as a anti-cancer agent.

Anti-inflammatory effects: One of the many factors contributing to chronic disease is chronic inflammation within the body; this can contribute to heart disease, diabetes, arthritis and much more. Many compounds have anti-inflammatory benefits in the body and the berry has a large number of them such as quercetin and ellagic acid. These compounds can help minimize the formation of molecules in the body that cause inflammation; this can ease some symptoms of an inflammatory condition.

Heart health: Regularly eating this berry could be beneficial to your heart due to its high quantity of dietary fiber; this will help lower cholesterol levels within your body and consequently reduce the risk of atherosclerosis. The anthocyanins present in raspberries will also improve blood vessel function while lowering the risk of cardiovascular diseases, this is due to their preventative action on the oxidation of LDL cholesterol; which would lead to the buildup of plaque within your arteries.

Digestive health: Consuming this berry regularly is also known to assist with the healthy functioning of the body; the fiber it contains will ensure your bowels function normally and help keep your gut microflora healthy. The soluble fiber found in raspberries can also control blood sugar levels; therefore this is a suitable food for those with diabetes.

Weight Management: Due to their high fiber content and low calorie count, raspberries can aid in weight management by promoting feelings of fullness and reducing overall calorie intake. They are a nutritious snack option for those seeking to maintain or lose weight, as they provide essential nutrients without excess calories [12-14].

The medicinal use of fruits and berries has been developed in traditional medicine systems for thousands of years. Not a single nutritionist, not a single doctor, or even any sane person, even those not burdened with a higher or secondary medical education, will deny the enormous benefits, nutritional and medicinal value of fruits and berries [15, 16].

Rubuscaesius L., commonly known as the European dewberry or the bramble, is a member of the Rosaceae family. It is a deciduous shrub native to Europe and Asia, where it has been a part of the natural landscape for centuries. Much like its close relative, Rubusidaeus [the raspberry], Rubuscaesius produces edible fruit and has a rich history of use in both food and traditional medicine. Though less widely cultivated than the raspberry, Rubuscaesius still holds ecological, cultural, and economic value. This referat will provide an in-depth examination of Rubuscaesius L., including its botanical characteristics, cultivation, uses, health benefits, and the role it plays in its native ecosystems [17].

Rubuscaesius L is a perennial shrub that can grow in many different environments; including clearings, woods, hedgerows, roadsides and waste grounds. This persistence has led to its proliferation in the British and European and also Asia landscapes.

Rubuscaesius L. Is a straggling or trailing semi erect shrub that can grow up to 2m long. In structure it can be identified as arching thorny canes which, when immature, are green, or reddish, becoming woody and brown when they are fully developed. The thorns are stiff and sharp; picking the berries could therefore be an unpleasant activity. The propagation occurs via suckers, which emerge from the root system and give it its strong presence in its habitats [16].

Leaves of Rubuscaesius are compound and alternate along the stems. These consist of 3-5 leaflets. Leaflets are lancolate or ovate in shape, and have serrated margins. They are bright green on the top and covered by a silver, fine pubescence on the underside. This gives the leaves their unique appearance which are often used in herbal remedies, such as infusions or teas.

Flowers are generally white or pale pink in colour, they have 5 petals and bloom at the end of the canes. Typically blooming during late spring and early summer [May, June], they have male and female sexual parts therefore are hermaphroditic. Pollination depends on insects, as it has no other way of producing fruit.

Rubuscaesius is a species commonly found in temperate regions, especially across Europe, from southern Scandinavia to the Mediterranean, and in parts of Asia. Its ecological role is significant, both in its native habitats and in the context of its interactions with other species. The plant is particularly common in woodlands, hedgerows, scrublands, and wastelands [18]. While Rubuscaesius is not considered an invasive species in its native range, it has the potential to become invasive when introduced into areas where natural predators and competitors are less effective in controlling its spread. Some non-native regions, including areas in New Zealand, allow for thick stands of Rubus caesius to form which choke out native vegetation and thereby disrupt and affect local flora and fauna. Dense stands of R. Caesius form habitat for wildlife, including the presence of small mammals and numerous species of birds.

The plant has thorns that help defend it from predation by herbivores, and its berries are utilized as a food source by birds, small rodents, and large animals like deer.

It supports local diversity. The European dewberry, also called. Common bramble or raspberry bush, is a species of flowering plant in the rose family, and is widespread and often naturalised outside Europe. It usually grows in sunny and semi-shady situations in well-drained, nutritious soil, such as along forest edges, in sites of agricultural disturbance or on vacant lots, abandoned fields, or roadsides.

R.Caesius is adapted to a variety of soil conditions and exposure levels. The different parts of R. Caesius plant have also traditionally been utilized for medicine.

Traditionally, European dewberry leaves are utilized in herbal tea to manage issues like mild digestive problems, sore throats and discomfort in the GI system. European dewberry leaves are considered having inflammatory-retarding, drying, and also antioxidant capabilities. In addition, the fruit of R. Caesius is consumed in order to boost the body immune systems and thanks to its high Vitamin C web content [19, 20].

Due to the content of vitamins, antioxidants and also essential fats, Europedewberry fruit and leaves extracts might be employed in cosmetic item to get rid of aging of skin as well as provide the skin with moisturizing properties. Robuscaesius L has the following main health benefits thanks to its nutritional makeup: Packed With Nutrients: The European dewberry is rich in numerous vital vitamins, minerals, as well as fiber, consisting of vitamin C, which is crucial for a robust immune system, skin health, and the proper healing of wounds. The fruit is also packed with antioxidants, particularly anthocyanins, which are potent compounds known to aid in lowering oxidative damage throughout the body [20, 21]. Antiviral And Antibacterial Capabilities: The antioxidants present in R. Caesius, more especially anthocyanins, help neutralize harmful free radicals that harm body cells and are connected with conditions like cardiovascular disease, cancer cells as well as diabetes mellitus.

It may also play an important role in minimizing aging as they safeguard your skin from oxidative damages. Anti-inflammatory Properties: European dewberry leaves as well as fruits have an anti-inflammatory impact on the body. This makes them a valuable tool for treating various persistent conditions such as inflammatory heart problem or joint inflammation.

The high fiber content in dewberries makes them an excellent food for supporting digestive health. Fiber promotes regular bowel movements, aids in digestion, and helps prevent constipation. The fruit's high water content also supports overall hydration and gastrointestinal health.

Heart Health: The antioxidants and fiber in Rubuscaesius contribute to cardiovascular health by reducing the risk of high blood pressure, improving blood circulation, and lowering levels of "bad" LDL cholesterol. Regular consumption of dewberries may reduce the risk of heart disease and stroke.

The medicinal use of fruits and berries has been developed in traditional medicine systems for thousands of years. Not a single nutritionist, not a single doctor, or even any sane person, even those not burdened with a higher or secondary medical education, will deny the enormous benefits, nutritional and medicinal value of fruits and berries [22, 23].

Studying the natural forests of Uzbekistan and protecting forests is the main task of foresters. For this purpose, to identify and reproduce endangered species with nutritional properties, we studied the RubuscaesiusL., which is naturally distributed in the mountain and tugai forests of our republic [23].

The purpose of this study is to determine amino acids in Rubusidaeus L. and Rubuscaesius L. plants growing in the Fergana region of the Republic of Uzbekistan.

Fig.1. The root of the plant Rubusidaeus L.

2. MATERIALS AND METHODS

2.1. Plant Material Collection and Preparation

Stem [above-ground] and root [below-ground] parts of *Rubus idaeus* L. and *Rubus caesius* L. were collected from the Fergana region, Uzbekistan, during the winter season. The plant species were identified based on standard botanical characteristics.

Collected samples were thoroughly washed with distilled water to remove adhering soil and impurities, followed by air-drying under shade at ambient temperature until constant weight was achieved. The dried plant materials were ground into fine powder using a laboratory grinder and stored in airtight containers at room temperature until further analysis.

2.2. Extraction of Free Amino Acids

Free amino acids were extracted following protein precipitation using trichloroacetic acid [TCA]. Briefly, 1 mL of plant extract was mixed with 1 mL of 20% [w/v] TCA in centrifuge tubes. The mixture was allowed to stand for 10 min to ensure complete precipitation of proteins and peptides.

The samples were then centrifuged at 8000 rpm for 15 min, and the clear supernatant was collected. An aliquot [0.1 mL] of the supernatant was subjected to lyophilization. The dried residue was dissolved in a triethylamine–acetonitrile–water [TAW] mixture [1:7:1, v/v/v] and evaporated to dryness. This neutralization step was repeated twice to remove residual acid [24].

2.3. Derivatization of Amino Acids

The dried samples were derivatized using phenylisocyanate to obtain phenylthiocarbamyl [PTC] derivatives of amino acids, following the method described by Cohen and Strydom with minor modifications. This derivatization enhances chromatographic separation and detection sensitivity of amino acids.

2.4. HPLC Analysis

Quantitative analysis of amino acids was performed using a high-performance liquid chromatography [HPLC] system [Agilent Technologies 1200 series] equipped with a diode array detector [DAD].

Separation was achieved using a Discovery HS C18 column [75 × 4.6 mm, particle size 5 µm]. The mobile phase consisted of:

- Solvent A: 0.14 M sodium acetate buffer containing 0.05% triethylamine [pH 6.4]
- Solvent B: Acetonitrile

Chromatographic analysis was carried out at a flow rate of 1.2 mL/min with detection at 269 nm. The column temperature was maintained at ambient conditions.

2.5. Identification and Quantification

Amino acids were identified by comparing their retention times with those of standard amino acid mixtures analyzed under identical chromatographic conditions. Quantification was performed using external calibration curves constructed from standard solutions of known concentrations.

Results were expressed as milligrams of amino acid per gram of dry weight of plant material [mg/g DW].

2.6. Statistical Analysis

All analyses were performed in triplicate, and the results are presented as mean ± standard deviation [SD]. Statistical comparisons between species and plant parts were conducted using appropriate statistical methods [one-way ANOVA], with significance considered at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Identification and Quantification of Free Amino Acids

High-performance liquid chromatography [HPLC] analysis enabled the identification and quantification of 20 free amino acids in the stem and root of *Rubus idaeus* L. and *Rubus caesius* L. The chromatographic method provided clear separation and reliable detection of amino acids under the specified analytical conditions, confirming the suitability of the method for plant metabolite profiling. The total amino acid content was found to be **8.15 mg/g** in *R. idaeus* and **9.256778 mg/g** in *R. caesius*, indicating a comparatively higher accumulation of free amino acids in *R. caesius* figure 2,3.

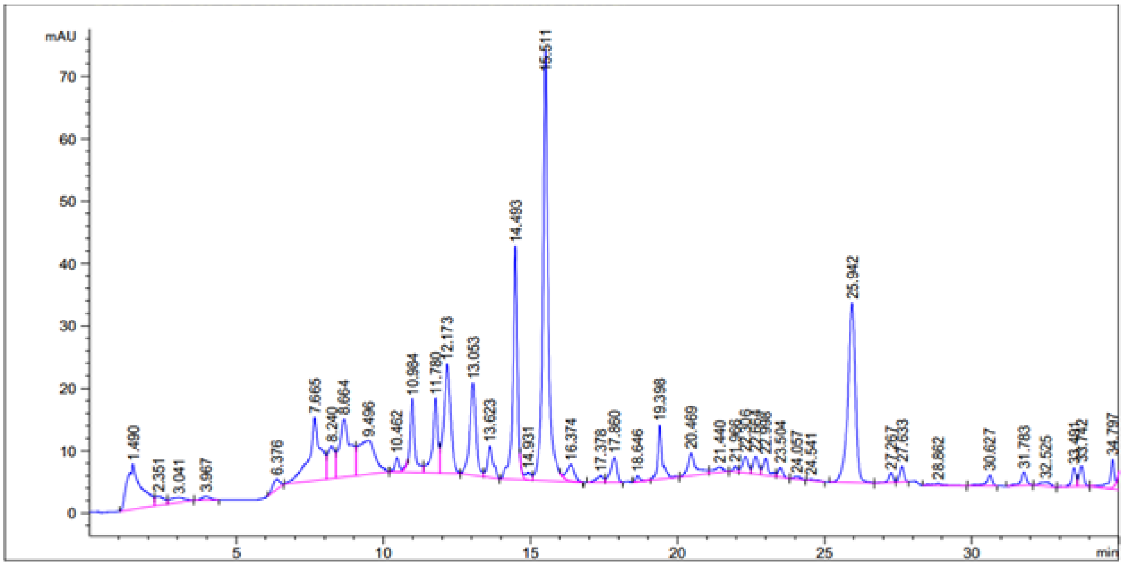

Figure 2. HPLC chromatogram of amino acids in Rubus idaeus L. Separation was performed using a Discovery HS C18 column [75 × 4.6 mm, 5 µm] with sodium acetate buffer–acetonitrile mobile phase at a flow rate of 1.2 mL/min and UV detection at 269 nm. Peaks 1–20 correspond to identified amino acids.

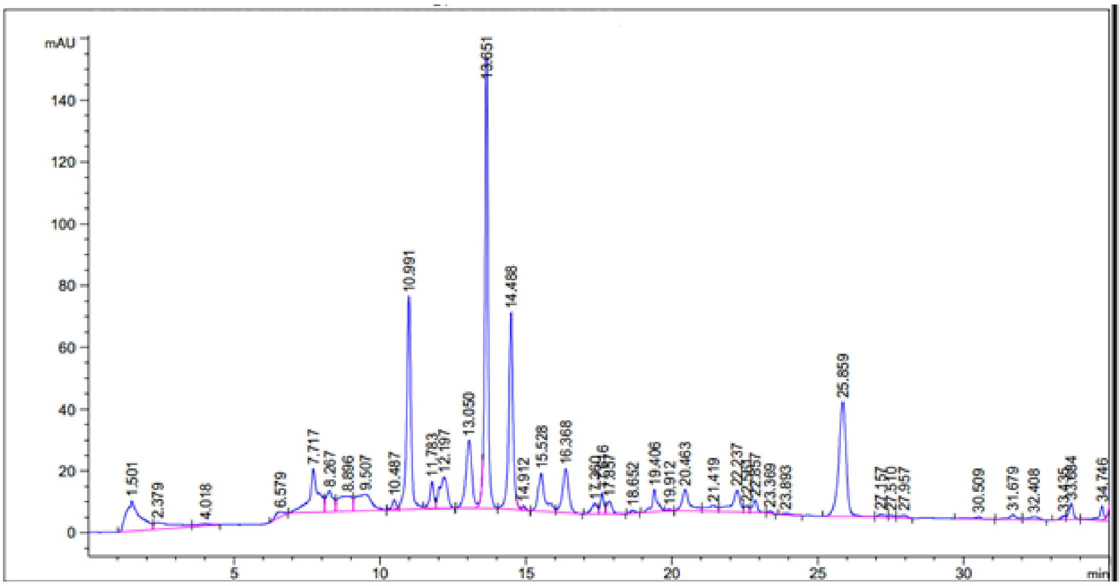

Figure 3. HPLC chromatogram of amino acids in Rubus caesius L. Separation was performed using a Discovery HS C18 column [75 × 4.6 mm, 5 µm] with sodium acetate buffer–acetonitrile mobile phase at a flow rate of 1.2 mL/min and UV detection at 269 nm. Peaks 1–20 correspond to identified amino acids.

3.2. Amino Acid Composition of *Rubus idaeus* L.

The amino acid profile of *R. idaeus* revealed a predominance of cysteine [2.396 mg/g], followed by threonine [0.931 mg/g], proline [0.910 mg/g], glutamine [0.816 mg/g], and arginine [0.659 mg/g]. Moderate concentrations of serine, asparagine, and tyrosine were also observed, while essential amino acids such as leucine, isoleucine, and tryptophan were present in relatively lower amounts. The high cysteine content suggests a strong potential for antioxidant activity and detoxification processes, whereas elevated levels of proline and glutamine indicate possible roles in stress adaptation, tissue repair, and metabolic regulation table 1.

Natural Resources for
Human Health
eISSN: 2583-1194

Table 1. Free amino acids in the Rubus idaeus L. plant composition

No.	Amino Acid	Concentration [mg/g dry weight] [Mean ± SD]	Significance
1	Aspartic acid	0.083 ± 0.004	****
2	Glutamic acid	0.275 ± 0.014	****
3	Serine	0.459 ± 0.023	****
4	Glycine	0.121 ± 0.006	****
5	Asparagine	0.241 ± 0.012	****
6	Glutamine	0.816 ± 0.041	****
7	Cysteine	2.397 ± 0.120	****
8	Threonine[E]	0.931 ± 0.047	****
9	Arginine	0.659 ± 0.033	****
10	Alanine	0.105 ± 0.005	****
11	Proline	0.910 ± 0.046	****
12	Tyrosine	0.401 ± 0.020	****
13	Valine[E]	0.213 ± 0.011	****
14	Methionine[E]	0.106 ± 0.005	****
15	Histidine	0.107 ± 0.005	****
16	Isoleucine[E]	0.041 ± 0.002	****
17	Leucine[E]	0.082 ± 0.004	****
18	Tryptophan[E]	0.079 ± 0.004	****
19	Phenylalanine[E]	0.058 ± 0.003	****
20	Lysine[E]	0.066 ± 0.003	****
Total		8.150 ± 0.407	

Values represent mean ± SD [n = 3]. DW = dry weight. Amino acids are listed in order of chromatographic elution. [E] **Essential amino acid. In graph **** is represented by #.**

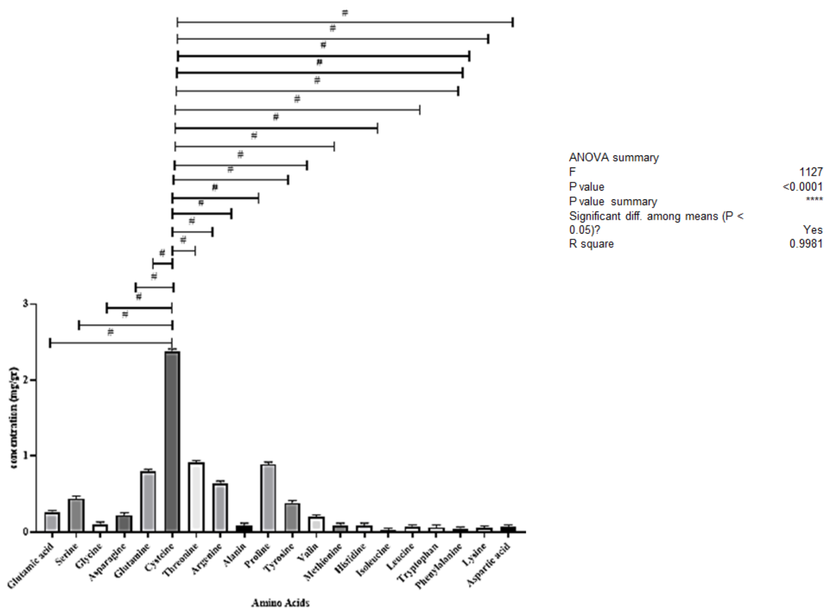

Figure 4. Amino acid profile of *Rubus idaeus* L. Quantitative distribution of free amino acids identified in the stem and root of *Rubus idaeus* L. using high-performance liquid chromatography [HPLC]. Results are expressed as concentration [mg/g dry weight, DW] and presented as mean ± SD [n = 3]. Cysteine, threonine, proline, glutamine, and arginine were identified as the predominant amino acids in *R. idaeus* L.

3.3. Amino Acid Composition of *Rubus caesius* L.

In *R. caesius*, the main amino acids identified were serine [1.525 mg/g], glutamine [1.125 mg/g], Valinee [1.068 mg/g], asparagine [0.971 mg/g], and aspartic acid [0.775 mg/g]. Proline [0.728 mg/g] and cysteine [0.573 mg/g] were also present in notable amounts. Compared to *R. idaeus*, *R. caesius* exhibited higher levels of serine, Valine, and asparagine, suggesting differences in nitrogen metabolism and protein biosynthesis pathways. The relatively high Valine content also highlights its potential contribution to energy metabolism and muscle-related functions table 2 figure 5.

Table 2. Free amino acids in Rubuscaesius L. plant Composition

No.	**Amino Acid**	**Concentration [mg/g dry weight] [Mean ± SD]**	**Significance**
1	Aspartic acid	0.775 ± 0.039	**
2	Glutamic acid	0.203 ± 0.010	****
3	Serine	1.525 ± 0.076	****
4	Glycine	0.484 ± 0.024	ns
5	Asparagine	0.971 ± 0.049	****
6	Glutamine	1.125 ± 0.056	****
7	Cysteine	0.573 ± 0.029	-
8	Threonine^E	0.079 ± 0.004	****
9	Arginine	0.403 ± 0.020	*
10	Alanine	0.126 ± 0.006	****
11	Proline	0.728 ± 0.036	ns
12	Tyrosine	0.255 ± 0.013	****
13	Valine^E	1.068 ± 0.053	****
14	Methionine^E	0.110 ± 0.005	****
15	Histidine	0.354 ± 0.018	*
16	Isoleucine^E	0.085 ± 0.004	****
17	Leucine^E	0.030 ± 0.002	****
18	Lysine^E	0.228 ± 0.011	****
19	Tryptophan^E	0.041 ± 0.002	****
20	Phenylalanine^E	0.180 ± 0.009	****
Total		9.342 ± 0.467	

Values represent mean ± SD [n = 3]. DW = dry weight. Amino acids are listed in order of chromatographic elution. ^{E} **Essential amino acid. In graph **** represented by #.**

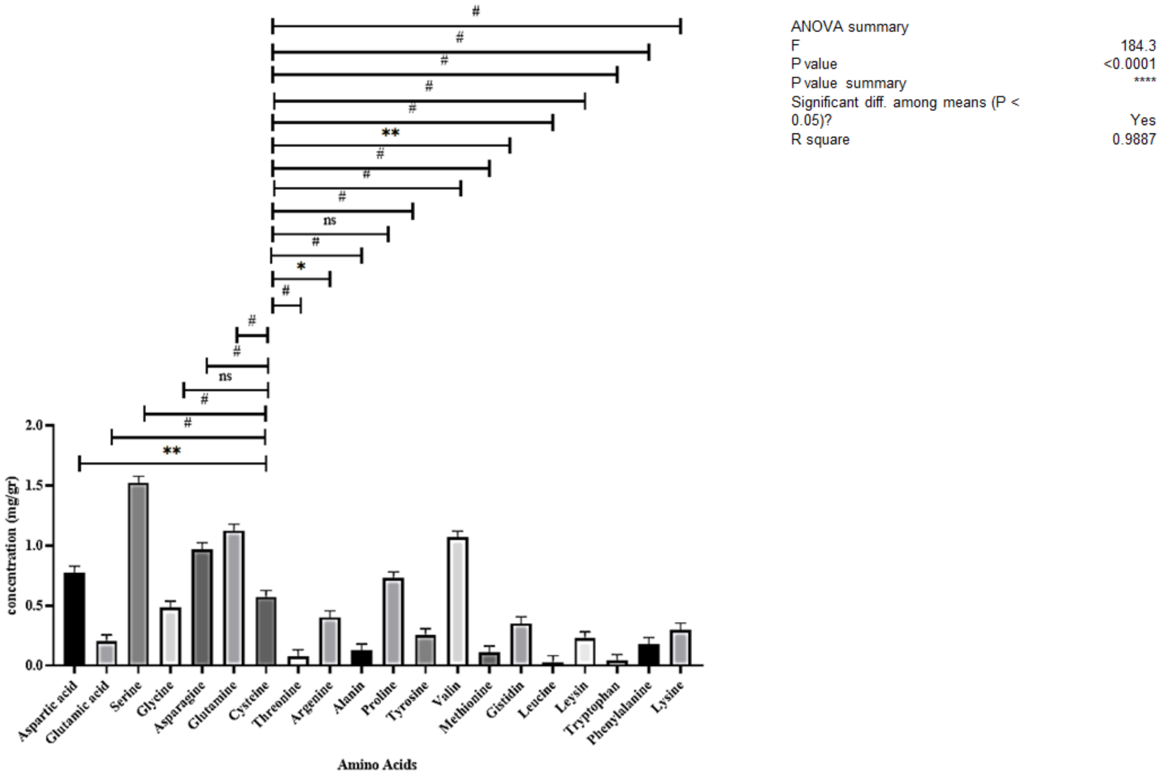

Figure 5. Amino acid profile of *Rubus caesius* L. Quantitative distribution of free amino acids identified in the stem and root of *Rubus caesius* L. using high-performance liquid chromatography [HPLC]. Results are expressed as concentration [mg/g dry weight, DW] and presented as mean ± SD [n = 3]. Serine, glutamine, valine, asparagine, and aspartic acid were the predominant amino acids detected in *R. caesius* L.

3.4. Comparative Analysis of Amino Acid Profiles

The two species were compared and we observed that there were variations between the two samples regarding amino acid composition, and that R. Idaeus was rich in cysteine, threonine and arginine while R. Caesius was rich in serine, Valine and asparagine. It was interesting to note that proline and glutamine remained dominant in both species which means that these amino acids are a key component for plants. The differences in amino acid distribution could be attributed to physiology, biochemistry and the metabolism within each plant depending on their environment. Regarding foodomics, these two species contain essential and conditionally essential amino acids which are crucial for various biological activities such as antioxidant activity, modulation of the immune system and tissue repair and metabolism. The comparative representation of the two species regarding the amino acid distribution is shown below.

The results shown in Table 1 suggest that stem and root of Rubus idaeus L are rich in cysteine, threonine, proline, glutamine and arginine while the stem and root of Rubus caesius L [Table 2] are rich in serine, glutamine, Valinee, asparagine and aspartic acid. Amino acids are important biological compounds, they are the building blocks for proteins, and they participate in several metabolic processes. It is likely that R. Idaeus isrich in cysteine in order to perform antioxidant and detoxicifying functions, and R. Caesius is rich in serine and Valinee in order to perform metabolically relevant functions. The presence of proline and glutamine both at considerable levels within the samples indicated that both amino acids are important for stress, for the repair of tissues and for nitrogen metabolism. The profile of the samples indicated that both plant species were a good source of food compounds.

4. DISCUSSION

A detailed comparative analysis of the contents of free amino acids of stem and root of Rubus idaeus L. And Rubus caesius L. Is provided. A high amount of various free amino acids can be found in both the stem and the root of the two Rubus species and it could be an underutilized source of metabolically active compounds of nutritional significance. The analysis identified 20 amino acids in both species, indicating a good amino acid composition suitable for a food matrix based on a plant derived compound.

A significantly higher concentration of total free amino acids was determined in the stem of R. Caesius [9.56 mg/g] compared to R. Idaeus [8.15 mg/g], possibly depending on differences between the two species in nitrogen metabolism or in the pathways of amino acids biosynthesis influenced by genetically determined differences, by the environment and by physiological condition. This between-species variation in amino acids content was already detected in many other plant matrices. Therefore the species selection is important in the functional foods area.

Regarding the content in amino acids in each part analyzed, Cysteine was the highest content in R. Idaeus, that seems related with its antioxidant system of the cells. Cysteine could be a precursor of glutathion and therefore would play a protection role of the cells against oxidative stress. In R. Caesius higher content of Serine, Valinee and Asparagine could suggest different metabolic processes. The presence of high quantity of Valinee may indicate an important role in muscle related functions or in energy metabolism.

An high amount of Proline and Glutamine was detected in both the Rubus species, indicating their essential role in plant stress response and metabolic stability, Proline acts as an osmoprotector and stabilizer of macromolecules, whereas Glutamine is one of the key component in nitrogen metabolism.

Considering the foodomics aspect of the free amino acids determined in the different parts of both species. Thr, Valinee and Trp were the essential amino acids present in the Rubus tissues considered. While Thr, Valinee and Trp are essential amino acids for human metabolism and they should be introduced with diet, other amino acids as Arg and Gln can be conditionally essential for the cell physiology depending on the different conditions. Therefore introducing Rubus parts into the diet can be nutritionally advantageous.

It should be stressed also the potential differences among stems and roots to be used as the stem is more commonly referred to agricultural waste but represents a source of interesting compounds. The nutritional potential of these stems and roots as well as in the fruit in a future may lead to food applications or even in the formulation of nutritional supplements or of nutraceuticals.

This analysis is a confirmation that many secondary compounds are present in non fruits of Rubus species and foodomics investigation on the whole plant tissue may bring many useful data regarding its nutritional contribution. Future study should be carried out regarding bioavailability and physiological impact of these metabolites in human health.

CONCLUSIONS

This study reveals a comprehensive foodomic assessment of free amino acids in the stems and roots of Rubus idaeus L. And Rubus caesius L., thus identifying these plant organs as a rich, but underexplored, resource of biologically important compounds. All twenty-seven determined amino acids are present in both plant species, indicating the plants' well-developed and metabolically relevant composition profile. There is an interesting differentiation in the composition profile between the two species: R. Idaeus showed increased amounts of cysteine, threonine, proline, glutamine, and arginine, whereas R. Caesius exhibited significant levels of serine, Valinee, asparagine and aspartic acid. Among them, the occurrence of proline and glutamine in both species proves the existence of an underlying functional metabolic profile and human health benefits. From the point of view of foodomics, the presence of several essential and conditionally essential amino acids proves the feasibility of their application as functional food and nutraceutical ingredients. Most importantly, the use of untraditional plant organs as Rubus stems and roots proves the potential benefit of using sustainable resources and adding new bioactive components to our diet. Future works should address the bioavailability and functionality, potential health impact, and processing/environmental factors influencing their composition, to add evidence to their consumption and applicability in healthy eating patterns. Both Rubus idaeus L. [red raspberry] and Rubus caesius L. [European dewberry] are good source of biologically significant amino acids both in above ground and under ground parts; it supports the possibility of them being important nutrient and health relevant parts. Rubus idaeus is found to have significantly high concentration of cysteine, threonine, proline, glutamine and arginine, while R. Caesius showed higher amounts of serine, Valinee, glutamine, asparagine and aspartic acid. Both species share proline and glutamine in their composition and thus share benefits related to immunity and tissue repairing. Proline is associated to immunity, protein repair, and skin function, as well as to neuroprotective activity and resistance to stress. Glutamine is a building block of proteins and provides energy to immune cells, gut and nerve cells. The amino acid content could explain their importance on maintaining tissue structures, like skin and muscle [Valinee, proline], immune response, neural equilibrium and detoxification [aspartic acid, asparagine, Valinee, proline and glutamine]; in fact, threonine, and Valinee are considered essential amino acids, which means that human body does not produce them and it has to be taken through diet, enhancing the biological value of Rubus species. Due to their content in essential and conditionally essential amino acids, whose functions are critical for immune system modulation, tissue renovation, and energy metabolic processes, both species Rubus idaeus and Rubus caesius can be regarded as candidate for the development of functional food, dietary supplement or as fitotherapy resource. Its amino acid composition justify that further research should be conducted on its bioactivity, functionality and in the identification of clinical potential and dietary related applications to help people with a low protein supply.

List of Abbreviations

HPLC – High-performance liquid chromatography

DAD – Diode array detector

TCA [TCAA] – Trichloroacetic acid

PTC – Phenylthiocarbamyl

TEA – Triethylamine

ACN [CH_3CN] – Acetonitrile

C18 – Octadecylsilane column

TAW – Triethylamine–acetonitrile–water mixture

UV – Ultraviolet

mg/g – Milligrams per gram

Declarations

Disclaimer

Declaration of generative AI and AI-assisted technologies in the writing process: During manuscript preparation, the authors used Paperpal [Editage] to enhance language clarity and grammar. Following this assistance, all content was carefully reviewed and revised by the authors, who take full responsibility for the final published version.

Acknowledgments

None

Author contributions

S.K, I.A, N.A: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing original draft, Writing review & editing. Y.A, M.I, D.A: Conceptualization, Resources, Validation, Supervision, Writing review & editing. Both authors read and approved the submitted version.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Funding

This work did not receive any specific grant for the conduct of the study.

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