Original Research Article

Amino acid, fatty acid, and mineral compositions of fruit, stem, leaf and root of *Rubus amabilis* from the Qinghai-Tibetan Plateau

Rezeng Caidan, Limao Cairang, Bin Liu, Yourui Suo

**A R T I C L E   I N F O**

Article history:
Received 18 February 2012
Received in revised form 31 August 2013
Accepted 17 September 2013

**Keywords:**
Rubus amabilis  
Nutritional composition  
Qinghai-Tibetan Plateau  
Himalayan Plateau  
Food analysis  
Amino acid  
Fatty acid  
Mineral element  
Traditional foods  
Indigenous foods  
Wild foods  
Biodiversity and nutrition  
Food composition

**A B S T R A C T**

The amino acid, fatty acid, and mineral content of *Rubus amabilis* harvested from the Qinghai-Tibetan Plateau were analyzed. Results revealed that the total amino acids in the leaves, fruits, roots, and stems of *R. amabilis* were 17.1, 7.5, 6.5, and 5.7 g, respectively. Further analysis of the amino acids showed that the protein contained nutritionally useful quantities of essential amino acids. The total essential amino acids in the leaves of *R. amabilis* were 9.3 g EAAAs/100 g. Total fatty acids varied in different parts of *R. amabilis*. Stearic acid, linolenic acid, linoleic acid, and palmitic acid in the leaf samples were 41.4%, 13.7%, 11.9%, and 6.7%, respectively. Lauric acid, oleic acid, docosahexaenoic acid, and eicosenoic acid were present only in small quantities. Potassium, magnesium, and calcium were the most abundant minerals in the leaf samples. Among the essential trace mineral elements, Fe exhibited the highest content in different parts of *R. amabilis*.

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1. Introduction

Daily intake of fruit, vegetables, and fatty acids may reduce by approximately 20% to 30% the risk of cardiovascular disease and extend the lifespan of a 40-year-old individual by approximately one year (Peter et al., 2010). In many parts of the Qinghai-Tibetan Plateau (also known as the Himalayan Plateau in the Tibet Autonomous Region and Qinghai Province in western China), fruits and tea plants are seldom grown; thus, individuals rely mainly on wild plants to supplement their diet because of the long-term shortage of fruits. As a result, individuals who consume animal meat and animal-derived products as staple food in the western plateau rely on wild plants such as *Rubus amabilis* to satisfy their nutritional requirements.

*R. amabilis* belongs to the Rosaceae family and Rosoideae subfamily. Rosaceae comprises approximately 500 species distributed worldwide (Meng and Finn, 2002; Liu and Wang, 2006; Brown, 2002). *R. amabilis* is a deciduous shrub widely spread in China ( Kunming Institute of Botany, 2003). In the Qinghai-Tibetan Plateau, *R. amabilis* is highly appreciated and consumed particularly by local populations. *R. amabilis* fruits are characterized by a distinct and pleasant smell. As source of food and medicine, *R. amabilis* fruits are eaten fresh or canned by local individuals when in season. The leaves of the plant are also dried and consumed as tea. Roots, stems, and leaves exhibit antiphlogistic, analgesic, antitodal, and antitumor effects (Liu and Wang, 2006). In previous studies, compounds from the genus *Rubus* and their pharmacutical functions have been described (Cen et al., 2001; Lu, 2007).

Many *Rubus* species bear fruits. In most countries, fruits derived from *Rubus* species are commonly used in food and beverage...
industries to produce wine, beer, soft drinks, preserved and canned foods, and desserts. Dried leaves from *Rubus* species can be used to prepare tea and herbal tea blends (Brown, 2002). *Rubus* extract can be applied in traditional medicine as an antimicrobial (Rauha et al., 2000; Tan et al., 2002), anticonvulsant, muscle relaxant (Nogueira and Vassiliouf, 2000), and radical scavenging (Moyer et al., 2002) agents.

However, a detailed analysis of the nutrient content of this plant is unavailable. This study aimed to determine the amino acid, fatty acid, and nutrient concentrations in the fruits, leaves, and roots of *R. amabilis* from the Himalayan Plateau in western China.

2. Materials and methods

2.1. Materials

Fruits, stems, roots, and leaves of *R. amabilis* were collected in September 2009 in the mountain valleys of Huzhu in the eastern region of Qinghai Province in western People’s Republic of China. The collected plant parts were transported to the laboratory and grouped accordingly. Each part was individually washed with tap water and deionized water. Afterward, the parts were dried at 55 °C until a constant weight was obtained. A total of 120 samples of different parts of *R. amabilis* were analyzed. All of the samples (5.0 g of each) were ground into powder by using a glass mortar and stored at −4 °C until use. The crude protein and crude fat contents of the samples were analyzed according to Micro-Kjeldahl and Soxhlet methods, respectively; total carbohydrates were calculated based on standard methods (AOAC, 1995). Moisture was determined by drying at 105 °C until a constant weight was achieved (AOAC, 1995).

2.2. Amino acid analysis

The following amino acid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA): arginine (Arg); aspartic acid (Asp); serine (Ser); glutamic acid (Glu); threonine (Thr); glycine (Gly); alanine (Ala); 4-amino-butyric acid (GABA); proline (Pro); methionine (Met); valine (Val); phenylalanine (Phe); tryptophan (Trp); nor-leucine (Leu); isoleucine (Ile); cystine (Cys); histidine (His); ornithine (Orn); lysine (Lys); and tyrosine (Tyr); 2-[2-(Dibenzocarbazole)-ethoxy] ethyl chloroformate (DBCEC) was synthesized in our laboratory. HPLC-grade acetonitrile was purchased from Jining Reagent Co. (Shandong, China). Analytical grade formic acid was purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Borate buffer was prepared from 0.2 mol/L of boric acid solution and adjusted to pH 8.5 with 4 M potassium hydroxide solution. All of the other reagents were of analytical grade unless otherwise stated.

Each sample (50 mg) was extracted ultrasonically using acetonitrile–methanol (1:1, v:v) for 30 min to obtain free amino acids. The extract was filtered and dried; derivatization reagent and borate buffer (pH 8.5) were added. Each sample (100 mg) was hydrolyzed with 1 mL of 6 M hydrogen chloride at 110 °C for 24 h under a nitrogen atmosphere to obtain the total amino acids. The products were filtered and dried; derivatization reagent, DBCEC, and borate buffer (pH 8.5) were added.

Each derivatization was conducted at 45 °C for 10 min and then filtered using a 0.45 μm membrane filter prior to analysis. DBCEC-amino acid derivatives were analyzed by Agilent H1100 Series high-performance liquid chromatography (Agilent Technologies, Santa Clara, CA, USA). The derivatives were separated in an Eclipse XDB-C8 column (150 mm × 4.6 mm, 5 μm, Agilent) by gradient elution. Mobile phase A comprised 30% acetonitrile with 30 mM formic acid buffer (pH 3.7); B contained acetonitrile–water (50:50; v/v), and C contained acetonitrile–water (95:5, v/v). A constant flow rate was set at 1.0 mL/min and the column temperature was set at 30 °C. The fluorescence excitation (λex) and emission (λem) wavelengths were 300 and 390 nm, respectively.

The amino acid composition of each sample was determined by pre-column derivatization method (Sun et al., 2010). This procedure was conducted in triplicate. The correlation coefficients were >0.9991. The detection limits, counted with a signal-to-noise ratio (S/N) of 3, ranged from 12.85 fmol to 55.29 fmol (Table 1). The results showed that the amino acid recoveries (n = 5) ranged from 90.4% to 100.6%. This result indicated that the method could yield high recovery.

2.3. Fatty acid analysis

The fatty acids (C10–C22) used as standards were of chromatographic grade and purchased from Shanghai Chemical Reagent Co. (Shanghai, China); 2-(12-Oxobenzyl)[6]cycloindene-5(12H)-yl)

Table 1

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Regression equation</th>
<th>Correlation coefficients</th>
<th>Detection limit (fmol)</th>
<th>Migration time</th>
<th>Peak area RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intraday (3 replications)</td>
</tr>
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<td>Arg</td>
<td>Y = 78.96X - 67.88</td>
<td>0.9995</td>
<td>36.75</td>
<td>12.85</td>
<td>1.24</td>
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<tr>
<td>Asp</td>
<td>Y = 38.58X - 43.19</td>
<td>0.9994</td>
<td>29.26</td>
<td>15.25</td>
<td>0.48</td>
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<tr>
<td>Ser</td>
<td>Y = 57.71X - 11.64</td>
<td>0.9999</td>
<td>46.98</td>
<td>15.90</td>
<td>1.17</td>
</tr>
<tr>
<td>Glu</td>
<td>Y = 36.62X - 42.88</td>
<td>0.9994</td>
<td>23.08</td>
<td>16.79</td>
<td>1.21</td>
</tr>
<tr>
<td>Thr</td>
<td>Y = 59.90X + 1.55</td>
<td>0.9997</td>
<td>36.57</td>
<td>19.36</td>
<td>2.09</td>
</tr>
<tr>
<td>Gly</td>
<td>Y = 57.00X + 7.58</td>
<td>0.9998</td>
<td>20.89</td>
<td>21.28</td>
<td>1.58</td>
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<tr>
<td>Ala</td>
<td>Y = 66.82X + 7.32</td>
<td>0.9999</td>
<td>51.68</td>
<td>14.32</td>
<td>0.83</td>
</tr>
<tr>
<td>GABA</td>
<td>Y = 78.66X + 23.57</td>
<td>0.9994</td>
<td>18.61</td>
<td>25.43</td>
<td>0.59</td>
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<tr>
<td>Pro</td>
<td>Y = 62.05X + 9.29</td>
<td>0.9998</td>
<td>41.09</td>
<td>17.82</td>
<td>1.80</td>
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<tr>
<td>Met</td>
<td>Y = 97.66X + 11.03</td>
<td>0.9995</td>
<td>13.86</td>
<td>29.42</td>
<td>1.51</td>
</tr>
<tr>
<td>Val</td>
<td>Y = 77.58X + 15.38</td>
<td>0.9998</td>
<td>24.97</td>
<td>26.96</td>
<td>1.46</td>
</tr>
<tr>
<td>Trp</td>
<td>Y = 50.30X + 0.26</td>
<td>0.9999</td>
<td>15.72</td>
<td>38.21</td>
<td>1.87</td>
</tr>
<tr>
<td>Phe</td>
<td>Y = 83.02X + 6.98</td>
<td>0.9999</td>
<td>4.52</td>
<td>19.97</td>
<td>0.72</td>
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<tr>
<td>Ile</td>
<td>Y = 69.06X + 13.46</td>
<td>0.9998</td>
<td>21.33</td>
<td>40.88</td>
<td>1.21</td>
</tr>
<tr>
<td>Leu</td>
<td>Y = 73.62X + 14.08</td>
<td>0.9997</td>
<td>35.86</td>
<td>31.50</td>
<td>1.59</td>
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<tr>
<td>(Cys)_{3}</td>
<td>Y = 21.56X - 13.88</td>
<td>0.9991</td>
<td>43.94</td>
<td>48.95</td>
<td>1.87</td>
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<tr>
<td>His</td>
<td>Y = 40.72X - 22.83</td>
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<td>35.38</td>
<td>33.64</td>
<td>2.04</td>
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<tr>
<td>Orn</td>
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<td>0.9996</td>
<td>12.85</td>
<td>24.21</td>
<td>1.29</td>
</tr>
<tr>
<td>Lys</td>
<td>Y = 85.28X - 8.20</td>
<td>0.9997</td>
<td>51.32</td>
<td>55.29</td>
<td>1.83</td>
</tr>
<tr>
<td>Tyr</td>
<td>Y = 101.52X + 23.40</td>
<td>0.9996</td>
<td>43.51</td>
<td>50.53</td>
<td>2.10</td>
</tr>
</tbody>
</table>

Y: peak area; X: injected amount (pmol).
The ammonium injection under was formate neutralized at wavelengths of (Shanghai, and 50 (Millipore 28 C10 C22 C20 C17 C22:6 C18:3 purchased eluent area; fatty acids. For was mg equations, 80%; separated filtered the sample = 12.03 17.29 16.67 15.88 99.96 12.84 18.28 25.99 24.04 18.38 59.78 29.66.

The derivatized fatty acids ranged from 18.42 pmol to 32.39 pmol (Table 2). The results showed that the experimental recoveries (n = 5) of the free fatty acids and the total fatty acids ranged from 95.8% to 102.5% with the largest mean RSD (%) < 2.0% and from 93.7% to 103.3% with the largest mean RSD (%) < 28%, respectively.

2.4. Mineral analysis

For mineral analysis, 10 μg mL⁻¹ of an environmental calibration standard (Agilent), 1 μg mL⁻¹ of Rh, 1 μg mL⁻¹ of Re, 5% HNO₃ (Merck), and H₂O₂ (MOS) were used. The samples were digested in a Model CEM Mars 5 (CEM, Matthews, NC, USA) microwave oven. Each sample (1 g) was weighed precisely in a clean Teflon® digestion oven and wet oxidized in a microwave digestion system with 2 mL of H₂O₂ and 5 mL of ultrapure HNO₃. The operating program of the microwave digestion system used for the samples is shown in Table 3. One randomly selected vessel filled with reagents was utilized as a blank sample during the entire procedure. The sample solutions were cooled to room temperature and quantitatively transferred to 50 mL polyethylene flasks. The digestion vessels were cleaned with 5 mL of concentrated HNO₃ in the microwave vessel at 800 W for 15 min and then at 0 W for 10 min of cooling. The combined samples were diluted in ultrapure water until the final volumes were reached before these samples were subjected to inductively coupled-plasma mass spectrometry (ICP-MS) analysis. Individual mineral element was determined in the solution by using an Agilent 7500a ICP-MS (Agilent Technologies Co., Santa Clara, CA, USA) equipped with a Babington nebulizer, a Peltier cooled quartz spray chamber, and a standard torch (2.5 mm i.d.). Before each experiment, ICP-MS was tuned using an aqueous multi-element standard solution (Agilent, Las Rozas, Madrid, Spain) consisting of 10 ng mL⁻¹ each of Li, Y, Co, Ce, and others.
and Tl to obtain consistent sensitivity (7Li, 89Y, and 205Tl) as well as minimum doubly charged and oxide species levels (140Ce). The operating conditions are shown in Table 4. The elements in each sample were determined according to a previously described method (Wang et al., 2006). These procedures were performed in triplicate. The detection limits ranged from 0.002226 ng/g to 1.4300 ng/g. The results showed that element recoveries (n = 3) ranged from 82.0% to 112%. This result indicated that the method yielded high recovery.

2.5. Statistical analysis

The results were expressed as mean ± S.D. of triplicate data. Data were analyzed by one-way ANOVA. Significance level was set at p < 0.05. IBM SPSS Statistics 14.0 software was used for data analysis.

3. Results and discussion

3.1. Proximate composition

The proximate composition of the different parts of R. amabilis grown in the Qinghai-Tibetan Plateau is presented in Table 5. The crude protein contents of the leaves and fruits were 9.3% and 11.2%, respectively; the crude lipid contents were 1.6% and 2.9%, respectively.

3.2. Free amino acids

The composition and amount of free amino acids varied among the different parts (Table 4). Free amino acids, which exist naturally in food, determine the taste, flavor, and quality of various foods (Kabelova et al., 2008; Barylko-Pikielna and Kostyra, 2007; Sinesio et al., 2009). The most abundant essential and non-essential free amino acids in different parts of R. amabilis were Met and Cys or GABA. Fruits, leaves, stems, and roots contained 0.20, 2.66, 6.66, and 0.161 g/Met/100 g of sample, respectively. As the major non-essential amino acid, Cys content ranged from 0.17 g/100 g of sample to 0.21 g/100 g of sample. GABA, Ala, and Lys were more abundant in fruits than in other parts. GABA is an important compound in tea leaves and functions as a sedative that reduces excitability, thereby leading to restlessness, insomnia, and other disruptive conditions.

For the total amino acid contents (Table 4), Met exhibited the highest concentration with the highest mean content among the tested amino acids (Asp, Glu, Ser, His, Arg, Thr, Ala, Tyr, Val, and Lys). By contrast, other fruits and vegetables, such as Brazil nuts (1.008 g/100 g), oat (0.312 g/100 g), peanuts (0.309 g/100 g), corn (0.197 g/100 g), and beans (0.117 g/100 g), only contain a small amount of Met. Considering that Met has been successfully used to treat depression, inflammation, liver diseases, and muscle pains, we found that R. amabilis fruits and leaves can also be utilized as a feasible pharmaceutical source, in addition to food source.

Among the different plant parts used in this study, the roots and the leaves contained the highest essential free amino acid (EFAA) and essential total amino acid (ETAA). The fruits contained 0.27 g EFAA/100 g and 3.03 g ETAA/100 g. The stems, roots, and leaves contained 6.94, 0.90, and 0.03 g EFAs/100 g and 2.58, 2.49, and 9.31 g ETAs/100 g, respectively. The amount of free amino acids was also higher in the roots (8.75 g/100 g) than in the fruits (1.11 g/100 g) and stems (1.55 g/100 g). Total amino acid content (17.10 g/100 g) was the highest in the leaves followed by the total amino acid contents of the fruits (7.47 g/100 g), roots (6.37 g/100 g) and stems (5.70 g/100 g). Therefore, many amino acid components are mainly concentrated in R. amabilis leaves and fruits.
3.3. Fatty acids

Eighteen fatty acids, including saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs), were determined in different parts of R. amabilis (Table 6), and the characterized fatty acids in SFAs and UFAs were 16:0 and 18:2, respectively. The compositions of free fatty acids (FFAs) and total fatty acids (TFAs) are shown in Table 6. The percentages of unsaturated FFA were 5.0%, 25.0%, 34.1%, and 36.5% in the fruits, roots, stems, and leaves, respectively.

Although the fatty acid profiles varied in each proportion of TFAs, these profiles contained similarly high proportions of linoleic acid, palmitic acid, and stearic acid. The main TFAs components in stems and leaves were linoleic acid, linolenic acid, stearic acid, and palmitic acid. Linoleic acid and linolenic acid belong to the essential fatty acids that humans and animals are unable to synthesize and must be acquired from other food sources. Essential fatty acids also have a crucial function in the life and death of cardiac cells (Reiffl and Mcdonald, 2006; Herbaut, 2006).

Linoleic acid and linolenic acid, the major UFAs of plant lipids, significantly benefit human health. In the present study, R. amabilis contained stearic acid, palmitic acid, and linoleic acid. This finding provided information to elucidate the medical functions and nutritional properties of R. amabilis. R. amabilis could also be used as a potential supplement of essential fatty acids.

3.4. Minerals and trace elements

Heavy metal concentrations in plants should be determined and evaluated to promote nutritional and safety considerations. Essential mineral elements such as iron, copper, zinc, and manganese have critical functions in biological systems. Toxic elements, including mercury, lead, and cadmium, are also present in trace amounts. However, excessive concentrations of essential elements can also induce toxicity (Lekouch et al., 2001). The average contents of the elements in the different parts of R. amabilis are shown in Table 7. Macrolelement and trace element concentrations in the leaf samples were analyzed by ICP-MS, and all data were expressed as mg/g of dry basis; each sample of four different plant parts was determined in triplicate; LOD: 0.000002226–0.0014300 μg/g. Different letters in the same row refer to significant differences at p < 0.05 levels between different parts.
revealing the following trend: K > Ca > Mg > Fe > Al > Na > B > Mn > Zn > Hg > Cu > Pb > Ni > V > Mo > Cr > Se > Co > As. These elements were also detected in fruit samples according to the following order: K > Ca > Mg > Fe > Al > Na > Mn > Zn > B > Cu > Ni > Hg > V > Cr > Mo > Pb > Se > As > Co. In the stem samples, the following concentrations were observed: Ca > K > Mg > Na > Fe > Al > Zn > Mn > B > Cu > Cr > V > Ni > Mo > Pb > Hg > Co > As > Se. In the root samples, the following result was obtained: Ca > K > Mg > Fe > Al > Mn > Na > B > Zn > Cu > Ni > V > Cr > Mo > Pb > Co > Pb > Se > Hg. Considering these elements, researchers have identified the required concentrations of an individual’s daily intake of elements (mg/day) (Anke et al., 2002). The mean concentrations of Mg, As, and Pb analyzed in *R. amabilis* ranged below the maximum permissible levels.

Fig. 1 shows that K, Ca, and Mg are higher in the leaves than in the other parts of *R. amabilis*. In the stems, Na was higher than K, Ca, and Mg. Fe was the most abundant essential trace mineral element in different parts. The trace element distribution in the fruits is consistent with that in the leaves; the distribution in stems was similar to that in roots. Fruits and leafy vegetables contribute moderate trace elements because of their high water content. In the present study, *R. amabilis* contained various trace elements, such as Fe, Mn, B, Zn, Cu, Ni, V, Cr, Co, As, and S, in which Fe, Mn, Cu, Se, and Mo are essential minerals. Zn, Fe, Mn, and Cu are also necessary for the formation of antioxidants similar to those found in green tea. Therefore, *R. amabilis* contains several types of minerals compared with commonly consumed teas, such as green tea and red tea.

4. Conclusions

Fatty acids, amino acids and mineral elements were found in *R. amabilis* collected from the Qinghai-Tibetan Plateau. The relationship between the composition of *R. amabilis* and nutrients was investigated. Diverse amino acids, fatty acids, and mineral elements that are essential to human health were abundantly found in *R. amabilis*. Met, Cys, linoleic acid, linolenic acid, K, Na, Ca, and Mg were found in relatively high levels. High Fe content was found in all plant parts of *R. amabilis*. Considering these results, we found that *R. amabilis* fruits and leaves can contribute to some nutritional requirements in human diet.

Acknowledgement

This study was supported by the Research Fund for Self-Selected Topic of Beijing University of Chinese Medicine (Grant No. 2009JYBZZ-XS037) and the National Natural Science Foundation of China (Grant No. 81260684). The authors thank You Jin-Mao of Qufu Normal University for his support.

References


Fig. 1. Logarithmic diagram of elements in different parts of *R. amabilis*.