

**Exotic fruits as a source of important phytochemicals: Improving the traditional  
use of *Rosa canina* fruits in Portugal**

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**Short Communication**

## **ABSTRACT**

Several exotic fruits are used in folk medicine as potential sources of healthy compounds. *Rosa canina* L. (dog rose) fruits and other parts used to be widely consumed in rural areas from Portugal. The present work intends to highlight the presence of bioactive compounds in those different parts, in order to improve their use based on scientific studies. The antioxidant activity was screened through: radical scavenging effects, reducing power, and inhibition of lipid peroxidation in brain homogenates. Phytochemical characterization included determination of sugars by HPLC-RI, fatty acids by GC-FID, tocopherols by HPLC-fluorescence, phenolics, flavonoids, carotenoids, chlorophylls and ascorbic acid, by spectrophotometric techniques. Galls revealed the highest antioxidant potential, ripen hips showed the highest tocopherols and  $\beta$ -carotene contents, as also the most adequate n-6/n-3 fatty acids ratios. Unripe hips gave the highest levels of ascorbic acid and petals revealed the highest concentration of sugars. Ethnobotanical studies conducted have mentioned different use-reports for seeds, petals, flowers and galls, as well as for fruits in different stages of maturity and, therefore, the comparison between chemical compounds and antioxidant properties of those different parts is a key-point of the present study. Furthermore, the levels of antioxidants found would make them suitable sources of compounds to be used commercially to retard rancidity in fatty materials in food manufacturing, to reduce the effects of ageing and to help to prevent oxidative-stress related diseases such as cancer and heart disease.

**Keywords:** *Rosa canina*; Wild fruits; Phytochemicals; Antioxidant properties

## **1. Introduction**

In several Portuguese regions, traditional uses of wild fruits have become an interesting issue, despite a twenty-year period in which its consumption has decreased. Our research group has been interested in the chemical characterization of wild fruits traditionally consumed in Portugal by their medicinal and edible properties. Fruits of roses (*Rosa* sp. pl.), strawberry-tree (*Arbutus unedo* L.), blackthorn (*Prunus spinosa* L.) and hawthorn (*Crataegus monogyna* Jacq.) are empirically recognized as a source of healthy compounds, as some ethnobotanical surveys have reported (Carvalho, 2010; Carvalho & Morales, 2010). Recent phytochemical studies performed by us revealed that these species are important sources of antioxidants for food, pharmaceutical, or cosmetic applications (Barros, Carvalho, & Ferreira, 2010; Barros, Carvalho, Morais, & Ferreira, 2010; Guimarães, Barros, Carvalho, & Ferreira, 2010).

Dog rose (*Rosa canina* L.) fruits are very popular and its use is increasing due to their prophylactic and therapeutic activities against a wide range of ailments (Rein, Kharazmi, & Winther, 2004; Orhan, Hartevioğlu, Küpeli, & Yesilalada, 2007; Kharazmi, 2008). Despite the high popularity of these wild fruits in Portugal, data regarding a complete nutritional and phytochemical characterization are missing. Furthermore, the present work highlights the antioxidant potential and phytochemical composition of *Rosa canina* fruits in different stages of maturity, comparing the bioactivity results with other parts also having folk uses.

## **2. Materials and methods**

### *2.1. Samples*

Previous research focused on over ripened fruits of dog rose ([Barros et al., 2010a](#)). Herein immature and mature fruits, and their seeds, as well as fertilized flowers and petals removed from flower buds were analysed. The samples were collected in sequence, during 2009 spring, summer, and autumn, synchronized with the species' development of flowers and fruits, according to Portuguese traditional uses.

## *2.2. Antioxidant activity assays*

A fine freeze-dried powder (20 mesh; ~1g) was extracted by stirring with 30 mL of methanol at 25 °C at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with one additional 30 mL portion of methanol. The combined methanolic extracts were evaporated at 35 °C under reduced pressure, re-dissolved in methanol at a concentration of 5 mg/mL, and stored at 4 °C for further use.

Total phenolics and flavonoids were estimated based on procedures described by the authors ([Guimarães et al., 2010](#)). Gallic acid (0.05-0.8 mM) and (+)-catechin (0.0156-1.0 mM) were used to calculate the standard curves. The results were expressed as mg of gallic acid equivalents (GAE) and mg of (+)-catechin equivalents (CE), respectively for phenolics and flavonoids, per g of extract.

The antioxidant activity was evaluated by DPPH radical-scavenging activity, reducing power, inhibition of  $\beta$ -carotene bleaching in the presence of linoleic acid radicals and inhibition of lipid peroxidation using TBARS in brain homogenates ([Guimarães et al., 2010](#)). The extract concentrations providing 50% of antioxidant activity ( $EC_{50}$ ) were calculated from the graphs of antioxidant activity percentages against extract concentrations (for each assay). Trolox was used as standard.

### 2.3. Composition in phytochemicals

Tocopherols content was determined by HPLC/fluorescence following a procedure previously described by the authors (Barros et al., 2010b), using tocol as internal standard. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method. Tocopherol contents in the samples are expressed in mg per 100 g of dry sample.

Ascorbic acid was determined according to Barros et al. (2010b). Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.006-0.1 mg/mL), and the results were expressed as mg per 100 g of dry sample.

Liposoluble pigments were determined according to Barros et al. (2010b). Contents of liposoluble pigments were calculated according to the following equations: lycopene (mg/100 mL) =  $-0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453}$ ;  $\beta$ -carotene (mg/100 mL) =  $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$ ; Chlorophyll a (mg/100 mL) =  $0.999 \times A_{663} - 0.0989 \times A_{645}$ ; Chlorophyll b (mg/100 mL) =  $-0.328 \times A_{663} + 1.77 \times A_{645}$ . The results were expressed as mg per 100 g of dry weight.

Free sugars were determined by HPLC/RI as described by Barros et al. (2010b), using melezitose as internal standard. The results are expressed in g/100 g of dry weight, calculated by internal normalization of the chromatographic peak area. Sugar identification was made by comparing the relative retention times of sample peaks with standards.

Fatty acids were determined by GC/FID as described previously by the authors (Barros et al., 2010b). Fatty acid identification was made by comparing the relative retention

times of FAME peaks from samples with standards. The results were expressed in relative percentage of each fatty acid.

#### 2.4. Statistical analysis

For each part, three samples were analysed and the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (SD), and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with  $\alpha = 0.05$  (SPSS v. 16.0 program).

### 3. Results and discussion

The chemical composition and antioxidant properties of *Rosa canina* fruits were compared with other wild fruits also traditionally consumed in Portugal such as those from *Rosa micrantha*, *Arbutus unedo*, *Prunus spinosa* and *Crataegus monogyna*. *Rosa canina* and *Rosa micrantha* are the most widely used in Trás-os-Montes region (Portugal). Nevertheless, ethnobotanical surveys have documented that *Rosa canina* has more general use, as it is easily recognized by everybody, instead of *Rosa micrantha* that is recommended mostly by healers who are able to distinguish the two species. Furthermore, these surveys mentioned different use-reports for seeds, petals, flowers and galls, as well as for fruits in different stages of maturity (Carvalho, 2010) and, therefore, the comparison between chemical compounds and antioxidant properties of those different parts is also a key-point of the present work.

Four different assays were used for the evaluation of the antioxidant properties of *Rosa canina* hips, seeds, petals, flowers and galls. The results of scavenging activity on DPPH radicals, reducing power, inhibition of  $\beta$ -carotene bleaching, and inhibition of

lipid peroxidation in brain tissue homogenates, and also phenolic and flavonoids contents are shown in **Table 1**. Galls proved to have the most promissory antioxidant activity (the lowest EC<sub>50</sub> values, ranging from 0.02 to 0.08 mg/mL), with the highest phenolic (495.89 mg GAE/g extract) and flavonoids (22.81 mg CE/g extract; **Table 1**) contents which is according with its traditional use for gastrointestinal disorders and inflammatory processes. Moreover, EC<sub>50</sub> values obtained in radical scavenging activity and reducing power of this sample had the same magnitude of the results obtained for the standard trolox (0.04 and 0.03 mg/mL, respectively). Petals and hips also showed antioxidant properties, and seeds proved to have the lowest potential. Dog rose ripened hips revealed better lipid peroxidation inhibition properties than overripe hips, also studied by us in a previous report ([Barros et al., 2010a](#)). Furthermore, they proved to have higher antioxidant potential than strawberry-tree and blackthorn fruits. Dog rose (*Rosa canina*) unripe hips showed higher antioxidant activity than *Rosa micrantha* Borrer ex Sm fruits in the same stage of maturity ([Guimarães et al., 2010](#)).

Despite some studies report correlations between phenolics and flavonoids contents and antioxidant capacity of vegetables ([Faller & Fialho, 2009](#)) and other matrixes ([Quirós, Lage-Yusty, & López-Hernández, 2009](#)), herein those correlations were not observed. Therefore, it can be concluded that other antioxidants, such as reducing sugars or ascorbic acid, might be present in the methanolic extracts being also responsible for the antioxidant activity observed in *Rosa canina* samples.

In fact, the studied samples revealed to be a source of important phytochemicals, mostly antioxidants such as tocopherols, ascorbic acid, carotenoids and sugars (**Table 2**).

$\alpha$ -Tocopherol was the major compound in all the samples, and  $\gamma$ - and  $\delta$ -tocopherols were not detected in ripened hips seeds. Ripened hips presented the highest content of

tocopherols (79.73 mg/100 g of dry weight), with the highest levels of  $\alpha$ - and  $\gamma$ -tocopherols. Dog rose ripened hips revealed higher tocopherols content than its overripe hips (8.33 mg/100 g; [Barros et al., 2010a](#)), and also than strawberry-tree (23.46 mg/100 g), blackthorn (9.25 mg/100 g) and *Rosa micrantha* (19.64 mg/100 g; [Guimarães et al., 2010](#)) fruits.

Ascorbic acid was the most abundant vitamin in all the studied parts. Unripe and ripened hips were the parts with highest levels of ascorbic acid (262.09 mg/100 g and 213.83 mg/100 g, respectively). Unripe hips showed higher concentration than the same part of hawthorn (130.33 mg/100 g; [Barros et al., 2010b](#)). Dog rose ripened hips revealed higher levels than its overripe hips (68.04 mg/100 g), and than strawberry-tree (15.07 mg/100 g) and blackthorn (15.69 mg/100 g) fruits ([Barros et al., 2010a](#)).

Unripe hips gave the highest concentration of  $\beta$ -carotene (97.77 mg/100 g dry weight), while petals, that did not present  $\beta$ -carotene, gave the highest concentration of lycopene and chlorophylls. Dog rose hips proved to have higher  $\beta$ -carotene contents than hawthorn ([Barros et al., 2010b](#)) and *Rosa micrantha* ([Guimarães et al., 2010](#)) hips, and than strawberry-tree and blackthorn fruits ([Barros et al., 2010a](#)).

Petals revealed the highest total sugars content (29.32 g/100 g dry weight), with the highest levels of fructose (14.70 g/100 g), glucose (11.82 g/100 g) and trehalose (1.17 g/100g). This is in agreement with the results obtained for hawthorn whose petals also revealed highest levels than unripe and ripened hips ([Barros et al., 2010b](#)). Otherwise, ripened hips showed the highest levels of sucrose (3.77 g/100 g) which is certainly related to their sweet taste. In general, dog rose revealed higher sugars content than *R. micrantha* ([Guimarães et al., 2010](#)), and unripe hips showed highest sugars levels than the same part of hawthorn ([Barros et al., 2010b](#)).

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and the ratios of PUFA/SFA and n-6/n-3 of the studied parts are shown in **Table 3**. Twenty three fatty acids were identified and quantified. The major fatty acids found were linoleic acid (C18:2n6) and  $\alpha$ -linolenic acid (C18:3n3) and contributing to the prevalence of PUFA. Oleic acid (C18:1n9) was a main fatty acid in galls, while palmitic acid was also present in considerable amounts in petals and ripened hips. Unripe hips showed the highest PUFA contents (78.97%), as also the highest ratio of PUFA/SFA (8.20). All the parts presented n-6/n-3 fatty acids ratios lower than 4.0, as desirable (Kanu et al., 2007).

Accumulating chemical, biochemical, clinical and epidemiologic evidence supports the chemoprotective effects of phenolic antioxidants against oxidative stress-mediated disorders. The pharmacological actions of phenolic antioxidants stem mainly from their free radical scavenging and metal chelating properties as well as their effects on cell signalling pathways and on gene expression (Sobratee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005). In fact, besides their hydrogen-donating capacity they may exert modulatory actions in cells through actions at protein kinase and lipid kinase signalling pathways (Williams, Spencer, & Rice-Evans, 2004). Lipid-flavonoid and protein-flavonoid interactions can directly mediate a decrease in oxidant (free radical) production and/or oxidative damage to both cell and extracellular components (Galleano, Verstraeten, Oteiza, & Fraga, 2010). In the lipid peroxidation process, carotenoids and tocopherols act as chain-breaking antioxidants by donating a hydrogen atom and therefore, scavenging free radicals. Ascorbic acid reacts with tocopherols

radicals regenerating tocopherols molecules (Ferreira, Barros, & Abreu, 2009). All the mentioned antioxidants could contribute to the antioxidant activity of the samples, since the assays used in the present work measured mainly free radical capacity and inhibition of lipid peroxidation.

Overall, dog rose galls revealed the highest antioxidant potential, ripen hips showed the highest tocopherols and  $\beta$ -carotene contents, as also the most adequate n-6/n-3 fatty acids ratios. Unripe hips gave the highest levels of ascorbic acid and petals revealed the highest concentration of sugars.

Synthetic antioxidants are being questioned while natural antioxidants such as tocopherols, polyphenols and carotenoid pigments are having a greater relevance in the protection against lipid oxidation. Therefore, the levels of vitamins C and E, and  $\beta$ -carotene found in *Rosa canina* would make it a suitable source of these antioxidants that might be used commercially to retard rancidity in fatty materials in food manufacturing, to reduce the effects of ageing and to help to prevent oxidative-stress related diseases such as cancer and heart disease (Dewick, 2002). Furthermore, it is urgent to recover/improve the use of exotic species such as wild fruits and plants traditionally consumed by our ancestors, but with a scientific support based on phytochemical characterization of these matrixes.

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**Table 1.** Antioxidant Activity (EC<sub>50</sub> values) and composition in phenolics and flavonoids of *Rosa canina* (Mean ± SD; n=9). In each row, different letters mean significant differences ( $p < 0.05$ ).

|   | Unripe hips     | Unripe hips seeds | Ripened hips    | Ripened hips seeds | Petals           | Fertilized flowers | Galls            |
|---|-----------------|-------------------|-----------------|--------------------|------------------|--------------------|------------------|
| Extraction yields (%)                   | 37.90 ± 1.22    | 16.18 ± 1.02      | 43.19 ± 2.34    | 13.85 ± 0.99       | 40.38 ± 2.63     | 30.90 ± 1.85       | 29.98 ± 1.56     |
| DPPH scavenging activity (mg/mL)        | 0.49 ± 0.02 e   | 1.10 ± 0.07 b     | 0.75 ± 0.05 f   | 3.93 ± 0.10 a      | 0.22 ± 0.01 c    | 0.64 ± 0.07 d      | 0.08 ± 0.00 g    |
| Reducing power (mg/mL)                  | 0.23 ± 0.00 c   | 0.92 ± 0.04 b     | 0.18 ± 0.03 d   | 1.19 ± 0.05 a      | 0.24 ± 0.03 c    | 0.13 ± 0.01 e      | 0.06 ± 0.00 f    |
| β-carotene bleaching inhibition (mg/mL) | 0.12 ± 0.00 dc  | 0.83 ± 0.13 b     | 0.10 ± 0.00 dc  | 1.47 ± 0.05 a      | 0.12 ± 0.03 dc   | 0.15 ± 0.00 c      | 0.04 ± 0.00 d    |
| TBARS inhibition (mg/mL)                | 0.06 ± 0.00 d   | 0.14 ± 0.01 b     | 0.03 ± 0.00 e   | 0.34 ± 0.01 a      | 0.03 ± 0.00 e    | 0.10 ± 0.01 c      | 0.02 ± 0.00 e    |
| Phenolics (mg GAE/g extract)            | 104.73 ± 1.28 d | 63.76 ± 2.60 e    | 149.35 ± 6.64 c | 23.54 ± 0.32 f     | 270.28 ± 35.54 b | 123.93 ± 2.19 d    | 495.89 ± 19.18 a |
| Flavonoids (mg CE/g extract)            | 7.42 ± 0.14 d   | 7.78 ± 1.23 d     | 9.80 ± 0.21 c   | 2.12 ± 0.35 e      | 18.41 ± 1.19 b   | 6.88 ± 1.53 d      | 22.81 ± 1.35 a   |

EC<sub>50</sub> values for the standard trolox: 43 µg/mL (DPPH scavenging activity); 30 µg/mL (Reducing power); 3 µg/mL (β-carotene bleaching inhibition) and 4 µg/mL (TBARS inhibition).

**Table 2.** Composition (dry weight basis) in vitamins, liposoluble pigments and sugars of *Rosa canina* (Mean  $\pm$  SD; n=9). In each row, different letters mean significant differences ( $p < 0.05$ ).

| Compounds                    | Unripe hips         | Unripe hips seeds    | Ripened hips        | Ripened hips seeds  | Petals             | Fertilized flowers  | Galls               |
|------------------------------|---------------------|----------------------|---------------------|---------------------|--------------------|---------------------|---------------------|
| $\alpha$ -tocopherol         | 9.93 $\pm$ 0.05 b   | 1.52 $\pm$ 0.17 d    | 52.13 $\pm$ 4.22 a  | 1.55 $\pm$ 0.06 d   | 11.28 $\pm$ 0.24 b | 7.60 $\pm$ 0.28 cb  | 4.67 $\pm$ 0.04 cd  |
| $\beta$ -tocopherol          | 0.14 $\pm$ 0.01 c   | 0.11 $\pm$ 0.02 c    | 0.19 $\pm$ 0.07 cb  | 0.15 $\pm$ 0.01 c   | 0.30 $\pm$ 0.08 b  | 0.09 $\pm$ 0.01 c   | 0.43 $\pm$ 0.05 a   |
| $\gamma$ -tocopherol         | 8.30 $\pm$ 0.08 b   | 3.47 $\pm$ 0.08 c    | 27.19 $\pm$ 0.38 a  | nd                  | 0.92 $\pm$ 0.10 e  | 2.53 $\pm$ 0.11 d   | 0.36 $\pm$ 0.01 f   |
| $\delta$ -tocopherol         | 0.43 $\pm$ 0.01 b   | 0.20 $\pm$ 0.00 b    | 0.21 $\pm$ 0.02 b   | nd                  | 1.31 $\pm$ 0.25 a  | 0.26 $\pm$ 0.02 b   | 0.08 $\pm$ 0.01 b   |
| Total tocopherols (mg/100 g) | 18.79 $\pm$ 0.06 b  | 5.29 $\pm$ 0.27 d    | 79.73 $\pm$ 3.74 a  | 1.70 $\pm$ 0.05d    | 13.80 $\pm$ 0.03 c | 10.48 $\pm$ 0.16 c  | 5.54 $\pm$ 0.11 d   |
| Ascorbic acid (mg/100 g)     | 262.09 $\pm$ 1.87 a | 134.53 $\pm$ 18.58 d | 213.83 $\pm$ 9.49 b | 105.95 $\pm$ 1.96 e | 72.18 $\pm$ 6.07 f | 187.65 $\pm$ 3.66 c | 106.15 $\pm$ 2.28 e |
| $\beta$ -carotene (mg/100 g) | 25.88 $\pm$ 0.05 c  | 1.19 $\pm$ 0.02 f    | 97.77 $\pm$ 0.04 a  | 3.67 $\pm$ 0.00 d   | nd                 | 37.57 $\pm$ 0.05 b  | 2.67 $\pm$ 0.07 e   |
| Lycopene (mg/100 g)          | 0.02 $\pm$ 0.00 c   | 0.02 $\pm$ 0.00 c    | 0.41 $\pm$ 0.00 b   | 0.02 $\pm$ 0.00 c   | 8.72 $\pm$ 0.42 a  | nd                  | 0.09 $\pm$ 0.00 c   |
| Chlorophyll a (mg/100 g)     | 0.59 $\pm$ 0.00 c   | 0.11 $\pm$ 0.00 ed   | 0.16 $\pm$ 0.00 ed  | 0.01 $\pm$ 0.00 e   | 16.94 $\pm$ 0.49 a | 0.96 $\pm$ 0.00 b   | 0.33 $\pm$ 0.00 d   |
| Chlorophyll b (mg/100 g)     | 0.20 $\pm$ 0.00 b   | 0.08 $\pm$ 0.00 b    | 0.22 $\pm$ 0.00 b   | 0.01 $\pm$ 0.00 b   | 24.01 $\pm$ 1.02 a | 0.36 $\pm$ 0.00 b   | 0.26 $\pm$ 0.00 b   |
| Fructose                     | 2.14 $\pm$ 0.07 c   | 0.40 $\pm$ 0.02 d    | 8.89 $\pm$ 0.06 b   | 0.97 $\pm$ 0.10 d   | 14.70 $\pm$ 0.55 a | 1.63 $\pm$ 0.00 c   | 0.60 $\pm$ 0.00 d   |
| Glucose                      | 1.17 $\pm$ 0.04 c   | 0.33 $\pm$ 0.03 f    | 7.46 $\pm$ 0.02 b   | 0.92 $\pm$ 0.07 de  | 11.82 $\pm$ 0.49 a | 1.36 $\pm$ 0.00 dc  | 0.67 $\pm$ 0.01 fe  |
| Sucrose                      | 1.18 $\pm$ 0.02 b   | 0.53 $\pm$ 0.01 c    | 3.77 $\pm$ 0.32 a   | 0.62 $\pm$ 0.00 c   | 1.47 $\pm$ 0.09 b  | 1.31 $\pm$ 0.00 b   | 0.14 $\pm$ 0.04 d   |
| Trehalose                    | 0.94 $\pm$ 0.02 b   | 0.20 $\pm$ 0.00 e    | 0.34 $\pm$ 0.00 d   | 0.04 $\pm$ 0.01 f   | 1.17 $\pm$ 0.05 a  | 0.74 $\pm$ 0.00 c   | 0.21 $\pm$ 0.02 e   |
| Raffinose                    | 1.28 $\pm$ 0.03 a   | 0.46 $\pm$ 0.05 bac  | nd                  | nd                  | 0.15 $\pm$ 0.01 bc | 1.26 $\pm$ 0.00 ba  | nd                  |
| Total sugars (g/100 g)       | 7.25 $\pm$ 0.11 c   | 1.93 $\pm$ 0.02 d    | 20.46 $\pm$ 0.24 b  | 2.55 $\pm$ 0.17 d   | 29.32 $\pm$ 1.20 a | 6.30 $\pm$ 0.01 c   | 1.63 $\pm$ 0.07 d   |

nd- not detected.

**Table 3.** Composition in fatty acids (percentage) of *Rosa canina* (Mean  $\pm$  SD; n=9). In each row, different letters mean significant differences ( $p<0.05$ ).

|          | Unripe hips      | Unripe hips seeds | Ripened hips     | Ripened hips seeds | Petals           | Fertilized flowers | Galls            |
|----------|------------------|-------------------|------------------|--------------------|------------------|--------------------|------------------|
| C6:0     | 0.02 $\pm$ 0.00  | 0.03 $\pm$ 0.00   | 0.09 $\pm$ 0.01  | 0.19 $\pm$ 0.05    | 0.25 $\pm$ 0.01  | 0.14 $\pm$ 0.01    | 0.26 $\pm$ 0.07  |
| C8:0     | 0.10 $\pm$ 0.00  | 0.02 $\pm$ 0.00   | 0.50 $\pm$ 0.06  | 0.42 $\pm$ 0.02    | 0.42 $\pm$ 0.04  | 0.26 $\pm$ 0.03    | 0.18 $\pm$ 0.04  |
| C10:0    | 0.08 $\pm$ 0.00  | 0.02 $\pm$ 0.00   | 0.70 $\pm$ 0.04  | 0.32 $\pm$ 0.02    | 0.33 $\pm$ 0.04  | 0.18 $\pm$ 0.03    | 0.12 $\pm$ 0.00  |
| C12:0    | 0.53 $\pm$ 0.02  | 0.07 $\pm$ 0.01   | 5.43 $\pm$ 0.06  | 0.63 $\pm$ 0.06    | 0.95 $\pm$ 0.02  | 0.75 $\pm$ 0.06    | 0.39 $\pm$ 0.04  |
| C14:0    | 0.40 $\pm$ 0.00  | 0.11 $\pm$ 0.01   | 2.44 $\pm$ 0.14  | 0.84 $\pm$ 0.12    | 1.30 $\pm$ 0.06  | 0.85 $\pm$ 0.05    | 1.76 $\pm$ 0.02  |
| C14:1    | 0.08 $\pm$ 0.01  | 0.01 $\pm$ 0.00   | 0.11 $\pm$ 0.01  | 0.05 $\pm$ 0.01    | 0.04 $\pm$ 0.00  | 0.15 $\pm$ 0.02    | 0.04 $\pm$ 0.00  |
| C15:0    | 0.15 $\pm$ 0.00  | 0.08 $\pm$ 0.02   | 0.48 $\pm$ 0.04  | 0.37 $\pm$ 0.01    | 0.55 $\pm$ 0.05  | 0.50 $\pm$ 0.03    | 0.24 $\pm$ 0.03  |
| C15:1    | nd               | nd                | nd               | nd                 | nd               | 0.65 $\pm$ 0.09    | nd               |
| C16:0    | 7.50 $\pm$ 0.11  | 5.34 $\pm$ 0.15   | 17.05 $\pm$ 0.96 | 10.13 $\pm$ 0.46   | 18.16 $\pm$ 0.07 | 12.86 $\pm$ 0.74   | 10.25 $\pm$ 1.86 |
| C16:1    | 0.12 $\pm$ 0.00  | 0.05 $\pm$ 0.00   | 3.55 $\pm$ 0.50  | 0.39 $\pm$ 0.02    | 0.08 $\pm$ 0.00  | 0.11 $\pm$ 0.01    | 0.21 $\pm$ 0.06  |
| C17:0    | 0.30 $\pm$ 0.02  | 0.13 $\pm$ 0.01   | 0.63 $\pm$ 0.00  | 0.62 $\pm$ 0.01    | 0.70 $\pm$ 0.01  | 0.65 $\pm$ 0.06    | 2.40 $\pm$ 0.45  |
| C17:1    | 0.02 $\pm$ 0.00  | nd                | 0.02 $\pm$ 0.00  | nd                 | 0.01 $\pm$ 0.00  | 0.01 $\pm$ 0.00    | 0.33 $\pm$ 0.06  |
| C18:0    | 3.23 $\pm$ 0.23  | 1.92 $\pm$ 0.03   | 5.68 $\pm$ 0.12  | 4.64 $\pm$ 1.16    | 13.84 $\pm$ 0.05 | 5.16 $\pm$ 0.45    | 6.44 $\pm$ 0.99  |
| C18:1n9c | 8.37 $\pm$ 0.40  | 10.89 $\pm$ 0.14  | 11.82 $\pm$ 0.86 | 18.96 $\pm$ 2.78   | 6.35 $\pm$ 0.32  | 6.93 $\pm$ 0.51    | 32.77 $\pm$ 1.08 |
| C18:2n6c | 43.91 $\pm$ 0.61 | 54.27 $\pm$ 0.09  | 15.91 $\pm$ 1.42 | 43.48 $\pm$ 0.21   | 27.53 $\pm$ 0.15 | 34.31 $\pm$ 1.59   | 23.80 $\pm$ 0.97 |
| C18:3n6  | nd               | nd                | 0.63 $\pm$ 0.02  | nd                 | nd               | nd                 | nd               |
| C18:3n3  | 30.41 $\pm$ 0.17 | 24.41 $\pm$ 0.24  | 26.92 $\pm$ 0.68 | 14.44 $\pm$ 1.67   | 18.55 $\pm$ 0.47 | 28.01 $\pm$ 0.59   | 17.77 $\pm$ 1.74 |
| C20:0    | 1.70 $\pm$ 0.04  | 1.39 $\pm$ 0.03   | 1.38 $\pm$ 0.03  | 1.41 $\pm$ 0.39    | 3.60 $\pm$ 0.09  | 2.83 $\pm$ 0.17    | 1.13 $\pm$ 0.06  |
| C20:1    | 0.34 $\pm$ 0.01  | 0.44 $\pm$ 0.00   | 0.17 $\pm$ 0.04  | 0.30 $\pm$ 0.03    | 0.66 $\pm$ 0.03  | 0.13 $\pm$ 0.01    | 0.10 $\pm$ 0.00  |

|               |                |                |                 |                |                |                |                |
|---------------|----------------|----------------|-----------------|----------------|----------------|----------------|----------------|
| C20:2         | 0.26 ± 0.01    | 0.20 ± 0.00    | 0.88 ± 0.08     | 0.09 ± 0.01    | 0.17 ± 0.03    | 0.24 ± 0.02    | 0.05 ± 0.00    |
| C20:3n3+C21:0 | 0.16 ± 0.02    | 0.09 ± 0.01    | 0.23 ± 0.08     | 0.30 ± 0.03    | 0.54 ± 0.05    | 0.31 ± 0.04    | 0.11 ± 0.02    |
| C22:0         | 1.13 ± 0.00    | 0.35 ± 0.01    | 1.84 ± 0.17     | 1.25 ± 0.29    | 2.71 ± 0.30    | 2.80 ± 0.31    | 0.87 ± 0.16    |
| C24:0         | 1.21 ± 0.03    | 0.20 ± 0.03    | 3.55 ± 0.30     | 1.18 ± 0.14    | 3.27 ± 0.11    | 2.15 ± 0.23    | 0.77 ± 0.17    |
| Total SFA     | 16.34 ± 0.40 e | 9.64 ± 0.27 f  | 39.76 ± 0.96 b  | 21.99 ± 0.79 d | 46.07 ± 0.27 a | 29.14 ± 2.40 c | 24.82 ± 2.78 d |
| Total MUFA    | 8.93 ± 0.38 ed | 11.39 ± 0.14 d | 15.67 ± 1.33 c  | 19.71 ± 2.79 b | 7.14 ± 0.33 e  | 7.34 ± 0.48 e  | 33.45 ± 0.99 a |
| Total PUFA    | 74.74 ± 0.78 b | 78.97 ± 0.13 a | 44.58 ± 2.28 fe | 58.31 ± 2.02 d | 46.79 ± 0.61 e | 62.88 ± 2.03 c | 41.73 ± 1.79 f |
| PUFA/SFA      | 4.58 ± 0.16 b  | 8.20 ± 0.24 a  | 1.12 ± 0.08 f   | 2.65 ± 0.01 c  | 1.02 ± 0.02 f  | 2.17 ± 0.25 d  | 1.70 ± 0.26 e  |
| n-6/n-3       | 1.44 ± 0.01 c  | 2.22 ± 0.00 b  | 0.61 ± 0.04 d   | 2.97 ± 0.35 a  | 1.44 ± 0.02 c  | 1.21 ± 0.04 c  | 1.34 ± 0.13 c  |
| Total fat     | 1.55 ± 0.15    | 0.79 ± 0.02    | 0.67 ± 0.01     | 0.34 ± 0.05    | 1.16 ± 0.12    | 0.71 ± 0.09    | 1.60 ± 0.13    |

nd- not detected.