Original Articles

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *CHAENOMELES MAULEI* FRUIT JUICE

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Summary

Chaenomeles maulei is the name of the cultivar of *Chaenomeles japonica*, which was introduced in Europe. The present study aimed to investigate the chemical composition and the antioxidant activity of *Chaenomeles maulei* fruit juice (CMFJ). The ingredients of the juice sample were measured by spectrophotometric, high-performance liquid chromatography (HPLC) and gravimetric methods. The antioxidant activity was measured by the oxygen radical absorbance capacity (ORAC) and hydroxyl radical averting capacity (HORAC) assays. The results showed that CMFJ was extremely rich in polyphenolic substances, amongst which the highest was the concentration of procyanidin oligomers, followed by phenolic acids (vanillic > caffeic > chlorogenic > neochlorogenic > p-coumaric > ellagic > ferulic > 4-dihydroxy-benzoic) and flavonoids (epicatechin > catechin > quercetin-3-β-glucoside > quercetin > rutin > naringin > kaempferol > myricetin). The sour taste of the juice and its low pH were due to the high content of organic acids (malic > quinic > citric > shikimic > ascorbic > oxalic). The carbohydrates were presented by glucose > fructose > galactose > xylose > rhamnose > arabinose. The antioxidant activity measured by ORAC and HORAC was very high. It could be attributed to the high content of polyphenolics in the juice.

Key words: Chaenomeles maulei fruit juice, chemical composition, polyphenolic substances, organic acids, antioxidant activity

Introduction

The genus *Chaenomeles* belongs to the *Rosaceae* family and comprises four species: *C. cathayensis, C. japonica, C. speciosa* and *C. thibetica* [1]. *C. japonica*, known as Maule's quince or Japanese quince, was introduced to Europe in 1869 by Messrs Maule, nursery workers in Bristol, England. *Chaenomeles maulei* is the name of the cultivar introduced by the Maules in Europe, and the full scientific name is *Chaenomeles japonica* (Thunb.) Lindl [2].

Chaenomeles maulei is a dwarf shrub (0.6–1.2 m). Because of the beautiful flowers, it has long been appreciated for its ornamental value. Due to the high yield of fruits, *Chaenomeles maulei* is currently being developed as a fruit crop in Europe with importance for

food industry [3]. The valuable properties of the fruits are the distinctive aroma, the high contents of organic acids, vitamin C, phenolic compounds and dietary fiber [3-5]. Quince fruits are used for the production of juice, syrups, liqueurs, salad dressings, carbonated soft drinks, puree and jam in China [6], the Baltic States [7] and some other countries including Bulgaria.

In recent years, the scientific interest to *Chaenomeles* species has been increasing. Experimental studies have investigated the beneficial effects of the plant. The fruits have been shown to possess antioxidant, antimicrobial, anti-inflammatory, cytotoxic and anti-metastatic activities [6, 8-10].

The study aimed to investigate the chemical composition and in vitro antioxidant activity of *Chaenomeles maulei* fruit juice (CMFJ).

Materials and Methods

Chaenomeles maulei fruit juice (CMFJ)

Chaenomeles maulei was grown in the Balkan Mountains, Bulgaria, in the region of Troyan. After handpicking, the fresh fruits were ground, crushed and squeezed. The juice was filtered, preserved with potassium sorbate (1.0 g/l) and stored at 0 °C till the analyses.

Determination of total phenols

The spectrophotometric Folin-Ciocalteu assay was used to determine the total content of phenolic compounds [11]. Absorbance was read at 760 nm. Gallic acid was used as a standard. The results are presented as mg gallic acid equivalents per liter of juice (mg GAE/l).

Total proanthocyanidin content determination

Total proanthocyanidin content was determined by the method of Sarneckis et al. (2006) [12]. Methyl cellulose solution (0.04%, 1 ml) was added to properly diluted juice (0.5 ml) and the mixture was stirred several times. Saturated ammonium sulfate solution (1 ml) was added to this mixture and the total volume was made up to 5 ml with deionized water. The solution was allowed to stand for 10 min at room temperature and then centrifuged for 5 min at 4000 x *g*. The absorbance of the solution was recorded at 280 nm. Total proanthocyanidin content was calculated from a calibration curve with catechin solutions and was expressed as catechin equivalent per liter of juice (mg CE/l).

HPLC determination of procyanidin oligomers, phenolic acids, and flavonoids

High-performance liquid chromatography (HPLC) analyses were performed using an Agilent 1220 HPLC system (Agilent Technology, Palo Alto, Ca), equipped with a binary pump and UV-Vis detector. A wavelength of 280 nm was used. Phenolics separation was performed using Agilent TC-C18 column (5 мm, 4.6 mm x 250 mm) at 25° C. Mobile phases constituted of 0.5% acetic acid (A) and 100% acetonitrile (B) at a flow rate of 0.8 ml/min. The gradient condition started with 14% B, between 6 min and 30 min linearly increased to 25% B, then to 50% B at 40 min.

HPLC determination of organic acids

HPLC determination of organic acids was performed using the same HPLC system. A wavelength of 210 nm was used. Organic acids separation was achieved using Agilent TC-C18 column (5 μm, 4.6 mm x 250 mm) at 25 ºC. The mobile phase was 25 mM phosphate (K_2HPO_4/H_3PO_4) buffer (pH 2.4) flowing at 0.8 ml/min.

HPLC analysis of carbohydrates

Samples were centrifuged (6 000 x *g)* and the supernatants were used for HPLC analysis of sugars. HPLC determination was performed on Waters 484 system, connected to a refractometric Waters R401 detector and Aminex HPX – 87H column (300 x 7.8 mm, BioRad). The eluent was 0.004 mol/l sulfuric acid at a flow rate of 0.5 ml/min and temperature 23 °C.

Isolation of pectic polysaccharides

CMFJ was vacuum-concentrated and filtrated through Büchner funnel. Further, one volume of the concentrated juice was mixed with two volumes of cold 96% ethanol and left overnight in a refrigerator. Then the mixture was filtered through a paper filter and the content of pectic polysaccharide was determined gravimetrically by weighing the precipitated polysaccharide on the filter.

Oxygen radical absorbance capacity (ORAC) assay

ORAC was determined using the method developed by Ou et al. (2001) [13] with some modifications described in details by Denev et al. (2010) [14]. This method measures the ability of an antioxidant to neutralize peroxyl radicals. The free radical damage to fluorescein results in a downward change of fluorescent intensity. The presence of a radical scavenger results in an inhibition of the free radical damage to fluorescein resulting in a preservation of the fluorescent signal. The concentration of an antioxidant in the sample is proportional to the area under the fluorescence decay curve. Peroxyl radicals are generated by thermal decomposition of 2,2'-azobis (2-amidino-propane) dihydrochloride. Trolox solutions are used to construct a standard curve. An ORAC unit is the area under the fluorescence decay curve of a Trolox solution with a concentration of 1 μM. Our results are expressed in μmol Trolox equivalents per liter (μmol TE/l). Measurements were performed on the FLUOstar OPTIMA Fluorometer (BMG LABTECH, Offenburg, Germany). An excitation wavelength of 485 nm and an emission wavelength of 520 nm were used.

Hydroxyl radical averting capacity (HORAC)

The method was developed by Ou et al. (2002) [15]. It measures the complexing ability of an antioxidant under Fenton reaction conditions induced by the interaction between Co (II) and hydrogen peroxide (H_2O_2). The method is based on the oxidation of fluorescein from hydroxyl radicals generated by H_2O_2 as a result of which fluorescence decreases over time. Antioxidants block this oxidation of fluorescein. The area under the fluorescence decay curve is used to determine the antioxidant activity of the sample. Gallic acid solutions are used to construct a standard curve. The area under the fluorescence decay curve of a one μM gallic acid solution is considered as one HORAC unit. The results are expressed in μmol of gallic acid equivalents per liter (μmol GAE/l). Measurements were performed on the FLUOstar OPTIMA Fluorimeter (BMG LABTECH, Offenburg, Germany). An excitation wavelength of 485 nm and an emission wavelength of 520 nm were used.

Results

Chemical composition of CMFJ

The spectrophotometric Folin-Ciocalteu assay showed that the total content of phenolic substances was extremely high – 890.00 mg GAE/l (Table 1).

The HPLC analysis revealed a very high content of procyanidin oligomers and also the presence of several phenolic acids and flavonoids in CMFJ (Figure 1).

3-β-glucoside, quercetin, rutin and naringin. The least was the contents of kaempferol and myricetin (Table 1).

In CMFJ, six organic acids were detected (Table 2). The highest was the content of malic acid followed by quinic acid. Lower were the contents of citric acid, shikimic acid, ascorbic acid and oxalic acid.

The phenolic acids were presented by eight acids. The highest was the content of vanillic acid, followed by caffeic acid and chlorogenic acid. Lower were the contents of neochlorogenic acid, p-coumaric acid, ellagic acid, ferulic acid and 4-dihydroxy-benzoic acid (Table 1).

The most abundant flavonoids in CMFJ were epicatechin and catechin, followed by quercetin-3-β-glucoside, quercetin, rutin and naringin. The least was the contents of kaempferol and myricetin (Table 1).

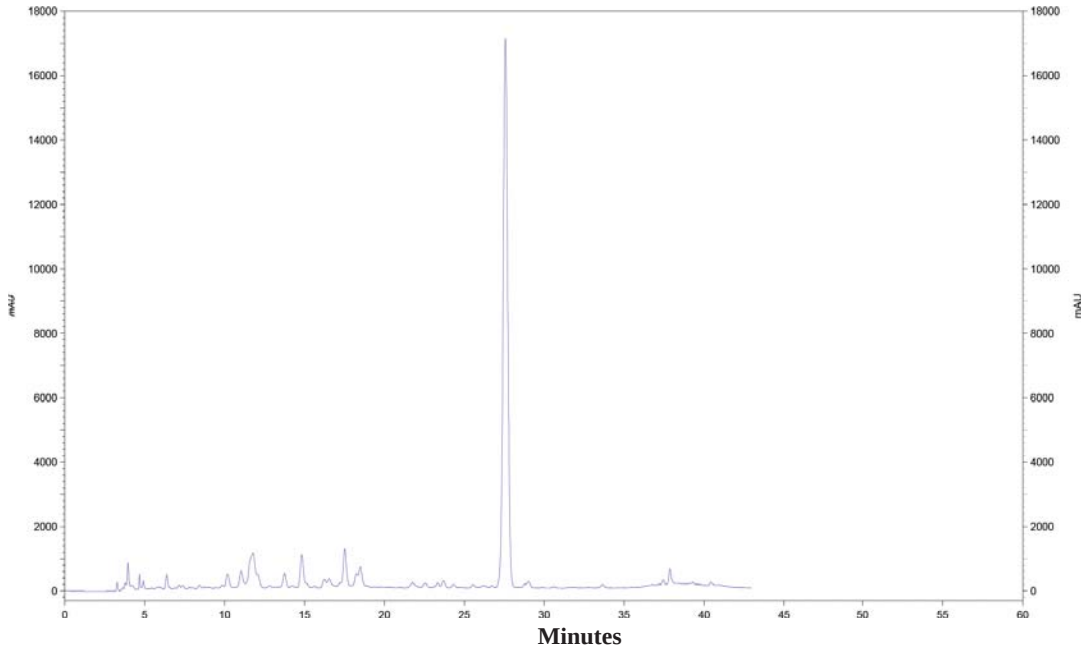
In CMFJ, six organic acids were detected (Table 2). The highest was the content of malic acid followed by quinic acid. Lower were the contents of citric acid, shikimic acid, ascorbic acid and oxalic acid.

Seven monosaccharides were detected and measured as seen from the chromatogram on Figure 2 glucose, fructose, galactose, sucrose, xylose, rhamnose and arabinose.

Amongst them, the highest was the content of glucose, followed by fructose. Considerably lower were the contents of the other monosaccharides: galactose, sucrose, xylose, rhamnose and arabinose. There was also one unidentified disaccharide. The search for polysaccharides in CMFJ did not detect such (Table 3).

Table 1. Contents of phenolic substances in *Chaenomeles maulei* fruit juice; catechin equivalents (CE), gallic acid equivalents (GAE)

Phenolic substances	Content (mg/100 ml)
Total phenols	890.00 GAE
Total proanthocyanidins	253.29
Procyanidin oligomers	280.52 CE
Phenolic acids	
Vanillic acid	14.91
Caffeic acid	14.48
Chlorogenic acid	11.00
Neochlorogenic acid	2.44
p-Coumaric acid	1.52
Ellagic acid	1.29
Ferullc acid	1.26
4-dihydroxy-benzoic acid	1.23
Flavonoids	
Epicatechin	5.59
Catechin	5.25
Quercetin-3-β-glucoside	3.58
Quercetin	3.43
Rutin	2.72
Naringin	1.46
Kaempferol	0.42
Myrecetin	0.23

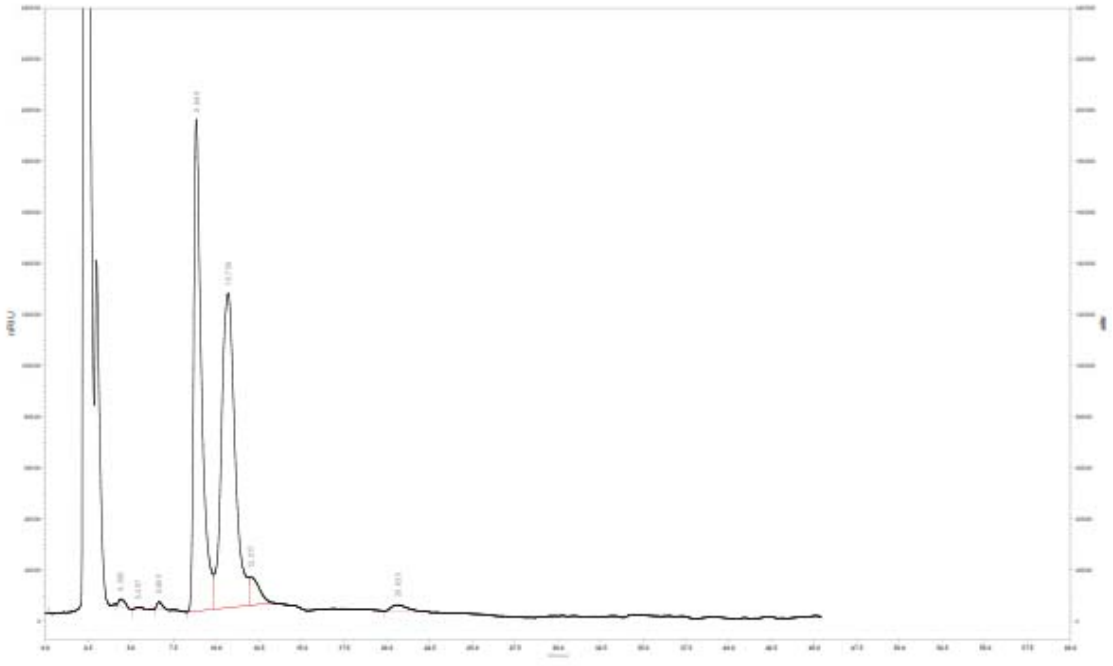


***** Peaks are identified as follows: Retention time (RT) 7.0 min – neochlorogenic acid; RT 7.8 min – 3,4-dihydroxy-benzoic acid; RT 9.8 min – chlorogenic acid; RT 10.2 min – catechin; RT 12.7 min – vanillic acid; RT 13.7 min – caffeic acid; RT 14.9 min – epicatechin; RT 20.8 min – p-coumaric acid; RT 23.8 min – ferulic acid; RT 24.1 min – rutin; RT 25.2 min – ellagic acid; RT 26.2 min – quercetin-3-glicoside; RT 27.5 min – procyanidin oligomers; RT 30.3 min - naringin; RT 35.2 min – myrecetin; RT 40.4 min – quercetin; RT 43.6 min – kaempferol.

Figure 1. HPLC chromatogram of phenolic compounds in CMFJ

Table 2. Contents of organic acids in *Chaenomeles maulei* fruit juice

Organic acid	Content (mg/100 ml)
Malic acid	3647.0
Quinic acid	1034.0
Citric acid	51.0
Shikimic acid	30.0
Ascorbic acid	22.0
Oxalic acid	17.0



***** Peaks are identified as follows: Retention time (RT) 4.4 min – rhamnose; RT 5.4 min – xylose; RT 6.7 min – arabinose; RT 8.9 min – fructose; RT 10.7 min – glucose; RT 12.0 min – galactose; RT 20.8 min – sucrose.
Figure 2. HPLC chromatogram of sugars in CMFJ

Table 3. Carbohydrate content of *Chaenomeles maulei* fruit juice

Carbohydrate	Content (mg/100 ml)
Glucose	1713.0
Fructose	1237.0
Galactose	320.0
Sucrose	189.0
Xylose	35.0
Rhamnose	18.0
Arabinose	8.0
Unidentified disaccharide	2.7
Polysaccharides	not detected

ORAC and HORAC of CMFJ

The results of the ORAC and HORAC assays showed very high values. They are presented in Table 4.

Table 4. Oxygen radical absorbance capacity (ORAC) and hydroxyl radical averting capacity (HORAC) of *Chaenomeles maulei* fruit juice; Trolox equivalents (TE), gallic acid equivalents (GAE)

Assay	Result
ORAC	84401.4 ± 1934.2 μmol TE/l
HORAC	18167.8 ± 938.8 μmol GAE/l

Discussion

Because of their high acidity and tough pulp, the fruit of *Chaenomeles maulei* are not suitable for direct consumption but might be a valuable source of bioactive substances.

The current investigation of the chemical composition of CMFJ demonstrated an extremely high amount of phenolic compounds. The total phenolic content was 890 mg GAE/100 ml. For comparison, the content of phenolic substances in *Aronia melanocarpa* fruit juice ranged from 546.1 to 665.2 mg GAE/100 ml [16]. Literature data show that the content of phenolic compounds measured in orange juice was 75.5 mg/100 ml, in grapefruit juice 53.5 mg/100 ml, in pineapple juice 35.8 mg/100 ml, in apple juice 33.9 mg/100 ml [17]. The high content of phenolic substances is an essential property of CMFJ as the total phenolic content of fruit juices correlates with their antioxidant activity [18]. Polyphenols have been demonstrated to act as antioxidants [19] and to affect intracellular signaling molecules [20]. As a result, plant products rich in polyphenols have shown protective effects in experimental models of organ damage and toxicity [21-25] as well as therapeutic effects in inflammation and cancer [26, 27]. Due to their ability to improve memory polyphenols are possible new approaches to treat neurodegenerative diseases [28-30]. They also possess antimicrobial, antiviral and antifungal activities [31]. The high content of polyphenols in CMFJ is a prerequisite for future investigation of its health benefits.

The review of the literature shows that the current investigation presents in full detail the polyphenolic profile of a chaenomeles juice. The most abundant phenolic acid was vanillic acid. It probably contributes to the pleasant flavor of the juice. From the flavonoids, the highest was the content of epicatechin and catechin. In the analyzed CMFJ sample, six organic acids were identified, and the highest was the amount of malic acid. Hellín et al. (2003) [32] established three organic acids in chaenomeles juices: malic acid, quinic acid, and succinic acid. The highest was the content of malic acid, followed by quinic acid. The very high content of organic acids accounts for the acidity of CMFJ. The pH of the investigated CMFJ sample was 2.58. The high acidity makes CMFJ undrinkable if not sweetened. At the same time, it is suitable as a natural acidifying agent in foods like lemon juice. Malic acid is currently used in food industry as an acidifying additive (E-296).

The content of HPLC-detected ascorbic acid in CMFJ was 22 mg/100 ml. Hellín et al. (2003) [33] reported a high amount of vitamin C in chaenomeles juices: 45-109 mg as ascorbic acid/100 ml. These authors did not detect ascorbic acid by HPLC analysis, suggesting that all vitamin C was in the dehydroascorbic acid form. In the current investigation, the ascorbic acid content was identified by HPLC. For comparison, the content of vitamin C in lemon juice was reported to be 50.82 mg/100 ml, in orange juice 39.25 mg/100 ml and in grapefruit juice 35.23 mg/100 ml [34].

Amongst the seven monosaccharides detected in CMFJ, the highest was the content of glucose (1.713 g/100 ml) followed by fructose (1.237 g/100 ml). Other investigations of chaenomeles juices demonstrated that the highest content was that of fructose (0.73–2.29 g/100 ml), followed by glucose (0.31–1.07 g/100 ml) [33]. For comparison, in lemon juice and orange juice, the glucose content was respectively 0.5 g/100 ml and 2.4 g/100 ml, and the respective fructose content was 0.9 g/100 ml and 2.4 g/100 ml [35].

The antioxidant activity of CMFJ was investigated by the ORAC and HORAC assays. The ORAC assay measures the ability of an antioxidant to neutralize peroxyl radicals that have proven to be of highest physiological significance. The HORAC assay measures the ability of an antioxidant to prevent hydroxyl radical formation. Since the hydroxyl radicals are the most reactive forms of oxygen, it is of particular importance to assess the ability of an antioxidant or a sample to prevent their formation. The ORAC of CMFJ was 84401.4 ±1934.2 µmol TE/l, and the HORAC value was 18167.8±938.8 µmol GAE/l. The high antioxidant activity of CMFJ might be attributed to its high content of phenolic substances and vitamin C. For comparison, the ORAC of *Aronia melanocarpa* fruit juice ranged from 52045 to74045 µmol TE/l, and the HORAC ranged from 30560 to 51661 µmol GAE/l [16]. Thus, both juices showed very high antioxidant activities in vitro, but due to their different polyphenol profile, the

ORAC was higher for CMFJ while the HORAC was higher for *Aronia melanocarpa* fruit juice.

Conclusions

The investigation of the chemical composition of *Chaenomeles maulei* fruit juice showed a very high content of phenolic compounds, the predominant of which were procyanidins, phenolic acids, and flavonoids. The sour taste of the juice, attributed to the high content of organic acids, might prove useful in the food industry. The juice showed a very high antioxidant activity assessed by the ORAC and HORAC assays. The highly active ingredients of the juice possessing not only antioxidant but also many other beneficial activities make the juice a promising tool for future research.

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Conflict of Interest Statement

The Authors declare that there is no conflict of interest.

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