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Phytochemical characteristics and *in vitro* antioxidant activity of fresh, dried and processed fruits of Cornelian cherries (*Cornus mas* L.)

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In the current study, chemical and technological properties and antioxidant activity of fresh, dried and processed Cornelian cherry fruits were evaluated. Several fruit characteristics, such as total titratable acidity (TA), pH, total phenolic compounds, total monomeric anthocyanin (TMA), total antioxidant activity and sugar composition were studied. Average fruit mass and flesh ratio were as follows: for fresh fruits (1.53 g and 77.16%), for dry fruits (0.86 g and 67.61%) and for compôte (1.68 g and 63.15%), respectively. In fruits of Cornelian cherry dry matter varied from 18.7 to 81.4%, ash content from 0.5 to 2% and TA from 1.5 to 3.4%. The highest content of TMA - 32.1 mg cyd-3-glu/100 g fresh weight (fw), as well as of total phenolic compounds - 4.56 mg GAE/g fw was found in fresh fruits. Fresh Cornelian cherry demonstrated the highest antioxidant potential - 36.5 mM TE/g fw (DPPH assay) and 29.6 mM TE/g fw (FRAP assay). The sugar composition in all Cornelian cherry products mainly consisted of sucrose, glucose and fructose, as sucrose was in low levels from 0.15 to 0.30% fw. The polyuronic content was in the range from 1.1 to 1.3%. The current study demonstrated nutritional characteristics of Cornelian cherry fruits suitable for direct consumption and various forms as infusions or compôte. The analyzed products were evaluated as natural foods with high antioxidant activity and potential health benefits.

Key words: Cornelian cherry, Phytochemical compounds, Antioxidant activity

INTRODUCTION

Cornelian cherry (*Cornus mas* L.) is a species of the genus Dogwood (*Cornus* L.) and belongs to the family Cornaceae [1]. In the recent decades the Cornelian cherry has been recognized as an important source of safefood, and breeding programs on this kind of fruit have been launched in several countries as Ukraine, Slovakia, Turkey, Serbia, the Czech Republic, Bulgaria and Austria [2].

Cornelian cherry fruits are widely used in traditional and modern medicine, pharmacy and food industry [2-4]. At the stage of technological maturity, Cornelian cherries acquire a sweet-sour taste [3]. They are not only consumed fresh but also used in the production of jams, jellies, marmalade, stewed fruit [1, 3], as seasoning for meat and fish, in the form of various processed products: wine, brandy, candies, compôte, syrup, fruit juice, fruit yogurts, etc. Fresh processed fruits of the Cornelian cherry can be safely recommended as food for children and persons with impaired health [2].

In Bulgaria, Cornelian cherry is typically consumed fresh, dried as decoct, like marmalade or as compôte [5]. In Turkey its fruits are used for pestil (a locally dried fruit-pulp product), pickled like olives or even in tarhana production [6]. In traditional medicine Cornelian cherry fruits have been used to treat diarrhea, hemorrhoids, diabetes, sore throat, digestion problems, measles, chicken pox, anemia, rickets, liver and kidney diseases [2, 6]. It was reported that fruits also possess astrigent, anti-inflammatory, antioxidative and antihelmintic activity [7-10]. In Turkey, the Cornelian cherry is used for its antiallergenic, antimicrobial and antihistamine properties [4].

Recent research suggests that Cornelian cherry contains significant phytochemical properties and antioxidant capacity [10]. Fresh Cornelian cherry fruits contain many nutritional and bioactive compounds. Furthermore, fruits contain high amount of vitamin C (106.3 mg/100 g), they are rich in sugars (7.2-8.5%), organic acids, cellulose (0.75-1.00%), pectin (0.5-1.12%) and tannin [5, 11]. Jaćimović *et al*. [12] found pectin content in the range of 1.03 to 2.47%. Many studies reported the presence of significant amounts of bioactive substances including anthocyanins in Cornelian cherry fruits [2, 10, 13]. The study of Yilmaz *et al*. [10] also demonstrated high total phenolic content (74.8 mg GAE/g fw), anthocyanins (115 mg cyd-3-glu/100 g fw) and high antioxidant activity [10]. Bjorøy *et al*. [14] reported that anthocyanins from Cornelian cherry fruits demonstrated anticancer,

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anti-inflammatory and antioxidant effects and showed promising results for treatment of diabetes mellitus-related disorders.

Many of the reports discussed the chemical composition of fresh fruits from different *Cornus mas* L. genotypes [2, 4, 13, 15, 16]. The reports for the chemical composition of dried and processed fruits of Cornelian cherry were absent or not available.

The aim of the current study was to evaluate the chemical and technological properties and the antioxidant activity of fresh, dried and processed Cornelian cherry fruits. Several fruit characteristics, such as total titratable acidity (TA), pH, total phenolic compounds, total monomeric anthocyanin (TMA), total antioxidant activity and sugar composition were studied. Phytochemical characteristics and *in vitro* antioxidant activity of fresh, dried and processed fruits of Cornelian cherries were investigated as well.

EXPERIMENTAL

Plant material

Fruits of *Cornus mas* L. were collected in August 2016 from the region of Plovdiv, Bulgaria. Part of the collected fruits was air-dried in darkness at room temperature (25 °C) and then packed in close tight jars for further uses. Another part from the harvested fresh fruits was directly used. The last part of Cornelian cherry was processed for production of home-made drink with whole fruits, named ‘compôte’ without added sugar (250 g of Cornelian cherry and 750 ml of distilled water) and boiling for 30 min. All other solvents and chemicals were of analytical grade.

Fruit weight (g) and flesh ratio (%) were assessed. Fruit weight was measured by a digital balance with a sensitivity of 0.0001 g. Flesh ratio (%) was calculated considering fruit and seed weight [2, 4].

Moisture and ash content

Moisture content was determined by the weight difference after drying of the sample, following the official method of AOAC [17]. Ash content was determined according to the standard AOAC procedures [17].

Total acidity

The content of total acids was measured by potentiometric titration of aqueous fruit extract with sodium hydroxide to the pH value of 8.1 by using pH meter 7110 WTW (Germany). The obtained result was converted to the content of acids (expressed as maleic acid) [18].

pH determination

Fruit tissue (5 g) was homogenized with 25 ml of distilled H_2O. pH was determined using pH meter 7110 WTW (Germany) according to AOAC [17].

Fruit extraction

For the extraction of phytochemical compounds, fruits (5 g) were extracted with 70% (v/v) aqueous ethanol (solid to liquid ratio 1:20 (w/v)). The extraction procedure was performed in an ultrasonic bath (VWR, Malaysia, 45 kHz and 30 W) at 45 °C for 15 min [19]. Sugars were extracted from Cornelian cherry fruits with distilled H_2O under ultrasound-assisted irradiation at the above mentioned conditions.

Total soluble and reducing sugars

The total soluble sugars content in fruit samples was estimated by the phenol-sulfuric acid method [20]. The absorbance was measured at 490 nm against blank with distilled H_2O. The amount of present carbohydrates was determined from a calibration curve constructed with glucose [21]. The reducing sugars were evaluated by the PAHBAH method [22].

HPLC analysis of mono-and disaccharides

Chromatographic separation and determination of sugars present were performed on a HPLC instrument Elite Chrome Hitachi with refractive index detector (RID) Chromaster 5450. The separation was done on a Shodex® Sugar SP0810 (300 mm × 8.0 mm) with Pb^{2+} and a guard column Shodex SP - G (5 µm, 6 × 50 mm) operating at 85°C, mobile phase distilled H_2O with flow rate of 1.0 ml/min and injection volume of 20 µl [23].

Polyuronic acid content

The polyuronic acid content (PUC) in the plant materials was determined titrimetrically according to Owens *et al*. [24].

Total phenolic contents

Total phenolic contents were measured using a Folin-Ciocalteu reagent. Briefly, 1 ml Folin-Ciocalteu reagent diluted five times was mixed with 0.2 ml of sample and 0.8 ml of 7.5% Na_2CO_3. The reaction was performed at room temperature in darkness for 20 min. The absorbance was measured at 765 nm against blank. The results were expressed as mg equivalent of gallic acid (GAE) per g fresh weight (fw) [25].

Determination of total monomeric anthocyanins

Total anthocyanins content was determined according to the pH differential method [26]. Absorbance was measured at 520 and 700 nm. Data were reported as cyanidin-3-glycoside per 100 g of fw of fruit or 100 g of tissue for at least three replicates.

$$TMA = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}, \text{ mg/L}$$

where A=(A_{520nm} – A_{700nm})pH1.0 – (A_{520nm} – A_{700nm})pH4.5; MW (molecular weight) 449.2 g/mol cyanidin-3-glycoside (cyd-3-glu); DF – dilution factor, l = path length in cm; ε-molar coefficient of 26900 L × mol^{-1}× cm^{-1} for cyd-3-glu and factor 1000 for conversion from g to mg.

DPPH radical-scavenging ability

Cornelian cherry fruits ethanol extract (0.15 ml) was mixed with 2.85 ml of freshly prepared 0.1mM solution of DPPH in methanol. The sample was incubated at 37 °C in darkness for 15 min. The reduction of absorbance was measured at 517 nm against a blank containing methanol and % inhibition was calculated [25].

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to Benzie and Strain [27] with slight modification. The FRAP reagent was freshly prepared by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part of 20 mM $FeCl_3 \times 6H_2O$ in distilled H_2O. The reaction was started by mixing 3.0 ml of FRAP reagent with 0.1 ml of investigated extract. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm against a blank prepared with methanol. Antioxidant activity was expressed as mM Trolox® equivalents (TE) per g fresh weight (fw) [25].

Statistical analysis

All experimental measurements were carried out in triplicate and were expressed as average of three analyses± standard deviation.

RESULTS AND DISCUSSION

Physicochemical characteristics of fruits

The results for some physical parameters of Cornelian cherry fruits are summarized in Table 1. The average fruit weight was in the range of 0.86 to 1.68 g. Our results were lower than reported for Turkish and Serbian Cornelian cherry, where the average fruit weight was in range of 2.9 to 3.9 g [2, 3]. However, our results coincided with other reports for some Turkish and Slovakian Cornelian cherry fruit types (0.39-1.03 g) [13] and 0.5-3.4 g [28], respectively.

Table 1. Fruit characteristics of Cornelian cherry (*Cornus mas* L.)

Product	Number of fruits	Total fruit weight, g	Pulp, g	Stones, g	Fruit weight, g	Flesh/solid ratio, %
Fresh fruits	21	32.22	24.86	6.53	1.53	77
Dried fruits	21	17.97	11.97	5.99	0.86	67
Compôte fruits	29	48.76	30.79	17.97	1.68	63

Flesh/solid ratio varied from 77 to 63% in our study. The fresh Cornelian cherry fruits showed higher values of flesh ratio than processed fruits (Table 1). Many studies showed that fruit weight and fruit flesh ratio is the most variable characteristics of Cornelian cherries. Fruit weight and fruit flesh ratio of Cornelian cherry genotypes in Turkey were reported between 1.49-9.11 g [29-31] and 74-93% [10, 31]. For Serbian *Cornus mas* L. genotypes these values were in the range of 3.42 to 8.00 g and 79.32 and 88.55%, respectively [2, 16, 32]. The highest results for fruit weight and fruit flesh ratio were reported for Ukrainian *Cornus mas* L (5.0-8.0 g and 89-92%) [33]. The results in our study were near to Turkish Cornelian cherry genotypes, reported previously [29-31]. The contents of total dry matter in fruits (Table 2) varied in the range from 18.19% to 81.37%. Especially, fresh Cornelian cherry fruits had high dry matter content - 30.74%, which was near to the results reported for some Serbian genotypes [2, 16], but higher than previously reported values for Turkish genotypes [13, 18, 30, 31]. The reason for this increase could be explained by the different growing and environmental conditions, because the values of these parameters vary considerably not only as a result of the genotypes [30].

Ash content in pulp from fresh, dried and processed fruits of Cornelian cherry varied in the range of 0.49 to 2.83%. Our results coincided with previous reports for ash in fresh fruits of some Serbian and Turkish *Cornus mas* L. genotypes [2, 10, 16, 18].

In the present study the pH values of Cornelian cherry fruits were between 3.19 – 3.46 which was in agreement with other reports (2.5–3.53) [3, 10, 13, 18, 29].

Total acidity of Cornelian cherry fruits was found to be in the range of 0.95 to 1.49% (as maleic acid), while in the similar studies TA was between 1.10 to 4.69% [2-4, 10, 16, 30]. Our results demonstrated lower total acidity of Bulgarian fresh and dried Cornelian cherry fruits in comparison with fresh samples collected from territory of Serbia and Turkey [2, 16, 13].

Table 2. Physicochemical characteristics of Cornelian cherry (*Cornus mas* L.)

Product	Moisture content, %	Total dry matter, %	Ash content, %	pH	Total acidity, %
Fresh fruits	69.25±1.79	30.74±1.79	0.88±0.01	3.40	0.95±0.23
Dried fruits	18.62±0.22	81.37±0.22	2.83±0.35	3.46	1.05±0.20
Compôte fruits	81.81±0.74	18.19±0.75	0.49±0.01	3.19	1.49±0.22

Sugar content in the fruits of Cornelian cherry

The individual sugar contents present in aqueous extracts of fresh, dried and processed Cornelian cherry fruits were analyzed by HPLC-RID method. Only three sugars (sucrose, glucose and fructose) were detected (Fig. 1) in *Cornus mas* fruits.

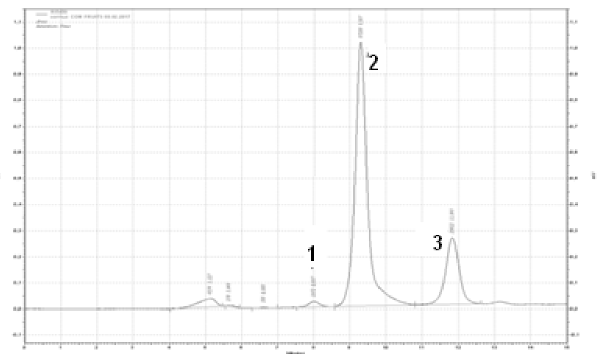

Fig. 1. HPLC-RID chromatograms of aqueous extracts from dry Cornelian cherry fruits, where 1 – sucrose, 2- glucose and 3-fructose

Glucose and fructose were the main monosaccharides detected in all fruits of Cornelian cherry. Their content was 12.26±0.36 and 1.90±0.21% dw, respectively. Sucrose content in fresh fruits was low - 0.17% fw. Sucrose content in dried fruits was 0.25% dw, whereas its content in fruits of compôte was 0.15% (Table 3).

The results reported by us were higher than the content of reducing sugars by Kalyoncu *et al.* [3] and near to some other Turkish genotypes [18]. The monosaccharides content in dry samples was near to reports for Bulgarian *Cornus mas* L. fruits [34]. Our results for total sugars were in agreement with Burmistrov [35], who reported that the content of sugars in fruits of wild genotypes varied between 9.4% and 17.4%. Cornelian cherry genotypes from Slovakia [28] contained from 6.5% to 15.1% of total sugars.

Table 3. Carbohydrate content in Cornelian cherry fruits

Product	Total soluble sugars, %	Reducing sugars, %	Sucrose, %	Glucose, %	Fructose, %	PUC %
Fresh fruits	16.88±0.54[b]	14.56±0.26[b]	0.17±0.09[b] 0.56±0.05[a]	12.26±0.36[b] 39.84±0.36[a]	1.90±0.21[b] 6.11±0.21[a]	1.3[a]
Dried fruits	15.50±0.36[b]	11.03±0.13[a]	0.25±0.07[a]	7.45±0.29a	2.63±0.64[a]	1.2[a]
Compôte fruits	13.78±0.66[b]	10.56±0.25[b]	0.15±0.09[b] 0.82±0.09[a]	8.43±0.53[b] 46.28±0.53a	0.88±0.25b 4.83±0.25a	1.1[a]

a – dry weight, b – fresh weight

The reducing sugars content for dry Cornelian cherry were similar to those in the reports of Didin *et al.* [36] and Kalyoncu *et al.* [3]. Cornelian cherry genotypes from Turkey had significantly lower contents of total and reducing sugars [10, 30] in relation to our results. Our results for total and reducing sugars coincided with some Serbian genotypes [2, 16], but sucrose levels in our research were lower than in previous studies [2, 16]. The explanation for the lower level of sucrose in Cornelian cherry fruit is that they are a rich source of acid and hydrolysis to glucose and fructose could be possible. Therefore, our study evaluated Cornelian cherry fruit as fruits with low sucrose content.

Cornus mas L. fruits were evaluated as a source of pectin as PUC content in fresh, dried and possessed fruits was in the range from 1.1 to 1.3% dw. The drying and heating caused a small decrease in pectin content to 1.1% that is due to enzyme or temperature partial hydrolysis of pectic polysaccharides. The well-proven properties of pectin as soluble dietary fibers find significant application in healthy and dietary nutrition. The pectin content in Serbian genotypes varied from 0.37 to 1.57% expressed as Ca-pectate [2, 16]. Therefore, our data showed significantly higher content of pectin in *Cornus mas* fruits in comparison with some Serbian varieties.

Phenolic compounds and antioxidant activity

Cornelian cherry fruits were evaluated as a good source of phenols and anthocyanin; therefore they are a food with high antioxidant capacity (Table 4). Cornelian cherry fruits are characterized with a significant content of colored substances. The content of total phenols ranged from 10.44 to 5.78 mg GAE/g dw and from 4.35 to 1.90 mg GAE/g fw (Table 4). The lowest levels were detected in dried fruits, the highest values were found in fresh fruits of *Cornus mas* L. The obtained results for total phenols in *Cornus mas* L. were in accordance with reported 432 mg GAE/100 g fresh weight [37]. Close to our results were also the reports of Tural and Koca [13] who reported that the total phenolic contents of Cornelian cherries ranged between 2.81 mg/g and 5.79 mg/g. Our results coincide also with reports for total phenols, especially for Turkish Cornelian cherry in dark red stages of ripening (4162 μg GAE/g fw) [29]. Moreover, the various factors such as genotype, agronomic practices, maturity level at harvest, postharvest storage, climatic and geographical locations influence the total phenolic content of the plant [2, 4, 16]

Table 4. Total phenols, total monomeric anthocyanins and antioxidant activity of fruits of *Cornus mas* L.

Product	Total phenols mg GAE[1]/g	TMA, mg cyd-3-glu/100 g	DPPH, mM TE[2]/g	FRAP, mM TE/g
Fresh fruits	4.35±0.19[b]	48.04±1.16[b]	29.91±0.76[b]	29.12±0.26[b]
	14.14±0.19[a]	136.63±1.16[a]	97.21±0.76[a]	94.64±0.26[a]
Dried fruits	5.78±0.01[a]	3.55±0.16[a]	46.58±3.34[a]	48.53±0.32[a]
Compôte	1.90±0.48[b]	20.44±1.15[b]	14.63±5.04[b]	13.12±0.14[b]
fruits	10.44±0.48[a]	112.22±1.15[a]	80.32±5.04[a]	72.03±0.14[a]

a – dry weight, b – fresh weight, [1]GAE –gallic acid equivalents, [2]TE – Trolox equivalents

The content of total monomeric anthocyanins in Cornelian cherry varied from 136.63 to 112.22 mg cyd-3-glu/100 g fw (Table 4). Similar findings have been published for Cornelian cherry genotypes grown in Turkey, with anthocyanins values from 107 to 292 mg cyd-3-glu/100 g fw [13, 15]. Our results for TMA were similar to those for some Serbian genotypes [2, 16]. In compôte the content of TMA decreased approximately twice on fresh weight, while in dry Cornelian cherry it decreased more than 45 times. Therefore, TMA remained in compôte and the latter is a proper approach for processing fruits, better than drying process.

The antioxidant activity of extracts from fruits of C*ornus mas* was determined using DPPH and FRAP methods, based on different mechanisms. The highest antioxidant activity evaluated by both methods possessed fresh fruits of Cornelian cherry followed by processed ones as compôte. The lowest antioxidant activity showed dried fruits of *Cornus mas* L. - 46-48 mM TE/g dw. Therefore, the preservation of Cornelian cherry as compôte demonstrated a promising antioxidant potential of fruits and presented a beverage with high content of phenolic compounds.

CONCLUSION

Our study evaluated the nutritional and antioxidant potential of fresh and dried Cornelian cherry fruits, as well as compôte. The results demonstrated that the highest content of phytochemical compounds was found in fresh fruits of *Cornus mas* L. The conservation of this fruits as compôte showed significantly higher levels of TMA and antioxidant activity. The lowest content of biologically active substances was found in dried fruits. Therefore, the current study recommends consumption of Cornelian cherry fruits in fresh state or as compôte.

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ФИЗИКОХИМИЧНА ХАРАКТЕРИСТИКА И *in vitro* АНТИОКСИДАНТНА АКТИВНОСТ НА СВЕЖИ, ИЗСУШЕНИ И ПРЕРАБОТЕНИ ПЛОДОВЕ ОТ ОБИКНОВЕН ДРЯН (*Cornus mas* L.)

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(Резюме)

В настоящето изследване са оценени химичните и технологичните свойства на свежи, изсушени и преработени плодове от обикновен дрян. Определени са редица характеристики на плода, като механичен състав, обща титруема киселинност, pH, общо полифенолно съдържание, общи мономерни антоциани, антиоксидантна активност и захарен състав. Средната маса на плода и процентът на месестата част са както следва: за свежи плодове (1.53 g и 77.16%), за изсушени плодове (0.86 g и 67.61%) и съответно за компот (1.68 g и 63.15%). Сухото вещество на плодовете варира от 18.7 до 81.4%, пепелно съдържание от 0.5 до 2%, а титруемата киселинност от 1.5 до 3.4%. Най-високо съдържание на общи антоциани и полифенолни компоненти беше намерено в свежите плодове – съответно 32.1 mg cyd-3-glu/100 g св. м. и 4.56 mg GAE/g св. м. Свежите плодове показаха най-висок антиоксидантен потенциал - 36.5 mM TE/g св. м. (DPPH метод) и 29.6 mM TE/g св. м. (FRAP метод). От захарите във всички плодове най-застъпени са захарозата, глюкозата и фруктозата, като захарозата е намерена в най-малки количества от 0.15 до 0.30% св. м. Полиуронидното съдържание се открива в границите от 1.1 до 1.3%. Настоящото изследване демонстрира хранителните характеристики на дренките, като те са подходящи за консумация в свежо състояние, а също така и под формата на запарка или компот. Анализираните продукти бяха оценени като естествена храна с висока антиоксидантна активност и потенциални здравни ползи.