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Freezing enhances the phytochemical content in cornelian cherry (*Cornus mas* L.) liqueur

OSKAR SZCZEPANIAK*, JOANNA KOBUS-CISOWSKA, DOMINIK KMIECIK

Department of Gastronomy Sciences and Functional Foods, Faculty of Food Science and Nutrition, Poznan University of Life Sciences, Poznań, Poland

*Corresponding author: oskar.szczepaniak@up.poznan.pl

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Abstract: Cornelian cherry liqueur is a traditional Polish alcoholic beverage. In the present study, two variants of the liqueur were prepared: one with fresh cornelian cherry (*Cornus mas* L.) fruits and the other with frozen fruits. The dry mass, total phenolic content and antioxidant potential were determined in the final products. The liqueurs prepared from frozen fruits had a higher antioxidant capacity and dry mass than the liqueurs produced traditionally. The freezing process resulted in no growth in the total phenolic content. Some other secondary metabolites other than the phenolic acids may have affected the difference in the antioxidant potential.

Keywords: antioxidants; extraction yield; maceration process; polyphenols

Liqueur is an alcoholic extract (macerate) prepared from plant raw material, i.e., fruits, herbs, blossoms, etc. Liqueurs vary in their sugar content, ethanol content and raw materials applied. They may have a positive multidimensional impact on the health and metabolism (Johnson et al. 2013; Pereira et al. 2018).

The cornelian cherry (*Cornus mas* L.) is a plant growing in Eastern and Southern Europe, whose edible parts are reddish fruits. They are rich in bioactive metabolites, such as iridoids, anthocyanins and flavonoids (Szczepaniak et al. 2019a). Cornelian cherry fruits have been reported to have significant anti-inflammatory, neuroprotective and protective effects against cardiovascular diseases both *in vitro* and *in vivo* (Francik et al. 2014; Świerczewska et al. 2019). Cornelian cherry alcoholic beverages possess a noticeably high

content of polyphenolic compounds, anthocyanins and iridoids. This corresponds to the high antioxidant capacity of these beverages (Sokół-Łętowska et al. 2014; Adamenko et al. 2019). Adamenko et al. (2019) showed that changes in the preparation method of the alcoholic beverage clearly affected the results of the final product. Fruit freezing before maceration causes mechanical damage to the plant tissue as a result of the ice crystal formation. The fruit juice extraction should be facilitated due to the damage, which should result in the higher extraction of the bioactive compounds and sugars into the extractant.

The aim of the study was to validate whether freezing the fruits before starting the maceration process would increase the antioxidant potential and phytochemical content of the finished product.

MATERIAL AND METHODS

All the parameters were tested in triplicate for each variant. Two individual specimens were used for each liqueur, thus, there were six measurement replicates. Four of them were taken into account for the statistical analyses and further interpretation, and the two most outlying were not considered.

Tested material

The raw materials were prepared based on the ripe fruits of the cornelian cherry cultivar (cv.) Szafer, collected at the “Szynsad” orchard in Dąbrówka, Nowa, Błędów, Mazowieckie, Poland (51°47'01"N 20°43'04"E). The final products examined were two variants of cornelian cherry liqueurs manufactured according to the scheme shown in Figure 1.

The tested liqueurs were produced in two variants. The first was prepared by the traditional maceration of the *C. mas* fruits at a ratio of 1.5 kg fruit per 1 L

70% ethanol with the addition of sugar at a ratio of 170 g per 1 L of extract. The preparation of the other also involved freezing the fruits before the maceration process. We prepared each liqueur in duplicate.

Ash content. The ash content was determined in a porcelain evaporator in a muffle furnace (Naberterm S 27; Naberterm, Germany) at 535 °C in the presence of air. The final results were calculated based on the percentage of the sample mass reduction, according to the method of Kobus-Cisowska et al. (2019).

Total phenolic content (TPC). The total phenolics were determined according to the method of Cheung et al. (2003) The method is based on measuring the absorbance of the coloured complex formed by the phenolic groups in the tested liqueur reacting with a Folin-Ciocalteu reagent (POCH, Poland). The absorbance was measured at 765 nm using a Metertech SP-830 spectrophotometer (Metertech, Taiwan). The final results were expressed in grammes gallic acid equivalents (GAE) per gram of dry matter (DM) of the liqueur.

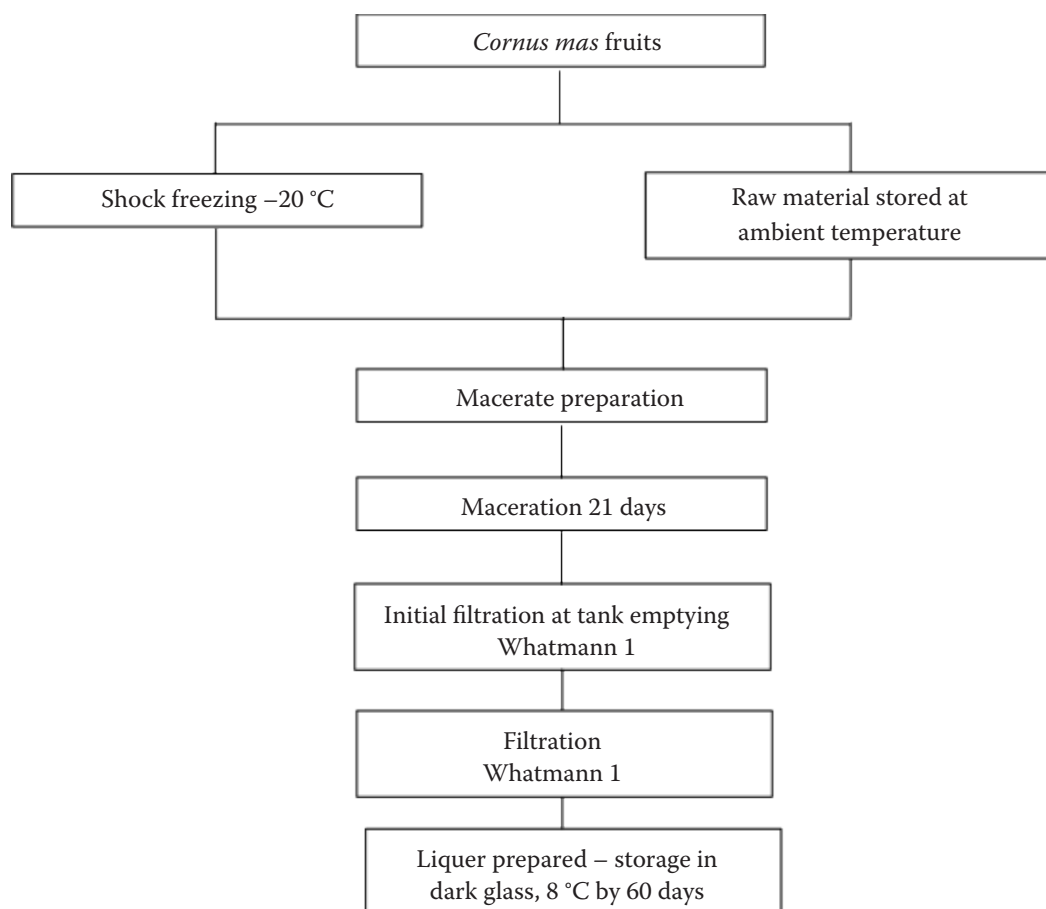


Figure 1. *Cornus mas* liqueurs' production scheme *Cornus mas* fruits

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Antioxidant capacity. The antioxidant capacity was determined individually against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals.

The antioxidant capacity against the DPPH radical was determined according to Amarowicz et al. (2000). Amount of 1 mL of the tested liqueurs was diluted in 2 mL of pure methanol (Honeywell, UK), followed by the addition of 250 μL of a 1 mM DPPH ethanolic solution. After 1 min of vortexing, the sample was incubated for 20 min in the dark. The absorbance was read at an analytical wavelength of 517 nm (Metertech SP-830; Metertech, Taiwan). The antioxidant capacity (AA) was determined according to Equation 1:

$$AA = 100 - \frac{(E_p - E_o)}{E_k} \times 100 (\%) \quad (1)$$

where:

E_p – absorbance of the tested sample;

E_o – absorbance of the zero sample;

E_k – absorbance of the control sample.

No DPPH reagent was added to the zero sample; the control sample contained the DPPH reagent and no addition of the tested sample. The calibration curve was based on the Trolox (Sigma-Aldrich, Poland) standard solutions: 2.0, 1.5, 1.0 and 0.5 mg mL^{-1} (r^2 0.9892). The final results were given in mg Trolox equivalents (Tx) per 100 mL of liqueur.

The ABTS scavenging ability was performed according to the method of Re et al. (1999). The changes in absorbance were measured at 735 nm using an SP-830 spectrophotometer (Metertech, Taiwan). The ABTS aqueous stock solution 7 mM (Sigma-Aldrich, Poland) and potassium peroxydisulfate 2.45 mM (POCH, Poland) were prepared. The mixture was kept in the dark for 12–16 h to react. On the day of the analysis, the ABTS working solution was diluted with ethanol to give a final absorbance of 0.70 ± 0.02 at 734 nm. All the measurements were performed according to the following scheme: 100 μL of the tested extract was transferred

to 2.0 mL of the ABTS working solution. The absorbance was recorded after 6 min against the control sample, i.e. 100 μL of ethanol instead of the sample. The calibration curve was based on the Trolox (Sigma-Aldrich, Poland) standard solutions: 2.0, 1.5, 1.0 and 0.5 mg mL^{-1} (r^2 0.9851). The final results were given in mg Tx per 100 mL of the liqueur.

Metal chelating. The chelating ability was assessed according to Tang et al. (2002) and it was based on changes in the absorbance at 562 nm of the Fe^{2+} -3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,p'*-disulfonic acid monosodium salt hydrate (ferrozine) complex. All the tested liqueurs (1 mL) were diluted two-fold with 70% ethanol; 0.1 mL of 2 mM FeCl_2 (Chempur, Poland) and 0.2 mL of a ferrozine reagent (Sigma-Aldrich, Poland) were added to a test tube. The mixture was vortexed for ~60 s and then left for 20 min at an ambient temperature. The absorbance was recorded using a Metertech SP 830 apparatus (Metertech, Taiwan). Deionised water was applied as the zero sample and the ferrozine solution was used as the control sample.

Statistical analysis

The collected results were analysed using Statistica software 13 (Statsoft, Poland). The descriptive statistics were calculated for all the tested parameters, and the significant differences between the variants were determined using Tukey's test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Significant differences in the dry matter and total ash content (Table 1) were found between the tested liqueurs. The liqueur prepared based on the frozen *C. mas* fruits had higher values. This showed that the freezing process enhanced the phytochemical migration. The higher extraction rate could have resulted from the presence of sugars, which have excellent solubility both in water and in a mixture of water and ethanol.

Table 1. Ash, dry mass and total phenolic content in the tested liqueurs (mean \pm SD, $n = 4$)

Variant	Ash (%)	Dry mass (%)	Total phenolic content (g GAE per 100 g DM)
TD	0.31 ^a \pm 0.01	11.32 ^a \pm 0.12	1.52 ^a \pm 0.12
TF	0.42 ^b \pm 0.00	21.43 ^b \pm 0.22	1.44 ^a \pm 0.11

SD – standard deviation; TD – liqueur based on fresh cornelian cherry fruits; TF – liqueur based on frozen cornelian cherry fruits; GAE – gallic acid equivalents; DM – dry matter; the mean values in the same column marked with different superscript lowercase letters differ significantly ($P \leq 0.05$)

Table 2. Antiradical capacity of the tested liqueurs (mean \pm SD, $n = 4$)

Variant	ABTS (mmol Tx per100 mL)	DPPH (mmol Tx per100 mL)	ChA (%)
TD	1.21 ^a \pm 0.12	1.45 ^a \pm 0.01	20.85 ^a \pm 0.86
TF	1.47 ^b \pm 0.22	1.65 ^b \pm 0.00	33.88 ^b \pm 0.54

SD – standard deviation; ABTS – antiradical activity against ABTS; DPPH – antiradical activity against DPPH; ChA – Chelating ability; TD – liqueur based on fresh cornelian cherry fruits; TF – liqueur based on frozen cornelian cherry fruits; Tx – Trolox equivalent; the mean values in the same column marked with different superscript lowercase letters differ significantly ($P \leq 0.05$)

The total phenolic content did not vary significantly. In the study of Szczepaniak et al. (2019a), the TPC approximated to 8.13 g GAE per 100 g DM cornelian cherry water-ethanolic extracts, and 5.95 g GAE per 100 g DM for the aqueous ones. In the case of the present liqueurs, the total phenolic content was about 13% and 7% dry mass for the liqueur prepared from fresh cornelian cherry fruits and the frozen ones, respectively. Sokół-Łętowska et al. (2014) determined the TPC level at 17.2 mg GAE per 100 mL for liqueur stored at 15 °C and 11.4 mg GAE per 100 mL for the variant stored at 30 °C. In addition, no significant differences were found in the latter study between the liqueurs prepared with and without the addition of sugar. In this study, the TPC approximately amounted to 172.1 mg GAE per 100 g product for the fresh cornelian cherry fruits (TD) and 308 mg GAE per 100 g product for the frozen cornelian cherry fruits (TF). The differences between our results and the referred study could be due to differences in the liqueur preparation models. In our study, fruits were added in a 3 : 2 ratio (m/v) and sugar was added along with the fruits at the start of the maceration, in contrast to the study of Sokół-Łętowska et al. (2014), in which the liqueurs were sweetened before pouring into the storing flasks, and their fruit: ethanol ratio was about 1 : 1 (m/v). The higher TPC noted in our work may have resulted from a higher fruit content in the macerate and/or a higher sugar content. In previous studies by the same authors (Kucharska et al. 2007), the TPC was estimated at 119.6–179.1 mg per 100 mL for 40% ethanolic liqueurs stored for 6 months, depending on whether the fruits had been jabbed before. These values can be compared with our findings in the current study.

In the present study, two spectrochemical methods were applied in order to confirm the antioxidant potential of cornelian cherry liqueurs. The scavenging ability of the liqueurs prepared with the frozen fruits was significantly higher (13–20%) for both the ABTS

and DPPH radicals compared to the liqueurs prepared with the fresh fruits (Table 2).

The predominate compounds in *Cornus mas* fruits include flavonoids, anthocyanins, iridoids and vitamin C. Anthocyanins and iridoids play a key role in the antioxidant capacity of fruits and products prepared on their basis (Kucharska et al. 2015; Adamenko et al. 2018). In the cited study (Szczepaniak et al. 2019b), in addition to the antioxidant properties of the *Cornus mas* fruits, fresh fruits of cv. Szafer also had an antiradical capacity against ABTS and DPPH (2.74 and 1.95 mM Tx per 100 g DM, respectively). This could indicate that the maceration process resulted in a 50% migration of the compounds reacting with the ABTS cation radical from the raw material, while the efficiency for the compounds scavenging the DPPH was higher (75–85%). This difference was associated with the various affinities of the phyto-compounds reacting with the tested radicals. The literature indicated that catechins, phenolic acids and kaempferol scavenged DPPH radicals, while phenolic acids and quercetin quenched the ABTS radicals (Nenadis et al. 2004; Villaño et al. 2007). For ice wines prepared and tested by Olejar et al. (2015), the collection and maceration of frozen *Vitis vinifera* L. fruits caused a higher TPC and DPPH scavenging ability (by 30%). In a similar work by Kucharska et al. (2007), the already mentioned *C. mas* liqueurs scavenged the ABTS and DPPH radicals at an efficiency of 1.60–2.01 and 1.03–1.3 mmol Tx mL⁻¹, respectively.

The preliminary freezing process also enhanced the metal chelating ability (Table 2). The results for the TF amounted to 50% of the liqueurs prepared in the traditional way.

The total phenolic content was estimated at 13% DM for the TD and 7% DM for the TF. Probably, the total antioxidant capacity of the liqueurs could have been dependent on the compounds not reacting preferably with the Folin-Ciocalteu reagent, i.e., anthocyanins and iridoids, as noted by other authors (Adamenko et al. 2018; Kawa-Rygielska et al. 2018).

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CONCLUSION

The freezing stage prior to the fruit maceration positively affected the efficiency of the fruits. Frozen fruit-based liqueurs had a higher dry mass and total ash content, but they did not differ in the TPC. The liqueur based on the frozen fruits was also more active as a free radical quencher than the traditional one, which could be affected by the plant's secondary metabolites other than the phenolic acids.

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