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INFLUENCE OF THE PREPARATION PROCEDURE ON THE ANTIOXIDANT ACTIVITY AND COLOUR OF LIQUEURS FROM CORNELIAN CHERRY (*CORNUS MAS* L.)

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Key words: cornelian cherry liqueur, antioxidant activity, colour

The aim of this work was to investigate the antioxidant activity and colour of liqueurs (cornelle) made from fruits of cornelian cherry (*Cornus mas* L.). The liqueurs were obtained with different efficiency from 42 to 73% in comparison to ingredients used. Their extract was at a level of 26-37%. The extraction of polyphenols from fruits, depending on the preparation variant, was from 73% to 35%. After six months, we noted the concentration of polyphenols at a level of 182 to 246 mg/100 mL. Liqueurs obtained after applying higher concentrations of alcohol (40 and 60%) were characterised with high contents of polyphenols. Their colour was bright (higher L*), less red (lower a*), and more yellow (higher b*). Jabbing the fruits substantially boosted polyphenols extraction and therefore enhanced the antioxidant activity (DPPH, ABTS, FRAP). Sugar added to the fruits at the beginning together with alcohol caused an increase in the efficiency, but lowered polyphenols extraction and antioxidant activity of the liqueurs. In the liqueurs made of fruits with stones, although the part of the pulp was (18%) lower, we have observed a higher activity and higher contents of polyphenols. As a result of storage of liqueurs (maturation), their colour changed to less red (lower a*) and more yellow (higher b*).

INTRODUCTION

Alcoholic beverages made of fruits or herbs are gaining increasing popularity. They are characterised with attractive taste, aroma and colour. They also contain many valuable compounds coming directly from plant raw material, belonging in particular to the group of polyphenols and other phenolic compounds, which are characterised with high antioxidant activity. Alamprese *et al*. [2005] have proved that the antioxidant activity of liqueur made of green, unripe walnuts (nocino liqueur) was directly correlated with the total phenol content and did not change during storage, even for many years. High activity of the nocino liqueur was confirmed also by Stampar *et al.* [2006].

Alcoholic beverages based on plant raw material were produced in Poland yet in the 18th and 19th century. For their preparation, many different fruits, roots and herbs were used, thanks to which miscellaneous tastes were obtained. The liqueurs were used both because of their taste and health-promoting properties.

Among many wild fruits, a valuable raw material for the preparation of liqueurs, particularly in the area of eastern Poland and Ukraine, were edible fruits of cornelian cherry (*Cornus mas* L.). Cornelian cherry fruits are a valuable source of vitamin C, anthocyanins, flavones, phenolic acids, dietary fiber and hydrolysable tannins [Pantelidis *et al.*, 2007; Cai *et al.*, 2004; Tian *et al.*, 2000]. There exists many old and traditional recipes and ways of preparation of liqueurs from cornelian cherry fruits. However, up till now neither such liqueurs nor their antioxidant properties have been investigated. Therefore, the aim of this work was to study the antioxidant activity and colour of liqueurs made of fruits of cornelian cherry (*Cornus mas* L.). By the analogy to French liqueur "Prunelle" made of plum fruits (*Prunus*) we propose the name "Cornelle" for cornelian cherry liqueurs.

MATERIALS AND METHODS

Materials. The raw material used in this research were ripe cornelian cherry fruits harvested in 2004, obtained from Arboretum and Institute of Physiography in Bolestraszyce.

Sample preparation. The liqueurs were prepared in seven variants: with the use of ethanol with concentrations of 20%, 40% and 60% (v/v), from fruits with and without stone, jabbed or not, and with the addition of sugar on various stages of product preparation (at the beginning and after 3 months of storage).

The following samples were prepared: (1) entire fruits with stones, without jabbing, 40% ethanol, sugar added after 3 months; (2) entire fruits with stones, without jabbing, 40% ethanol, sugar added together with ethanol; (3) entire fruits with stones, with jabbing, 40% ethanol, sugar added after 3 months; (4) entire fruits with stones, with jabbing, 40% ethanol, sugar added together with alcohol; (5) fruits without stones, 40% ethanol, sugar added after 3 months; (6) entire fruits with stones, without jabbing, 20% ethanol, sugar added after 3 months; (7) entire fruits with stones, without jabbing, 60% ethanol, sugar added after 3 months.

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The liqueurs were prepared from fruits, ethanol and sugar at a weight ratio of 2:2:1.The steeping process was carried out in the dark, at the room temperature.

Analytical methods. The total polyphenols were determined with the Folin-Ciocalteu's method [Slinkart & Singleton, 1977]. The results were calculated as gallic acid. Anthocyanin contents and the degradation index (ID) were determined with the method of Fuleki & Francis [1968]. Ferric reducing antioxidant power (FRAP) was measured according to Benzie & Strain [1996]. DPPH assay was carried out as described by Yen & Chen [1995]. ABTS assay was done according to the method of Re *et al*. [1999]. Results of antioxidant activity were expressed in μmol Trolox equivalents/cm^3. The colour of liqueurs was measured using a Color Quest XE colorimeter (HunterLab). L^*, a^*, b^*, chroma (C^*) and hue angle (h^o) were measured in triplicate in the total transmission mode using Illuminant D65 and 10^o observer angle.

Statistical analysis. Analysis of variance was performed by ANOVA procedures. Statistical analysis was performed using Statictica 7.0. Significant differences between means were determined by Duncan multiple range tests. Correlations among data were obtained using Pearson's correlation coefficient (r).

RESULTS AND DISCUSSION

The liqueurs were obtained with different yield, ranging from 42 to 73%, in comparison to ingredients used (Table 1). The highest yield was noted for the liqueur prepared from fruits with stones, and the lowest – from fruits without stones. It was observed that the increase of yield was additionally affected by sugar added in the later stage of liqueurs preparation, *i.e.* after separation of alcohol from above the fruits.

The extract of liqueurs was from 26 to 37% (Table 1). Its level depended mainly on the time when sugar was added. Liqueurs in which sugar was added at the beginning, together with alcohol, were characterised with high extract.

Cornelian cherry fruits, when compared to other red fruits, are a rich source of polyphenolic compounds [Pantelidis *et al*., 2007] which influence the antioxidant activity. Mean contents of total polyphenols in those fruits are at a level from 2693 to 4256 mg/kg [Kucharska *et al*., 2007]. The obtained products, in comparison to raw material, were characterised by relatively high total polyphenols contents. The extraction of polyphenols form fruits, depending on the liqueur preparation variant applied, was from 35 to 73%. After 6 months, the concentration of polyphenols was at a level from 1819 to 2457 mg/L (Table 1). Increasing the ethanol concentration to 40 and 60% influenced the increase of the polyphenols contents.

The extraction of polyphenols was considerably increased by jabbing the fruits, and also the presence of the stones. In liqueurs mad of fruits with stones, although the part of the pulp was 18% lower, the highest polyphenols contents was observed. The reason of this could be an additional extraction of active compounds from the stones. The addition of sugar together with alcohol deteriorated polyphenols extraction, therefore it was favorably to add it to fruits after preliminary liqueur separation.

In comparison to other alcoholic beverages from fruits (see for example [Heinonen *et al*., 1998]), the investigated liqueurs were characterised by the high polyphenols contents.

Heinonen *et al*. [1998] determined the total phenolic contents in berry and fruit wines and liquors at a level between 160 and 1820 mg/L.

Among liqueurs, a high concentration of polyphenols is found in cherry liqueurs (1080 to 1525 mg/L) [Heinonen *et al*., 1998; Rodijer *et al*., 2006] and in liqueurs made of green, unripe walnuts (nocino liqueur) (2001 – 3522 mg/L) [Alamprese *et al*., 2005].

Table 2 collates the results of determination of the antioxidant activity of investigated liqueurs, as the ability to scavenge cation radicals ABTS$^+$ and radicals DPPH, and the reducing power (FRAP). It was observed that the activity of liqueurs against ABTS was nearly two times larger than against DPPH.

After 6 months from preparation, the activity of liqueurs, measured as ABTS was from 16 to 31 μmol trolox/mL, and as DPPH and FRAP, respectively 10 to 19 μmol trolox/mL and 11 to 22 μmol trolox/mL. The highest activity was shown for liqueurs to which 40 and 60% alcohol had been applied, and the lowest – for the liqueur after applying ethanol with 20% concentration. Jabbing the fruits and the presence of stones considerably enhanced polyphenols extraction, and enhanced the activity against DPPH and ABTS radicals, and the reducing power (FRAP).

TABLE 1. Liqueur yield, and soluble solids, anthocyanins, polyphenols contents of cornelian cherry liqueur[a].

Sample	Liqueur yield (% ww.)[b]	Soluble solids (%)	Anthocyanins (mg/100 mL)		Anthocyanins Degradation index	
	6 months	6 months	6 months	9 months	6 months	9 months
1	73.2	34.9	0.716	0.271	2.4	5.3
2	64.3	36.9	0.806	0.361	2.1	3.9
3	48.4	31.4	0.155	0.310	2.3	4.8
4	54.6	36.0	1.052	0.484	2.1	4.1
5	42.4	25.7	1.032	0.916	2.6	3.2
6	59.2	26.5	0.484	0.219	2.8	6.2
7	49.1	33.2	0.155	0.419	2.3	3.8

[a] results are the means of three replications; [b] percentage liqueur yields in weight/weight wet ingredients (fruits, sugar, ethanol).

The activity of liqueurs against cation radicals $ABTS^{+}$ and radicals DPPH, as well as ferric reducing power, denoted as FRAP, were highly correlated with the contents of polyphenols (the correlation coefficient was 0.98, 0.83 and 0.95, respectively – see Table 2). The opposite situation was found, when considering anthocyanins, the contents of which in liqueurs was negatively correlated with the antioxidant activity (correlation coefficient from –0.62 to –0.80). A similarly high correlation coefficient between the total phenols content and the antioxidant activity of nocino liqueur ($r=0.966$) was obtained by Alamprese *et al*. [2005].

Ripe fruits of cornelian cherry contain from 45 to 120 mg of anthocyanins/100 g [Kucharska *et al*., 2007], and even to 223 mg/100 g [Pantelidis *et al*., 2007]. Liqueurs, in comparison to cornelian cherry fruits were characterised by a low content of anthocyanins. After only three months, their almost total degradation was observed (Table 1). The degradation index of anthocyanins was high, and ranged from 2.1 to 2.8 after 6 months, and from 3.2 to 6.2 after 9 months of storage. It was the reason why the colour of liqueurs was more yellow than red.

The colour of beverages is an important indicator of their quality, which plays a crucial role among consumers. Products from edible cornelian cherry are characterised by a nice, red colour. In the case of cornelian cherry liqueurs the situation is quite different. Their colour changes much in comparison to fruits used. Those changes depend on many factors, such as concentration and quality of anthocyanins, but also the method of liqueur preparation and storage. In Table 3 we show the results of measurements of liqueurs colour parameters in CIE L^* a^* b^* system. The L^* value is a measure of lightness, from completely opaque (0) to completely transparent (100); a^* is a measure of redness ($-a^*$ greenness) and b^* of yellowness ($-b^*$ blueness). After 6 months the value of L^* parameter was from 49 to 65, that of a* parameter – from 26 to 43, and that of b^* parameter – from 44 to 55. The colour of liqueurs obtained after applying higher concentrations of ethanol (40 and 60%) was lighter (higher L^* parameter) and less red (lower a^* parameter), and more yellow (higher b^* parameter). Hue angle (h^o) gives a numerical estimate of the colour. The hue sequence on a CIELab diagram is defined with red-purple (0^o), yellow (90^o), bluish-green (180^o) and blue (270^o). Cornelian cherry liqueurs have a high hue angle between 51 and 62 value (Table 4), corresponding to the yellow-orange region of the colour. As a result of liqueurs storage, their colour changed into less red (lower a^* parameter) and more yellow (higher b^* parameter). Also the hue of liqueurs changed (increase of hue angle h^o) as well as their chromaticity (increase of C^*).

A high correlation ($r = 0.89$) was observed between anthocyanin contents and a* parameter (Table 4). The opposite situation took place with L^* parameter and hue angle h^o, which were negatively correlated with red colorants concentration ($r = -0.78$ and -0.82). The value of a^* parameter was negatively correlated with lightness L* ($r = -0.95$) and hue

TABLE 2. Antioxidant activity of cornelian cherry liqueur as determined by the ABTS, DPPH, and FRAP.

Sample	Polyphenols (mg/L)		Antioxidant activity (µmol Trolox/mL)					
			DPPH		ABTS		FRAP	
	6 months	9 months	6 months	9 months	6 months	9 months	6 months	9 months
1	$1819^{c}\pm6$	$1874^{bc}\pm103$	$15.78^{b}\pm0.87$	$15.48^{c}\pm0.23$	$23.64^{d}\pm1.10$	$22.26^{c}\pm1.10$	$15.91^{d}\pm0.51$	$14.32^{d}\pm0.21$
2	$1196^{d}\pm57$	$1286^{d}\pm10$	$10.35^{d}\pm0.45$	$11.46^{e}\pm1.41$	$15.96^{f}\pm0.68$	$16.20^{e}\pm0.48$	$11.10^{f}\pm0.39$	$9.72^{g}\pm0.10$
3	$2400^{a}\pm12$	$2505^{a}\pm5$	$19.28^{a}\pm0.37$	$19.60^{a}\pm0.76$	$31.74^{a}\pm1.28$	$31.86^{a}\pm1.00$	$21.80^{b}\pm0.16$	$19.32^{b}\pm0.44$
4	$1791^{c}\pm14$	$1584^{cd}\pm257$	$13.00^{c}\pm0.50$	$13.92^{d}\pm0.31$	$20.10^{e}\pm0.89$	$20.22^{d}\pm1.28$	$14.54^{e}\pm0.14$	$13.26^{e}\pm0.31$
5	$1724^{c}\pm1$	$1737^{bc}\pm41$	$12.81^{c}\pm0.59$	$13.10^{d}\pm0.24$	$20.34^{e}\pm1.09$	$20.58^{cd}\pm0.10$	$14.32^{e}\pm0.32$	$12.63^{f}\pm0.16$
6	$2109^{b}\pm56$	$2059^{b}\pm187$	$16.69^{b}\pm0.50$	$17.39^{b}\pm0.47$	$27.00^{c}\pm0.36$	$26.52^{b}\pm1.30$	$19.11^{c}\pm0.18$	$17.48^{c}\pm0.12$
7	$2457^{a}\pm21$	$2458^{a}\pm264$	$18.97^{a}\pm0.13$	$19.39^{a}\pm0.12$	$29.94^{b}\pm0.81$	$30.18^{a}\pm1.17$	$22.89^{a}\pm0.21$	$20.10^{a}\pm0.27$

Results are the means ± SD. Statistically homogeneous (p value ≤0.05) groups are designated with the same letters.

TABLE 3. Color parameters (lightness (L^*), redness (a^*), yellowness (b^*) chroma (C^*), hue angle (h^o)) of cornelian cherry liqueur[a].

Sample	L*		a*		*b**		*C**		h°	
	6 months	9 months	6 months	9 months	6 months	9 months	6 months	9 months	6 months	9 months
1	60.11	62.10	34.88	30.10	44.96	54.97	56.90	62.67	52.20	61.29
2	62.20	63.13	33.22	29.03	46.54	54.58	57.18	61.82	54.48	61.99
3	64.04	64.17	28.88	26.92	53.40	61.47	60.71	67.11	61.59	66.35
4	56.48	56.49	39.74	36.66	53.40	59.77	63.45	70.12	51.22	58.47
5	49.55	50.40	43.59	40.43	49.46	64.01	70.67	75.71	51.91	57.72
6	62.51	61.24	30.09	28.69	55.62	55.95	55.61	62.88	57.25	62.85
7	65.95	65.45	26.69	26.12	44.96	61.86	58.31	67.15	62.76	67.11

[a] Results are the means of three replications.

TABLE 4. Pearson's correlation coefficients calculated between train characteristics (anthocyanins, total polyphenols, antioxidant activity, color parameters) of cornelian cherry liqueurs.

	1	2	3	4	5	6	7	8	9	10
1 Anthocyanins	1.00									
2 Polyphenols	-0.54*	1.00								
3 ABTS	-0.62*	0.98***	1.00							
4 FRAP	-0.55*	0.95***	0.95***	1.00						
5 DPPH	-0.80***	0.83***	0.87***	0.78***	1.00					
6 *L**	-0.78***	0.45^{ns}	0.53^{ns}	0.52^{ns}	0.69**	1.00				
7 *a**	0.89***	-0.54*	-0.62*	-0.55*	-0.80***	-0.95***	1.00			
8 *b**	-0.15^{ns}	0.25^{ns}	0.21^{ns}	0.02^{ns}	0.24^{ns}	-0.22^{ns}	-0.02^{ns}	1.00		
9 *c**	0.32^{ns}	-0.04^{ns}	-0.11^{ns}	-0.25^{ns}	-0.18^{ns}	-0.66**	0.48^{ns}	0.87***	1.00	
10 h^o	-0.82***	0.59*	0.63*	0.47^{ns}	0.79***	0.65*	-0.83***	0.58*	0.09^{ns}	1.00

^{ns} – non significant; *,**,*** – significant at $p<0.05$, 0.01, 0.001, respectively.

angle (r = –0.83), and the value of *b** parameter was positively correlated with chromaticity (r = 0.87).

The above data indicate that the method of preparation of cornelian cherry liqueurs influences substantially both their antioxidant activity and colour.

CONCLUSIONS

1. Sugar added to fruits at the beginning, together with alcohol caused enhancement of yield, but diminished polyphenols extraction and lowered antioxidant activity – therefore it is more favorable to add it at the later stages.

2. Liqueurs obtained after applying higher alcohol concentrations (40 and 60%) were characterised by high polyphenols contents and high antioxidant activity, as well as lower concentration of anthocyanins. Their colour was lighter (higher *L** parameter), less red (lower *a** parameter), and more yellow (higher *b** parameter).

3. Jabbing the fruits considerably enhanced polyphenols extraction and therefore enhanced activity against DPPH and ABTS radicals, and the reducing power (FRAP).

4. As a result of an additional extraction of active compounds from stones, in liqueurs made from entire fruits, the higher antioxidant activity and higher polyphenols contents was observed, although the part of the pulp was 18% lower.

5. The storage of liqueurs (maturing) changed their colour to less red (lower *a** parameter) and more yellow (higher *b** parameter).

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WPŁYW METODY PRZYGOTOWANIA NA AKTYWNOŚĆ ANTYOKSYDACYJNĄ I BARWĘ NALEWEK Z OWOCÓW DERENIA WŁAŚCIWEGO (*CORNUS MAS* L.)

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Celem pracy była ocena aktywności antyoksydacyjnej i barwy nalewek (dereniówek) sporządzonych z owoców derenia właściwego (*Cornus mas* L.). Nalewki otrzymano z różną wydajnością od 42 do 73% w stosunku do użytych składników. Ich ekstrakt był na poziomie 26-37%. Ekstrakcja polifenoli z owoców, w zależności od zastosowanego wariantu przygotowania nalewki, wynosiła od 73% do 35%. Po sześciu miesiącach odnotowano stężenie polifenoli na poziomie od 182 do 246 mg/100 mL. Nalewki otrzymane po zastosowaniu wyższych stężeń alkoholu (40 i 60%) charakteryzowały się wysoką zawartością polifenoli. Ich barwa była jaśniejsza (wyższy parametr L^*) oraz mniej czerwona (niższy parametr a^*) i bardziej żółta (wyższy parametr b^*). Nakłucie owoców istotnie zwiększyło ekstrakcję polifenoli i tym samym zwiększyło aktywność wobec rodników DPPH i ABTS i siłę redukującą (FRAP). Cukier dodawany do owoców na początku razem z alkoholem wpłynął na zwiększenie wydajności, jednak pogorszył ekstrakcję polifenoli i obniżył aktywność antyoksydacyjną nalewek, dlatego korzystniej było dodawać go w późniejszym czasie. W nalewkach sporządzonych z owoców z pestką, pomimo, że udział miąższu był mniejszy (o 18%), obserwowano wyższą aktywność i wyższą zawartość polifenoli. Przyczyną tego mogła być dodatkowa ekstrakcja związków aktywnych z pestek. W wyniku przechowywania nalewek (dojrzewania) ich barwa zmieniła się na mniej czerwoną (niższy parametr a^*) i bardziej żółtą (wyższy parametr b^*).