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Polyphenols in Traditional Sour Cherry Liqueurs - Beverages with Health Benefits

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Abstract

The polyphenolic compounds of two traditionally obtained sour cherry liqueurs were evaluated. Liqueurs were prepared of ripe fruits, with addition of sucrose and food grade ethanol. The maceration process was performed with exposure on direct sunlight for the liqueur LA, and in dark at room temperature for the second one (LB), in period of 40 days. After aging of 6 months in dark fruits were separated from the liquid. The obtained liqueurs were analyzed on HPLC-DAD system, and the individual components were identified with LC-ESI-MS system. In both sour cherry liqueurs the presence of 36 phenolic compounds was identified. However, the phenolic profiles of both liqueurs differed significantly due to preparation conditions, and they were also different from that of sour cherries used as raw material. It was estimated that in the moment of analysis the total phenolics recovery was only 15.72% for the liqueur prepared with exposure to sunlight during maceration, and 20.65% for the one where maceration was carried in dark.

Key words

Liqueur, Sour cherry, Polyphenols, HPLC, LC-MS

1. INTRODUCTION

Liqueurs have been prepared and consumed as part of the European culture for more than five centuries [1]. Nowadays liqueurs of many different types are widespread and very popular beverages among the consumers. Their production involves maceration of fruits and/or herbs into hydroalcoholic solutions during a certain period of time, with or without addition of sweetener. This process ends with maturation period of at least three months [2]. Fruits and herbs are rich sources of aromatic and polyphenolic compounds. When raw material is introduced into alcohol these compounds are extracted, passing into the liquid and enriching it [3]. The contents of phenolics in finished product depend on the composition of raw material, applied preparation technique, presence of other substances, and finally the storage conditions. The fruit maturity also has great impact on polyphenolic composition of liqueurs, since the contents of polyphenols decrease with fruit ripening [4].

The polyphenols are important and one of the most present classes of secondary metabolites in higher plants, especially in medicinal and edible plants [5]. They are responsible for the color and flavor of fresh and processed

foods. Moreover, today's tendency is these phytochemicals to become significant part of common human diet, because of their documented antioxidant, anti-inflammatory, anticancer and cardio-protective effects [6], [7]. In that way, fruits are proven and reliable sources of polyphenolics, and one of the well-known fruits worldwide are sour cherries. They are rich with sugars, acids, vitamins, minerals, and different polyphenols [8]. Besides consumption in fresh and processed form, sour cherries are also used for preparation of liqueurs. Polyphenolics in sour cherry fruits and their respective products are represented by two main groups of substances, namely non-flavonoid (phenolic acids, basically hydroxycinnamic derivates) and flavonoid (anthocyanins, flavonols and flavan-3-ols) compounds [8], [9], [10]. Anthocyanins are the main phenolics responsible for the color of red fruit beverages. Their astringency and bitterness are result of the phenolic acids and flavan-3-ols. On the other hand, hydroxycinnamic acids and flavanols, and also flavonols, act as co-pigments of anthocyanins [11]. Overall, all present phenolic compounds participate in numerous chemical reactions during the fruit processing (e.g. maturation process) and product storage, undergoing many different transformations [12].

The objectives of this study were to evaluate the polyphenolic compounds present in two traditionally obtained sour cherry liqueurs, and to investigate the influence of preparation conditions on polyphenols in both liqueurs.

2. MATERIALS AND METHODS

The liqueurs were prepared from sour cherry fruits of Oblachinska variety (OS), from the harvest season 2015. The ethanol (food grade quality) was purchased from Alkaloid Ltd (Skopje, Macedonia), and the sucrose (food grade) from the local food supplying store. Chemicals for preparation of sour cherry extract with quality of analytical grade were purchased from Alkaloid Ltd (Skopje, Macedonia) and Sigma-Aldrich GmbH (Steinheim, Germany). The chromatographic analysis was performed using standards (cyanidin-3-glucoside; cyanidin-3-rutinoside; peonidin-3-glucoside; pelargonidin-3-glucoside; quecetin-3-rutinoside; quecetin-3-galactoside; kaempferol glucoside; isorhamnetin glucoside; catechin; epicatechin; procyanidin B1; procyanidin B2; chlorogenic acid; neochlorogenic acid; 4-caffeoylquinic acid) and reagents of HPLC quality grade purchased from Sigma-Aldrich GmbH (Steinheim, Germany) and Fluka GmbH (Buchs, Switzerland).

Sour cherry extract prepared according the procedure presented by [13] was used for quantification of the phenolics (HPLC analysis) in the raw material. The eight-step extraction was carried out using 25 g of plant material and solvent in ratio 1:1, and the extracts were collected in 250 ml flask. A mixture containing methanol and distilled water in ratio 60:40, acidified with 1% w/v hydrochloric acid was used as solvent. Sour cherry extract was prepared in five repetitions. For the purpose of polyphenolics identification (LC-MS analysis) another extract was prepared. Namely, 1 g sample was mixed with 10 mL of extraction solution (methanol acidified with 3% w/v formic acid). The mixture was placed in cooled ultrasonic bath for 1 h, then centrifuged at 10000 rpm for 7 min at 4°C, and finally the supernatant was separated. This procedure was repeated five times. Both types of extracts were filtered through 0.2 μm pore size polyamide filter (Cromafil AO-20/25), and transferred into vials prior to analysis.

Two types of sour cherry liqueurs were prepared according to a traditional recipe. Namely, ripe sour cherry fruits were placed in jars together with sucrose (in proportion 2:1), during the summer season 2015. Immediately, ethanol (50% v/v) in quantity sufficient to cover the content in the jar was added. The jars were sealed, and exposed to direct sunlight in first case (liqueur LA), and in the second they were stored in dark at room temperature (liqueur LB). The maceration process took place 40 days in both cases. After 6 month period of aging at dark place, the fruits were separated from the liquid. The obtained liqueurs were filtered through 0.2 μm pore size polyamide filter (Cromafil AO-20/25) and transferred into vials, prior to analysis.

The analysis of phenolic compounds in the sour cherry liqueurs was performed on Dionex Ultimate 3000 UHPLC system (Thermo Scientific, San Jose, CA) with diode array detector at 280 nm (flavan-3-ols), 350 nm (flavonols and phenolic acids), and 530 nm (anthocyanins), using a Gemini C18 column (150 x 4.6 mm, 3 μm; Phenomenex) operated at 20 °C. The elution solvents were prepared of 0.1% (w/v) formic acid and 3% (w/v) acetonitrile in double distilled water for solvent A, and 0.1% (w/v) formic acid and 3% (w/v) double distilled water in acetonitrile for the solvent B. The elution was carried out using a linear gradient from 5 to 20% B in the first 15 min, followed by a linear gradient from 20 to 30% B for 5 min, then an isocratic gradient for next 5 min, followed by a linear gradient from 30 to 90% B for 5 min, and then an isocratic mixture for 15 min before returning to the initial conditions. The injection volume was 20 μL, and the flow rate 0.6 mL min^{-1}.

The identification of the phenolic compunds was performed using a mass spectrometer (Thermo Electron LCQ Deca XP MAX, Thermo Finigan, San Jose, CA) with an electrospray ionization (ESI) operating in negative (all phenolic groups execpt for anthocyanins) and positive (for anthicyanins) ion mode, according to the method described by [14].

Statistics was performed using Minitab 17 Statistical Software (Minitab Inc., USA), conducting the one-way ANOVA.

3. RESULTS AND DISCUSSION

Liqueurs are alcoholic extracts of different fruits and herbs, and could be perceived as cocktails of polyphenols [4]. Sour cherry liqueurs were prepared according two different traditional recipes, and then analyzed for their polyphenolic profile. Liqueurs were prepared during the summer season of 2015. The main difference in preparation procedure among both liqueurs was the maceration process. In the first case maceration was performed by exposing the mixture of sour cherries, alcohol and sucrose on direct sunlight (liqueur LA), and in the second the same mixture was placed in the dark place at room temperature (liqueur LB). During the 40 days of maceration process the average exposure of the mixture (LA) to direct sunlight was 8 hours, at average temperature of about 47 °C. On the other hand, the preparation of liqueur with maceration in dark (LB) was characterized by almost constant temperature (25 °C) during the entire period of 40 days. The aging for both liqueurs was carried out in same conditions in dark, from September to February, with minor fluctuations in temperature (it dropped gradually from 22 °C in September to 20 °C in February). All conditions during the processes of preparation and aging of liqueurs contribute to changes of the present components. Numerous factors affect the stability of flavonoids and phenolic acids, including pH, temperature, and the presence of co-pigments, metal ions and sugars, but also the nature of the extracting matrix [15].

*Table 1. Total polyphenolic contents and relative proportions (%) of the different groups of phenolic compounds in the sour cherry liqueurs (LA and LB) and sour cherry fruits (OS)**

Sample	Total polyphenolics content (mg/mL)	Relative proportions of the phenolic compounds (%)			
		Anthocyanins	Flavonols	Flavan-3-ols	Phenolic acids
LA	450.78 ± 11.58^{c}	20.37 ± 0.28^{c}	21.09 ± 0.79^{a}	27.54 ± 0.87^{a}	31.00 ± 0.65^{a}
LB	592.37 ± 17.91^{b}	41.05 ± 1.34^{b}	12.41 ± 0.27^{b}	19.68 ± 0.94^{b}	26.86 ± 0.71^{b}
OS	2868.46 ± 61.89^{a}	73.27 ± 0.54^{a}	4.25 ± 0.07^{c}	18.18 ± 0.50^{c}	4.30 ± 0.18^{c}

* *Data are presented as mean value ± standard deviation (n=5); different superscript letters (a, b, c) within same column indicate significant difference between mean values (HSD test, α=0.05).*

The analysis revealed the presence of 36 different polyphenolic components in both traditionally prepared sour cherry liqueurs. Namely, they contained 7 anthocyanins (cyanidin-3-*O*-sophoroside; cyanidin-3-*O*-(2'-glucosyl) rutinoside; cyanidin-3-*O*-glucoside; cyanidin-3-*O*-rutinoside; peonidin-3-*O*-(6'-*p*-coumaroylglucoside) glucoside; peonidin-3-*O*-rutinoside; pelargonidin pentoside), 8 flavonols (kaempferol trihexoside; dihydroxikaempferol dihexoside; quercetin-3-*O*-rutinoside hexoside 1; quercetin-3-*O*-rutinoside hexoside 2; quercetin-3-*O*-rutinoside; quercetin-3-*O*-galactoside; kaempferol-3-*O*-rutinoside; isorhamnetin-3-*O*-rutinoside), 12 flavan-3-ols (procyanidin B1; procyanidin trimer 1; procyanidin B2; procyanidin tetramer 1; epicatechin; procyanidin trimer 2; procyanidin tetramer 2; procyanidin trimer 3; procyanidin tetramer 3; procyanidin dimer 1; procyanidin dimer 2; procyanidin trimer 4), and 9 phenolic acids (dicaffeoylquinic acid 1; dicaffeoylquinic acid 2; 5-caffeoylquinic acid; 3-*O*-*p*-coumaroylquinic acid; 3-caffeoylquinic acid; 4-caffeoylquinic acid; *p*-coumaroylquinic acid 1; *p*-coumaroylquinic acid 2; 3,5-dicaffeoylquinic acid). Generally, the results regarding to polyphenolic profiles are in accordance with the available data for the polyphenols in sour cherries [8], [9], sour cherry wines [10], and sour cherry juice [16], despite some differences due to the used variety of sour cherry, product type, techniques employed for preparation/ processing, and possible differences in the method used for analysis.

However, our both investigated liqueurs had different polyphenolic profiles, and also different from that of the sour cherry fruits, used for liqueur preparation. The contents of total polyphenolics and the relative proportions (%) of each group of polyphenolic components (anthocyanins, flavonols, flavan-3-ols, and phenolic acids) contained in both liqueurs and the sour cherry fruits (OS) are presented in Table 1. The most noticeable are the differences of the anthocyanins proportion. Evidently, anthocyanins were dominant polyphenolic fraction in fresh fruits (73.27%), but their proportions in both liqueurs were significantly lower (20.37% in LA, and 41.05% in LB). These obvious changes certainly contribute to the increase of proportions of other three polyphenolic fractions. The proportions of flavonols were higher in liqueurs compared to the raw material, and the same tendency could be observed for phenolic acids. The proportions of flavan-3-ols in sour cherries (OS) and the liqueur prepared in dark (LB) were insignificantly different (18.18% and 19.68%, respectively), but significantly higher (27.54%) in

the liqueur LA. These differences have shown that the conditions for preparation of liqueurs had strong influence on transformations of polyphenolic compounds, wherein some of them were probably degraded or interacted with other present compounds. Degradation reactions of polyphenols are initiated by the enzymes present in the raw material, and continue in liqueurs during storage in form of non-enzymatic process. On the other hand, polyphenolic compounds undergo polymerization and condensation reactions with other polyphenols [17].

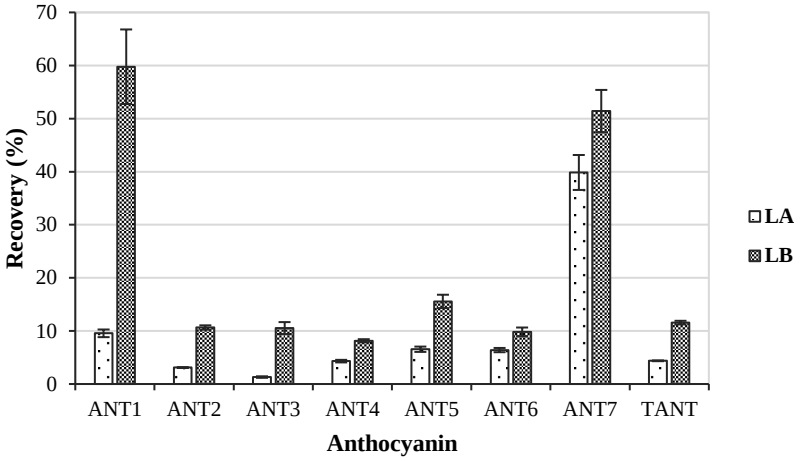

Figure 1. Recovery (%) of identified anthocyanins in the sour cherry liqueurs: cyanidin-3-O-sophoroside (ANT1); cyanidin-3-O-(2'-glucosyl) rutinoside (ANT2); cyanidin-3-O-glucoside (ANT3); cyanidin-3-O-rutinoside (ANT4); peonidin-3-O-(6'-p-coumaroylglucoside) glucoside (ANT5); peonidin-3-O-rutinoside (ANT6); pelargonidin pentoside (ANT7); total anthocyanins (TANT); (data are presented as means ± standard deviation, n=5)

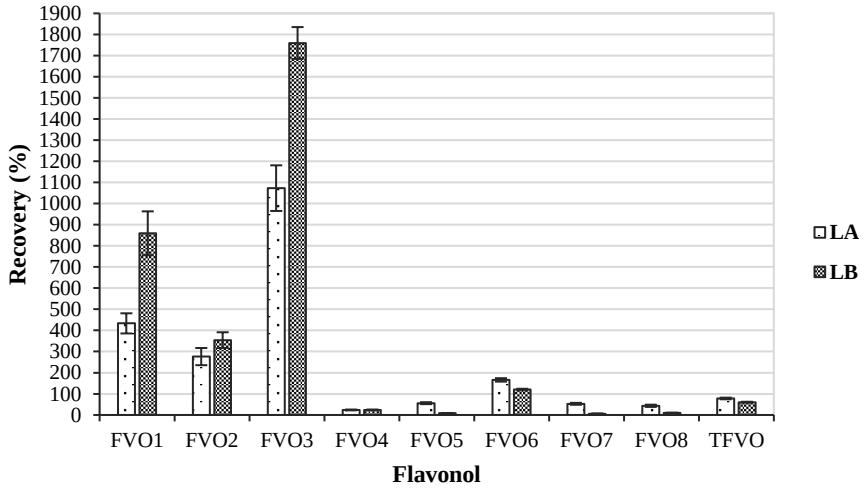

Figure 2. Recovery (%) of identified flavonols in the sour cherry liqueurs: kaempferol trihexoside (FVO1); dihydroxikaempferol dihexoside (FVO2); quercetin-3-O-rutinoside hexoside 1 (FVO3); quercetin-3-O-rutinoside hexoside 2 (FVO4); quercetin-3-O-rutinoside (FVO5); quercetin-3-O-galactoside (FVO6); kaempferol-3-O-rutinoside (FVO7);isorhamnetin-3-O-rutinoside (FVO8); total flavonols (TFVO); (data are presented as means ± standard deviation, n=5)

In order to get a more detailed overview on the polyphenolic profile of investigated sour cherry liqueurs, the recovery of each identified polyphenol regarding the content of respective compound in the raw material was

calculated. The higher content of the particular compound in the liqueurs means the higher percentage of recovery, however recovery value higher than 100% indicated on a higher content in a liqueur compared to the sour cherry fruits. The recovery of anthocyanins ranged between 1.31 and 39.85% for LA liqueur, and 8.14 to 59.77% in LB liqueur, as shown on Figure 1. As it was expected, the recovery of all identified anthocyanins was higher for the liqueur prepared in dark, compared with the liqueur prepared with exposure to sunlight. The highest recovery values had cyanidin-3-*O*-sophoroside (~60%, ANT1) in LB liqueur and pelargonidin pentoside (ANT7) for both liqueurs, and the recovery below 20% was established for the other anthocyanins in both liqueurs. Anthocyanin contents tend to decrease during maceration probably due to the influence of temperature, co-pigmentation reactions and interactions with sugars [18]. The co-pigments could inhibit degradation of anthocyanins when subjected to UV light [19]. Elevated concentrations of ethanol increase the rate of degradation, and in the same time reduce the co-pigmentation leading to decreased anthocyanin stability [20].

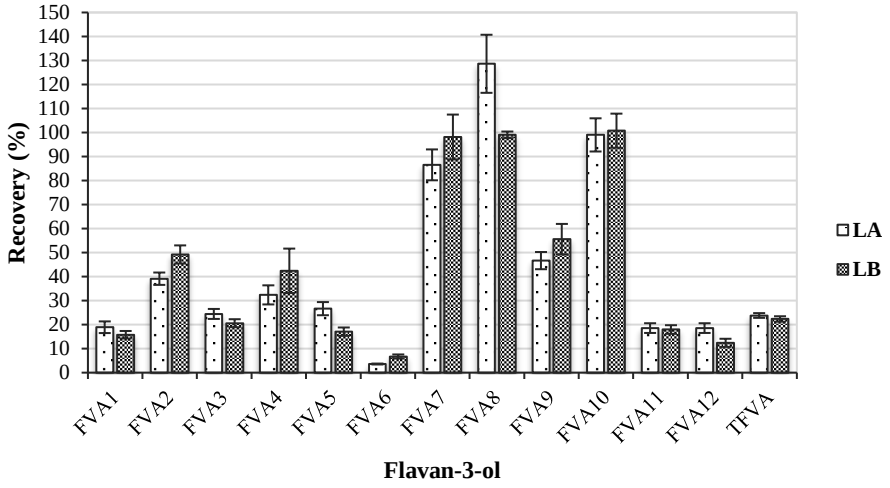

Figure 3. Recovery (%) of identified flavan-3-ols in the sour cherry liqueurs: procyanidin B1 (FVA1); procyanidin trimer 1 (FVA2); procyanidin B2 (FVA3); procyanidin tetramer 1 (FVA4); epicatechin (FVA5); procyanidin trimer 2 (FVA6); procyanidin tetramer 2 (FVA7); procyanidin trimer 3 (FVA8); procyanidin tetramer 3 (FVA9); procyanidin dimer 1 (FVA10); procyanidin dimer 2 (FVA11); procyanidin trimer 4 (FVA12); total flavan-3-ols (TFVA); (data are presented as means ± standard deviation, n=5)

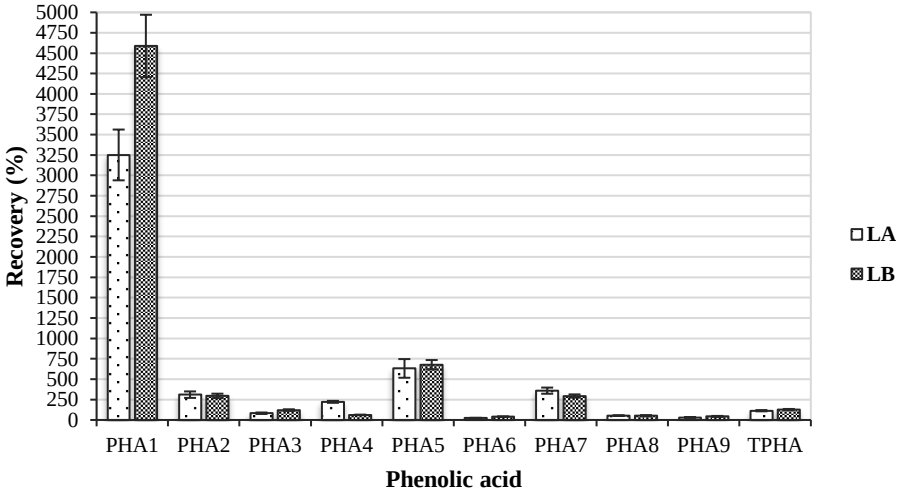

Figure 4. Recovery (%) of identified phenolic acids in the sour cherry liqueurs: dicaffeoylquinic acid 1 (PHA1); dicaffeoylquinic acid 2 (PHA2); 5-caffeoylquinic acid (PHA3); 3-O-p-coumaroylquinic acid

(PHA4); 3-caffeoylquinic acid (PHA5); 4-caffeoylquinic acid (PHA6); p-coumaroylquinic acid 1 (PHA7); p-coumaroylquinic acid 2 (PHA8); 3,5-dicaffeoylquinic acid (PHA9); total phenolic acids (TPHA); (data are presented as means ± standard deviation, n=5)

In contrast to colored phenolics the colorless phenolic compounds were present in higher contents in both liqueurs than in sour cherries. The recovery values for flavonols in the sour cherry liqueurs are presented on Figure 2. Noticeably high recovery percentage had quercetin-3-*O*-galactoside (FVO6), dihydroxikaempferol dihexoside (FVO2), kaempferol trihexoside (FVO1), and particularly the quercetin-3-*O*-rutinoside hexoside 1 (FVO3). The remaining 4 flavonol compounds were significantly less recovered, 23.77-55.84% in the liqueur prepared with sunlight exposure (LA), and 6-24.05% for the liqueur where maceration was performed in dark (LB). In addition, the recovery values were greater for LB liqueur than for the LA liqueur.

On the other hand, the colorless flavan-3-ols, except procyanidin trimer 3 (FVA8) in LA liqueur, had recovery values up to 100% (Figure 3). The procyanidin B1 (FVA1), procyanidin B2 (FVA3), epicatechin (FVA5) and procyanidin trimer 4 (FVA12) showed a higher level of recovery in LA liqueur (prepared on sunlight), compared with the LB liqueur (prepared with maceration in dark). In regards to phenolic acids it is characteristic that 5 components had a very high recovery in both liqueurs (Figure 4). For the liqueur prepared by maceration on sunlight (LA) dicaffeoylqunic acid 2 (PHA2), 3-*O*-*p*-coumaroylquinic acid (PHA4) and *p*-coumaroylquinic acid 1 (PHA7) had higher percentage of recovery than in liqueur prepared by maceration in dark conditions (LB). But, dicaffeoylquinic acid 1 (PHA1), 5-caffeoylquinic acid (PHA3) and 3-caffeoylquinic acid (PHA5) had greater recovery in LB liqueur compared to LA liqueur.

These findings clearly indicate the possible transformations of the present compounds in liqueurs during the maceration and aging towards formation of some of the identified polyphenols. But the fact that the liqueurs were prepared with maceration of whole sour cherry fruits (without pitting), indisputably suggest that stones contributed to polyphenolic profiles of these beverages. Sweet cherry stones are reach sources of flavonols, flavanols, flavones, flavanones and hydroxycinnamic acids, and their extraction depend on used water/organic solvent proportion. So, for example quercetin glycosides are more soluble in organic solvents which is one of the reason for being among the most abundant components in the liqueurs [21]. The content of flavanols is related with their participation in polymerization reactions that occur during maceration process, and the used extraction medium [15]. It was found that during the aging of wines in bottles the contents of hydroxycinnamic acids increased, process associated with disappearance of the anthocyanins, especially *p*-coumaroyl derivates [22].

4. CONCLUSIONS

This study is a contribution to the even more raising interest for traditional foods. The presence of 36 different polyphenolic compounds in sour cherry liqueurs prepared in two different traditional ways was confirmed. A strong influence over polyphenolic profiles had the processing conditions. Liqueur prepared in dark during the maceration had more favorable polyphenolic contents and overall higher recoveries of polyphenolics, compared to the liqueur prepared with exposure to direct sunlight. Anthocyanins fraction was dominant in LB liqueur, and its proportion was 50% higher than in LA liqueur. The colorless polyphenolic fractions (flavonols, flavan-3-ols, and phenolic acids) are more prevalent in the both liqueurs, compared to the raw material. In further investigation the evolution of polyphenolic profiles during maceration and aging should be evaluated, as well as the antioxidant capacity of the liqueurs.

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