Formulations of Novel Liqueurs from Juice Industry Waste: Consumer Acceptance, Phenolic Profile and Preliminary Monitoring of Antioxidant Activity and Colour Changes During Storage

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SUMMARY

Research background. Apple juice is one of the most popular and liked beverages worldwide. Due to the increased health consciousness among consumers, beetroot and chokeberry juices have also rising consumption trends. Despite representing a considerable percentage of the processed fruit and rich source of bioactive compounds, fruit pomace, remaining after juice production, has still been underutilized. Here, the possibility of using apple, beetroot and chokeberry pomace in liqueurs formulation was investigated.

Experimental approach. Apple and chokeberry liqueurs (LA and LC) were produced from apple and chokeberry pomace extracts, respectively. Apple/chokeberry (LAC) and apple/beetroot liqueur (LAB) were obtained by combining apple pomace with chokeberry and beetroot pomace extracts in ratios 50:50 and 70:30, respectively. The sensory quality and acceptability of freshly prepared liqueurs were evaluated by experts and consumers. Sugars and phenolics were identified and quantified by HPAEC-PAD and HPLC–DAD–MS/MS, respectively. Storability was preliminarily evaluated based on monitoring of total phenolic content, antioxidant activity and colour each month
during 6 months of storage at 4 and 22 °C.

Results and conclusions. The experts and the consumers testing indicated that apple and chokeberry pomace could be used as raw materials without any flavour corrections while LAB would require modification. High total phenolic content and antioxidant activity were found in all freshly prepared liqueurs, with LC being by far superior. Among identified phenolics, ellagic acid and phlorizin were quantified as the most prominent, except in LC in which phlorizin was not quantified. Despite the decrease in total phenolic content and antioxidant activity upon 6 months, liqueurs still represented a rich source of phytochemicals. The highest phenolics retention and antioxidant activity maintenance were observed in LC. Also, the appealing colour was retained despite the changes detected in chromatic characteristics.

Novelty and scientific contribution. The possibility of apple, beetroot and chokeberry pomace restoration into the food chain by the production of liqueurs was demonstrated for the first time. Functional and sensorial properties of newly developed liqueurs indicated that the selected pomaces represent the promising raw material for liqueurs production. The applied approach represents a contribution to the circular economy in juice production.

Key words: pomace liqueurs, antioxidant activity, phenolic profile, sensory analysis, circular economy in juice production

INTRODUCTION

One of the most promising waste materials from the food industry is pomace, a by-product in juice production, which mainly contains skins, pulp, seeds, and stalks of the fruit. Phenolic compounds are mainly found in fruit skin as natural plant protection from environmental factors, so pomace is a valuable source of polyphenols, especially if taking into account that most of the antioxidants tend to stay in the pomace rather than transfer into juice (1,2). According to data for 2016, the EU-28 countries produced roughly 2.1 billion litres of apple juice (3). Apple and beetroot are commonly used, while chokeberry use in juice production is constantly increasing.

Apple pomace makes up to 25–35 % of the processed fruit (4). Phenolic compounds (catechins, procyanidins, phloridzin, phloretin glycosides, caffeic and chlorogenic acid, quercetin and cyanidin glycosides) and dietary fibres (soluble pectins, β-glucans, galactomannan gums, nondigestible oligosaccharides including inulin and insoluble lignin, cellulose and hemicelluloses) of apple pomace exhibit antioxidative, cardioprotective, antidiabetic and antilipemic effects and improve the function of the gastrointestinal tract. Only 3-10 % of the overall antioxidant activity of an apple
remains in the apple juice. However, apple pomace is still used only as animal feed in Serbia (5,6). Despite numerous health benefits and high potential for utilization as a substrate, source of bioactive compounds or ingredient of various food products, this abundant, available and renewable natural resource is still underutilized.

Beetroot is one of the 10 most powerful vegetables in terms of antioxidant capacity. Beetroot juice production yields about 15–30 % of beetroot pomace. Beetroot pomace obtained from different cultivars from Serbia was reported to contain ferulic, vanillic, p-hydroxybenzoic, caffeic and protocatechuic acids and betalains (betanin, isobetanin, vulgaxanthin I) (7). These compounds possess many properties beneficial to health, including free radical scavenging ability. Total phenolic content decreases in the order: peel (50 %), crown (37 %) and flesh (13 %), is evidence of a considerable amount of beneficial substances in beetroot pomace.

Black chokeberry is among the richest sources of anthocyanins responsible for various health-beneficial properties. The majority of chokeberries are used for the production of juice with extremely potent antioxidant activity (8). Chokeberry fruit is rich in dietary fibre (up to 5.6 % of fresh mass) and chokeberry pomace is a good source of cellulose, hemicellulose and lignin (8). Among various chokeberry products, including fresh fruit and juices, the highest total phenolic content and anthocyanin content were found in chokeberry pomace, containing skin and seeds (9). Therefore, there is a realistic possibility to use chokeberry pomace as a raw material for the isolation of bioactive compounds or as an ingredient of functional food.

Food waste management has become a challenging task for the food processing industry due to a growing concern about environmental issues in recent years as well as the adoption of sustainable development goals (10). The large quantity of pomace produced, especially apple pomace, suggests that one route of its utilization would not resolve the problem entirely. The development of a single product is not economically feasible. Also, diversification of products based on pomace from the juice industry would lead to better exploitation of underutilized sources of valuable phytochemicals. It is, therefore, worthwhile to explore the production of alcoholic beverages using pomace as raw material.

According to epidemiological studies, the impact of moderate consumption of alcoholic beverages on lipid metabolism and the prevention of coronary artery diseases and colon cancer is related to polyphenol compounds and antioxidant activity (11). Rodríguez Madrera et al. (12) produced a spirit with an alcoholic strength of 60 % (V/V) from dry apple pomace and selected yeasts strains, whereas Zhang et al. (13) evaluated the influence of pectinase treatment on fruit spirits produced from apple mash, juice, and pomace. An increasing trend in the development of new fruit-
based liquors was already noted by Santos et al. (14), but according to our knowledge, the possibility of using pomace in the production of liqueurs with a high phenolic content and AO activity has not been investigated yet. This study seeks to address this gap.

The main aim of this research was to examine the possibility for application of apple, beetroot and chokeberry pomace, both individually or in combination, in liqueurs production. In that regard, sugar content, fixed and volatile acidity, pH and turbidity were analyzed in obtained liqueurs. Sensory quality and consumer acceptability of the freshly prepared liqueurs were also evaluated. Additionally, the composition of individual phenolic compounds in fresh products was assessed. Changes in total phenolic content, antioxidant activity and chromatic characteristics of freshly prepared liqueurs were followed during 6 months of storage at refrigeration and room temperature (4 and (20±2 °C respectively) to provide preliminary insight in the produced liqueurs' stability during storage and to elucidate appropriate storage conditions that would ensure good retention of phenolics as bioactive compounds responsible for beneficial health effects and preservation of colour, as an important aspect for the acceptance of novel products.

MATERIALS AND METHODS

Chemicals and raw materials

Folin-Ciocalteau reagent, sodium carbonate, sodium acetate trihydrate, acetic acid, hydrochloric acid, sodium hydroxide and phenolphthalein were obtained from Merck (Darmstadt, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl) from Fluka (Buchs, Switzerland), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), 2,4,6-tripyridyl-S-triazine (TPTZ), and gallic acid from Sigma-Aldrich (Munich, Germany), ethanol 96 % (V/V) from Ada Vrenje (Belgrade, Serbia), glycerol from Oleohemija (Belgrade, Serbia) while citric acid was purchased on the local market.

Glucose, fructose and sucrose were acquired from Tokyo Chemical Industry, TCI (Europe, Belgium). All aqueous solutions were prepared using ultrapure water (Thermofisher TKA MicroPure water purification system, 0.055 µS/cm). Phenolic standards (protocatechuic acid, p-hydroxybenzoic acid, ellagic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, rutin, naringin, pterostilbene, aesculin, quercetin, quercetin-3-O-rhamnoside, quercetin-3-O-galactoside, isorhamnetin and isorhamnetin-3-O-rutinoside) were supplied by Sigma-Aldrich (Steinheim, Germany).

The company Healthy Organic (Selenča, Serbia) provided apple and beetroot pomaces while chokeberry pomace was acquired from the family farm of D. M. Perić (Belgrade, Serbia). Wet pomaces, collected immediately after juice production, were dried at the industrial scale level at 55 °C
using the dehydrator ‘Solaris’ (NTIM Tehnology, Belgrade Serbia) and ground in an industrial mill to produce a fine powder that is easy to preserve, store, transport and use as a food ingredient (15,16).

**Liqueur preparation**

For the preparation of the liqueurs, a traditional procedure involving a pilot-scale maceration system was used, comprised of two blending tanks with agitation systems (30 L), located at the experimental farm "Radmilovac" (Faculty of Agriculture, University of Belgrade). In the first tank, powdered pomaces were macerated in a water-ethanol mixture (40 % V/V) for 24 h at (20±2) °C. The pomace to solvent ratio was 1:10 (m/V). Extracts obtained after decanting and filtration through disc filters Filtrox Fibrafix AF31H (12-5.0 μm retention rate; Saint Gallen, Switzerland) were transferred into the second tank and commercial sugar (Crvenka a.d., Crvenka, Srbija) and water were added to obtain 150 g/L of sugar and a final alcohol strength of 20 % V/V of alcohol content. Citric acid (1 g/L) was added to achieve a sweetness-sourness balance and glycerol (2 ml/L) was used as a body enhancer. Apple pomace liqueur (LA), chokeberry pomace liqueur (LC), a mixture of LA and LC in a 50:50 ratio (LAC) and a combination of beetroot pomace liqueur with LA in the 30:70 ratio (LAB) were formulated. Beetroot was used only in combination to mitigate its pronounced, undesirable earthy flavour.

**Physico-chemical properties of fresh pomace liqueurs**

The turbidity of liqueurs analyzed was determined with a portable turbidimeter (Hach 2100Q, Loveland, Colorado, USA). The results of turbidity measurement are expressed in a Formazin Nephelometric Units (FNU), with a reading range between 0 and 1000 NTU (Nephelometric Turbidity Units). Fixed and volatile acidity (g/L) was determined by standard AOAC methods (17), while pH was measured by WTW Multi 9310 apparat (WTW, Weilheim, Germany).

**Determination of sugars in fresh pomace liqueurs**

The liqueurs were filtered through 0.22 μm and the filtrate was analysed using the HPAEC-PAD technique on an ISC 3000 DP liquid chromatograph (Dionex, Sunnyvale, CA, USA) equipped with a quaternary gradient pump (Dionex, Sunnyvale, CA, USA), according to the procedure reported by Vasić et al. (18). The total amount of each sugar compound was calculated from the corresponding calibration curve and expressed as g/L. The linear range was 10-100 ppm with correlation coefficients over 0.998. The recovery was between 92 and 108 %. The limit of detection was from 1.2 to 3.4 ppm.
and the limit of quantification was between 4.0 and 10 ppm. The precision was lower than 3 % and accuracy was around 97 %.

**Sensory quality rating of fresh pomace liqueurs**

The sensory quality of the freshly prepared liqueur samples was assessed in the sensory testing laboratory by a 6-member panel (35-60 years old) consisting of staff members from the University of Belgrade – Faculty of Agriculture, experienced in alcoholic beverages quality judging. The samples were labelled with random 3-digit codes and presented to the panellists monadically in random order. Low sodium bottled water was used for palate cleansing. Overall sensory quality was assessed by evaluating five selected sensory characteristics: colour, clarity, distinction, odour (orthonasal olfaction), and flavour, which were rated using category scales with score ranges 0-1, 0-1, 0-2, 0-6, and 0-10, respectively. The quality of the beverages was rated as follows: excellent quality (quality score>18.0); very good quality (16.0<score≤18.0); good quality (14.0<score≤16.0); poor/unsatisfactory quality (12.0<score≤14.0); very poor quality (score≤12.0). The overall quality score, with a maximum value of 20, was calculated by adding the quality scores of the five individual characteristics. The panel evaluated all of the samples once.

**Consumer sensory testing of pomace fresh liqueurs**

Consumer acceptance tests were performed in a sensory testing laboratory by 143 students (21-25 years old) from the University of Belgrade – Faculty of Agriculture. The students were randomly selected, provided that they were relatively frequent (at least occasional) consumers of alcoholic beverages. The samples were labelled with random 3-digit codes and presented to the panellists monadically in random order. Low sodium bottled water was used for palate cleansing. Overall acceptance, appearance acceptance, odour acceptance and flavour acceptance were assessed using the 9-point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9=like extremely). The just-about-right (JAR) scales (1=too little, 5=JAR, 9=too much), were used to evaluate the samples for accepted intensities of ‘colour’ (too light/pale – JAR – too dark), ‘sweetness’ (not sweet enough – JAR – too sweet), ‘sourness’ (not sour enough – JAR – too sour), and ‘alcoholic strength’ (too weak– JAR – too strong). In addition, 9-point attribute-intensity scales were used to assess consumer perception ‘fullness of flavour’ (1=empty, 5=medium, 9=full) and ‘distinctiveness of flavour’ (1=not at all, 5=medium, 9=completely characteristic).
Identification and quantification of phenolics in fresh pomace liqueurs by HPLC–DAD–MS/MS

After filtration of samples through a 0.22 µm filter, individual phenolic compounds were identified and quantified in the filtrate using a Dionex Ultimate 3000 UHPLC system equipped with a diode array detector connected to a TSQ Quantum Access Max triple quadrupole mass spectrometer (Thermo Fisher Scientific, Basel, Switzerland) with the ion source in the form of electrospray ionisation (200 °C) in the negative mode (from 100 to 1000 m/z); triple quadrupole (UHPLC–DADMS/MS), according to previously published procedure (19). The total amount of each compound was calculated from the corresponding calibration curve and expressed as mg/L. The linear range was 1-100 ppm, whereas the correlation coefficients were from 0.9945 to 0.9996. The recovery of the method was between 85-115 %. The limit of detection was from 0.01 to 0.12 ppm, whereas the limit of quantification was in the range of 0.05 to 0.21 ppm. The precision was lower than 5 % and accuracy was in the range of 91 to 105 %.

Determination of total phenolic content and antioxidant activity in fresh and stored pomace liqueurs

The total phenolic content of the prepared liqueurs was determined by the Folin-Ciocalteu (FC) method described by Singleton et al. (20). Properly diluted samples (0.5 mL) were mixed with 0.1 M FC reagent (2.5 mL), 2.5 ml of sodium carbonate solution (75 g/L) was added after 6 min in the dark, the mixture was left for 2 h in the dark, after which absorbance at 740 nm was measured using spectrophotometer (Thermo Scientific Evolution 600, Thermo Fisher Scientific Ins., Germany), using distilled water as a blank. The results were expressed as gallic acid equivalents (GAE) per litre of liqueur. The antioxidant capacity was determined by DPPH and FRAP (Ferric Reducing Antioxidant Potential) assays, using procedures described by Blois (21) and Benzie and Strain (22) respectively. Diluted samples (0.2 mL) were mixed with 2.8 mL of the ethanolic solution of DPPH (0.1 mM) mixed with acetate buffer (0.1 M) in the ratio 2:1, and the mixture was allowed to react 30 min in the dark before absorbance measurement at 517 nm against distilled water. Diluted samples (0.1 mL) were mixed with distilled water (0.3 mL) and freshly made FRAP reagent (3 mL), incubated 40 min at 37 °C and absorbance was measured against the reagent blank at 593 nm. The results of DPPH and FRAP were expressed as mM Trolox equivalent (TE) per litre of the sample. Measurements were performed on the first day (no storage) and upon each month during six months of liqueurs storage at (20±2) °C in a dark place and in a refrigerator (4 °C). All measurements were performed in triplicate.

Colour measurements in fresh and stored pomace liqueurs

Colour intensity (Cl) and hue (h) were determined according to Glories (23). Liqueurs were diluted to 1:10 with 20 % ethanol, centrifuged 5 min at 3000 × g using centrifuge Tehtnica Železniki
(Železniki, Slovenia) and absorbance was measured at 420 nm, 520 nm and 620 nm in a 1-cm cell path length using a spectrophotometer (Thermo Scientific Evolution 600, Thermo Fisher Scientific Ins., Germany). Colour intensity was calculated as the sum of $A_{420}$ nm, $A_{520}$ nm and $A_{620}$ nm, whereas hue was calculated as the ratio of $A_{420}$ nm to $A_{520}$ nm. Measurements were performed on the first day (no storage) and upon each month during six months of liqueurs storage at $(20\pm2) ^\circ C$ in a dark place and in a refrigerator $(4 ^\circ C)$.

Statistical analysis

The measurements of total phenolic content, antioxidant capacity, colour intensity and hue were performed in triplicate and the results are presented as mean values: standard deviation (S.D.). The data related to total phenolic content, antiradical activity (DPPH), total reducing power (FRAP), and analytical colour measurements (colour intensity and hue), were subjected to principal component analysis (PCA). PCA was performed on the unfolded data matrix which included all replicate measurements. Upon dimension reduction, when it was clear that the first extracted principal component (PC1) was completely enough to satisfactorily explain the variations in the data matrix, PC1-scores for samples were subjected to 3-way ANOVA (PC-ANOVA) (24) with ‘products/pomaces’, ‘storage time’, and ‘storage temperature’ taken as fixed factors. Also, another PC-ANOVA model, followed by Tukey’s honestly significant difference (HSD) test, was applied in order to separate the mean PC1-scores for ‘samples’.

Sensory quality and acceptance (hedonic and attribute-intensity) data were subjected to 2-way ANOVA with ‘samples’ as a fixed factor, and ‘assessors’ as a random factor. Tukey’s HSD test was used to separate the mean values for samples.

Mean drop analysis was performed by combining the JAR data with the overall hedonic data, as described in (25), in order to assess the potential impact of being off from just-about-right on the overall acceptability of the liqueurs. Raw JAR scores were grouped into three categories as follows: 1, 2 and 3=’below JAR’; 4, 5 and 6=’at JAR’; 7, 8 and 9=’above JAR’. Mean drop values were calculated by subtracting the overall mean hedonic scores of each below/above-JAR category from the hedonic mean of the JAR-category. ANOVA and Tukey’s HSD test were used in order to compare the overall hedonic means of the JAR and non-JAR categories. Minimum percentage skew for ‘not just right’ (the cut-off) was set at 20 % of the total consumer panel.

Statistical analyses were performed using SPSS Statistics v. 17.0 (26). The level of statistical significance was set at 0.05.
RESULTS AND DISCUSSION

Physicochemical properties and sugar content of fresh pomace liqueurs

The results of physicochemical analysis (turbidity, pH, fixed, and volatile acidity) and quantitative sugar profile for liqueurs analyzed were summarized in Table 1. The highest value of turbidity was determined in sample LAB (250.4 FNU), followed by samples LA (240.0 FNU) and LAC (229.8 FNU), whereas the lowest value was ascribed to LC (102.6). Apple pomace is a rich source of compounds with colloidal properties (i.e. dietary fibers), so as expected, liqueurs produced with this raw material showed higher turbidity (27).

Herein, obtained pH values of prepared liqueurs were between 3.30 (LA) to 3.72 (LAB). Corroborating to obtained results, the pH values of differently prepared apple wines, reported by Won et al. (28), were lower than 4. Also, pH values of a variety of chokeberry products ranged from 3.31 to 4.28 (29). The pH value of liqueur LAB (3.72) was similar to pH of beetroot-based wines reported by Singh et al. (30) (3.56-4) and by Soibam et al. (31) (3.45-3.8).

No marked difference was evident in the obtained values for fixed and volatile acidity in all liqueurs analyzed. The fixed acidity ranged from 2.48 to 2.84 g/L of malic acid, whereas the volatile acidity was from 0.16 to 0.33 g/L of acetic acid. These results are in line with the values for the total acidity of apple liqueurs (1.16-5.82 g/L) reported by Marques et al. (32). Liqueurs are alcoholic beverages produced without fermentation, so, expectedly, the volatile acidity is low. On the other side, acidity regulators such as citric acid, added in the tested samples, in addition to malic acid and citric acid originating from the apple, also contribute to the pronounced total acidity (32).

As expected, the most abundant sugars in freshly prepared liqueurs detected by HPAEC-PAD technique were glucose, fructose, and sucrose. Due to the significant amount of sugar added (150 g/L), the content of sucrose was expectedly the highest in all samples when compared to glucose and fructose. As shown in Table 1, there is no significant difference between the values for total sugar content of all liqueurs analysed, which can be explained by the different ratios between individual sugars (glucose, fructose, and sucrose) in contained apple, chokeberry, and beetroot pomace. Indeed, the absence of sucrose in cultivated black chokeberries is an important characteristic of its sugar profile (33), while beetroot is a valuable source of sucrose and a scarce source of glucose and fructose (34). In the case of the apple-based samples (LA, LAC, and LAB), the content of fructose was higher when compared to glucose, which is in agreement with a previous study (35). The fresh chokeberry fruit contains a slightly higher content of glucose than fructose (8), which is also found in liqueur (LC) prepared with chokeberry pomace.
Sensory properties of fresh pomace liqueurs

According to the results of the sensory quality rating of the liqueurs (Table 2), it seems that selected sensory characteristics were rated in a similar way over the spectrum of the evaluated products. The mean odour quality score for LAB (4.4±0.5) was significantly lower (p<0.05) compared to the other three liqueurs placed within the same homogenous subset. The main defects regarding LAB odour were the undesirable aroma and flavour linked to an earthy note known to be caused by the volatile bicyclic alcohol geosmin (trans-1,10-dimethyl-trans-(9)-decalol) (36). The lowest mean overall sensory quality score was obtained for the LAB (16.6±0.7), noting that the value differed significantly (p<0.05) from the LC only, which was the best-rated (17.6±0.9). The uniqueness of LC can be explained by the sensory expert’s additional notes that its flavour and odour were characterized by an appealing and desirable sour cherry aroma. Regardless of the difference, all mean overall quality scores (16.6-17.6) were in the range of ‘very good quality’.

The results of testing the likeability of the liqueurs are shown in Table 3. The overall, odour and flavour mean hedonic scores of LAB (4.5, 4.5 and 4.3, respectively) were in the range of neutral consumer attitude - ‘neither like nor dislike’ and were significantly lower (p<0.05) when compared with the scores of the other liqueurs that were found in the range of ‘liking’ (≥6.0). On the other hand, by using a 9-point attribute-intensity scale, consumers perceived ‘fullness’ and ‘distinctiveness’ of LAB flavour at the same intensity level (p>0.05). These results, together with the results of sensory quality testing, indicate that lower hedonic scores for LAB were not the result of a lack of flavour, but can most probably be directly linked to the acceptability of the typical earthy, leafy and neutral flavour of beetroot in alcoholic spirits. This conclusion is also supported by the results of mean drop analysis (Fig. 1). Among the consumers tested, there were three large groups (≥20 %) for LAB, with significant mean drops of the overall hedonic scores (p<0.05), with the opinion that the product was ‘not sweet enough’ (29.4 %), ‘not sour enough’ (23.8 %), or ‘too weak’ in terms of alcohol level (25.2 %). When compared to the other liqueurs, no large consumer groups with significant mean drops were observed for the liqueurs with chokeberry pomace, whereas for the LA sample, it can be seen that consumers complained the product was ‘not sour enough’ and ‘too sweet’.

The phenolic profile of fresh pomace liqueurs

The content of individual phenolics in liqueurs is shown in Table 4. Chokeberry-based liqueurs (LC and LAC) seem to be the richest source of tested phenolics. In the study of Sokoł-Łętowska et al. (37) chokeberry liqueur also showed the predominance in the amount of phenolic compounds when compared to cornelian cherry, black rose, blackcurrant, blackberry, raspberry, mahonia, sloe,
strawberry, and sour cherry liqueurs. The predominance of ellagic acid in LC, LAC and LAB can be easily observed, with LC and LAC containing by far superior concentrations. A high concentration of phlorizin was evident in all liqueurs containing apple pomace, as expected since it was suggested to be used as an apple pomace marker (19). Quercetin and its sugar derivatives were also present in significant amounts in all liqueurs, with LC and LAC being the most endowed. According to previous studies, quercetin was the predominant flavonol in chokeberry (38). Also, chokeberry wine contained quercetin as the most abundant flavonoid, and represented the richest source of this flavonol, as well, when compared to some fruit wines such as blackberry wine, sour cherry wine, etc. (39). Corroborating with results obtained for LA, quercetin was one of the major flavonols in apple pomace from several cultivars in which the presence of quercetin glycosides was observed, as well as the prevalence of galactoside over rhamnoside (19). Other phenolic compounds found in liqueurs in notable amounts are ferulic acid, 5-o-caffeoylquinic acid, protocatechuic acid, phloretin, p-hydroxybenzoic acid, rutin, p-coumaric acid, and pterostilbene.

Total phenolic content and antioxidant capacity of fresh pomace liqueurs

All liqueurs produced showed notable total phenolic content at the time of preparation, with the following descending order of activities: LC>LAC>LA>LAB (Table 5). TPC value for LC ((3473.3±33.3) mg GAE/L) was in line with the previous findings of Sokol-Łętowska et al. (37), who showed chokeberry liqueur to be among the richest sources of substances reacting with the Folin-Ciocalteu reagent (3292 mg GAE/L). The total phenolic content of sour cherry liquors was reported at comparable values (3360 mg GAE/L, 40). In comparison to the total phenolic content of commercial Terras Madeirenses Portuguese red wines (1724-1871 mg GAE/L) (41), LC and LAC showed almost double values, as well as far higher values than those of various red and white wines from the Serbian market (164 to 2314 mg GAE/L) (42). Similarly, when compared to the herbal bitter liqueur based on medicinal plant extracts (43) that contained a conclusively larger amount of phenolics (1500 mg GAE/L) than similar commercial herbal spirits, LC and LAC showed twice as high total phenolic content, as well as several times stronger antioxidant activity measured by DPPH and FRAP assays.

It can be noticed that the total phenolic content determined by FC was higher than the sum of individual phenols quantified by HPLC. This is in line with previous studies which explained such result by the interference of various substances other than phenols (organic acids, residual sugars, amino acids, proteins and other hydrophilic compounds) in the FC assay, various responses of individual phenols, presence of only low molecular weight phenols in extracts (5,19), as well as missing values of unidentified polyphenols by HPLC/MS.
A similar rank of order of antioxidant activity of liqueurs measured by DPPH and FRAP assays was obtained as for total phenolic content, with LC being by far the strongest radical scavenger ((28.0±0.7) and (58.9±0.5) mmol/L, respectively). However, in the case of results obtained by FRAP assay, it can be observed that LAB had slightly higher antioxidant potential than LA.

Preliminary evaluation of liqueur storability based on changes of total phenolics and antioxidant activity

The results of total phenolic content and antioxidant capacity trends of analysed liqueurs during 6 months of storage at two different temperatures are presented in Table 5. As evident, total phenolic content and antioxidant activity decreased during storage at 20 and 4 °C. However, the decrease differs among liqueurs. After 6 months, the amount of initial total phenolic content of LA, LC, LAC and LAB, stored at 4 and 20 °C decreased by about 75, 50, 70 and 80 %, and by 70, 50, 70 and 77 %, respectively. Throughout the entire storage period of 6 months the highest phenolics retention was observed in LC, which had preserved a much higher value at the end of storage than the initial total phenolic content of LA and LAB.

In all cases, with exception of LC stored at 4 °C, there were no significant differences in total phenolic content values upon 5 and 6 months of storage, leading to the assumption that the decomposition of phenolic compounds is complete after 5 months.

The decrease of antioxidant activity of LC and LAC during storage, measured by DPPH, was also the least prominent (by approximately 50 %) in comparison to LA and LAB, where drops greater than 60 % were observed. At the same time, antioxidant capacity reduction determined by FRAP method was between 65-85 % for all liqueurs analysed.

There is scarce literature data on the possibility of utilization of apple, beetroot and chokeberry pomace in the production of antioxidant-rich alcoholic or non-alcoholic beverages. In a study dealing with antioxidant activity of liqueurs made from ten red fruits, in the majority of samples, the content of phenolic compounds decreased over the periods considered (37). The same study demonstrated that chokeberry liqueur was among those possessing the highest phenolic content and antioxidant activity, and when stored at a temperature of 30 °C for 6 months it showed a significant reduction in activity (assayed with the DPPH test) of approx. 50 % of the initial value (37). Walkowiak-Tomczak (44) reported that after 20 days under facultative anaerobic conditions, black chokeberry juice concentrate solutions antioxidant activity decreased by 7-12 % at 10 °C, 12-15 % at 20 °C and by 16-35 % at 30 °C, whereas under aerobic conditions the changes ranged from 63 to 76 % after 10 days and from 64 to 79 % after day 20. Furthermore, phenolic compounds in myrtle liqueur showed considerable
changes even when stored with constant headspace. The anthocyanins in particular, both total and free, tended to decrease (45).

The majority of spirits, including liqueurs, are commonly stored safely at room temperature since alcohol provides microbiological stability. Studies on a half-year period of sour cherry liqueur storage showed that their characteristic features are almost unchanged if stored at 15 °C and without sugar added, but organoleptic properties were better in samples stored at 30 °C (46). Here, different storage temperatures did not have a significant influence on the total phenolic content of LC and LAC, but the total phenolic content of LA and LAB was significantly higher after 6 months of storage at room temperature than in the refrigerator. It seems that the lower temperature might slightly decrease the solubility of phenolics inducing their precipitation. However, the differences in values obtained are not so prominent to enable a conclusion of room temperature being the most appropriate conditions for storage of analysed liqueurs.

The strong correlation between total phenolic content and antioxidant capacity measured by DPPH and FRAP was confirmed by high correlation coefficients (0.978 and 0.966, respectively). Such a result indicates that the potent antioxidant capacity of the liqueurs is highly influenced by phenolics present in apple, beetroot and chokeberry pomace, as well as in prepared mixtures, which corroborates the previous reports (40,42,47).

Preliminary evaluation of liqueurs storability based on colour changes

Colour is one of the most important quality features of liqueurs with a huge influence on consumers’ preferences. The determination of the optimal storage conditions can prevent colour changes that consumers associate with food spoiling and can thus be crucial in preventing economic losses, especially in sales of new products. According to literature, the intensive red colour of chokeberry liqueurs depends on the structure and concentration of anthocyanins (37). Apple skin colour is influenced by chlorophyll and carotenoids, anthocyanins, flavonols, and proanthocyanidins, whereas the beetroot pomace is a rich source of red-coloured betacyanins and yellow pigments betaxanthin (48,49).

The colour intensity and hue of the analysed liqueurs are presented in Table 6. The significant difference in the chromatic characteristics of liqueurs is primarily due to the type and quantity of pomace pigment compounds.

The colour intensity of LAC, LC and LAB decreased throughout the evaluated storage period, although the reduction was non-linear. No particular trend of change in CI over time can be observed for LA, at both tested temperatures. Comparing the results obtained for the two tested temperatures,
it can be observed that the lower temperature did not prevent the degradation of colour during storage. Except in the case of LA, an increase in hue was observed during the evaluated storage period, indicating the growth in the percentage of the yellow colour and/or loss of the red colour. The colour of myrtle liqueur, evaluated according to the classic spectrophotometric parameters of intensity and hue, showed marked variability during storage in the bottles with increasing headspace, while values remained almost constant in unopened ones (45). The increase in values obtained for the yellow component and hue angle with the aging time of berry fruit syrup wines with different pH values was previously linked to the formation of anthocyanin-derived yellow-orange pigments like pyranoanthocyanins, as well as to the oxidation of pigments. Also, the red component percentage in these wines decreased after 6 months of storage. The decrease was associated with the possible precipitation of insoluble polymeric anthocyanin-derived pigments, and/or the degradation of free anthocyanins caused by oxidation (50).

**Principal Component Analysis (PCA)**

The results of PCA applied to the unfolded data matrix, derived from the antioxidant activity (total phenolic content, FRAP, DPPH) and colour measurements (hue and CI), showed that only the first extracted principal component had an eigenvalue larger than one, and according to both the Kaiser criterion and scree plot (51), PC1 was retained for describing objects in the new PC-space explaining 87.8% of the variance in the data matrix values. All five initial variables had high PC1 loadings, indicating strong correlations of these attributes with PC1. The antioxidant activity variables (total phenolic content, FRAP, DPPH), together with ‘colour intensity’ (CI), showed strong positive correlations (loading values 0.98, 0.96, 0.99, and 0.96, respectively), while ‘hue’ showed strong negative correlation (-0.79) with PC1. Therefore, taking into account that greater values for total phenolic content, FRAP, DPPH and CI (i.e. greater positive values of PC1) indicated lower levels of the oxidation processes, the PC1 was referred to as ‘antioxidant activity’ axis.

The results from PC1-ANOVA showed that antioxidant activity was significantly affected (p<0.05) by all examined factors: type of pomace used, storage time and temperature. Also, all interactions among the factors were statistically significant. The factor-interactions profile plots are shown in Fig. 2a and Fig. 2b. Trends of the type of pomaces used for liqueurs preparation are consistent over different levels of the ‘storage time’ factor (LC>LAC>LA>LAB), indicating a stronger influence of chokeberry pomace presence on the antioxidant potential of the liqueurs over the storage period. Chokeberry is among the richest plant sources of anthocyanins and possesses one of the highest antioxidant activities among plant species (8). Sokol-Łetowska et al. (37) reported that the
chokeberry liqueur was the richest in anthocyanins (1674 mg/L) among red fruit liqueurs. This trend for antioxidant activity over the storage period correlated with the level of antioxidant potential recorded for control (freshly prepared) samples. Mean PC1 scores-values for the samples after preparation differed significantly (p<0.05) from each other, in the same descending order as observed during the period of storage (LC>LAC>LA>LAB). Also, regardless of the storage temperature, the curves for LC and LAC had slightly steeper slopes than LA and LAB, which remained milder (Fig. 2). Although compared to initial values, LC and LAC showed lower total phenolic content and antioxidant activity reduction, according to PC1-ANOVA the greater initial antioxidant activity expressed through total phenolic content, FRAP and DPPH, the greater was the rate of their loss during six months of storage at both 4 °C and 20 °C. Although it has been noticed that sugar moiety stabilizes anthocyanin stability (46), anthocyanins are unstable pigments, easily oxidized, particularly in the presence of ascorbic acid (the most abundant vitamin in the black chokeberry fruit (8)) and products of its degradation. Polymerization and condensation of polyphenols are also believed to be involved in these processes during prolonged storage (37). However, due to the variety of compounds interacting simultaneously, it is difficult to establish the exact mechanism of degradation of anthocyanins and other polyphenols.

CONCLUSIONS

An innovative way of powdered apple, beetroot and chokeberry pomace utilization was demonstrated. As a source of bioactive molecules, pomaces were employed to obtain liqueurs with notable functional and acceptable sensorial properties. According to our knowledge, this is the first study that deals with the application of powdered pomace from industrial juice production in liqueurs development. Sensorial properties of freshly produced liqueurs indicated the possibility of chokeberry and apple pomace exploitation in the production of liqueurs without flavour correction, while further research aimed at finding a way to improve sensorial properties of LAB and at sensory analysis of liqueurs during storage are required. Analysis of individual phenolic compounds revealed the predominance of ellagic acid and phlorizin in freshly prepared liqueurs, except in LC in which phlorizin was not quantified. The high total phenolic content and antioxidant activity of freshly prepared liqueurs prove that apple, beetroot and chokeberry pomace can be used as a source of bioactive molecules and also indicates the liqueurs’ potential contribution to bridging the antioxidants gap in the modern diet. The storability of liqueurs during the initial 6 months of storage, estimated based on antioxidant activity and total phenolic content, showed that they remained a rich source of bioactive compounds despite the significant decrease of surveyed parameters. Measurable changes in colour
characteristics were also detected but the appealing colour was retained. Acceptable sensorial properties of freshly prepared liqueurs as well as notable total phenolic content and antioxidant activity during 6 months of storage, along with the growing market demand for natural products, indicate the developed products might be an additional source of phytochemicals. The suggested pomace application can also contribute to the adoption of circularity into the fruit and vegetable processing industry.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTION

M. Petrović designed the work, drafted the manuscript, performed liqueurs preparation and analysis of total phenolic content and antioxidant activity, and took part in sensory analysis. S. Veljović performed colour measurements, took part in antioxidant activity determination and sensory analysis and performed statistical analysis of the data obtained. N. Tomić organized and performed sensory analysis, collected the data, performed data analysis and data interpretation. S. Zlatanović took part in samples preparation and participated in sensory analysis. T. Tosti performed and described sugar and polyphenol profile analysis of the samples. P. Vukosavljević organized the raw material supply, participated in sensory analysis, its critical revision and data interpretation. S. Gorjanović planned the research, participated in the drafting of the manuscript and performed critical revision and final approval of the version to be published.

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Fig. 1. Mean drop analysis ($N$(consumer)=143) for the freshly prepared liqueurs (L) samples from apple (A), chokeberry (C) and beetroot (B) pomaces. A point in the plot that shows statistically significant mean drop and a large percentage of consumers (above 20% in this case) is a cause for concern and suggests that the product be modified in the appropriate direction. LA=liqueur from apple pomace, LC=liqueur from chokeberry pomace, LAC=liqueur from apple and chokeberry pomace (50:50), LAB=liqueur from apple and beetroot pomace (70:30), mean drop=the drop of the mean hedonic score calculated as the difference between the 'just-about-right' consumer group and the 'too much' or 'not enough of an attribute' consumer groups.
Fig. 2. 'Storage time' by 'type of product/pomace' (by 'storage temperature') interaction profile plots as a result of three-way PC1-ANOVA (N=3), for the liqueur (L) samples with apple (A), chokeberry (C) and beetroot (C) pomaces stored for six months at 4 °C (I) or 20 °C (II). PCA included the chemical and objective colour variables. PC1 loading values were 0.98, 0.96, 0.99, -0.79, and 0.96, for total phenolic content, FRAP, DPPH, hue, and CI, respectively (the combination of these variables was referred to as “antioxidant activity”). Scores-values marked with the same lower-case letter within a liqueur sample are not statistically different (α=0.05). PC1 scores-values for control (freshly prepared) samples were 2.65, 1.29, -0.64, and -0.57, for LC, LAC, LA, and LAB, respectively. LA=liqueur from apple pomace, LC=liqueur from chokeberry pomace, LAC=liqueur from apple and chokeberry pomace (50:50), LAB=liqueur from apple and beetroot pomace (70:30).
Table 1. Physicochemical parameters and sugar content of fresh liqueurs produced from apple, chokeberry and beetroot pomace

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LA</th>
<th>LC</th>
<th>LAC</th>
<th>LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physico-chemical properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turbidimetry/FNU</td>
<td>240.0±1.0</td>
<td>102.6±0.9</td>
<td>229.8±4.4</td>
<td>250.4±2.0</td>
</tr>
<tr>
<td>pH</td>
<td>3.30±0.02</td>
<td>3.60±0.02</td>
<td>3.50±0.02</td>
<td>3.72±0.02</td>
</tr>
<tr>
<td>Fixed acidity as γ(malic acid)/(g/L)</td>
<td>2.48±0.01</td>
<td>2.84±0.03</td>
<td>2.64±0.01</td>
<td>2.80±0.01</td>
</tr>
<tr>
<td>Volatile acidity as γ(acetic acid)/(g/L)</td>
<td>0.32±0.02</td>
<td>0.33±0.03</td>
<td>0.32±0.02</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>Sugar content as γ(sugar)/(g/L)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>(8±1)a</td>
<td>(31±3)b</td>
<td>(21±2)c</td>
<td>(13±1)d</td>
</tr>
<tr>
<td>Fructose</td>
<td>(16±2)a</td>
<td>(24±2)b</td>
<td>(22±2)bc</td>
<td>(19±2)ac</td>
</tr>
<tr>
<td>Sucrose</td>
<td>(195±18)a</td>
<td>(148±11)b</td>
<td>(159±13)a</td>
<td>(177±16)a</td>
</tr>
<tr>
<td>Total</td>
<td>(219±17)a</td>
<td>(203±11)a</td>
<td>(202±16)a</td>
<td>(208±16)a</td>
</tr>
</tbody>
</table>

L=liqueur, A=apple, C=chokeberry, B=beetroot. *Values marked with the same letter within the same row are not statistically different (α=0.05)

Table 2. Sensory quality scores for the fresh liqueurs produced from apple, chokeberry and beetroot pomace

<table>
<thead>
<tr>
<th>Samples</th>
<th>Color' (max. 1)</th>
<th>Clarity' (max. 1)</th>
<th>Distinction' (max. 2)</th>
<th>Odor'' (max. 6)</th>
<th>Flavor'' (max. 10)</th>
<th>Overall score'' (max. 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>(5.3±0.6)b</td>
<td>8.0±0.4</td>
<td>(17.3±0.9)a,b</td>
</tr>
<tr>
<td>LC</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>(5.3±0.1)b</td>
<td>8.4±0.3</td>
<td>(17.6±0.3)b</td>
</tr>
<tr>
<td>LAB</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>(4.4±0.5)a</td>
<td>8.2±0.2</td>
<td>(16.6±0.7)a</td>
</tr>
<tr>
<td>LAC</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>(5.3±0.5)b</td>
<td>8.4±0.5</td>
<td>(17.6±0.9)a,b</td>
</tr>
</tbody>
</table>

L=liqueur, A=apple, C=chokeberry, B=beetroot. 'Values are modes (6 assessors, 1 repetition). ''Values are arithmetic mean±standard deviation (6 assessors, 1 repetition). Values marked with the same letter under the same type of spirit are not statistically different (α=0.05)
Table 3. Sensory acceptance of the fresh liqueurs produced from apple, chokeberry and beetroot pomace

<table>
<thead>
<tr>
<th>Samples</th>
<th>Overall acceptance*</th>
<th>Appearance acceptance*</th>
<th>Odour acceptance*</th>
<th>Flavour acceptance*</th>
<th>Fullness of flavour**</th>
<th>Distinctiveness of flavour**</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>(6.2±2.2)b</td>
<td>(7.0±1.9)b</td>
<td>(6.6±2.0)c</td>
<td>(6.0±2.3)b</td>
<td>6.2±1.9</td>
<td>6.2±2.3</td>
</tr>
<tr>
<td>LC</td>
<td>(6.1±2.3)b</td>
<td>(7.4±1.9)b</td>
<td>(6.0±2.3)b</td>
<td>(6.0±2.4)b</td>
<td>6.3±1.9</td>
<td>6.4±2.0</td>
</tr>
<tr>
<td>LAB</td>
<td>(4.5±2.5)a</td>
<td>(6.3±2.1)a</td>
<td>(4.5±2.6)a</td>
<td>(4.3±2.7)a</td>
<td>5.9±2.2</td>
<td>6.7±2.3</td>
</tr>
<tr>
<td>LAC</td>
<td>(6.3±2.4)b</td>
<td>(7.0±1.9)b</td>
<td>(5.9±2.3)b</td>
<td>(6.2±2.4)b</td>
<td>6.4±2.0</td>
<td>5.9±2.3</td>
</tr>
</tbody>
</table>

L=liqueur, A=apple, C=chokeberry, B=beetroot. *Ratings on the 9-point hedonic scale.* Ratings on the 9-point attribute-intensity scale (a consumer concept). Values are arithmetic mean±standard deviation (N=143). Values marked with the same letter within the same column are not statistically different (α=0.05).

Table 4. Phenolic profile of the fresh liqueurs produced from apple, chokeberry and beetroot pomace

<table>
<thead>
<tr>
<th>γ/(mg/L)</th>
<th>LA</th>
<th>LC</th>
<th>LAC</th>
<th>LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellagic acid</td>
<td>34.82±0.31</td>
<td>293.25±7.02</td>
<td>191.32±6.30</td>
<td>128.33±0.08</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>93.26±0.04</td>
<td>n.d.</td>
<td>62.32±0.89</td>
<td>51.83±0.04</td>
</tr>
<tr>
<td>Phloretin</td>
<td>10.12±0.03</td>
<td>n.d.</td>
<td>5.33±0.01</td>
<td>4.04±0.00</td>
</tr>
<tr>
<td>Quercetin</td>
<td>10.50±0.0</td>
<td>19.56±0.01</td>
<td>16.79±0.04</td>
<td>14.64±0.04</td>
</tr>
<tr>
<td>Quercetin-3-O-galactoside</td>
<td>7.66±0.03</td>
<td>9.97±0.09</td>
<td>9.64±0.12</td>
<td>8.85±0.02</td>
</tr>
<tr>
<td>Quercetin-3-O-rhamnoside</td>
<td>4.72±0.04</td>
<td>4.79±0.06</td>
<td>4.21±0.05</td>
<td>3.76±0.03</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>11.46±0.08</td>
<td>12.36±0.12</td>
<td>11.98±0.45</td>
<td>8.63±0.04</td>
</tr>
<tr>
<td>5-O-Caffeoylquinic acid</td>
<td>12.39±0.07</td>
<td>11.36±0.05</td>
<td>11.25±0.14</td>
<td>3.46±0.05</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>4.95±0.04</td>
<td>7.13±0.10</td>
<td>7.05±0.07</td>
<td>4.35±0.01</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>5.37±0.07</td>
<td>3.75±0.01</td>
<td>3.67±0.07</td>
<td>0.023±0.00</td>
</tr>
<tr>
<td>Rutin</td>
<td>2.81±0.04</td>
<td>4.18±0.11</td>
<td>3.13±0.04</td>
<td>3.35±0.05</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>1.94±0.03</td>
<td>4.09±0.06</td>
<td>3.98±0.06</td>
<td>4.35±0.05</td>
</tr>
<tr>
<td>Pterostilbene</td>
<td>1.44±0.00</td>
<td>1.56±0.10</td>
<td>1.21±0.11</td>
<td>0.23±0.00</td>
</tr>
<tr>
<td>Aesculin</td>
<td>0.86±0.03</td>
<td>0.69±0.03</td>
<td>0.76±0.05</td>
<td>0.74±0.05</td>
</tr>
</tbody>
</table>
Isorhamnetin-3-O-rutinoside & 0.66±0.02 & 0.86±0.03 & 0.74±0.02 & 0.60±0.03 \\
Isorhamnetin & 0.38±0.00 & 0.53±0.00 & 0.45±0.02 & 0.43±0.04 \\
Caffeic acid & 0.26±0.01 & 0.63±0.02 & 0.62±0.03 & 0.01±0.00 \\
Naringin & 0.36±0.00 & 0.38±0.00 & 0.37±0.01 & 0.46±0.02 \\
Sinapic acid & 0.23±0.01 & 0.25±0.01 & 0.24±0.01 & 0.11±0.00 \\
Taxifolin & 0.26±0.01 & 0.27±0.01 & 0.29±0.00 & 0.15±0.00 \\

L=liqueur, A=apple, C=chokeberry, B=beetroot, n.d.=not determined
Table 5. Changes in total phenolic content and antioxidant activity determined by DPPH and FRAP occurring during 6 months of storage of apple, chokeberry and beetroot pomace liqueurs at two different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>LA</th>
<th>LC</th>
<th>LAC</th>
<th>LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>no storage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>y(TPC as GAE)/(mg/L)*</td>
<td>c(DPPH)/(mmol/L)**</td>
<td>c(FRAP)/(mmol/L)**</td>
<td>y(TPC as GAE)/(mg/L)*</td>
</tr>
<tr>
<td>4°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(871.3±31.7)*</td>
<td>(3.1±0.0)*</td>
<td>(3473.3±33.3)*</td>
<td>(28.0±0.7)*</td>
</tr>
<tr>
<td>2</td>
<td>(646.0±24.2)*</td>
<td>(2.8±0.0)*</td>
<td>(3205.0±42.1)*</td>
<td>(21.9±0.4)*</td>
</tr>
<tr>
<td>3</td>
<td>(645.0±9.2)*</td>
<td>(2.6±0.1)*</td>
<td>(3218.3±20.8)*</td>
<td>(19.3±0.6)*</td>
</tr>
<tr>
<td>4</td>
<td>(552.2±6.5)*</td>
<td>(2.0±0.2)*</td>
<td>(2941.9±10.9)*</td>
<td>(19.6±0.2)*</td>
</tr>
<tr>
<td>5</td>
<td>(409.4±3.2)*</td>
<td>(1.5±0.1)*</td>
<td>(2658.4±26.4)*</td>
<td>(16.3±0.7)*</td>
</tr>
<tr>
<td>6</td>
<td>(256.2±4.0)*</td>
<td>(0.9±0.0)*</td>
<td>(2500.2±19.5)*</td>
<td>(17.6±0.6)*</td>
</tr>
<tr>
<td>7</td>
<td>(226.18±2.4)*</td>
<td>(2.3±0.2)*</td>
<td>(1729.1±14.9)*</td>
<td>(14.4±0.3)*</td>
</tr>
<tr>
<td>20°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(640.3±2.1)*</td>
<td>(2.9±0.0)*</td>
<td>(3301.7±33.3)*</td>
<td>(23.8±0.5)*</td>
</tr>
<tr>
<td>2</td>
<td>(618.7±5.5)*</td>
<td>(2.2±0.0)*</td>
<td>(3400.0±10.0)*</td>
<td>(21.9±0.2)*</td>
</tr>
<tr>
<td>3</td>
<td>(542.4±2.3)*</td>
<td>(3.4±0.1)*</td>
<td>(2996.5±25.0)*</td>
<td>(21.0±0.5)*</td>
</tr>
<tr>
<td>4</td>
<td>(423.6±2.8)*</td>
<td>(1.4±0.2)*</td>
<td>(2647.9±29.3)*</td>
<td>(18.5±0.5)*</td>
</tr>
<tr>
<td>5</td>
<td>(255.3±4.4)*</td>
<td>(0.8±0.1)*</td>
<td>(2034.5±20.9)*</td>
<td>(14.5±0.8)*</td>
</tr>
<tr>
<td>6</td>
<td>(268.2±5.7)*</td>
<td>(0.9±0.0)*</td>
<td>(1717.7±7.1)*</td>
<td>(14.6±0.2)*</td>
</tr>
</tbody>
</table>

L=liqueur, A=apple, C=chokeberry, B=beetroot; TPC= total phenolic content. *Expressed as gallic acid equivalent per litre of liqueur. **Expressed as Trolox equivalent per litre of liqueur. Values marked with the same letter within the same column are not statistically different (α=0.05).
Table 6. Changes of colour intensity (CI) and hue (h) values during 6 months of storage of apple, chokeberry and beetroot pomace liqueurs at two different temperatures

<table>
<thead>
<tr>
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<th>t /month</th>
<th>CI</th>
<th></th>
<th></th>
<th>h</th>
<th></th>
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<td>LA</td>
<td>LC</td>
<td>LAC</td>
<td>LAB</td>
<td>LA</td>
<td>LC</td>
<td>LAC</td>
<td>LAB</td>
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<td>1.213h</td>
<td>0.391!j</td>
<td>2.784!e</td>
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<td>1.162d</td>
<td>0.732g</td>
<td>0.327l</td>
<td>3.118!g</td>
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</table>

L=liqueur, A=apple, C=chokeberry, B=beetroot. Values are arithmetic means (standard deviation values of triplicates were zero or negligible). Values marked with the same letter within the same column are not statistically different (α=0.05; Tukey HSD test)