Food Control 50 (2015) 714–721

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Evaluation of volatile compound and food additive contents in blackberry wine

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ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form
6 October 2014
Accepted 9 October 2014
Available online 18 October 2014

Keywords:
Blackberry wine
Volatile compounds
Food additive
GC-FID
HPLC

ABSTRACT

In addition to the biologically nutritious components present in wine, there can be substances that are harmful to human health. For this reason, the purpose of this study was to evaluate the contents of volatile compounds and food additives in blackberry wine samples produced from conventionally and organically grown blackberries and compare their concentrations with the maximum acceptable levels in wines for the first time. Volatile compounds were analyzed in 15 blackberry wines using a GC-FID method, while the contents of food additives were determined using a newly developed HPLC method. Both methods were validated for their linearity, sensitivity, repeatability, and intermediate precision. The major volatile compound in blackberry wine samples was ethanol (7.11–15.33%). The second most prevalent volatile compound found in all blackberry wine samples was methanol (174.2–641.4 mg/L), which is highly toxic. The amount of ethyl acetate in the investigated samples ranged from 53.8 to 188.4 mg/L, while propan-1-ol was found in three of the organic samples. Although isoamyl alcohol was found in all samples, the measured values ranged from 56.7 to 226.9 mg/L. The obtained results demonstrated that the concentrations of benzoic acid in the analyzed blackberry wines varied from 4.36 to 18.55 mg/L. The presence of sorbic acid has was detected in seven samples, and the values were in the range of 1.43–54.48 mg/L. Salicylic acid is a natural component of blackberry fruit, and the contents of this phytochemical in the studied wines were between 1.99 and 13.55 mg/L. The saccharin concentrations were detected in three samples, and the highest value was 31.51 mg/L. The concentrations of amyl alcohol, acesulfame, aspartame and caffeine were found to be below the quantitation limits of the method in the investigated blackberry wines. Furthermore, the obtained data provide evidence that the contents of methanol in blackberry wines were above the permissible limits, except for four conventional blackberry wines. Thus, the concentrations of analyzed volatile substances measured in blackberry wines necessitate more extended investigations of these volatile compounds in this popular fruit wine.

1. Introduction

In recent years, there has been intense interest in fruit wines that are produced from different types of fruit and an increased diversity of commercially available wines. Non-grape fruit wine is a complex mixture of water, alcohol, and other different components that are initially present in the fruit and formed during the fermentation process. Blackberry wine is a product of yeast fermentation of natural sugars present in blackberry juice. This popular fruit wine is usually served as a dessert wine and is enjoyed with meals in moderate quantities. Daily consumption of blackberry wine in recommended quantities (approximately 250 mL) can be a significant dietary source of essential minerals (Amidžić Klarić, Klarić, Velić, Vedrina Dragojević, 2011). Additionally, this non-grape fruit wine is of particular interest because of its high anthocyanin pigment and phenolic contents and its high antioxidant properties (Amidžić Klarić, Klarić, Mornar, 2011; Arozarena et al., 2012; Gao et al., 2012; Johnson & Gonzalez de Mejia, 2012; Ortiz, Marín-Arroyo, Noriega-Domínguez, Navarro, & Arozarena, 2013).

In addition to biologically nutritious components, substances that can be harmful to human health can also be found in wine.

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http://dx.doi.org/10.1016/j.foodcont.2014.10.013
0956-7135/

Today, the evaluation of wine quality is important for manufacturers, merchants and consumers. The gas chromatographic analysis of volatile compounds in blackberry wine is a very important tool that is useful for wine classification, quality control and sensory evaluation. The aroma of blackberry wine is produced by a complex balance of several volatile compounds, such as hydrocarbons, alcohols, terpene alcohols, esters, aldehydes, ketones, acids, ethers, lactones, and sulfurous and nitrogenous compounds. The composition of the volatile fraction of wine depends on factors such as blackberry variety, soil, climate, vintage year and winery procedures. Furthermore, wines are taxed, in large part, based on their alcohol levels (Rebolo et al., 2000; Zoecklein, Fugelsang, Gump, & Nury, 1995).

Additionally, food additives are widely used in the food industry, and their potential adverse effects on heath and consumer safety has been established. Food additives, the non-nutrient components of foods, are substances or mixtures of substances that are added to foods and involved in their production, processing, packaging and/or storage. Additives are used to increase: (i) the nutritional value of foods (vitamins, minerals, amino acids and their derivatives), (ii) the sensory properties (pigments, flavoring components or flavor enhancers), (iii) the shelf life of foods (antimicrobial additives and buffer additives). Additives or their degradation products generally remain in food, although in some cases they can be removed during processing. It is implicitly assumed that food additives and their degradation products should be non-toxic in their recommended doses during use. This equally applies to both acute and chronic toxicity, especially regarding potential teratogenic, mutagenic and carcinogenic effects. It is generally recommended that additives are only used when necessary for the sensory or nutritional value of foods and their production or handling. The use of additives is regulated by the Food and Drug Administration or Health and Welfare Administration in most countries. Legislation varies from country to country, but there is an ongoing effort to align based on toxicological studies and the requirements of modern food technology (Belitz, Grosch, & Schieberle, 2004).

In recent years, the production of blackberry wine has considerably increased in Croatia. This fruit wine is mostly produced from conventionally and organically grown blackberries because these two cultivation types are the most widespread. Although blackberries have a long history of consumption, and there have been numerous studies on their chemical composition and pharmacological activity (Elisia, Hua, Popovich, & Kittsa, 2007; Kafkas, Koşar, Türemiş, & Başer, 2006; Kaume, Howard, & Devareddy, 2012; Marinova & Ribarova, 2007; Wang & Xu, 2007), research studies related to this fruit wine are only in the initial stages, and there is no data available on its contents of volatile compounds and food additives. For this reason, the aims of this work were: (i) to evaluate the content of volatile compounds (amyl alcohol, ethanol, ethyl acetate, isoamyl alcohol, methanol and propan-1-ol) and food additives (acesulfame, aspartame, benzoic acid, caffeine, saccharin, salicylic acid and sorbic acid) in representative Croatian blackberry wine samples produced from conventionally and organically grown blackberries, (ii) to compare their concentrations with the maximum acceptable levels in wines, and (iii) to correlate the concentrations of all compounds.

2. Materials and methods

2.1. Blackberry wine samples

The contents of volatile compounds and food additives in blackberry wine were evaluated in 15 samples of blackberry wine originating from small Croatian producers. The samples were divided into two groups, conventional and organic, according to the blackberry cultivation method. The conventional group consisted of seven samples (CBW 1–CBW 7), while eight samples (OBW 8–OBW 15) were produced from organically grown blackberries that were cultivated according to the EU organic cultivation legislation (Commission Implementing Regulation (EU) No 203/2012; Commission Regulation (EC) No 889/2008).

2.2. Chemicals

Methanol, ethanol and ethyl acetate standards were purchased from Sigma–Aldrich Chemie GmbH (Germany), while propan-1-ol, amyl alcohol and isoamyl alcohol were from Riedel-de Haën (Seelze, Germany). Benzoic and sorbic acid were obtained from Supelco (Bellefonte, PA, USA). Aspartame, caffeine and saccharin were purchased from AcrosOrganics (Geel, Belgium), while standards of acesulfame and salicylic acid were purchased from Fluka (Buchs, Switzerland). Acetonitrile, ethanol, KH_2PO_4, K_2HPO_4 and H_3PO_4 were purchased from Sigma–Aldrich Chemie GmbH (Germany). All reagents used in this work were of analytical reagent grade or better. Double deionized water was used in all experiments.

2.3. Gas chromatographic quantification of volatile compounds in blackberry wines

Methanol, ethanol, ethyl acetate, propan-1-ol, amyl alcohol and isoamyl alcohol were analyzed in 15 blackberry wines using an Agilent GC-6850 gas chromatograph equipped with a flame ionization detector (FID). A bottle of each wine was opened just before analysis, and a sample with no pre-treatment was immediately injected in triplicate. If the concentration of analyte was above the linearity range of the proposed method, the samples were diluted with ultrapure water. The chromatographic conditions were as follows: column, HP-1 (30 m × 0.32 mm I.D., film thickness, 0.25 μm, Agilent Technologies, Santa Clara, USA); the injection volume was set at 1.0 μL, the split ratio was 1:5; and the temperature of the inlet was 230 °C. The column head pressure was 2.33 psi and the nitrogen carrier gas flow rate was 0.5 mL/min. The average nitrogen gas velocity was 10 cm/s. The oven temperature was held at 26 °C for 7 min, then increased from 26 °C to 50 °C at a rate of 1 °C/min, and then increased to a final temperature of 200 °C (rate of 15 °C/min) and held for 4 min isothermally. The FID temperature was 250 °C and the flow rate of nitrogen carrier gas was 25 mL/min. Stock standard solutions of the analytes (10%) were prepared in ultrapure water and stored at 4 °C under refrigeration. Furthermore, working solutions were prepared by dilution of the stock solutions with ultrapure water in the appropriate quantities.

2.4. High performance liquid chromatographic determination of food additives in blackberry wine

The contents of acesulfame, aspartame, benzoic acid, caffeine, saccharin, salicylic acid and sorbic acid were determined in fifteen samples of blackberry wine. All samples were filtered through Minisart RC4, 0.45 μm filters (Sartorius, Germany), which did not retain any of the analytes. The chromatographic system consisted of a Dionex (USA) HPLC Pump P680, an Automated Sample Injector ASI-100, a Thermostatted Column Compartment TCC-100 and a UVD170S Detector. The separation of compounds was completed on an Acclaim™ C18 column (4.6 × 250 mm, 5 μm, Dionex, USA) with suitable guard column. The mobile phase consisted of solvent A (acetonitrile, 12%) and solvent B (10 mM phosphate buffer (pH 5.0), 88%). The flow rate was kept at 1.2 mL/min with an isocratic mode and the column temperature was maintained at 25 °C. The total running time was 25 min for each sample with an injection