Food Chemistry 167 (2015) 387–395

Contents lists available at ScienceDirect

Food Chemistry

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

Technological aspects as the main impact on quality of quince liquors

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ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form 1 July 2014
Accepted 3 July 2014
Available online 11 July 2014

Keywords:
Maceracion
Antioxidant activity
Cydonia oblonga Miller
Flavan-3-ols
Hydroxycinnamic acids
Flavonols
LC–MS analysis
Polyphenols

ABSTRACT

Phytochemical profiles of 24 quince liquors were studied as the combination of technologically variant. Liquors were obtained after macerating quinces from three varieties (*Vranja*, *ALM3* and *ZM2*), with or without skin, at two ratios of quince:ethanol (50:50 and 25:75), and at two alcohol content (60% and 30%). Polyphenols were identified by LC-PDA-QTOF/MS and quantified by UPLC-PDA and UPLC-FL. A total of 18 polyphenolic compounds were identified and classified as 5 flavan-3-ols, 5 phenolic acids, and 8 flavonols. Flavan-3-ols were the most abundant group followed by hydroxycinnamates and flavonols. The highest contents of total polyphenols (~1000 mg/100 mL) and antioxidant activity (37.1 mmol Trolox/100 mL) were found in the liquors prepared using fruits with skin and 50:50 quince:ethanol ratio. The skin of quinces was the main source of phenolic acids and especially flavonols. The high antioxidant activity and polyphenolic content of quince liquor may be deemed as a promising new alcoholic beverage.

1. Introduction

Quince (*Cydonia oblonga* Miller) belongs to the Maloideae subfamily of the Rosaceae family, which includes commercially important fruits, such as apples and pears (Wojdyło, Oszmiański, & Bielicki, 2013). Quince fruits are mainly used in the manufacture of marmalade, jam, jelly, cakes, and liquors (Silva et al., 2002; Silva, Andrade, Gonçalves, et al., 2004; Silva, Andrade, Ferreres, et al., 2005; Silva, Andrade, Martins, et al., 2005; Silva, Andrade, Martins, Seabra, & Ferreira, 2006); the most popular quince product in Spain is a jam called "quince sweet". However, their consumption as fresh fruit is not too popular mainly due to the high and unpleasant intensities of sourness, bitterness, astringency and of woodiness in the most widely grown quince cultivars.

Quinces have received attention in the last ten years because of their high content in biologically active phytochemicals (proanthocyanidins, hydroxycinnamic acid etc.) and antioxidant capacity. Several studies have recently described antioxidant (Legua et al., 2013), antimicrobial (Fattouch et al., 2007; Silva & Oliveira, 2013), antiallergic (Shinomiya, Hamauzu, & Kawahara, 2009), antihemolytic (Costa et al., 2009), and antiproliferative (Márcia, Silva, Renata, Patrícia, & Andrade, 2010) properties of quince phenolics (Wojdyło, Oszmiański, & Bielicki, 2013). Besides, quince has low fat content and it is an important source of organic acids, sugars, crude fiber and minerals, such as potassium, phosphorous and calcium (Rodríguez-Guisado et al., 2009; Sharma, Joshi, & Rana, 2011; Shinomiya et al., 2009).

The polyphenolic compositions of quince fruits (Wojdyło, Oszmiański, & Bielicki, 2013; Silva et al., 2002), leaves (Oliveira et al., 2007), jams (Silva, Andrade, Seabra, & Ferreira, 2001; Wojdyło, Oszmiański, Teleszko, & Sokół-Łętowska, 2013), juice (Wojdyło, Teleszko, & Oszmiański, 2014) and jellies (Silva et al., 2000) have been properly studied. Besides, the effects of the manufacturing process on the composition and quality of final commercial products have been evaluated; for example, the effects of jam processing on the contents of phenolics, organic acids, and free-amino-acids were examined (Silva, Andrade, Valentão et al., 2004). However, there are no studies in the scientific literature dealing with detailed information on the polyphenolic profiles and antioxidant activity of quince liquors.

Herbs spirits have been made and consumed for centuries in different European countries, especially in cold regions. These liquors are generally prepared by macerating different aromatic herbs or/and fruits in fermented grape marc distillate, distilling

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http://dx.doi.org/10.1016/j.foodchem.2014.07.012
0308-8146/

the fermented grape marc in the presence of herbs, adding herbal extracts to the distilled alcohol, or combining some of these methodologies (Vázquez-Araújo, Rodríguez-Solana, Cortés-Diéguez, & Domínguez, 2013).

In Spain there are several Geographical Designation of Spirits, e.g. Spirits and Traditional Liquors from Galicia, GDSTL from Galicia (http://www.orujodegalicia.org) and from Alicante (http://www.licoresdealicante.com). The products protected by these Geographical Designations must fulfill some requirements; for instance, and among others: (i) the liquors should be prepare using aromatic herbs typical and endemic of specific regions, (ii) the liquors must have between 20% and 40% alcohol by volume (abv), and (iii) the liquors need to meet certain sensory characteristics: translucent and clean appearance, color between pale yellow to dark brown, intense, fine, delicate, tasty and complex aroma, with floral and balsamic notes, and reminding the herb notes. Some of these Geographical Designations are opening their spectrum of herbs and perhaps typical fruits could be included in a near future; for instance, the GDSTL of Galicia has very recently authorized the use of any kind of herb which is suitable for human consumption.

In this study, it was investigated how the varieties [one quince cultivar: *Vranja* and two quince clones: *ALM3* and *ZM2*] and technological aspects [(i) maceration in alcohol, (ii) use of fruits with or without skin, and (iii) the ratio of fruit to alcohol] affect the content of polyphenol compounds and antioxidant activity in quince liquors. The identities of polyphenolics were confirmed using LC–MS.

2. Materials and methods

2.1. Chemicals

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), acetic acid, phloroglucinol, and methanol were purchased from Sigma–Aldrich (Steinheim, Germany). (−)-Epicatechin, (+)-catechin, procyanidin B1, B2 and C, quercetin and kaempferol -3-*O*-glucoside, -3-*O*-galactoside and -3-*O*-rutinoside were purchased from Extrasynthese (Lyon, France). Chlorogenic acid (3-caffeoylquinic acid), neochlorogenic acid (5-caffeoylquinic acid), cryptochlorogenic acid (4-caffeoylquinic acid), and 3,5-dicaffeoylquinic acid were purchased from TRANS MIT GmbH (Giessen, Germany). Acetonitrile for UPLC (Gradient grade) and ascorbic acid were from Merck (Darmstadt, Germany). UPLC grade water, prepared by using an HLP SMART 1000s system (Hydrolab, Gdańsk, Poland), was additionally filtered through a 0.22 μm membrane filter immediately before use. Extra pure ethanol (96% abv, alcohol by volume) was from Scharlau, (Barcelona, Spain).

2.2. Quince fruits

One quince cultivar (*Vranja*) and two clones (*ALM3* and *ZM2*) were used in this research; all of them were appropriate for food manufacturing and widely grown in Spain. The selected plant materials belong to the quince gene bank located at the experimental field station of the Universidad Miguel Hernández de Elche (Orihuela, Alicante, Spain). Trees were planted at a spacing of 4 × 3 m. The experiment was established in a randomized block design with four single-tree replications and grafted onto quince *BA-29* rootstock. Fruits were harvested at commercial ripening stage (total soluble solids > 15 °Brix and maturity index > 30) at the end of September and first October 2012, and 20 homogeneous fruits (based on color, size and absence of defects) were selected from each variety (5 fruits from each tree) and used for manufacturing of liquors; about 6 kg of fresh quinces per each variety was used in this study.

2.3. Liquor manufacturing

Extra pure ethanol [96% abv (alcohol by volume)] was used for the manufacturing of the liquors. Ethanol was first adjusted to 60% abv for maceration with quince fruits. Three parameters were considered in this study: (i) quince cultivar/clone, (ii) fruits with or without skin, and (iii) ratio quince:ethanol (60% abv). As previously mentioned, 3 quince cultivars/clones were evaluated: *Vranja*, *ALM3*, and *ZM2*. Half of the quince fruits were manually peeled, and maceration was conducted with whole or peeled fruits. Finally, two different quince:ethanol ratios were used: (i) 50:50 and (ii) 25:50. A total of 3 L of each quince liquor was macerated during 3 months at approximately 20 °C and in darkness, using 3 different 1 L glass jars (3 replications), and avoiding leaving headspace in the jars. After appropriate maceration period (3 months), the alcohol percentage was adjusted to 30% abv by proper addition of sucrose and distillate water; this alcohol level was selected to obtain products with similar characteristics to Spanish commercial liquors.

2.4. Identification of polyphenols by the LC-PDA-MS method

Identification and quantification of polyphenols from quince liquors was carried out using an Acquity ultraperformance LC system equipped with a photodiode detector (PDA; UPLC) with binary solvent manager (Waters Corp., Milford, MA, USA) series with a mass detector G2 QTOF Micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative modes. Separation of polyphenols was carried out using a UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm; Waters Corp., Milford, MA, USA) at 30 °C.

Samples (5 μL) were injected, and elution was completed within 15 min using a sequence of elution modes: linear gradients and isocratic. The flow rate was 0.45 mL/min. The mobile phase was composed of solvent A (4.5% formic acid) and solvent B (100% of acetonitrile). The program began with isocratic elution with 99% A (0–1 min), and then a linear gradient was used until 12 min, lowering A to 0%; from 12.5 to 13.5 min, returned to the initial composition (99% A); and then held constant to re-equilibrate the column. Analysis was carried out using full scan, data-dependent MS scanning from *m*/*z* 100 to 1000. The mass tolerance was 0.001 Da, and the resolution was 5.000. Leucine enkephalin was used as the mass reference compound at a concentration of 500 pg/μL at a flow rate of 2 μL/min, and the $[M-H]^-$ ion at 554.2615 Da was detected over 15 min of analysis during ESI-MS accurate mass experiments, which was permanently introduced via the LockSpray channel using a Hamilton pump. The lock mass correction was ±1.000 for Mass Window. The mass spectrometer was operated in a negative ion mode and set to the base peak intensity (BPI) chromatograms and scaled to 12,400 counts per second (cps) (=100%). The optimized MS conditions were as follows: capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 100 °C, desolvation temperature of 300 °C, and desolvation gas (nitrogen) flow rate of 300 L/h. Collision-induced fragmentation experiments were performed using argon as collision gas, with voltage ramping cycles from 0.3 to 2 V. The characterization of the single components was carried out via the retention time and the accurate molecular masses. Hydroxycinnamic acid, flavan-3-ols and flavonols compound were optimized to their estimated molecular masses $[M-H]^-$ in the negative mode before and after fragmentation. The data obtained from LC–MS were subsequently entered into MassLynx 4.0 ChromaLynx Application Manager software. On the basis of these data, the software is able to scan different samples for the characterized substances.