Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 100–105
Food Chemistry
www.elsevier.com/locate/foodchem

How much do cultivar and preparation time influence on phenolics content in walnut liqueur?

Jerneja Jakopic *, Mateja Colaric, Robert Veberic, Metka Hudina, Anita Solar, Franci Stampar

University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Chair for Fruit Growing, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Received 29 March 2006; received in revised form 14 September 2006; accepted 6 November 2006

Abstract

The influence of cultivar and picking date on the phenolic content of walnut liqueur was investigated using HPLC with a PDA detector. Ten phenolic compounds, namely gallic, protocatechuic, ellagic, chlorogenic (5-caffeoylquinic), syringic, *p*-coumaric and sinapic acids, as well as (+)-catechin, 1,4-naphthoquinone and juglone were detected. The walnut liqueur under analysis was made of the cultivars 'Franquette' and 'Elit', on two sampling dates (June 30th and July 7th). A close interaction between cultivar and sampling date was noticed for most of the phenolics analyzed. The content levels of the main phenolic compounds under investigation were highest in 'Franquette' at the end of June and lowest in 'Elit' on the second or both sampling dates, except for syringic acid. A strong influence of cultivar choice and picking date was observed. The content levels of most phenolics were higher in liqueur prepared from the cultivar 'Franquette', than in 'Elit'.

Keywords: Walnut liqueur; Walnut; *Juglans regia* L.; Phenolic compounds; Cultivar; Green walnut fruits; Picking time

1. Introduction

Walnut is a traditionally important and widely spread deciduous tree in all parts of Slovenia (Solar, Ivancic, Stampar, & Hudina, 2002). Its ripe fruit have been used for fresh consumption and in confectionery, while unripe fruit serve to make liqueur. For many years, green unripe walnuts have been picked just before hardening of the endocarp, then sliced and steeped in alcohol; thus the delicious beverage was made. In Italy, a similar alcoholic drink from green walnuts, called *nocino*, is prepared (Alamprese & Pompei, 2005; Alamprese, Pompei, & Scaramuzzi, 2005).

Walnut liqueur, a dark brown, bitter and tasteful beverage, is often served as an aperitif or sometimes taken to treat stomach ache. The astringency and bitterness of foods and beverages depend on their contents of phenolic compounds (Bravo, 1998). Therefore, the bitter taste of walnut liqueur could also be due to the phenolic content, since Stampar, Solar, Hudina, Veberic, and Colaric (2006) reported that the liqueur is a cocktail of phenolics.

Several studies have been carried out to determine total phenol content. Nuts serve as a good source of phenolics with a high antioxidant potential, especially walnuts, pistachios, pecans, almonds with hulls, hazelnuts and peanuts (Kornsteiner, Wagner, & Elmadfa, 2006). Experimental investigations reported by Anderson et al. (2001) support this assumption. Kornsteiner et al. (2006) have analyzed total phenol and total tocopherol content in 10 types of nuts. They have found that both total phenol and total tocopherol content were highest in walnuts, pistachios and pecans. Likewise, Gunduc and El (2003) reported that walnut kernels had the highest total phenol concentration and the highest antioxidant ability among 25 types of commonly consumed foods.

Although phenolic compounds have no known nutritional function, they may be important for human health. It has been reported that phenolic compounds have

* Corresponding author. Fax: +386 1 423 10 88.
E-mail address: jerneja.jakopic@bf.uni-lj.si (J. Jakopic).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.11.008

antioxidant, antimutagenic, and free radical-scavenging properties (Bravo, 1998). The phenolic compounds (such as caffeic, ellagic and ferulic acid) also exhibit anticarcinogenic activity and inhibit atherosclerosis (Craig, 1999); (+)-catechin delays oxidation of human plasma and some phenolic compounds, such as *p*-coumaric acid, inhibit LDL oxidation (Lee, Koo, & Min, 2004).

The levels of phenolic compounds are influenced by various factors. They greatly depend on light, they are influenced by genetic factors, environmental conditions and storage, and they vary greatly, even between cultivars of the same species (Bravo, 1998). Colaric, Veberic, Solar, Hudina, and Stampar (2005) have determined the levels of nine phenolic compounds in ripe walnut fruit. They observed considerable differences in phenolic content among 10 walnut cultivars. The influence of cultivar was also confirmed in the phenolic content of apples (Veberic et al., 2005) and in the genus *Prunus* (Veberic & Stampar, 2005). In previous studies, the influence of maturation degree on the phenolic content was proved. Stampar et al. (2006) have studied certain phenolics in green walnut husks, Jay-Allemand et al. (2001) in the young leaves of the walnut and Solar, Colaric, Usenik, and Stampar (2006) in annual walnut shoots.

It has been mentioned that both cultivar and maturation stage of the walnut influence the phenolic content in various parts of the walnut. But how much do they influence the level of certain phenolic compounds in traditionally prepared walnut liqueur? The aim of our study was to answer this question.

2. Materials and methods

2.1. Preparation of samples

Green walnuts from the cultivars 'Franquette' and 'Elit' were picked in the experimental orchard in Maribor (Slovenia) on two sampling dates: June 30th (1st sampling) and July 7th (2nd sampling). Walnut liqueur was prepared according to the traditional method. Six hundred grams of fruits were cut into pieces, put into a glass jar, immersed in 1 l of 40% food-grade ethanol (see also Alamprese et al., 2005) and left to steep for three weeks.

After this time, the liqueur was filtered, diluted with methanol in the ratio liqueur: methanol 1:4 (v/v), and filtered though a 45 μm polyamide filter Chromafil® AO-45/25 (Machery-Nagel) prior to injection into the HPLC system. Four replications were made for each cultivar on each sampling date.

2.2. HPLC analyses

Certain phenolics were detected by the Thermo Finningan Surveyor HPLC system with a photodiode array PDA detector, scanning spectra of wavelength in the range 220–380 nm. Separations were carried out using a Chromsep HPLC Column SS (250 × 4.6 mm, Hypersil 5 ODS), coupled with a Chromsep guard column SS (10 × 3 mm) from Crompack. The system was controlled by the CromQuest™ 4.0 Chromatography workstation software system.

The chromatographic conditions followed the method described by Schieber, Keller, and Carle (2001). The injection volume of a sample was 20 μl, and the flow rate was 1.0 ml per min. The column temperature was 25 °C. Solvent A was 2% acetic acid in bidistilled water, and solvent B was 0.5% acetic acid in bidistilled water and acetonitrile (ratio 1:1, v/v). The gradient used began with 90% of solvent A and introduced a gradient to obtain 45% A at 50 min, 0% at 60 min and again 90% of solvent A at 65 min. The total run time was 65 min, with 15 min of equilibration treatment (90% A) performed between each analysis.

Phenolic compounds were detected at a wavelength of 280 nm. A specimen chromatogram is shown in Fig. 1. The identification of phenolics was achieved through the following: comparison of the retention times of standard solutions with the retention times of compounds in samples, absorption maxima of compounds in the scanned spectrum, and the addition of standards to samples.

The concentrations of certain phenolic compounds were calculated with the help of a corresponding external standard, based on the comparison of peak areas from the samples with those of the standard solution.

2.3. Chemicals

The ethanol used to prepare the liqueur was food-grade 96% (from Merck) diluted with bidistilled water.

The following standards were used to determinate the phenolic compounds: gallic, syringic and protocatechuic acids, 1,4-naphthoquinone and juglone from Merck; ellagic, chlorogenic (5-caffeoylquinic) and sinapic acids from Fluka; and (+)-catechin from Roth.

The chemicals for mobile phases were acetonitrile and methanol from Sigma–Aldrich, and acetic acid from Merck.

The water used in sample preparation, solutions and analyses was bidistilled and purified with a Milli-Q water purification system.

2.4. Statistical evaluation

The results were statistically analyzed with the programme Statgraphics Plus for Windows 4.0, using one-way analysis of variance (ANOVA). The differences in the phenolic contents were estimated with the *t*-test or Duncan's test. *P*-values of less than 0.05 were considered as statistically significant.

3. Results and discussion

In the liqueur prepared from green walnut fruit, the following phenols were detected: gallic, syringic, ellagic, protocatechuic, *p*-coumaric, chlorogenic and sinapic acids, as