Phenolic Characterization of Some Hazelnut Cultivars from Different European Germplasm Collections

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Abstract

Some main national varieties or endangered old cultivars from six countries (13 from France, 12 from Greece, 10 from Italy, 6 from Portugal, 8 from Spain and 8 from Slovenia) were analysed for phenolic composition within the framework of the SAFENUT (Safeguard of hazelnut and almond genetic resources: from traditional uses to novel agroindustry opportunities) European project. Using a high-performance liquid chromatograph (HPLC) with a diode array (PDA) detector, the content of phenolic compounds was analyzed in extracts from the kernels. Gallic, chlorogenic, sinapic, p-coumaric, ferulic and ellagic acid, as well as epicatechin and routine, were individually identified in all tested cultivars. Large variations of phenolic contents were noted between cultivars. Intercultivar variability was the most evident in sinapic, ellagic and p-coumaric acid, as well as in routine. The cultivar 'Casina' from France had the highest sum (251.01 mg/kg) of all the identified phenolics. In contrast, the Greek cultivar 'Polycarpos' was the poorest with phenolic compounds et al (49,16 mg/kg). Ellagic acid, one of the most important polyphenolic antioxidants, appeared in concentrations between 0.56 mg/kg ('Polycarpos', Greece) and 18.55 mg/kg ('Porpurea', Portugal). On the chromatograms, two additional phenolic compounds were found, expressed as the equivalent of chlorogenic acid. They were the most abundant phenolics in all the studied cultivars. An improved method, MS-HPLC will be used next year with the aim of identifying them individually.

INTRODUCTION

Hazelnut kernels are an important source of several health-promoting bioactive and nutraceutical compounds, such as unsaturated fatty acids, proteins, carbohydrate, dietary fibre, minerals, vitamins, phytosterols, and phenolics. Due to their chemical and nutritional composition, hazelnuts are recognized as beneficial to human health (US FDA, 2003).

During the disease prevention and health promotion, an important role is played by phenolic compounds that have antioxidant and antiradical potential (Sang et al., 2002; Fukuda et al., 2003; Goli et al., 2005; Wijeratne et al., 2006), as well as anticancerogenic function (Surh, 2003, Shahidi, 2005). They are able to reduce the risk of cardiovascular diseases (Ness and Powles, 1997; Jiang et al., 2006). They are also known as antimicrobial compounds (Puupponen-Pimiä et al., 2001; Sousa et al., 2006; Pereira et al., 2007).

Total phenolic contents have already been reported for hazelnut fruits (Kornsteiner et al., 2006; Cristofori et al., 2008; Oliveira et al., 2008), as well as in green involucrum (Alasalvar et al., 2006), and lignified shells (Stévigny et al., 2007). Individual phenolic compounds have been described in hazelnut leaves (Amaral et al., 2005; Shahidi et al., 2007; Oliveira et al., 2007), hard shell, green involucrum (Shahidi et al., 2007), and buds (Peev et al., 2007). However, only a few previous studies have been conducted in which individual phenolics were determined in edible hazelnut kernels (Senter et al., 1983; Alasalvar et al., 2006; Shahidi et al., 2007). In addition, data on the extent of phenolics' biodiversity in the germplasm pool has been unavailable until now.

The objective of the present study is to determine individual phenolic compounds in hazelnut fruits of numerous cultivars originated in six European countries, and to contribute to their chemical characterization.

MATERIAL AND METHODS

Plant Material

13 cultivars from France (F), 12 from Greece (G), 10 from Italy (I), 6 from Portugal (P), 8 from Spain (S), and 8 from Slovenia (SI) were analysed. Most of them are the main national varieties, new varieties or some endangered old cultivars. 40 inshell nuts per variety, dried according standard procedure with 12 % of humidity were collected at the Biotechnical Faculty in Dec 2007. Before being analysed, they were stored in cold room, at app. 10^{o}C.

Chemical Analysis

1. Sample preparation. Nuts were cracked by hand. Whole nuts (kernel + pellicle) were ground into fine powder. 1 g of the sample was measured into a test tube. The sample was extracted with 10 ml methanol, containing 1 % BHT in ultrasonic bath for 60 minutes. Then the samples were centrifuged for 7 minutes at 10 000 rpm. The supernatant was filtered through polyamide filter Chromafil AO-45/25, transferred into vial, prior to analyses.

2. Determination of polyphenolics' content. The content of individual phenolics in extracts was analyzed on the Thermo Finningan Surveyor HPLC system equipped with photodiode array detector (PDA detector). A Gemini 3µm C18 column (150 x 4.6 mm, Phenomenex) protected with a security guard column was used. The system was controlled using the ChromQuest^{TM} 4.0 Chromatography workstation software system. The chromatographic conditions (mobile phase, gradient program, temperature of column) were similar to those described by Marks et al (2007).

Chromatograms were observed at 280 and 350 nm. Identification of individual phenolic compound was qualitatively achieved using a method of external standards and quantitatively comparing peak area on chromatograms of samples with those of diluted standard solutions.

Data Analysis

The differences among the cultivars, phenolic compounds, and origin were evaluated with multifactor ANOVA using Statistica 8 (StatSoft, Inc. 1984-2008) software.

RESULTS AND DISCUSSION

Phenolic Profile

Six phenolic acids, i.e. gallic, chlorogenic, sinapic, p-coumaric, ferulic and ellagic acid, and two flavonoids (epicatechin and routine) were individually identified in hazelnut kernels of all cultivars tested. Gallic, p-coumaric, ferulic and sinapic acid have already been identified in 'Tombul' kernels together with their pellicles, and green involucrum (Alasalvar et al., 2006), as well as in nut shell, kernel pellicle and leaves (Shahidi et al., 2007). Considering the origin of the 'Tombul' variety was Turkey, not Greece as in other study, and the extraction method used by Alasalvar et al. (2006), and Shahidi et al. (2007) was different than ours, the concentrations of identified phenolic acids could not be compared. This is in agreement with Yurttas et al. (2000) and Stévigny et al. (2007) who reported that a variety and extraction exerted a great deal of influence on the concentration and variability of phenolic acids present.

Additionally, two interesting and distinctive peaks were expressed on the chromatograms. Using the listed standards, it was impossible to determine compounds that belong to those two peaks. They were called a and b and they were expressed as equivalent of chlorogenic acid. With the aim to identify them individually, an improved method, MS-HPLC will be used next year.

Intercultivar Variations of Phenolics

The highest sum (251.01 mg/kg) of all identified phenolics (SP) was determined in 'Casina'/F. Close to it, was 'Longue d'Espagne'/F with 212.71 mg/kg. In thirteen cultivars, the SP exceeded 150 mg/kg, thirty cultivars had between 100 and 150 mg/kg of SP, in 15 cultivars the SP reached between 50 and 100 mg/kg. With 49,16 mg/kg, cultivar 'Polycarpos'/G was the poorest with phenolic compounds et al. P-coumaric acid was the less abundant compound (mean value for all cultivars was 0.30 mg/kg). It was followed by sinapic acid with 1.65 mg/kg average. Ellagic acid which is one of the most important polyphenols with antioxidant and cancer chemopreventive activities (Cerda, 2005), appeared in concentrations between 0.56 mg/kg ('Polycarpos'/G) and 18.55 mg/kg ('Porpurea'/P.

Intercultivar variability was the more expressed in sinapic, ellagic and p-coumaric acid as well as in routine. The contents of gallic acid were the most homogenous and varied between 5.13 and 3.77 mg/kg. Non-identified phenolics a and b were the most abundant in all studied cultivars (mean value for 'a' was 38.95 mg/kg, and for 'b' was 39.74 mg/kg). In the case of compound 'b', the highest concentration (120.02 mg/kg) was detected in the nuts of 'Casina'/F. Phenolic compound 'a' reaching the maximum concentration (100.03 mg/kg) in 'Castelo de Paiva 9'/P.

Phenolic Contents in Relation to the Origin of the Cultivars

In the cultivars from French germplasm collection, the SP ranged from 76.31 mg/kg in 'Segorbe' to 251.01 mg/kg in 'Casina' (Fig. 1). Among Greek cultivars, the lowest SP was measured in 'Polycarpos' (Drama) (49.16 mg/kg), and the highest one in 'Karydato (Katerini)' (195.4 mg/kg). Portugal cultivars varied between 65.05 mg/100 g ('Comum'), and 189.43 mg/kg ('Castelo de Paiva 9'), and Italian between 66.31 mg/100 g ('TGDL' clone PD6), and 191.98 mg/kg ('Camponica'). Spanish cultivars expressed the lowest variability with minimum of 80.26 mg/kg ('Negret'), and maximum of 124.27 mg/kg ('Trenet'). In the cultivars of Slovene origin, the SP ranged from 105.10 mg/kg ('Istrska dolgoplodna leska') to 159.04 mg/kg ('BF-66').

CONCLUSIONS

In the study, no qualitative variations (presence/absence) among cultivars were found. On the other hand, the quantitative variability (concentrations of phenolic compounds related to the cultivars and their origin) was strong. Based on different polyphenolic contents, it can be concluded that the tested cultivars vary in their antioxidant capacity, too. Consequently, they also vary in their beneficial impacts on human health. The antioxidant activity of the kernels in different cultivars will be determined over the following two years.

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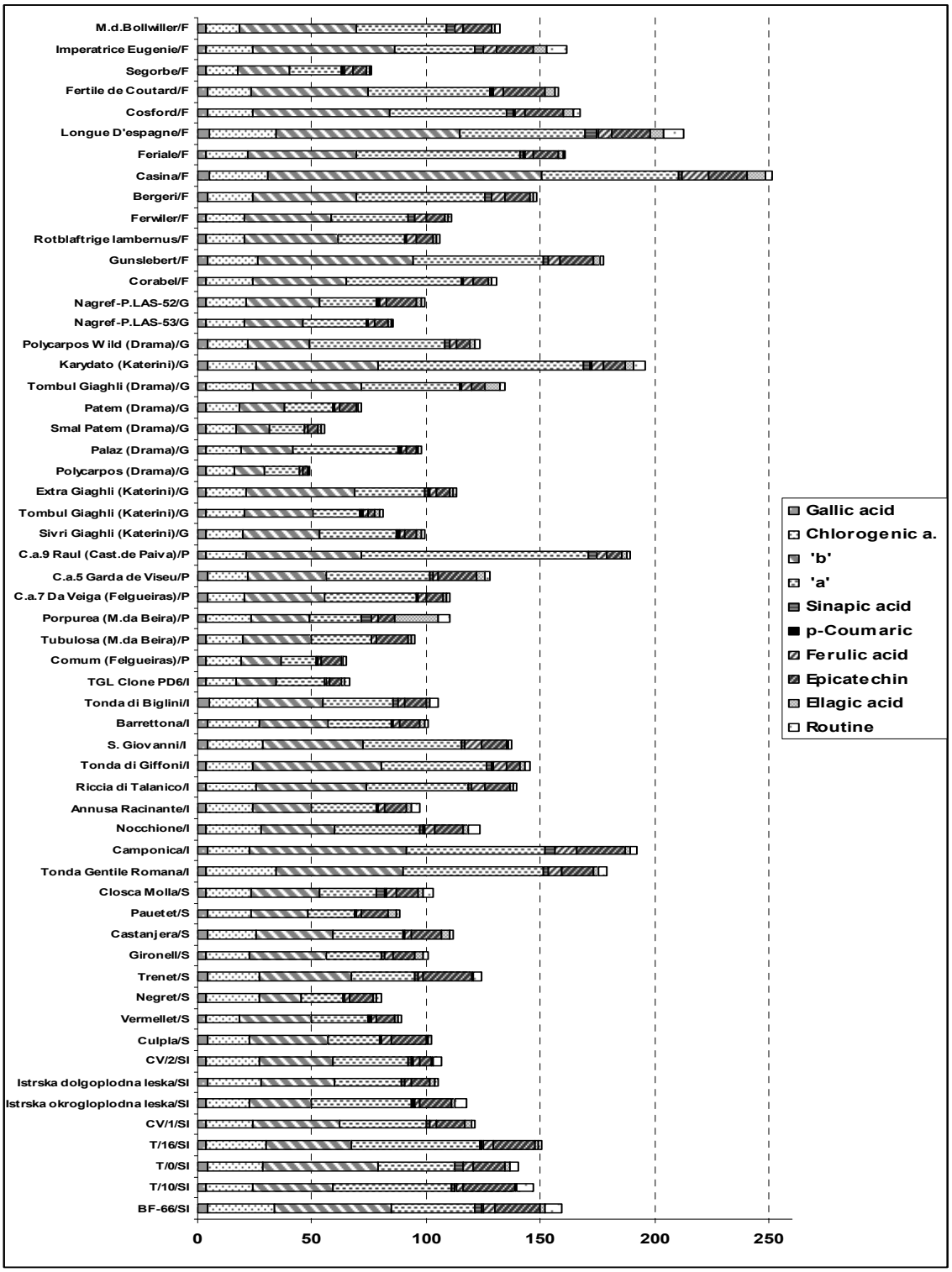

Fig. 1. The content (mg/kg) of ten phenolics in kernels of different hazelnut cultivars.