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1. Landscape of the Monti Cimini hazelnut area (courtesy of S. Gasbarra).
2. Hazelnut in vitro propagation (courtesy of L. Bacchetta).
3. Typical hazelnut orchard in Viterbo area (courtesy of V. Cristofori).
4. Hazelnut harvesting machines (coutesy of D. Monarca).
5. Hazelnut dieback (courtesy of L. Varvaro).
6. Industry hazelnut products (Courtesy of Stelliferi & Itavex Spa).

Identification of Soluble Phenolic Acids in Hazelnut (*Corylus avellana* L.) Kernel

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Keywords: phenolic, phenolic acids, hazelnut, kernel quality, HPLC

Abstract

Phenolic acids are a subclass of a large category of compounds commonly referred to as "phenolics". They are a very important group of secondary plant metabolites whose roles are still unknown. Due to their antioxidant behaviour and the potential health benefits associated with these simple phenolic compounds, many authors have proposed different techniques to extract these compounds from vegetable foods. Therefore, the aim of this work is to compare the experimental conditions commonly used to detect soluble phenolic acids (both free and esterified) in order to investigate the phenolic constituents in hazelnut kernel extracts. Phenolic compounds present in defatted samples were extracted using different solvent mixtures under reflux conditions at different temperatures; afterwards, the extraction and hydrolysis of phenolic acids was performed. HPLC analysis of the extracts obtained highlighted the presence of twelve phenolic acids. The main compounds identified were gallic acid, caffeic acid, *p*-cumaric acid, ferulic acid and sinapic acid. In all extracts, gallic acid was the most abundant, in both the free and esterified form. Ethanol solution (80% v/v) at 80°C was the most effective solvent for the quantitative extraction of benzoic acid derivatives, but extract obtained with acetone solution (80% v/v) at 50°C showed the highest number of identified phenolic acids. All the other suggested methods showed low extraction capacity for these compounds. Using these optimized methods, new research is in progress to define the effect of storage and roasting on these compounds.

INTRODUCTION

A diet rich in vegetables and fruit is commonly associated with lower risk for chronic degenerative diseases such as neuro-degenerative diseases, heart disease, cancer and so forth, so it has been assumed that dietary antioxidant may explain this protective effect. Antioxidants are compounds that act as free radical scavengers. In this context, plant-derived products (edible and non-edible) contain a large variety of phytochemicals that possess antioxidant and antiradical activities, anti-carcinogenic and anti-mutagenic effects and anti-proliferative potential. Phenolic acids are a subclass of these compounds commonly referred to as "phenolics". They are a very important group of secondary plant metabolites whose roles are still unknown (Robbins, 2003). Due to their antioxidant behaviour and the potential health benefits associated with these simple phenolic compounds, many authors have proposed different techniques to extract these compounds from vegetable foods. Therefore, the aim of this work is to compare the experimental conditions commonly used to detect soluble phenolic acids (both free and esterified) in order to investigate the phenolic constituents in hazelnut kernel extracts.

MATERIALS AND METHODS

Samples of 'Tonda Gentile delle Langhe' (TGL) hazelnut cultivar of (*Corylus avellana* L.) that had been harvested in 2006 were bought from a local manufacturer in Cortemilia (Cuneo, Italy). Shelled kernels, packed in vacuum bags, were kept in a storeroom at 4°C until the preparation of extracts.

Raw kernels were ground in a blender and then defatted with hexane in a rotary shaker at room temperature (1:10, w/v, 3×5 min). Preparation of extracts of defatted hazelnut raw kernel was performed in accordance with the methods described by Alasalvar et al. (2006) and Shahidi et al. (2007). Moreover, an extraction system at room temperature with two different solvents (ethanol/water and acetone/water mixture 80:20 v/v) was assessed (Fig. 1). Extraction and hydrolysis of phenolic acids were performed as shown in Figure 2. The identification of phenolic acids in both free and esterified fractions was conducted using a Thermo HPLC-DAD system. Separation was achieved with a Lychrosphere C18 column. The analyses were performed in triplicate.

RESULTS AND DISCUSSION

HPLC analysis of the extracts obtained highlighted the presence of twelve phenolic acids (Table 1). Unlike data previously reported (Alasalvar et al., 2006), free phenolic acids were identified. The main compounds identified were gallic acid (a hydroxylated derivative of benzoic acid), caffeic acid, *p*-cumaric acid, ferulic acid and sinapic acid (cinnamic acid derivatives). The third extraction procedure enabled the identification of cinnamic acid derivatives, while in the extract with ethanol at 80°C and 50°C some hydroxylated benzoic acids were identified. For the hydrolyzed fraction, the third extraction condition was in accordance with the method described by Alasalvar et al. (2006).

The performance of the different extraction methods was evaluated quantitatively (Table 2). In all extracts, gallic acid was most abundant, in both free and esterified forms. Ethanol solution (80% v/v) at 80°C was the most effective solvent for the quantitative extraction of benzoic acid derivatives, but the extract obtained with acetone solution (80% v/v) at 50°C showed the highest number of identified phenolic acids. All the other suggested methods showed low extraction capacity for these compounds.

Using these optimized methods, new research is in progress to define the effect of storage and roasting on these compounds.

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Tables

Table 1. Free and esterified phenolic acids identified in the hazelnut extracts.

	Extracts	Extraction condictions	Phenolic acids
Free phenolic acids	A	Ethanol 80% - 80°C	gallic, 2,4-dihydroxybenzoic, 3,4-dihydroxyibenzoic, 3-hydroxyicinnamic
	B	Ethanol 80% - 50°C	gallic, 2,4-dihydroxybenzoic, 3,4-dihydroxybenzoic, 2-hydroxybenzoic
	C	Acetone 80% - 50°C	gallic, siringic, sinapic, ferulic, 3-hydroxy-4-methoxycinnamic
	D	Ethanol 80% - room temperature	nd
	E	Acetone 80% - room temperature	gallic, 4-hydroxybenzoic, 2-hydroxybenzoic
Esterified phenolic acids	A	Ethanol 80% - 80°C	vanillic, sinapic, 3-hydroxycinnamic
	B	Ethanol 80% - 50°C	caffeic, sinapic, 2,4-dihydroxybenzoic, 3-hydroxycinnamic
	C	Acetone 80% - 50°C	gallic, sinapic, 3-hydroxycinnamic
	D	Ethanol 80% - room temperature	gallic, vanillic, siringic, 4-hydroxybenzoic, 3-hydroxycinnamic
	E	Acetone 80% - room temperature	gallic

Table 2. Content of free and esterified phenolic acids identified in the hazelnut extracts.

		Phenolic acids (μg/g)				
	Extracts	gallic	caffeic	*p*-cumaric	ferulic	sinapic
Free phenolic acids	A	34,50	-	5,14	-	-
	B	7,40	-	-	-	-
	C	11,40	-	-	8,10	9,09
	D	-	-	-	-	-
	E	4,53	-	-	-	-
Esterified phenolic acids	A	-	-	3,44	-	3,87
	B	-	2,18	3,71	-	7,70
	C	5,17	-	2,32	-	4,98
	D	7,15	-	2,80	-	-
	E	18,40	-	-	-	-

Figures

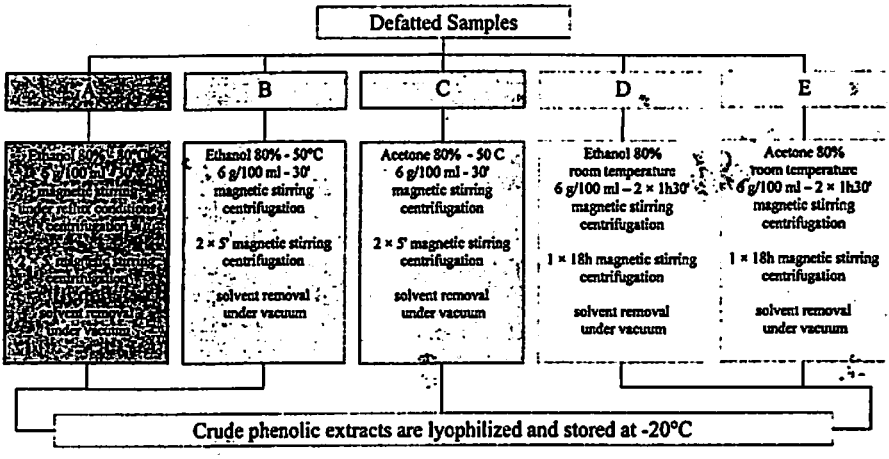

Fig. 1. Methods used for phenolics extraction from hazelnuts.

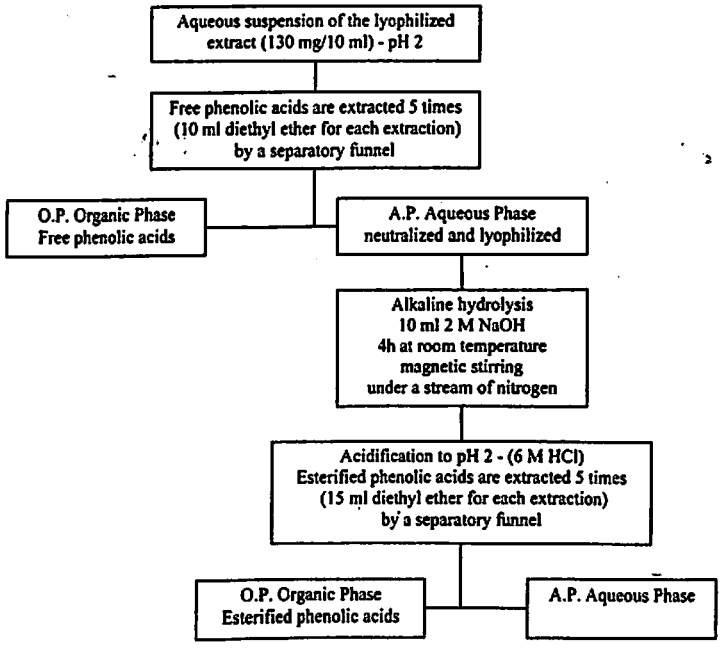

Fig. 2. Procedure for the extraction, hydrolysis and separation of free and esterified phenolic acids of hazelnuts.