Optimization of extraction of phenolic content from hazelnut shell using response surface methodology

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Abstract: Response surface methodology was applied to predict the optimum conditions for extraction of phenolic compounds in hazelnut shell. The phenolic content in the shell extract was determined spectrophotometrically according to the Folin-Ciocalteu method and expressed as gallic acid equivalent (mg GAE g^{-1}). Two central composite designs were used to investigate the effects of two independent variables, namely solvent composition (%) and extraction time (min), on phenolic extraction. In a first series of repeated batch extractions, the solvent consisted of different methanol percentages in distilled water at pH 4, while in a second series methanol was substituted by ethanol. The highest phenolic content (6.67 mg GAE g^{-1} of shell) was predicted at the extraction conditions of 55.7% ethanol and 108.7 min. These best conditions, obtained and applied to 13 different cultivars, showed values varying from 9.18 mg GAE g^{-1} of shell for Barcelona to 3.00 mg GAE g^{-1} of shell for Tonda di Giffoni.

Keywords: *Corylus avellana* L.; by-product valorization; hazelnut shell; phenolic extraction; CCD; RSM

INTRODUCTION

Agricultural and industrial residues are an attractive source of natural antioxidants, such as phenolic compounds.[1,2] Today there is strong evidence that polyphenols play a role in the prevention of age-related diseases, including cardiovascular disease and cancer. The action of polyphenols on health is also to protect against environmental stresses.[3] Numerous methods are used to evaluate and estimate the antioxidant capacity *in vitro*[1,4] and *in vivo*[1,5] of polyphenols inside food.

Nuts are an important source of by-products and the antioxidant activity of shell and skin of diverse nuts such as pistachio,[6] almond[7,8] and peanut[9,10] have been studied as sources of phenolic compounds.

Among edible nuts, hazelnuts represent one of the most cultivated. During the period 2000–2004 the world production of in-shell hazelnuts (*Corylus avellana* L.) was about 759 000 t.[11] Turkey is the world's largest hazelnut producer (70%), followed by Italy (13%). The in-shell consumption of hazelnuts accounts for only 10%; the rest is shelled and mainly used for industrial purposes.[12] Hard nutshells represent one of the major by-products from hazelnut industrial processing and signify a huge amount of discarded material available at very low cost. Nowadays, hazelnut shell is mostly used as fuel for burning, mulching and as raw material for production for furfural in the dye industry.[13]

Some publications have dealt with the antioxidant activity and phenolic constituents of hazelnut kernel,[14] its brown skin or testa,[15,16] its green leafy cover[17] and its hard shell.[18]

Owing to the complex nature of the phytochemicals present, there is not a single universal method or extraction solvent system for fruit or vegetable phenolic material.[1]

Therefore, the purpose of this work was to investigate the phenolic content of the hard hazelnut shell and to find an accurate extraction. The phenolic content was determined according to the Folin–Ciocalteu method. Central composite design (CCD) was used to investigate the effects of two independent variables, namely solvent composition (%) and extraction time (min), on phenolic content. Two solvents, methanol and ethanol, in different proportions in acidified water were tested. Response surface methodology (RSM) was then applied to predict optimum conditions for a standardized and significant extraction of phenolics. Furthermore, to study the phenolic content among different cultivars, the optimum conditions predicted by RSM were applied to diverse hazelnut varieties.

MATERIALS AND METHODS

Samples

Nuts of Tonda Gentile delle Langhe (TGL) hazelnut variety (*Corylus avellana* L.) were used to determine

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the experimental conditions for the phenolic extraction method. The optimum extraction method was then applied to 13 hazelnut varieties: Barcelona, Butler, Casina, Culplà, Daria, Ennis, Merveille de Bollwiller (MB), Pauetet, Ribet, Royal, Tonda Gentile delle Langhe (TGL), Tonda Gentile Romana (TR) and Tonda di Giffoni (TG). In the harvest of 2004, nuts of three plants of each variety were individually collected from an experimental orchard with a completely randomized design was located in Cravanzana at 550 m above sea level (Cuneo district, northwest Italy).

The nuts were manually harvested directly from the ground when the natural drop reached 80–90% and were then sun dried until they reached an in-shell moisture content of 6–8% w.b. The moisture content of nutshell samples was determined according to the AOAC 925.40 method.[19] Samples were kept at 20–22 °C until analysis and hand-shelled just prior to the extraction procedure.

Chemicals

All chemicals and solvents were of analytical grade and obtained from Sigma-Aldrich (Milan, Italy).

Extraction protocol

The hazelnut shells were ground using a mill (Tecator mill, Hoganas, Sweden) and passed through a sieve to select particles smaller than 0.5 mm. Each shell powder sample (0.5 g) was macerated with 5 mL of extraction solvents in a capped glass tube on an agitating plate (Asal s.r.l., Milan, Italy) at a constant stirring rate (100 rpm) at 20–22 °C. The glass tube was wrapped in aluminum foil to prevent light degradation during extraction. Extraction solvents used were methanol, ethanol and distilled water acidified with hydrochloric acid (pH 4). Afterwards, the extracts were centrifuged (5 min at 3000 rpm) and then filtered on a 0.45 μm membrane. Each solvent extraction was carried out in triplicate.

Determination of phenolic content

The amount of phenolics was assayed spectrophotometrically by means of the Folin–Ciocalteu method, as modified by Singleton and Rossi.[20] The phenolic content was expressed as gallic acid equivalent (GAE mg g^{-1} of shell).

2.5 mL of 10-fold diluted Folin–Ciocalteu reagent, 2 mL of 75 g kg^{-1} sodium carbonate solution and 0.5 mL phenolic extract were mixed well.[8] After heating for 15 min at 45 °C the absorbance was measured with a UV-visible spectrophotometer 1601 (Shimadzu, Osaka, Japan). A mixture of solvent extract and reagents was used as a blank.

Experimental design

Two factorial 2^2 CCDs were developed to optimize the phenol extraction method. Solvent composition ($\mathbf{X_1}$, %) and extraction time ($\mathbf{X_2}$, min) were chosen as the independent variables. In a first series of repeated batch extractions, the solvent contained different methanol percentages in distilled water at pH 4. In a second series, methanol was substituted by ethanol. Extraction time varied between 30 and 150 min. Variables were codified such that their values ranged between +1.414 and −1.414, taking the zero value as central point.

The variables were coded according to the following equation:

$$x_i = (\mathbf{X_i} - \text{mean } \mathbf{X_i})/\Delta\mathbf{X_i}$$

where x_i is the coded value of an independent variable, $\mathbf{X_i}$ is the real value of an independent variable, mean $\mathbf{X_i}$ is the real value of an independent variable at the centre point and $\Delta\mathbf{X_i}$ is the step change value.

Thus:

$$x_1 = (\mathbf{X_1} - 50)/35.36$$

$$x_2 = (\mathbf{X_2} - 90)/42.43$$

Table 1 shows the factorial design matrix, with variables in both coded and non-coded form, with a total of 13 experiments including five replicates of the centre point.

Mean values of triplicate determinations were analysed to fit the following second-order polynomial model:

$$Y = b_0 + b_1\mathbf{X_1} + b_2\mathbf{X_2} + b_{11}\mathbf{X_1}^2 + b_{22}\mathbf{X_2}^2 + b_{12}\mathbf{X_1}\mathbf{X_2}$$

Table 1. Experimental matrix of CCD

Experiment	Solvent composition (coded value) (x_1)	Extraction time (coded value) (x_2)	Solvent composition (real value) ($\mathbf{X_1}$[a], %)	Extraction time (real value) ($\mathbf{X_2}$, min)
1	−1	−1	15	48
2	1	−1	85	48
3	1	1	15	132
4	−1	1	85	132
5	−1.4141	0	0	90
6	1.4141	0	100	90
7	0	−1.4141	50	30
8	0	1.4141	50	150
9–13	0	0	50	90

[a] $\mathbf{X_1}$ is the percentage of methanol or ethanol in acidified water.

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where Y is the predicted response (Y_1 = phenolic content using methanol in different percentages in acidified water; Y_2 = phenolic content using ethanol in different percentages in acidified water), $\mathbf{X_1}$ and $\mathbf{X_2}$ correspond to independent variables, b_0 is the value in the central point conditions, b_1 and b_2 represent the principal effects associated with each variable, b_{11} and b_{22} are the squared effects and b_{12} is the interaction effect.

The second-degree polynomial equations were calculated with STATISTICA, data program version 7.0 (Statsoft Inc., Tulsa, OK, USA) and expressed as surface plots using RSM in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions. A statistical analysis system from STATISTICA was used to predict models through regression analysis (R^2) and analysis of variance (ANOVA).

Experimental data obtained from extraction of phenolic content of the 13 hazelnut cultivars were analysed using ANOVA and significant differences among means from triplicate analysis at $P < 0.01$ were determined by Tukey's test.

RESULTS AND DISCUSSION

Phenolic content

Table 2 shows reported phenolic content obtained for each set of variable combinations. The regression analysis for both responses indicated that the results were highly significant ($P < 0.001$), which suggested that they adequately explained the responses observed.

The ANOVA of phenolic content (Y_1) extracted using methanol as the solvent showed that the regression model had low dispersion ($R^2 = 0.9845$). The methanol concentration had linear and quadratic effects on phenolic content ($P < 0.001$), while for extraction time only the quadratic effect was significant ($P < 0.05$) (Fig. 1). The independent variables did not show any effect of interaction. The predicted model for Y_1 was

$$Y_1 = 5.7304 + 1.3194\mathbf{X_1} - 0.0803\mathbf{X_2} - 1.3756\mathbf{X_1X_1} - 0.0432\mathbf{X_1X_2} - 0.2418\mathbf{X_2X_2}$$

Figure 1 shows that the maximum phenolic content is predicted as 6.00 GAE mg g^{-1} of shell under extraction conditions of 67.1% methanol content in acidified water and 81 min of extraction time.

The regression equation obtained using ethanol as the solvent was highly significant ($P < 0.001$) and showed a good coefficient of determination ($R^2 = 0.9911$). The predicted model was

$$Y_2 = 6.5659 + 0.6501\mathbf{X_1} + 0.2210\mathbf{X_2} - 2.2804\mathbf{X_1X_1} + 0.1858\mathbf{X_1X_2} - 0.2842\mathbf{X_2X_2}$$

The ethanol concentration had significant linear and quadratic effects on phenolic content ($P < 0.001$) as well as the extraction time, but with less powerful effects ($P < 0.05$). No interaction effect between the two independent variables was shown. The relationship between independent and dependent variables is illustrated by a three-dimensional representation of the response surface (Fig. 2), in which it is shown that the increment of phenolic content depended mostly on ethanol concentration.

The maximum phenolic content under these conditions was predicted as 6.67 GAE mg g^{-1} of shell under extraction conditions of 55.7% ethanol content in the solvent and 108.7 min of extraction time.

The solvents used in this study had a clear ability to extract phenolic substances from these residues; lower results were obtained when acidified water was used alone as the extracting solvent (Table 2). The results obtained showed that ethanol was the more adequate extraction solvent.

Table 2. Phenolic content obtained from shell samples, following experimental design conditions

Experiment	Methanol/water, pH 4 (Y_1)	Ethanol/water, pH 4 (Y_2)
1	2.49 ± 0.35	3.14 ± 0.22
2	5.52 ± 0.34	4.24 ± 0.80
3	5.32 ± 0.57	4.89 ± 0.19
4	2.46 ± 0.27	3.06 ± 0.13
5	1.50 ± 0.38	1.37 ± 0.12
6	4.79 ± 0.49	3.00 ± 0.40
7	5.56 ± 0.21	5.74 ± 0.44
8	5.27 ± 0.99	6.59 ± 0.46
9	5.71 ± 0.25	6.51 ± 0.28
10	5.65 ± 0.22	6.53 ± 0.36
11	5.80 ± 0.36	6.70 ± 0.60
12	5.75 ± 0.23	6.62 ± 0.35
13	5.75 ± 0.16	6.48 ± 0.35

Mean values and standard deviations of triplicate determinations are expressed in GAE mg g^{-1} of shell.

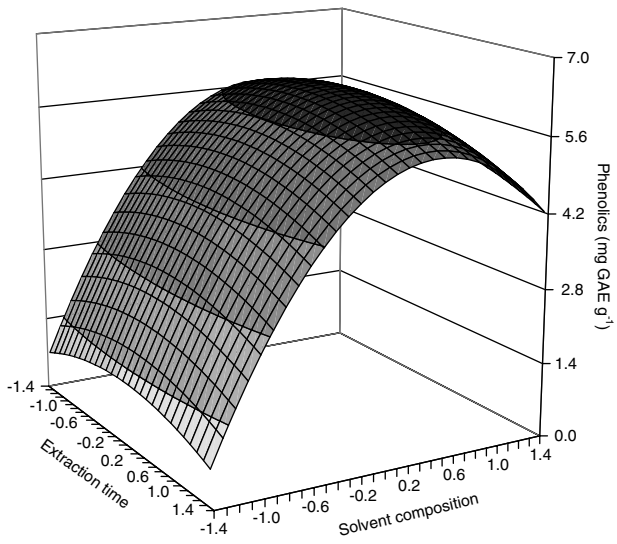

Figure 1. Response surface plot showing the effect of extraction time and solvent composition (methanol percentages in water pH 4) on phenolic content.

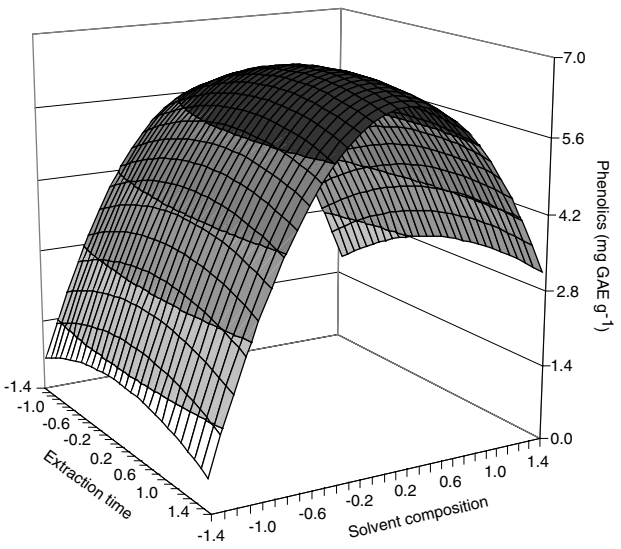

Figure 2. Response surface plot showing the effect of extraction time and solvent composition (ethanol percentages in water pH 4) on phenolic content.

It has been shown in previous studies of polyphenolic extraction that each material–solvent system showed different behaviour, which cannot be predicted, owing to the chemical characteristics of the solvent and also to the diverse structural compositions of the natural products.[8]

Because there is little data concerning phenols inside hazelnut shells, our results will be compared with those obtained from similar raw materials such as other nuts, taking into consideration only methanol and ethanol solvent extraction. However, the protocol of phenol extraction showed large variations (numbers of extraction, time of contact, solvent–solid ratio, particle size, etc.). The results obtained were expressed using different calibration standards, mainly gallic acid and catechin, and different methods of concentration expression (dry or fresh weight material, extract). These facts complicate the comparison of the results from one source to another.[21]

Alasalvar *et al.*[17] analysed the phenolic compounds inside hazelnut kernel (HK) and hazelnut green leafy cover (HGLC), extracted with 80% (v/v) acetone (HKa and HGLCa) and 80% (v/v) ethanol (HKe and HGLCe). HGLCa extract had the highest content of total phenolics (201 mg catechins equivalent (CE) g^{-1} of extract), followed by HGLCe (156 CE mg g^{-1}), HKa (103 CE mg g^{-1}) and HKe (23 CE mg g^{-1}) extracts, respectively. They concluded that both solvents are capable of extracting phenolics, but 80% acetone was a more effective solvent for the extraction process.

More recently, Shahidi *et al.*[18] analysed phenolic compounds and their antioxidant activity in hazelnut kernel and hazelnut by-products (skin, hard shell, green leafy cover, and tree leaf) using extraction with 80% (v/v) ethanol. Hazelnut by-products showed a larger content of phenolics in comparison with hazelnut kernel (14 mg CE g^{-1} of extract), in particular hazelnut skin (578 CE mg g^{-1}) and hazelnut hard shell (214 CE mg g^{-1}).

Regarding almond by-products, Pinelo *et al.*[8] reported that ethanol was the more adequate solvent for phenol extraction in almond hull. However, the total phenolic content determined was consistently less (0.23–0.61 GAE mg g^{-1} of dry matter) in comparison to those obtained from hazelnut shell. Siriwardhana and Shahidi[7] found a content of 87.8 mg CE g^{-1} in almond brown skin extract and 71.1 mg CE g^{-1} in almond green shell cover extract, using 80% (v/v) ethanol.

Regarding peanut by-products, Yen *et al.*[22] found levels between 33.4 and 71.3 mg CE g^{-1} peanut hull of varied maturities, using methanol solvent. 80% ethanol is the best extraction for peanut skin, according to Yu *et al.*[10] They found 90–125 GAE mg total phenolics g^{-1} dried peanut skin, depending on how the skin was removed from the peanut kernel. Nepote *et al.*[9] confirm this data. The maximum values of phenolic extraction were found in the range of 30–70% ethanol in the solvent, obtaining extraction values of 85.0–99.0 g phenol g^{-1} in peanut dry skin and 103.0–114.0 g phenol g^{-1} in defatted peanut dry skin.

Concerning pistachio hulls, Goli *et al.*[6] compared different extraction solvents and found the highest phenolic content of 32.8 and 34.7 mg TAE (tannic acid equivalent g^{-1} dry weight) using methanol and water, respectively.

Data obtained from different types of nuts showed that seed skin alone contains more phenolic compounds compared to nut hull. In particular, hazelnut skin showed a larger content of phenolics in comparison with hazelnut hard shell, but this last one is easily removed during processing and is available in huge quantities.

Influence of hazelnut cultivar on phenolic content extracted from shell

The 13 hazelnut varieties tested have different geographical origins and utilization (Table 3).

Total phenolic content was determined using the optimal conditions found in the previous experiment (56% ethanol and time extraction of 109 min). Samples of each cultivar were collected from the same experimental orchard, in which plants were grown with the same agronomical management, soil and climate, and nuts were harvested and stored under the same conditions. Values obtained varied significantly among hazelnut cultivars (Fig. 3). The maximum value was found for Barcelona (9.18 ± 0.51 GAE mg g^{-1} of shell), followed by Ribet (8.05 ± 0.61 GAE mg g^{-1} of shell). Tonda Romana and Tonda di Giffoni had the minimum phenol content (3.44 ± 0.16 and 3.00 ± 0.21 GAE mg g^{-1} of shell, respectively). Also Yen and Duh[23] found that the phenolic

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Table 3. Main characteristics of hazelnut varieties used for this study

Variety	Country of origin	Use	Nut weight (g)	Percent kernel (%)
Barcelona	Spain	In-shell market	3.11 ± 0.31	40.4 ± 2.2
Butler	USA	In-shell market	3.62 ± 0.15	46.3 ± 3.2
Casina	Spain	Industry	1.86 ± 0.14	53.3 ± 2.1
Culplà	Spain	Industry	2.56 ± 0.36	47.6 ± 2.9
Daria	Italy	Industry	2.25 ± 0.19	56.6 ± 2.9
Ennis	USA	In-shell market	4.22 ± 0.42	42.7 ± 1.9
MB	Germany	In-shell market	3.23 ± 0.27	38.9 ± 3.6
Pauetet	Spain	Industry	2.27 ± 0.16	50.3 ± 1.3
Ribet	Spain	Industry	1.87 ± 0.13	47.6 ± 3.2
Royal	USA	In-shell market	2.87 ± 0.44	39.2 ± 4.5
TGL	Italy	Industry	2.57 ± 0.26	48.3 ± 2.3
TG	Italy	Industry	2.76 ± 0.28	48.6 ± 3.1
TR	Italy	Industry	2.44 ± 0.28	44.8 ± 3.3

Data are expressed as means ± standard deviation ($n = 30$).

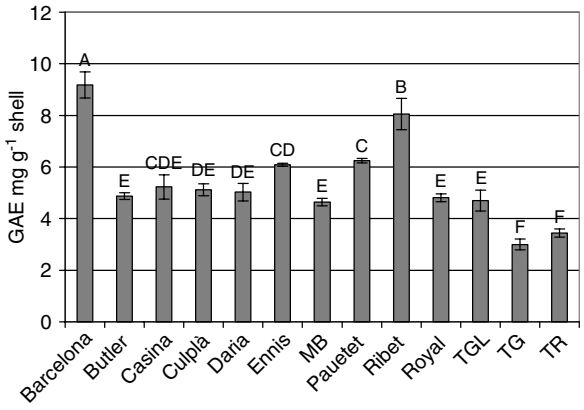

Figure 3. Phenolic content in hazelnut cultivar extracted using the best extraction conditions found with ethanol as solvent. Values are means of triplicate determinations with standard deviations indicated by vertical errors bars.Values not followed by the same letter are significantly different ($p \leq 0.01$).

content differed significantly among four different hull peanut cultivars (from 4.2 to 10.2 mg CE g^{-1} of hulls).

The wide range of data obtained suggested that phenol content in the hazelnut shell may be genetically controlled.[24]

CONCLUSION

Using CCD to study the effects of two independent variables, the results of this study indicated the following conditions of extraction as more efficient: 55.7% ethanol and 108.7 min of extraction time. These conditions, applied to 13 different cultivars, showed values varying from 9.18 mg GAE g^{-1} of shell for Barcelona to 3.00 mg GAE g^{-1} of shell for Tonda di Giffoni. This work showed that hazelnut hard shell could be a potential low-cost natural source of phenolics. The next step in our work will be to perform additional high-performance liquid chromatographic analysis in order to identify the major phenolic compounds inside our extracts and to evaluate their antioxidant activity in varied biological systems.

REFERENCES

1 Moure A, Cruz JM, Franco D, Domínguez JM, Sineiro J, Domínguez H, *et al*, Natural antioxidants from residual sources. *Food Chem* **72**:145–171 (2001).
2 Balasundram N, Sundram K and Samman S, Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chem* **99**:191–203 (2006).
3 Kroon P and Williamson G, Polyphenols: dietary components with established benefits to health. *J Sci Food Agric* **85**:1239–1240 (2005).
4 MacDonald-Wicks LK, Wood LG and Manohar LG, Methodology for the determination of biological antioxidant capacity *in vitro*: a review. *J Sci Food Agric* **86**:2046–2056 (2006).
5 Wood LG, Gibson PG and Manohar LG, A review of the methodology for the assessing *in vivo* antioxidant capacity. *J Sci Food Agric* **86**:2057–2066 (2006).
6 Goli AO, Barzegar M and Sahari MA, Antioxidant activity and total phenolic compounds of pistacchio (*Pistacia vera*) hull extracts. *Food Chem* **92**:521–525 (2005).
7 Siriwardhana S and Shahidi F, Antiradicals activity of extracts of almond and its by-products. *J Am Oil Chem Soc* **79**:903–908 (2002).
8 Pinelo M, Rubilar M, Sineiro J and Nunez MJ, Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*). *Food Chem* **85**:267–273 (2004).
9 Nepote V, Grosso NR and Guzman CA, Optimization of extraction of phenolic antioxidants from peanut skins. *J Sci Food Agric* **85**:33–38 (2005).
10 Yu J, Ahmedna M and Goktepe I, Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. *Food Chem* **90**:199–206 (2005).
11 FAO. [Online]. Available: http://www.fao.org [20 January 2005].
12 Tous J, World hazelnut production, in *Proceedings of the Ninth Australasian Conference on Trees and Nut Crops*, Perth, Australia (2001). http://www.aoi.com.au/acotanc/Papers/index.htm.
13 Ozdemir M, Critical evaluation of properties of Turkish hazelnuts. *Gida Teknolojisi* **2**:46–52 (1997). http://www.okyanus-bilgiambari.com.
14 Yurttas HC, Schafer HW and Warthesen JJ, Antioxidant activity of nontocopherol hazelnut (*Corylus* spp.) phenolics. *J Food Sci* **65**:276–280 (2000).
15 Senter SD, Horvat RJ and Forbus WR, Comparative GLC-MS analysis of phenolic acids of selected tree nuts. *J Food Sci* **48**:798–800 (1983).
16 Coisson JD, Papasso M, Travaglia F, Piana G, Arlorio M and Martelli A, Proprietà antiossidanti di estratti fenolici da sottoprodotti di lavorazione di cacao e nocciola, in *Atti del V*

congresso nazionale di chimica degli alimenti: Qualità e sicurezza degli alimenti, pp. 154–158 (2003).
17 Alasalvar C, Karamac M, Amarowicz R and Shahidi F, Antioxidant and antiradical activities in extracts of hazelnut kernel (*Coryllus avellana*) and hazelnut green leafy cover. *J Agric Food Chem* **54**:4826–4832 (2006).
18 Shahidi F, Alasalvar C and Liyana-Pathirana CM, Antioxidant phytochemicals in hazelnut kernel (*Coryllus avellana* L.) and hazelnut byproducts. *J Agric Food Chem* **55**:1212–1220 (2007).
19 AOAC, *Official Methods of Analysis* (17th edn). Association of Official Analytical Chemists, Washington, DC (2000).
20 Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am J Enol Vitic* **16**:144–158 (1965).
21 Stratil P, Klejdus B and Kuban V, Determination of total content of phenolic compounds and their antioxidant activity in vegetables: evaluation of spectrophotometric methods. *J Agric Food Chem* **54**:607–616 (2006).
22 Yen G, Duh P and Tsai C, Relationship between antioxidant activity and maturity of peanut hulls. *J Agric Food Chem* **41**:67–70 (1993).
23 Yen G and Duh P, Antioxidant activity of methanol extracts of peanut hulls from various cultivars. *J Am Oil Chem Soc* **72**:1065–1067 (1995).
24 Sarni-Manchado P and Cheynier C, *Les polyphenols en agroalimentaire*. TEC & DOC, Paris (2006).

J Sci Food Agric **87**:2817–2822 (2007)
DOI: 10.1002/jsfa