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Determination of Total Phenolic Content in Hazelnut Kernel Extracts and their Antioxidant Capacity – Evaluation of Spectrophotometric Methods

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KEYWORDS
Hazelnut kernel; antioxidant capacity; spectrophotometric assay.

SUMMARY
A study was performed on the content of phenolic compounds determined by the Folin-Ciocalteu reagent, the antioxidant capacity determined by TEAC, FRAP, CUPRAC and DPPH˙ assays and their correlations, in extracts of four hazelnut varieties. The total phenolic content (TPC) was significantly different among samples but only in raw kernel extracts and it averaged from 1.32 to 0.79 mg gallic acid equivalents (GAE)/g fresh mass. Comparable results were obtained for all antioxidant capacity assays and their correlations with TPC in raw hazelnut extracts were positively high ($0.83 \leq r \leq 0.97$, $p < 0.05$). When TEAC and FRAP methods were applied we obtained the highest regression coefficient between the values of TPC and the values of individual assay (r^2 = 0.93 and 0.88 respectively, $p < 0.01$). These results suggest that TEAC and FRAP assays, two of the most used spectrophotometric methods, could be used to analyze and assess the antioxidant potential of a wide number of hazelnut varieties.

INTRODUCTION
A diet rich in vegetable foods is commonly associated with lower risk for chronic degenerative diseases and it has been assumed that dietary antioxidant may explain this protective effect. Antioxidants are molecules or 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, anticarcinogenic and antimutagenic effects and antiproliferative potential.
Due to the complexity of food matrices, separation, identification and study of different antioxidant compounds present in vegetable foods, determined by usual separation methods (e.g. HPLC and GC) is generally difficult and costly. Therefore, the evaluation of content of total phenolic compounds and total antioxidant capacity, indices easily obtained from spectrophotometric measurements employing specific analitycal reagents, can be considered as measures of nutritional value of plant foodstuffs (Stratil *et al.*, 2006).
This work reported data relating to the total phenolics content (TPC) of four selected hazelnut kernel (raw and roasted) varieties (*Corylus avellana* L.) determined by Folin-Ciocalteu reagent, and the antioxidant capacity of the extracts assessed by the TEAC (Trolox equivalent antioxidant capacity), FRAP (ferric reducing antioxidant power), CUPRAC (cupric ion reducing antioxidant capacity) and DPPH˙ (using 2,2-diphenyl-1-picrylhydrazyl radical) assays. Due to the simplicity and cheapness of these assays, they are frequently used for studying antioxidant capacities in

vegetable and their products and foods, but the correct comparison of results and their interpretation are still a problem.
The aim of this research was to compare the efficiency of applied assays to estimate antioxidant capacities and their correlation with total phenolics content in raw and processed hazelnut kernels.

MATERIALS AND METHODS

Samples

Samples of shelled Tonda Gentile delle Langhe (TGL), Tonda di Giffoni, Ordu and Georgia hazelnut kernels (*Corylus avellana* L.), harvested in 2007, were purchased from a local shelling and processing hazelnuts company (Nocciole Marchisio S.r.l., Cortemilia, Italy). Hazelnuts were received in vacuum plastic bags and stored in a dark refrigerating room at 4 °C until they were processed and analyzed. All samples were roasted in laboratory (160 °C for 20 min) in a ventilated oven.

Extraction method

Raw kernels were extracted with ethanol and distilled water mixture at the extraction conditions of 50% v/v for 77 min, while roasted samples were extracted with acidified water with chlorhydric acid (pH 4) 100% for 90 min. The sample-solvent ration in both cases was 1:10 w/v (Ghirardello, 2007). Each solvent extraction was carried out in triplicate.

Total Phenolic Content (TPC) Determination

The total phenolics were assayed spectrophotometrically by means of the Folin-Ciocalteu method, as modified by Singleton and Rossi (1965). The phenolics content was expressed as gallic acid equivalent (GAE mg/g sample).

Total Antioxidant Capacity (TAC) Determination

The antioxidant capacity was determined according to the TEAC (Trolox Equivalent Antioxidant Activity) assay, following the procedure described by Re *et al.*, 1999 and was expressed as µmol of Trolox equivalent (TE)/ mg fresh mass.
The Ferric Reducing Antioxidant Power (FRAP) assay was estimated according to the procedure described by Benzie and Strain, 1996. FRAP values were expressed as mmol of Fe^{2+} equivalent (FE) per g fresh mass.
The Cupric Ion Reducing Antioxidant Capacity assay (CUPRAC) was performed as suggested by Prior *et al.*, 2005. All data were expressed as µmol TE/ mg fresh mass.
Finally, the DPPH$^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay was performed using the method described by Von Gadow *et al.*, 1997. The inhibition percentage (IP) of the DPPH$^{\cdot}$ by the extracts was calculated according the formula of Yen and Duh, 1994.

$$IP = [(A_{0min} - A_{60min})/ A_{0min}] \times 100$$

where A_{0min} is the absorbance of the control at t = 0 min; and A_{60min} is the absorbance of the samples at 60 min. The percentage of remaining DPPH$^{\cdot}$ is proportional to the antioxidants concentration in the extracts. A direct comparison of the results was obtained by applying the same common standard. Therefore, for all assays a Trolox calibration curve was prepared for a concentration range of 0-350 µmol.

Statistical analysis

Results were expressed as mean ± SD (n = 3) for each sample. Analyses of variance were performed using SPSS software (version 12.0 for Windows, SPSS Inc., Chicago, Illinois). Significant differences ($p < 0.05$) among means were determined using the Dunkan's test at a fixed level of $\alpha = 0.05$, and the Pearson correlation coefficient was used to determine the relationship among TPC and others variables.

RESULTS AND DISCUSSIONS

The amount of TPC was significantly different among hazelnut varieties but only in raw kernel extracts (Table 1). It averaged from 1.32 to 0.79 mg gallic acid equivalents (GAE)/g fresh mass. The same results were obtained for all antioxidant capacity assays that were able to discriminate significantly the hazelnut varieties. The roasting process interfered evidently on antioxidant capacity assessments. The amount of TPC in roasted kernels was more abundant, probably due to the action of melanoidines, and there were no significantly differences among samples.

Table 1. Total phenolic content (TPC) and antioxidant capacity of raw and roasted hazelnuts determined by the TEAC, FRAP, CUPRAC and DPPH• assays[1].

Samples	TPC mg GAE/g		TEAC µmol TE/mg		FRAP mmol $FeSO_4$/g		CUPRAC normal µmol TE/mg		CUPRAC incubated µmol TE/mg		DPPH• IP%	
Raw Hazelnuts												
TGL	$0,96^{ab}$	± 0,03	$0,008^{b}$	± 0,001	$0,011^{b}$	± 0,001	$0,007^{b}$	± 0,001	0,007b	± 0,001	$78,53^{b}$	± 0,93
Giffoni	$0,79^{a}$	± 0,09	$0,006^{a}$	± 0,001	$0,008^{a}$	± 0,000	$0,005^{a}$	± 0,001	0,006a	± 0,000	$64,03^{a}$	± 1,39
Ordu	$1,32^{c}$	± 0,18	$0,011^{c}$	± 0,001	$0,016^{c}$	± 0,002	$0,008^{c}$	± 0,000	0,008c	± 0,000	$92,14^{c}$	± 0,94
Georgia	$1,06^{b}$	± 0,05	$0,008^{b}$	± 0,001	$0,012^{b}$	± 0,001	$0,007^{b}$	± 0,000	0,008c	± 0,000	$80,09^{b}$	± 3,79
Roasted Hazelnuts												
TGL	$0,98^{a}$	± 0,05	$0,005^{a}$	± 0,001	$0,009^{a}$	± 0,000	0,009	± 0,000	0,012	± 0,001	$46,80^{a}$	± 0,36
Giffoni	$1,02^{ab}$	± 0,01	$0,006^{ab}$	± 0,000	$0,011^{a}$	± 0,000	0,009	± 0,001	0,012	± 0,002	$50,09^{b}$	± 0,08
Ordu	$1,11^{bc}$	± 0,06	$0,006^{ab}$	± 0,000	$0,010^{a}$	± 0,001	0,010	± 0,001	0,012	± 0,002	$50,19^{b}$	± 2,62
Georgia	$1,16^{c}$	± 0,06	$0,007^{b}$	± 0,001	$0,013^{b}$	± 0,002	0,009	± 0,001	0,011	± 0,001	$54,57^{c}$	± 0,41

[1] Data are expressed as mean ± SD (n = 3). Data in a column with different letters are statistically different ($p < 0.05$).

For all hazelnut varieties, all antioxidant assays gave the same profile. In particular, for raw hazelnuts, the antioxidant capacity (µmol TE/ mg fresh mass) decreased in the order DPPH• > TEAC > CUPRAC > FRAP (Figure 1).

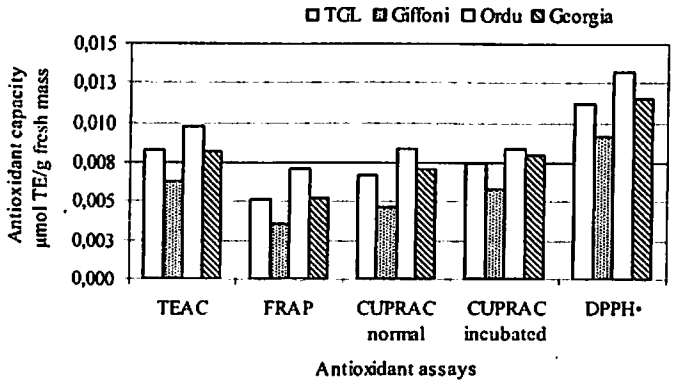

Fig. 1 Comparison of antioxidant capacity (µmol TE/ mg fresh mass) determined by TEAC, FRAP, CUPRAC and DPPH• assays.

Correlations among antioxidant capacity of all assays and TPC in raw hazelnut extracts were positively high ($0.83 \leq r \leq 0.97$, $p < 0.05$), especially between TPC and FRAP and TEAC assays (Table 2), with regression coefficients of 0.93 and 0.88 ($p < 0.01$) respectively.

Table 2. Pearson Correlation coefficient between Antioxidant Capacity and TPC in raw hazelnuts.

	TPC	TEAC	FRAP	CUPRAC normal	CUPRAC incubated	DPPH˙
TPC	1					
TEAC	0,937**	1				
FRAP	0,964**	0,984**	1			
CUPRAC normal	0,802**	0,885**	0,878**	1		
CUPRAC incubated	0,761**	0,789**	0,790**	0,911**	1	
DPPH	0,898**	0,945**	0,954**	0,955**	0,865**	1

** The correlation is significant at 0.01 level.

CONCLUSIONS

Measuring and reporting antioxidant capacity for fruits and other natural products requires selection of appropriate assays depending upon the hypothesis and types of potential antioxidants being tested (Verhagen *et al.*, 2003). The results of this study suggest that the FRAP assay, a simple technique, rapidly performed and best correlated with TPC, is a good choice to be combined with TEAC method. So, these two assays could be used to analyze and assess the antioxidant potential of a wide number of hazelnut varieties.

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