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Thermal Treatment of Eggplant (*Solanum melongena* L.) Increases the Antioxidant Content and the Inhibitory Effect on Human Neutrophil Burst

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The aim of this study was to compare the amount and activity of phytonutrients in raw, grilled, and boiled eggplant fruit using chemical measures and a biological assay of oxidative bursts in human neutrophils. The thermally treated samples showed various changes in their chemical composition (dry matter, soluble solids, acidity, and the amount of alcohol insoluble substances) due to the cooking processes and were much richer in the main phenolic compounds such as chlorogenic and caffeic acids, which are known to be antioxidants. Consequently, their free radical scavenging activity was significantly higher, especially that of superoxide anion. The biological assay of oxidative bursts from human neutrophils in the presence of *N*-formyl-methionyl-leucyl-phenylalanine confirmed the greater activity of extracts of the cooked eggplants with respect to raw eggplants. Successive extract dilutions showed a significant activity up to 1.25 μg/mL after cooking, while raw fruits resulted in an activity up to 10.00 μg/mL. These results showed that the thermal treatment commonly used before consumption can increase the content and biological activity of antioxidant compounds of eggplants.

KEYWORDS: Eggplant; cooking; free radical scavenging; human neutrophils; chemiluminescence

INTRODUCTION

Vegetables are rich in phytonutrients and are especially known for the antioxidant action by the scavenging activity against the free radicals generated by oxidative stress. It is generally believed that cooking vegetables leads to a loss in phytonutrients, but recent studies have demonstrated that this is not always true, especially in relation to the parameters associated with the antioxidant activity (*1*). Some authors (*2*) have reported a considerable loss of the usual antioxidant molecules, such as ascorbic acid, after cooking various vegetables or a significant increase in the free radical scavenging activity measured by 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) quenching. Other authors interpreted the increase in antioxidant capacity by three methods (Trolox equivalent antioxidant capacity, TEAC; total radical antioxidant parameter, TRAP; and ferric reducing antioxidant power, FRAP) with the formation of particularly active compounds or the tissue softening and disaggregation that increases extraction of the active principles (*3*).

Eggplant (*Solanum melongena* L.) fruits are particularly rich in antioxidant molecules and almost completely insensitive to ascorbate oxidase (*4*); therefore, these antioxidant molecules have been mainly ascribed to the class of polyphenols. Before they are eaten, eggplant fruits generally undergo thermal processing by means of various cooking techniques, after which their antioxidant capacity is retained or increased more than that of a wide range of other vegetables (*5*).

It has been pointed out that chemical assays of the free radical scavenging activity of phytonutrients do not fully inform one about their expected biological actions, but other in vivo tests involving human cells can enhance an understanding of the phenomena related to oxidative stress caused by antioxidant chain breakers in food. The literature on eggplant processing by cooking has only been partially accomplished by a description of the chemical profile and of the possible consequent antioxidant action. The aim of this study was to investigate the changes in the antioxidant burden of aqueous eggplant extracts before and after grilling and boiling. The antioxidant capacity assays were carried out by means of electron spin resonance (ESR) measurement of superoxide anion and hydroxyl radical scavenging. The interactions between eggplant and human polymorphonuclear neutrophils and the subsequent release of reactive oxygen species during respiratory bursts were studied by means of luminol-amplified chemiluminescence (LACL). Subsequent dilutions of the extract

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were made to establish the lowest concentration at which the aqueous extract still exerts a biological antioxidant activity.

MATERIALS AND METHODS

Plant Material. "Black Bell" eggplant fruits were harvested in the commercial ripening stage at the end of growing season at the experimental field of the Research Unit for Vegetable Crops of the Agricultural Research Council (CRA) in Montanaso Lombardo (Lodi, Italy). About 50 fruits were visually selected on the basis of their homogeneous size, shape, color, and apparent absence of diseases and were randomly divided into two batches, each of which was further subdivided into three portions: one left untreated (raw) and the others for processing (grilling and boiling). The fruits in each portion were washed with water and dried, and the edible parts were sliced into pieces of the same thickness (about 1 cm). Raw samples were immediately frozen in an air blast tunnel at −50 °C and then lyophilized.

The fruit samples for processing were either grilled for 4−5 min on either side (grilled) using a professional grilling apparatus equipped with a gas flame and a metal cooking plate or boiled in a stainless steel vessel on a moderate flame for 10 min (boiled) in tap water at a 1:10 ratio of slices and cooking water. The temperature profile during cooking was monitored with calibrated probes connected to the software ELLAB E-Val 2.10. The temperatures recorded were as follows: (a) grilling plate surface, stabilized at 190−210 °C; (b) inner part of slices during grilling, which reached 100 °C in 1 min; and (c) inner part of slices during boiling, which reached 75 °C in 1 min and 100 °C in next 4 min. A temperature of 100 °C in the inner parts of slices was maintained during the whole time for both cooking ways.

Just after cooking, boiled samples were drained off for 1 min. Both grilled and boiled samples were left at room temperature for 10 min, and then, they were frozen in an air blast tunnel at −50 °C and lyophilized. Lyophilized samples were obtained until a constant weight was reached, to calculate the dry matter (DM) value.

Chemical Assays. The soluble solids (SS) by refractometer, total acidity by titration with 0.1 M NaOH to pH 8.2, and pH were measured using the vortexed and subsequently centrifuged extracts of 300 mg of eggplant powder with 10 mL of cold H_2O at 2−4 °C. An aliquot of the aqueous extract was immediately filtered on glass wool, frozen at −80 °C, and further lyophilized until constant weight to calculate the yield of extraction for the biological assays. The remaining filtered extract was diluted 1:1 with cold 2 mM HCl and stored at −80 °C to analyze the antioxidant potential and the TPI.

The ethanol-insoluble residue (EIR) was calculated by weighing the residue of the lyophilized samples (2 g) after treating them twice with 75% EtOH (55 mL) at 60 °C and drying them with 20 mL of acetone until reaching a constant weight. The results were expressed as the percentage of DM.

The extractable fiber was evaluated by weighing the insoluble residue after treating it with 200 mg of the EIR with 15 mL of a 0.025 M potassium oxalate and 0.03 mM dimethylsulfoxide solution; the soluble fiber was extracted by means of vigorous shaking for 60 min and subsequent centrifugation (22000*g* for 30 min at 4 °C). The results were expressed as the percentage of EIR weight.

Phenolic Acids. Phenolic acids were extracted and analyzed according to Whitaker and Stommel (*6*) with minor modifications. Chlorogenic acid was quantified using a reverse phase high-performance liquid chromatography (RP-HPLC) separation, based on the absorbance at 325 nm in relation to the sesamol internal standard and an external standard of authentic chlorogenic acid (retention time, 24.7 min) and caffeic acid (retention time, 25.1 min). The results were expressed as mg/100 g of DM.

Anthocyanins. The anthocyanins contained in the peel were extracted and analyzed as described by Ichiyanagi et al. (*7*). Purified delphinidin-3-rutinoside was used as the external standard in the RP-HPLC separations. The standard was prepared by means of extraction from the eggplant peel with 10 mM HCl, cleaning by centrifugation, C_{18} SPE chromatography, elution with 30% aqueous ethanol, and quantification by spectrophotometry using the molar extinction coefficient of delphinidin. The results were expressed as mg/100 g of DM of whole fruit.

Total Polyphenols. The total polyphenol index (TPI) was assayed in an extract (see above) of lyophilized tissue by means of spectrophotometric analysis, using a modified Folin−Ciocalteu method (*8*). The results were expressed as mg per 100 g of DM of chlorogenic acid equivalent. Chlorogenic acid was used because it is the main phenolic compound in eggplant fruit.

Polyphenol Oxidase (*PPO*). The PPO activity was assayed as described by Fujita and Tono (*9*), using 30 mg of lyophilized fruit extracted with 1 mL of McIlvaine buffer (pH 5.0). The results were expressed as U/100 mg of DM (1 U = 0.01 absorbance unit increase per minute at 420 nm) using chlorogenic acid as a substrate (*10*, *11*).

Glycoalkaloids. The glycoalkaloids, solamargine and solasonine, were extracted from 0.5 g of lyophilized tissue using 95% ethanol as described by Birner (*12*) with some modifications. The analyses were performed by means of RP-HPLC according to Kuronen et al. (*13*), using partially purified solasonine and solamargine as the external standard. The data were expressed as mg/100 g of DM and represented the sum of solamargine and solasonine to make the measurement conform with the recommended limits for these compounds (*14*, *15*).

Antiradical Activity. The antiradical activity was assayed by measuring superoxide anions and hydroxyl radicals using ESR spectrometry, as described by Morelli et al. (*16*) and Valavanidis et al. (*17*), with some modifications. Free radical generation for superoxide anions (6.4 mM KO_2-crown-ether-18-6 1:1 in dimethylsulfoxide) was followed by spin trapping with 25 mM 5,5-dimethyl-1-pyrrolin-*N*-oxide (DMPO); for hydroxyl radicals, 2 mM Fenton system in 0.1 M phosphate buffer, pH 7.4, was used, followed by spin trapping with 10 mM DMPO. The reaction was allowed to take place for exactly 1 min at 25 °C, after which the ESR spectra were recorded in the presence and absence of eggplant extract (see above). To calculate the scavenging index, the main band amplitude was used applying the equation: $I = 100 - (I_x/I_0 \times 100)$, where I_x is the spectrum amplitude in the presence of eggplant extract and I_0 is the spectrum amplitude in its absence. The results were expressed as percentage quenching with different amounts of the extracts in relation to the control data obtained in the absence of eggplant extract.

Biological Assay. *Human Polymorphonuclear Neutrophil* (*PMN*) *Collection*. Peripheral venous blood was obtained from healthy adult blood donors at the Blood Donors Department of Niguarda Hospital (AVIS Comunale, Milan, Italy). The Ethics Committee of the hospital approved the study, and informed consent was obtained from each donor who had not received any medication for at least 2 weeks. The blood (5 mL) was stratified in 3 mL of a Polymorphprep cell separation medium (Axis-Shield, Oslo, Norway), and the PMNs were separated by means of density gradient centrifugation. After centrifugation, the upper mononuclear cell band was removed and discarded, and the lower PMN band was removed and washed in RPMI 1640 medium containing glutamine (Sigma Chemical Co., MO). Any residual erythrocytes in the granulocyte preparation were lysed using a 0.15 mol/L NH_4Cl solution (pH 7.4). After the aggregates were disrupted by being passed through a needle with an internal diameter of 150 μm, the PMNs were collected, washed in RPMI-1640, and tested for viability by means of Trypan blue exclusion. The number of cells in the final cell suspension used for each test was adjusted by means of counting in a Burker chamber (Normaski interference contrast microscopy).

LACL Measurement of Oxidative Burst Responses. PMN oxidative bursts were associated with the generation of superoxide anions, hydrogen peroxide, oxygen radicals, hydroxyl radicals, and hypochlorous acid. These reactive oxidant species (ROS) were not only microbicidal agents but also extremely toxic to human tissue. As luminol degradation by ROS is associated with luminescence, the inclusion of luminol in the reaction medium provided a sensitive means of detecting PMN respiratory bursts. LACL was investigated using the soluble stimulant *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), a bacterial tripeptide that is frequently used to stimulate PMN respiratory bursts and acts via a specific receptor. The measurements were made using a slightly modified version of the procedure described by Briheim and Dahlgren (*18*).

Briefly, 0.1 mL of a PMN suspension (1×10^6 cells/mL, previously incubated with or without eggplant extracts) plus 0.2 mL of 2×10^{-5} mol/L of luminol (Sigma) and 0.5 mL of Hanks' balanced salt solution (HBSS) were put into a 3 mL flat-bottomed polystyrene vial. The vial was placed in the light-proof chamber of a Luminometer 1250 (Bio Orbit, Turku, Finland), and the carousel was rotated to bring the sample in line with the photomultiplier tube to record background activity. A volume of

Table 1. General Chemical Parameters of the Raw and Processed Eggplant Samples[a]

	DM (%)	SS (°Brix)	total acidity (mEq % ww)	pH	extraction yield (mg/g of DM)	EIR (% of DM)	insoluble residue (% of EIR)
raw	10.9 b	4.9 b	2.54 b	5.47 b	464.3 b	56.9 ab	83.7 a
grilled	14.1 a	7.9 a	3.39 a	5.70 b	539.0 a	53.6 b	79.8 a
boiled	11.6 b	5.0 b	2.55 b	6.27 a	446.0 b	60.1 a	79.4 a

[a] The different letters indicate, for each column, significant differences according to Tukey's test ($p < 0.05$).

Table 2. Phenolic Profile and Glycoalkaloids of the Raw and Processed Eggplant Samples[a]

	total polyphenols (mg/100 g of DM)	chlorogenic acid (mg/100 g of DM)	caffeic acid (mg/100 g of DM)	delphinidin-3-rutinoside (mg/100 g of DM)	PPO activity (U/100 mg of DM)	total glycoalkaloids (mg/100 g of DM)
raw	910 cB	154 cC	12.8 cC	90.1 aA	13.3 aA	16.3 cA
grilled	2332 a	710 a	30.1 b	54.0 b	0.1 b	27.3 a
boiled	2119 b	467 b	36.1 a	89.5 a	0.9 b	20.0 b
			normalized data in relation to the DM of raw samples			
grilled	1803 A	549 A	23.3 B	41.7 B	0.1 B	21.1 A
boiled	1991 A	439 B	33.9 A	84.1 A	0.8 B	18.8 A

[a] The different letters indicate, for each column, significant differences according to Tukey's test ($p < 0.05$). Lowercase letters indicate the difference between non-normalized data, and capital letters indicate the differences between normalized data.

0.2 mL of fMLP was added at a concentration of 5×10^{-7} mol/L to reach a final volume of 1 mL. The resulting light output was continuously recorded in millivolts on a chart recorder and simultaneously by means of a digital printout set for recording intervals of 1−10 s. All of the constituents of the mixture were kept at 37 °C during the reaction by passing water from a thermostatically controlled circulation system through a polished hollow metal sample holder. No mixing took place during the recordings. The gain control was set to give a recording of 10 mV for a built-in standard. A background subtraction control zeroed the instrument before the addition of fMLP. The LACL response patterns were identified by calculating peak values (mV) and the times to peak values (min, s). The raw, boiled, and grilled lyophilized eggplant aqueous extracts were incubated for 30 min at 37 °C with PMNs at concentrations ranging from 160 to 0.62 μg/mL (log scale) of eggplant extract.

Statistical Analysis. The chemical assays were replicated three times, and the data were analyzed by means of analysis of variance using the GLM procedure of SAS Institute software, version 9.1. The statistical differences of means were indicated by different letters according to Tukey's test ($p < 0.05$).

For the biological data, four assays were made of each concentration, and the statistical significance of the differences was calculated by means of one-way analysis of variance, followed by multiple paired comparisons using Dunnett's test. The differences of means were considered statistically significant when the p value was ≤ 0.05.

The correlation between the amount of phytochemicals and the biological activity was performed by simple linear regression test, using Microsoft Excel 2003, and statistically analyzed with Statgraphics plus 5.0.

RESULTS

General Quality Parameters. The general chemical parameters (**Table 1**) revealed significant changes in the samples after the grilling and boiling.

The grilling effects may be mainly due to water loss and, partially, to the decomposition caused by different temperatures along the tissue of the eggplant slices. DM, SS, and total acidity significantly increased with respect to the raw sample by 29, 61, and 33%, respectively. The pH value was not significantly different from that of the raw samples, probably because of the contemporary concentration of organic acids and salts. The extraction yield in aqueous media was significantly higher (16%) in the grilled samples than in the raw samples. The EIR value was slightly lower than in the raw samples, although not significant.

The behavior of boiled samples was different and similar to that of the raw samples. The eggplant slices dipped in boiling water obviously reached a more homogeneous temperature (100 °C in 5 min) than the grilled slices (internally, it was 100 °C, and on the surface, it was around 200 °C, because of the contact with the cooking plate). The DM, SS, and total acidity values were not significantly different from those of the raw samples, but the pH was significantly higher, probably because of the leaching of the low molecular weight compounds, especially salts, in boiling water. The extraction yield and EIR were also not significantly different from those of the raw samples. The amount of extractable fiber from the EIR tended to be greater in the grilled and boiled samples than in the raw samples, as detected by the decrease in the insoluble residue (**Table 1**); however, the difference was not statistically significant.

Chemical Assays. Phenolic profiles are shown in **Table 2**. In general, the processed samples showed a considerable increase in polyphenol content. The data were also normalized to the DM in the raw samples, but this only slightly modified the general trend.

The TPI was more than doubled by both processes, whereas the increase in chlorogenic acid was greater in the grilled than in the boiled eggplant slices (3.6- vs 2.9-fold). The amount of caffeic acid was higher in the processed than in the raw samples, but the changes in the boiled and grilled samples were different, possibly because of the partial decomposition of caffeic acid. In fact, the molar caffeic/chlorogenic acid ratio was 0.16 in the raw samples, 0.08 in the grilled samples, and 0.15 in the boiled samples. Grilling induced a greater caffeic acid decomposition than boiling, as the ratio was similar in the boiled and raw samples. The eggplant pigment delphinidin-3-rutinoside showed an inverse trend, decreasing in the grilled samples but stabilizing after boiling.

Total glycoalkaloids (**Table 2**), as the sum of solamargine and solasonine, the most representative ones in eggplant, were also measured. The amount was significantly higher in the processed eggplants than in the raw samples, with a slower increase in the boiled samples than in the grilled ones. The normalized data showed the same trend but without statistical significance.

As expected, the PPO activity markedly decreased to very low levels in the processed samples (**Table 2**).

The antioxidant potential was evaluated by assaying the scavenging of superoxide anions and hydroxyl radicals and normalizing the data to the initial concentration of the raw eggplant extract. The superoxide anion scavenging data clearly showed that the thermally treated samples were more effective scavengers than the raw samples (**Figure 1**) at levels >99.5 μg/mL. At the maximum, the concentration assayed (equivalent to 995 μg/mL of

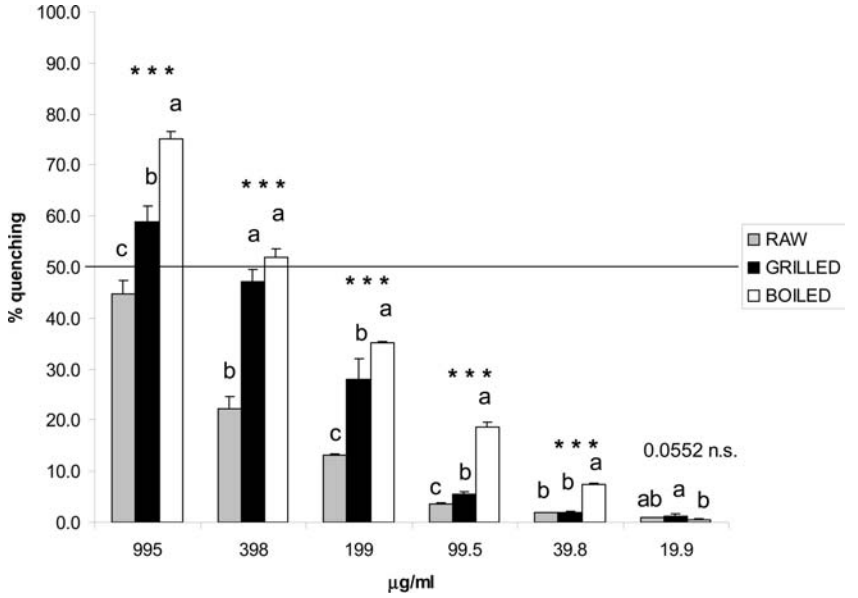

Figure 1. Free radical scavenging activity against 6.4 mM superoxide anion (1 min at 25 °C) in various concentrations of the differently processed eggplant extracts. GLM procedure: ***, $p < 0.001$; the reported number is the p value; n.s., not statistically different. The different letters indicate, for each group of data, significant differences according to Tukey's test ($p < 0.05$).

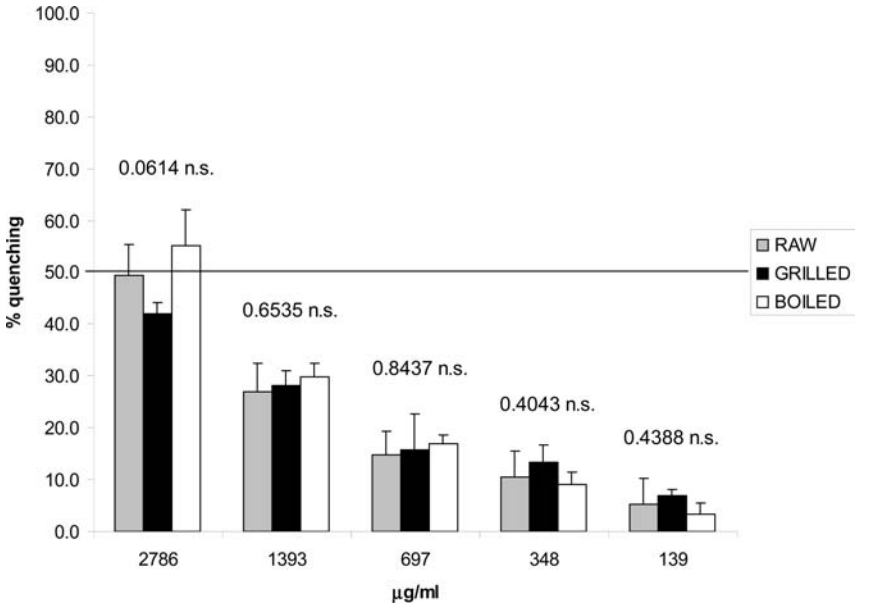

Figure 2. Free radical scavenging activity against 2 mM OH (1 min at 25 °C) at various concentrations of differently processed eggplant extracts. Reported numbers are the p values from the GLM procedure; n.s., not statistically different.

raw extract), the superoxide scavenging value was about 45% in the raw, about 60% in the grilled, and more than 70% in the boiled samples. Subsequent dilutions showed a significantly higher activity of both cooked samples with respect to raw samples up to 99.5 μg/mL. At 39.8 μg/mL, a significant positive value was found only in the boiled samples (about 7% scavenging). Finally, at 19.9 μg/mL, neither grilled nor boiled samples resulted in differences from raw ones. The pattern of hydroxyl radical scavenging was different (**Figure 2**). First of all, at the same reaction time and temperature, and even with a smaller molar amount of hydroxyl radical (2 mM) than the superoxide anion (6.4 mM), a larger quantity of extract was needed (2786 μg/mL) to reach the quenching index of about 50%. This can be explained by the very large difference in the reactivity of superoxide anions and hydroxyl radicals. No significant changes in eggplant activity were found between the differently processed samples and dilutions, except for a slight but not significant increase in the activity of boiled samples at 2786 and 1393 μg/mL. A residual protection of less than 10% was found at 139 μg/mL in all samples.

Biological Assays. The LACL data showed significant inhibition of oxidative bursts at much lower concentrations than the previous antioxidant assays. The initial concentration of 160 μg/mL almost completely inhibited the oxidative bursts (**Figure 3**). The quenching of about 50% obtained from the raw eggplant tissue, which needed, respectively, 995 and 2786 μg/mL for superoxide anions and hydroxyl radicals, was obtained at a concentration of 40 μg/mL. This means very high sensitivity for the LACL test. Further dilutions led to a linear diminution in the raw samples (significant up to 10 μg/mL), whereas both the grilled and the boiled samples surprisingly showed higher values, with a

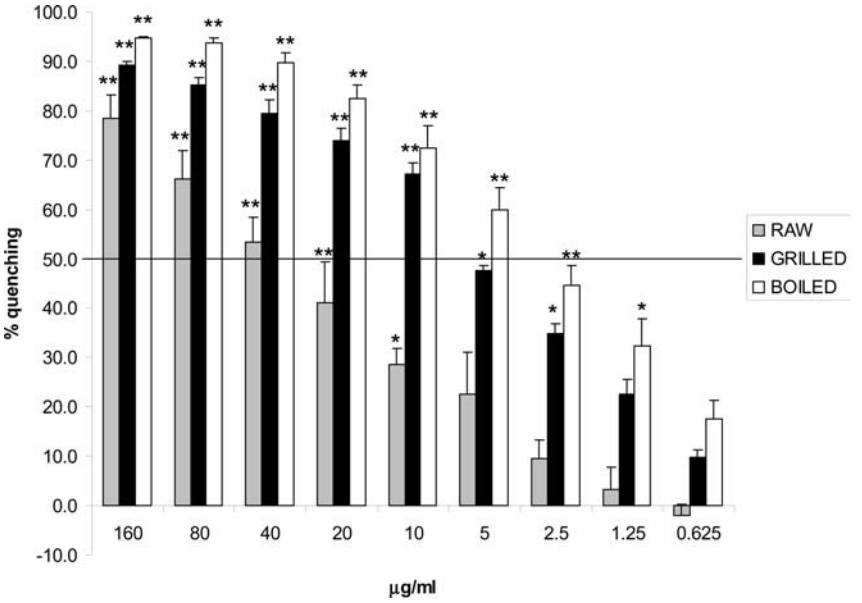

Figure 3. Comparative effects of various concentrations of differently processed eggplant samples on the LACL of the PMN respiratory bursts induced by fMLP (**, $p < 0.01$; *, $p < 0.05$).

tendency to saturation at higher concentrations of eggplant extract. The grilled extract showed significant activity up to 2.5 μg/mL, and the boiled extract showed significant efficacy at a further dilution (1.25 μg/mL). At each dilution, the LACL data from raw, grilled, and boiled samples were in close agreement with those of superoxide anion quenching.

Linear Regression of Phytochemicals Versus Activity. The role of individually monitored phytochemicals in scavenging activity was estimated by plotting the whole data set of LACL, superoxide anions, and hydroxyl radicals against the concentrations of individual phytochemicals classes (**Figure 4**). The ranking of the slopes was similar for all tests, in the order total polyphenols, chlorogenic acid, and delphinidin-3-rutinoside, and reflects their relative amounts in eggplants.

As seen in the case of the amount of extract necessary for significant scavenging, there was a clear difference in the reactivity of the three tests: the hydroxyl radical data plots always have a shallower slope than those of the other tests, meaning that more scavenger is needed to quench hydroxyl radical effectively, even at a lower concentration than the superoxide anion radical in the present system (2.0 vs 6.4 mM). Furthermore, the LACL data confirmed its greater sensitivity: for example, in the TPI plot, the slope of LACL was 6.5 and 28.3 times that of those of superoxide and hydroxyl radical.

TPI correlation showed a good linear relationship with the scavenging data, but affinity was highest for superoxide anions ($R^2 = 0.85$), followed by hydroxyl radicals ($R^2 = 0.80$) and LACL ($R^2 = 0.49$). The indices of correlation of chlorogenic acid were lower but again higher for superoxide than for hydroxyl radical or LACL scavenging. The correlation with eggplant pigment (delphinidin-3-rutinoside) showed an inverse trend: the closest correlation was with hydroxyl radical scavenging data ($R^2 = 0.87$), and the interaction with superoxide scavenging was relatively low ($R^2 = 0.65$) but always followed by LACL ($R^2 = 0.38$).

When divided by type of processing (**Figure 5**), the regression data showed that the raw samples for LACL were close to 0.80 and became very close to linearity in superoxide and hydroxyl data. On the contrary, the grilled and boiled data shifted out from linearity, decreasing the value of R^2, especially for LACL and superoxide data, meaning a tendency to a pleateau, while hydroxyl data remained well-fitted with the linearity of response.

The low regression value of the LACL probably is due to the presence of synergistic effects of the different eggplant antioxidant compounds at the upper concentrations used; this enabled plateau responses to be reached in grilled and boiled samples and not in raw ones (**Figures 3** and **5**). This fact further enforces the lower activity of raw eggplant with respect to the cooked eggplant.

DISCUSSION

In grilled eggplant slices, the grilling process induced an increase in DM and extraction yield. The exposure to high temperature caused water loss and, probably, the destructuring, which determined an increase of EIR. In boiled samples, the effect of boiling water on the leaching of solutes was remarkable only in the case of EIR in comparison with the grilled eggplants (**Table 1**).

The content of glycoalkaloids (**Table 2**) was slightly increased in processed eggplant, when measured on DM, while the normalized data gave no statistical differences between raw and cooked samples. This is in agreement with previous works, showing that cooking did not affect the level of glycoalkaloids in potato (*20*) and tomato (*21*).

The phenol content was greatly increased by cooking. Normalized data in **Table 2** show about a 3-fold increase in chlorogenic acid of thermally treated samples. The increased amount in chlorogenic acid, a conjugated derivative of caffeic acid, suggests its greater extractability after both cooking treatments. The higher surface temperature (200 °C) and the relative chlorogenic acid thermal stability may explain the higher amount found in grilled (3.6-fold) with respect to boiled samples (2.9-fold). The caffeic acid amount resulted increased in both cooked samples as well, but its increment was less in grilled (1.8-fold) than in boiled (2.6-fold) eggplant. This may be due to a reduced thermal stability of the free chlorogenic acid derivatives, which were subjected to a higher degradation rate. The evolution of the eggplant pigment, delphinidin-3-rutinoside, instead, was stable after boiling (0.9-fold), while the grilling process induced a clear depletion (0.5-fold). If the sum of single monomeric phenols is considered, the increase in processed with respect to raw eggplants was 2.3-fold after boiling and 3.1-fold after grilling.

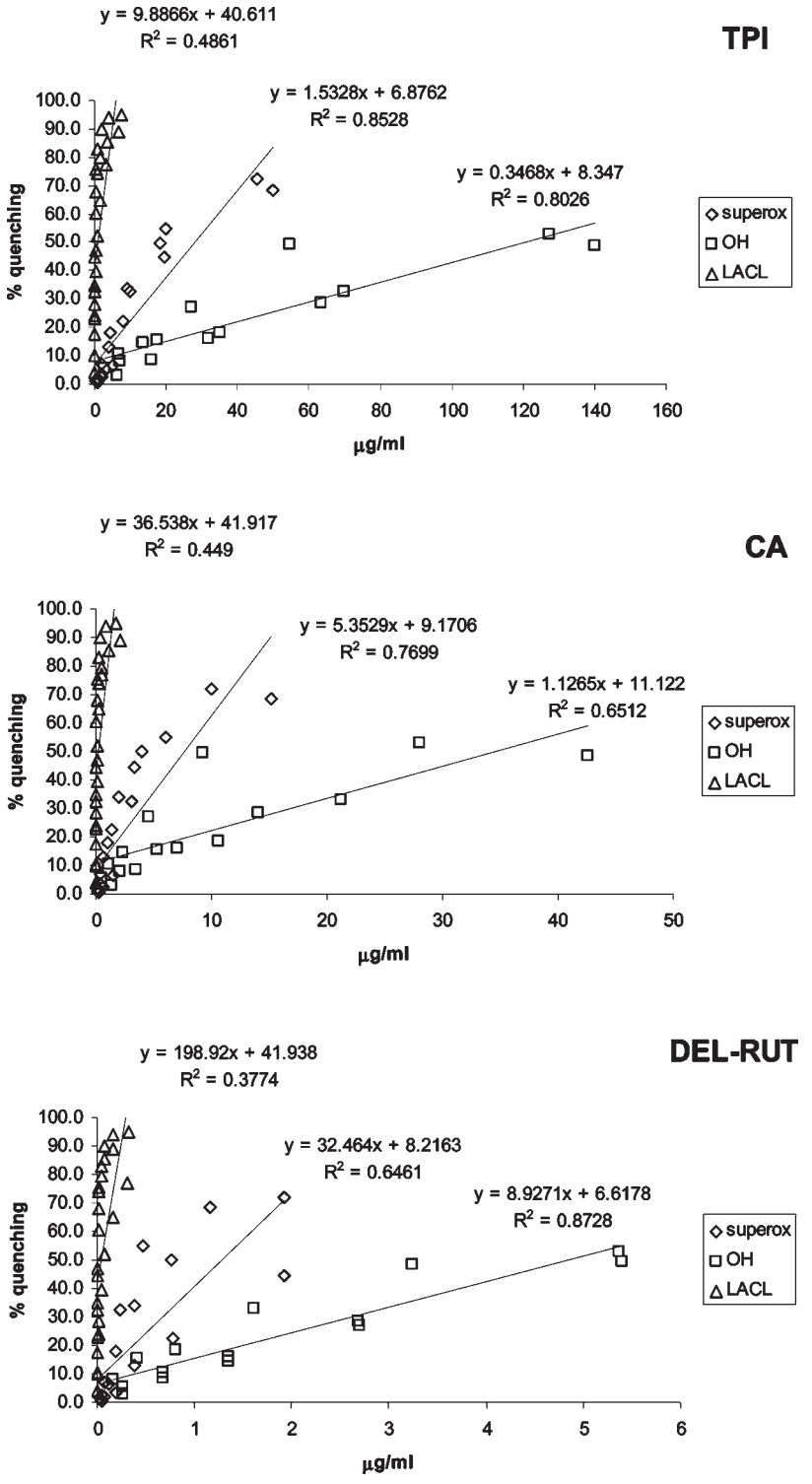

Figure 4. Simple linear regression of phytochemicals vs the scavenging activity of superoxide anions (superox), hydroxyl radicals (OH), and LACL in the raw and cooked eggplant samples. TPI, total polyphenols; CA, chlorogenic acid; and DEL-RUT, delphinidin-3-rutinoside. Each point is the average of the phytochemical and quenching data.

The increase in TPI was lower than that of chlorogenic acid: grilling and boiling have a 2.0- and 2.2-fold increase with respect to raw eggplant, respectively. The possible explanation of the difference between boiled and grilled eggplant slices may be that boiling mostly induces a matrix denaturation that leads to a more efficient extraction of the active components. On the contrary, grilling determines a higher temperature on the slice's surfaces with respect to boiling. Therefore, the grilling may induce, together with an improved extraction, degradation and de novo compounds production such as Maillard reaction products (MRP) as well. The occurrence of MRP, which are reactive in a Folin−Ciocalteu system (*19*), was evidenced by the brownish color of the extracts from grilled samples and not from the boiled ones.

It seems that boiled eggplant gave a better response than grilled eggplant in the antioxidant assays of superoxide anion and LACL but not in the hydroxyl radical assay. It can be hypothesized that grilling induces a deeper decomposition on some bioactive components that, contrarily, are retained intact in the boiled eggplant.

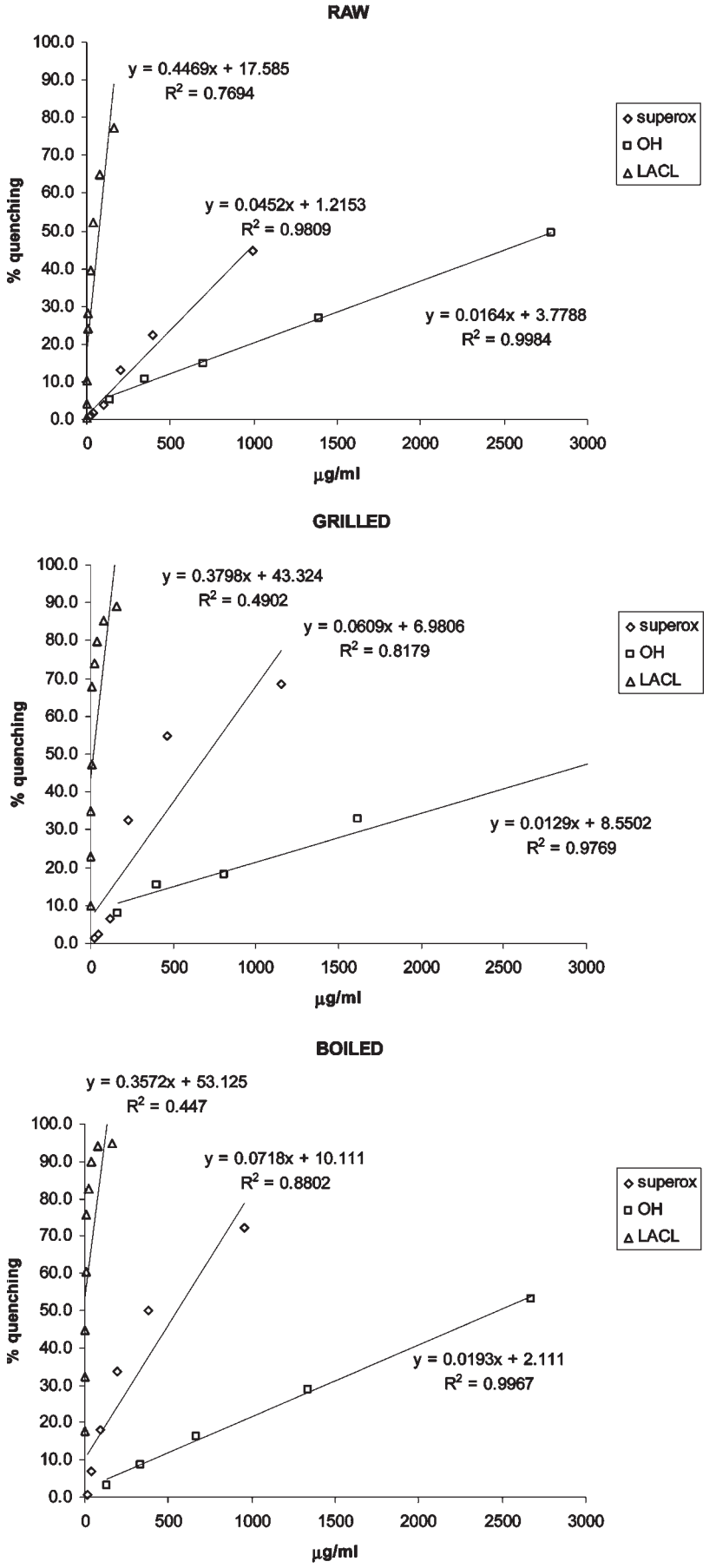

Figure 5. Simple linear regression of phytochemicals vs the scavenging activity of superoxide anions (superox), hydroxyl radicals (OH), and LACL in the raw, grilled, and boiled eggplant samples vs the extract amount. Each point is the average of the phytochemical and quenching data.

Our data may agree with this hypothesis when EIR (a potential source of phenolic acids), caffeic acid, and delphinidin-3-rutinoside are considered but not for total polyphenols and chlorogenic acid. Further studies are needed to better understand this different behavior.

Other authors (*3*) have detected significant losses of chlorogenic acid after the cooking of courgettes, carrots, and broccoli. However, they found a significant increase in antioxidant activity in the cooked samples with respect to raw ones. The increase in antioxidant activity is in line with our data, but our findings also revealed an increase in total polyphenols and chlorogenic acid after cooking. Our data are fully supported by the findings of Ferracane et al. (*22*), who found a very substantial increase in antioxidant capacity and the amount of caffeoylquinic esters, including chlorogenic acid, after the cooking of artichokes. Therefore, it seems that eggplant fruit tissue is particularly protective for chlorogenic acid. This can also be deduced from the inactivation of oxidizing enzymes, as demonstrated on PPO data, which revealed almost complete inactivation after the thermal treatments (**Table 2**). Eggplant PPO has its best substrate affinity for chlorogenic acid (*9*), and this could explain its considerable contribution to the chlorogenic acid increase after thermal treatments.

The antioxidant data of Yamaguchi et al. (*2*) are also in line with ours, as they found a significant loss in ascorbic acid after cooking but with a very small contribution (5%) to the antioxidant profile. Our study included an evaluation of the phytochemical profile, which extends previous results. Similar findings concerning the evolution of the chlorogenic acid during cooking have been reported by Kalogeropoulos et al. (*23*), who found a good retention after eggplant frying. Furthermore, this type of processing was effective in enhancing the antioxidant capacity of eggplant (*1*).

The major eggplant anthocyanin, which is known to be more easily decomposed than chlorogenic acid, underwent significant loss in our grilled but not in the boiled samples. The results on boiled samples partially agreed with the findings of Rossi et al. (*24*), who found that the blueberry juice obtained after the brief thermal treatment of the berries was richer in anthocyanins than that obtained from untreated berries, thus also affecting its antioxidant capacity.

Our LACL findings in human cells indicate that eggplant exerts its antioxidant action at very low concentrations not only in terms of chemical protection but also by reducing ROS release by human PMNs. These findings are interesting in relation to the strategy of improving the antioxidant burden and restoring redox balance in human cells using plant extracts. They can be very useful when the stress generated in living organisms favors free radicals as a result of the depletion of antioxidants.

The activity in LACL and superoxide assays was greatly increased in the cooked samples and seems to be strictly related to eggplant polyphenols, as demonstrated by the high correlation indices. The increased scavenging activity in the cooked samples was not so evident from the hydroxyl radical scavenging data, because it is well-known that this radical is highly and somewhat unspecifically reactive to plant components. However, it is worth noting that the thermal treatments did not induce any loss in activity in comparison with the raw samples.

In conclusion, the present study has highlighted the increase in and greater stability of antioxidant compounds and in the indices of antioxidant capacity after eggplant cooking. The LACL biological assay fully reflected superoxide scavenging, as it is associated with changes in the polyphenolic profile and with cooked eggplants that had more extractable antioxidant compounds than raw ones. Moreover, hydroxyl radical scavenging data showed no loss in activity after cooking. It must be noted that there is a great difference in reactivity between chemical and biological assays as the LACL system is about seven times more sensitive than the superoxide assay, as evaluated by an ESR method.

The antioxidant profile of eggplant potentially has a strong effect on living organisms, because, in a biological situation such as neutrophil activation, 80 μg of cooked eggplant extract was sufficient to completely inhibit oxidative bursts in a 10^6 suspension of human neutrophils. As this amount corresponds to 1−2 mg of fresh weight and the human neutrophil circulating pool is about 40×10^9 cells, an amount of about 40−80 g of eggplant may be sufficient to interact with all of the neutrophils present in the human body. Bearing in mind the bioavailability and other parameters not considered in our study, from a theoretical nutritional point of view, our findings support the fact that a serving of eggplant may contain sufficient antioxidants to neutralize neutrophil ROS release.

LITERATURE CITED

(1) Pellegrini, N.; Miglio, C.; Del Rio, D.; Salvatore, S.; Serafini, M.; Brighenti, F. Effect of domestic cooking methods on the total antioxidant capacity of vegetables. *Int. J. Food Sci. Nutr.* **2009**, *60* (S2), 12–22.

(2) Yamaguchi, T.; Mizobuchi, T.; Kajikawa, R.; Kawashima, H.; Miyabe, F.; Terao, J.; Takamura, H.; Matoba, T. Radical-scavenging activity of vegetables and the effect of cooking on their activity. *Food Sci. Technol. Res.* **2001**, *7*, 250–257.

(3) Miglio, C.; Chiavaro, E.; Visconti, A.; Fogliano, V.; Pellegrini, N. Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *J. Agric. Food Chem.* **2008**, *56*, 139–147.

(4) Kaneyuki, T.; Noda, Y.; Traber, M. G.; Mori, A.; Packer, L. Superoxide anion and hydroxyl radical scavenging activities of vegetable extracts measured using electron spin resonance. *Biochem. Mol. Biol. Int.* **1999**, *47*, 979–989.

(5) Jiménez-Monreal, A. M.; García-Diz, L.; Martínez-Tomé, M.; Mariscal, M.; Murcia, M. A. Influence of cooking methods on antioxidant activity of vegetables. *J. Food Sci.* **2009**, *74* (3), H97–H103.

(6) Whitaker, B. D.; Stommel, J. R. Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. *J. Agric. Food Chem.* **2003**, *51*, 3448–3454.

(7) Ichiyanagi, T.; Kashiwada, Y.; Shida, Y.; Ikeshiro, Y.; Kaneyuki, T.; Konishi, T. Nasunin from eggplant consists of cis-trans isomers of delphinidin 3-[4-(*p*-coumaroyl)-L-rhamnosyl (1→6)glucopyranoside]-5-glucopyranoside. *J. Agric. Food Chem.* **2005**, *53*, 9472–9477.

(8) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1974**, *299*, 152–178.

(9) Fujita, S.; Tono, T. Purification and some properties of polyphenoloxidase in eggplant (*Solanum melongena*). *J. Sci. Agric.* **1988**, *46*, 115–123.

(10) Concellón, A.; Añón, M. C.; Chaves, A. R. Characterization and changes in polyphenol oxidase from eggplant fruit (*Solanum melongena* L.) during storage at low temperature. *Food Chem.* **2004**, *88*, 17–24.

(11) Luthria, D. L.; Mukhopadhyay, S. Influence of sample preparation on assay of phenolic acids from eggplant. *J. Agric. Food Chem.* **2006**, *54*, 41–47.

(12) Birner, J. A method for the determination of total steroid bases. *J. Pharm. Sci.* **1969**, *58*, 258–259.

(13) Kuronen, P.; Väänänen, T.; Pehu, E. Reversed-phase liquid chromatographic separation and simultaneous profiling of steroidal glycoalkaloids and their aglycones. *J. Chromatogr. A* **1999**, *863*, 25–35.

(14) Jones, P. G.; Fenwick, G. R. The glycoalkaloid content of some edible solanaceous fruits and potato products. *J. Sci. Food Agric.* **2006**, *32*, 419–421.

(15) Krits, P.; Fogelman, E.; Ginzberg, I. Potato steroidal glycoalkaloid levels and the expression of key isoprenoid metabolic genes. *Planta* **2007**, *227*, 143–150.

(16) Morelli, R.; Russo-Volpe, S.; Bruno, N.; Lo Scalzo, R. Fenton-dependent damage to carbohydrates: Free radical scavenging activity of some simple sugars. *J. Agric. Food Chem.* **2003**, *51*, 7418–7425.

(17) Valavanidis, A; Nisiotou, C; Papageorgiou, Y; Kremli, I.; Satravelas, N.; Zinieris, N.; Zygalaki, H. Comparison of the radical scavenging potential of polar and lipidic fractions of olive oil and other vegetable oils under normal conditions and after thermal treatment. *J. Agric. Food Chem.* **2004**, *52*, 2358–2365.

(18) Briheim, G.; Dahlgren, C. Influence of antibiotics on formyl-methionyl-leucyl-phenylalanine-induced leukocyte chemiluminescence. *Antimicrob. Agents Chemother.* **1987**, *31*, 763–769.

(19) Summa, C.; Cordeiro Raposo, F.; McCourt, J.; Lo Scalzo, R.; Wagner, K. H.; Elmadfa, I.; Anklam, E. Effect of roasting on the radical scavenging activity of cocoa beans. *Eur. Food Res. Technol.* **2006**, *222*, 368–375.

(20) Friedman, M.; Si Dao, L. Distribution of glycoalkaloids in potato plants and commercial potato products. *J. Agric. Food Chem.* **1992**, *40*, 409–413.

(21) Friedman, M.; Levin, C. E. α-Tomatine content of tomato and tomato products determined by HPLC with pulsed amperometric detection. *J. Agric. Food Chem.* **1995**, *43*, 1507–1511.

(22) Ferracane, R.; Pellegrini, N.; Visconti, A.; Graziani, G.; Chiavaro, E.; Miglio, C.; Fogliano, V. Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. *J. Agric. Food Chem.* **2008**, *56*, 8601–8608.

(23) Kalogeropoulos, N.; Mylona, A.; Chiou, A.; Ioannou, M. S.; Andrikopoulos, N. K. Retention and distribution of natural antioxidants (α-tocopherol, polyphenols and terpenic acids) after shallow frying of vegetables in virgin olive oil. *Lebensm.-Wiss. Technol.* **2007**, *40*, 1008–1017.

(24) Rossi, M.; Giussani, E.; Morelli, R.; Lo Scalzo, R.; Nani, R. C.; Torreggiani, D. Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. *Food Res. Int.* **2003**, *36*, 999–1005.

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