



Cooking influence on physico-chemical fruit characteristics of eggplant (*Solanum melongena* L.)



Roberto Lo Scalzo^{a,*}, Marta Fibiani^a, Gianluca Francese^b, Antonietta D'Alessandro^b, Giuseppe L. Rotino^c, Pellegrino Conte^d, Giuseppe Mennella^b

^a Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, CREA-IAA Unità di Ricerca per i Processi dell'Industria Agroalimentare, via Venezian 26, 20133 Milan, Italy

^b Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, CREA-ORT Centro di Ricerca per l'Orticoltura, via Cavallotti 25, 84098 Pontecagnano-Faiano (Salerno), Italy

^c Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, CREA-ORT Unità di Ricerca per l'Orticoltura, via Paullese 28, 26836 Montanaso Lombardo (Lodi), Italy

^d Università degli Studi di Palermo, Dipartimento di Scienze Agrarie e Forestali, Viale delle Scienze 4, 90128 Palermo, Italy

ARTICLE INFO

Article history:

Received 25 February 2015

Received in revised form 14 August 2015

Accepted 18 August 2015

Available online 20 August 2015

Chemical compounds studied in this article:

Chlorogenic acid (PubChem CID: 1794427)

5-Hydroxy-methyl-furfural (PubChem CID: 237332)

Delphinidin-3-rutinoside (PubChem CID: 44256887)

Nasunin (PubChem CID: 44256909)

Putrescine (PubChem CID: 1045)

Spermidine (PubChem CID: 1102)

Solamargine (PubChem CID: 73611)

Solasonine (PubChem CID: 119247)

Keywords:

Eggplant

Cooking

Phenols

Antioxidants

NMR relaxometry

ABSTRACT

Physico-chemical traits of three eggplant genotypes ("Tunisina", "Buia" and "L 305") were evaluated before and after two cooking treatments (grilling and boiling). Different genotypes revealed different changes after cooking, with "Tunisina" showing a better retention of phytochemicals with respect to other two genotypes. The main physical phenomena were water loss during grilling, and dry matter loss after boiling. Chlorogenic acid, the main phenolic in eggplant, resulted higher in grilled samples, while delphinidin glycosides resulted more retained in boiled samples. Glycoalkaloids, thiols and biogenic amines were generally stable, while 5-hydroxy-methyl-furfural was found only in grilled samples. Interestingly, Folin–Ciocalteu index and free radical scavenging capacity, measured with three different assays, were generally increased after cooking, with a greater formation of antioxidant substances in grilled samples. NMR relaxation experiments clarified the hypothesis about the changes of eggplant compounds in terms of decomposition of larger molecules and production of small ones after cooking.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

The eggplant (*Solanum melongena* L.) fruit represents a very interesting plant system for its composition in phytochemicals and nutraceuticals; in particular polyphenols, such as phenolic acids (Whitaker & Stommel, 2003) and anthocyanins (Ichihara et al., 2005), are worthy to be considered as are well known for

their antioxidant properties (Kaneyuchi, Noda, Traber, Mori, & Packer, 1999).

Previous works have evidenced the possibility, displayed in genetic improvement programs, to obtain antioxidants (especially polyphenols) enriched eggplant breeding lines and germplasm (Hanson et al., 2006; Mennella et al., 2010; Plazas et al., 2013; Prohens, Rodríguez-Burruezo, Raigon, & Nuez, 2007; Prohens et al., 2013). The modifications of phytochemicals occurring in humans after ingestion of food are of difficult evaluation (Lafay & Gil-Izquierdo, 2008): such a problem increases in the case of plant products that have to be cooked before consumption (Ruiz-Rodríguez, Marín, Ocaña, & Soler-Rivas, 2008).

Some previous studies (Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009; Lo Scalzo et al., 2010; Pellegrini

* Corresponding author.

E-mail addresses: roberto.loscalzo@entecra.it (R. Lo Scalzo), marta.fibiani@entecra.it (M. Fibiani), gianluca.francese@entecra.it (G. Francese), antonietta.dalessandro@entecra.it (A. D'Alessandro), giuseppeleonardo.rotino@entecra.it (G.L. Rotino), pellegrino.conte@unipa.it (P. Conte), giuseppe.mennella@entecra.it (G. Mennella).

et al., 2009) demonstrated that eggplant possesses relevant antioxidant indexes after cooking due to the retention of antioxidant compounds with respect to the raw product. These phenomena can be essentially related to the thermostability of the phenolic fraction, with a very low contribution of the thermolabile fraction, mainly represented by ascorbic acid (Kaneyuchi et al., 1999; Yamaguchi et al., 2001). However, the identification of type and class of phytonutrients involved in the retention or increase of antioxidant properties after vegetable cooking needs further clarifications.

Previous studies, made on different vegetables other than eggplant, have demonstrated a cooking-related depletion in antioxidant compounds amount, followed by a contemporary increase of antioxidant indexes (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008). On the other hand, other works have shown a good agreement between the increase of caffeic acid esters, induced by cooking, and the variation of the antioxidant indexes (Ferracane et al., 2008; Lo Scalzo et al., 2010).

The present study is aimed to study the cooking-induced changes in the eggplant phytochemicals composition, before and after two commonly used cooking ways, grilling and boiling. The use of an approach made by a deep phytochemical analysis coupled with three different antioxidant assays together with a new spectroscopic technique for plant systems, such as the nuclear magnetic resonance (NMR) relaxometry (Fast-Field Cycling, FFC) on solid state (Conte, Bubici, Palazzolo, & Alonzo, 2009) could contribute to a progress in the resolution of these problems. Besides, to our best knowledge, previous works on this topic have been based on the cooking-induced changes made on a single plant genotype, with scarce consideration for a comparative evaluation between different genotypes of the same species. Here we highlighted a significant genetic effect by comparing eggplants belonging to three different genetic backgrounds.

2. Materials and methods

2.1. Plant material

Eggplant fruits of “Tunisina”, “Buia” and “L 305” genotypes, belonging to the “Round” (violet pale-purple), “Oval” (deep purple-black) and “Long” (deep purple) typologies, respectively, were harvested at the commercial ripening stage in an experimental field located in Montanaso Lombardo (Lodi, Italy) at the CREA, Unità di Ricerca per l’Orticoltura. The plants were grown next to each other in a very uniform soil using the usual agronomical techniques. Besides the external aspect and the genetic background, the assayed genotypes differentiate each other for the peel anthocyanin. In fact, the deep purple non-Japanese types “Buia” and “L 305” were characterized by delphinidin-3-rutinoside (D3R), while the pale purple Japanese type “Tunisina” by nasunin, a more complex delphinidin-3-(*p*-coumaroylrutinoside)-5-glucoside, occurring in *cis* and *trans* configurations (Azuma et al., 2008; Ichiyanagi et al., 2005). Then, “L 305” is a long-type eggplant spiny in the calyx, along the stem and leaf vein, it is taller than the other two genotypes and shows a weak anthocyanic coloration in the apex, its flesh is greenish next to the skin similar to “Buia”, which is a round-oval type eggplant with green calyx with less anthocyanic organ; “Tunisina” has anthocyanic stem, apex and leaf vein, a round-shaped fruit with evenly white flesh.

About 50 fruits for each genotype were visually selected based on their homogeneous size, shape, color, apparent absence of diseases and injury, and further subdivided into three aliquots: one left untreated (RAW), and the others for grilling and boiling processing. The fruits in each aliquot were cleaned and sliced parallel to fruit equator into pieces of the same thickness (1.0 ± 0.1 cm). RAW samples were immediately frozen in an air blast tunnel at -50 °C, and then lyophilised.

The aliquots for cooking were either grilled for 4–5 min (GRILL) on both sides, using a professional grilling apparatus, or boiled for 10 min (BOIL), in tap water at 1:10 ratio of eggplant slices:cooking water, monitoring the temperature with calibrated probes connected to the software ELLAB-E-Val 2.10. The grilling plate surface was stabilized at 190–210 °C, the inner part of slices during grilling reached 100 °C in 1 min, while the boiled slices reached 75 °C in 1 min and 100 °C in the next 4 min. The temperature was maintained at 100 °C during the remaining time in both cooking ways. After the cooking time, the GRILL and BOIL samples were kept on filter paper at room temperature for 15 min, draining out the water of BOIL samples, then both samples were frozen in air blast tunnel at -50 °C, and lyophilised.

Lyophilised samples were obtained until reaching a constant weight, in order to calculate the dry matter value (DM). They were successively reduced in powder by homogenization with a waring blender at 3–5 °C.

The rationale to evaluate the effect of cooking on the eggplant fruits components has been the simple ratio of each parameter in the cooked vs. the raw sample. The closer this value is to one, the more the component is unchanged under cooking treatment. The ratios are indicated in all tables with the given values of concentrations, all expressed by dry weight, this to avoid the variations in water content after cooking, that decrease after grilling and increase after boiling when compared to raw samples, as demonstrated by the changes in dry matter (DM, Table 1).

2.2. Chemical assays

Eggplant extracts were obtained from 300 mg of lyophilised powder by treating with 20 mL of 1 mM HCl at 2–4 °C, vortexing for 60 s and subsequently centrifuging at $25,000 \times g$, 10 min. The aqueous phase was immediately filtered on glass wool and frozen at -80 °C for the total phenols and antioxidant assays.

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

2.3. Simple sugars and organic acids

Both sugars and acids were analyzed using High Performance Liquid Chromatography (HPLC). The simple sugars sucrose, glucose and fructose were separated by a CarboSep Coregel 87C (Biorad) carbohydrate column with a 0.78×30 cm bed packed with a cation-exchange resin in the Ca^{2+} ionic form. The mobile phase was Milli-Q water at 0.7 mL/min, the elution was performed at 85 °C, and the signals were revealed by a refractive index detector. Samples, consisting of an aqueous extract from eggplant powder (300 mg in 10 mL water, shaken by vortexing, centrifuged and filtered before analysis) were 5-fold diluted with mobile phase before injection (10 μL). Sucrose, glucose and fructose solutions were used as external standards (retention times 8.3, 10.1 and 12.4 min, respectively) and the results were expressed as g/100 g DM for the sum of sucrose, glucose and fructose. The analyte concentrations were calculated from the experimental peak areas by analytical interpolation in a standard calibration curve for each compound.

As for hydroxy acids analysis, the separation was carried out on an Inertsil ODS-3 (GL Sciences) column, 5 μm of particle diameter and 0.46×25 cm dimensions. The elution was carried out at 30 °C with H_3PO_4 0.02 M in water as mobile phase at a flow rate of 0.6 mL/min. The detection was spectrophotometrically carried out at 214 nm. Samples, exactly made as for the simple sugars analysis, were injected in the HPLC system after 5-fold dilution

Table 1
Composition of three eggplant genotypes for main chemicals before (RAW) and after two cooking treatments (GRILL and BOIL); DM, dry matter; EIR, ethanol insoluble residue; TP, total phenols by Folin–Ciocalteu reaction; SCA, sum of glycoalkaloids; PPO, polyphenoloxidase activity. Different letters stand for statistically different values within each genotype ($p < 0.05$).

Genotype/thermal treatment	DM (%)	EIR (% of DM)	Simple sugars (g/100 g DM)	Organic acids (g/100 g DM)	TP (mg CA eq/100 g DM)	SCA (mg/100 g DM)	PPO (U/100 mg DM)
Tunisina							
RAW	7.8	45.4 ± 0.5 b	44.7 ± 2.4 a	1614 ± 12 b	1574 ± 287 b	27.2 ± 1.0 a	30.8 ± 5.1 a
GRILL	12.3	44.9 ± 0.3 b	45.0 ± 3.3 a	2322 ± 58 a	3008 ± 189 a	29.4 ± 0.8 a	0.0 ± 0.0 b
BOIL	5.6	58.3 ± 0.2 a	37.1 ± 2.5 b	1882 ± 199 b	1966 ± 50 b	17.5 ± 0.1 b	2.6 ± 0.0 b
Ratio GRILL/RAW	1.60	0.99	1.01	1.44	1.91	1.08	0.00
Ratio BOIL/RAW	0.72	1.28	0.83	1.17	1.25	0.65	0.08
Buia							
RAW	9.1	56.0 ± 0.8 b	37.1 ± 1.9 a	2299 ± 115 a	1578 ± 52 c	23.1 ± 1.0 b	21.0 ± 6.3 a
GRILL	14.4	55.4 ± 0.1 b	31.9 ± 1.9 b	2099 ± 326 a	2561 ± 204 a	47.8 ± 0.7 a	1.4 ± 0.3 b
BOIL	6.2	63.1 ± 0.6 a	26.3 ± 1.4 c	1992 ± 13 a	2065 ± 15 b	21.7 ± 0.7 b	2.0 ± 0.2 b
Ratio GRILL/RAW	1.57	0.99	0.86	0.91	1.62	2.07	0.07
Ratio BOIL/RAW	0.68	1.13	0.71	0.87	1.31	0.94	0.10
L 305							
RAW	8.0	62.1 ± 0.4 b	32.4 ± 2.9 a	2117 ± 251 a	1345 ± 113 b	43.0 ± 0.4 b	44.2 ± 5.3 a
GRILL	12.0	56.6 ± 0.1 c	24.2 ± 1.6 b	1718 ± 132 ab	2015 ± 66 a	73.6 ± 0.2 a	1.0 ± 0.3 b
BOIL	5.2	64.2 ± 0.2 a	24.4 ± 1.4 b	1575 ± 69 b	1826 ± 11 a	39.0 ± 0.1 c	2.4 ± 1.6 b
Ratio GRILL/RAW	1.44	0.91	0.75	0.81	1.50	1.71	0.02
Ratio BOIL/RAW	0.66	1.03	0.75	0.74	1.36	0.91	0.05

with mobile phase (10 µL). Quinic, oxalic, shikimic and citric acid solutions were used as external standards (retention times 5.4, 6.7, 8.8 and 12.7 min, respectively), and the results were expressed as mg/100 g DM for the sum of all identified acids.

2.4. Phenolic acids

Phenolic acids were extracted according to Whitaker and Stommel (2003) with minor modifications. All the extracts were analyzed using Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) coupled to a photodiode array detector and to an ion trap mass spectrometry system. Such system consisted of an Ultra Performance Liquid Chromatography Ultimate 3000 model (UPLC, Dionex) coupled to a LTQ XL mass spectrometer (ThermoFisher Scientific, Sunnyvale, CA, USA). A sample of 2 µL was injected on a Luna C₁₈ (100 × 2.0 mm, 2.5 µm particle size) column equipped with a SecurityGuard guard column (3.0 × 4.0 mm) from Phenomenex (Torrance, CA, USA). A binary mobile phase gradient of 0.05% aqueous formic acid and methanol was used as follows: 0–5.5 min, linear increase from 5% to 25% methanol; 5.5–9.5 min, linear increase from 25% to 50% methanol; 9.5–11 min, 50% methanol; 11–12 min, from 50% to 100% methanol; 12–15 min, 100% methanol; 15–16.5 min; linear decrease from 100% to 5% methanol. Flow rate was 0.2 mL/min. The analysis lasted for 28 min and the column temperature was set to 40 °C. Mass spectra were obtained in positive ion mode (ES⁺) over the range m/z 70–1500. The capillary voltages were set at 10 V and the source temperature was 34 °C. Quantitative determination of compounds was conducted by comparison with dose–response curves based on m/z data from authentic standards of chlorogenic acid (CA, retention time 12.52 min) and 5-hydroxymethyl-furfural (HMF, retention time 7.58 min) purchased from Sigma Aldrich (St. Louis, MO, USA). The results were expressed as mg/100 g of DM. Control of all instruments, data acquisition and data analyses were performed with Excalibur 2.2 software (ThermoFisher Scientific).

2.5. Anthocyanins

The anthocyanins contained in the peel were extracted from the whole fruit sample and analyzed as described by Ichiyanagi et al. (2005). Pure delphinidin-3-rutinoside (D3R) (Extrasynthese, France) was used as the external standard in the RP-HPLC separations. The standard of nasunin was prepared by means of extraction from the eggplant peel with 40 mM HCl, cleaning by centrifugation, C₁₈-SPE chromatography, elution with 30% aqueous ethanol, and quantification by spectrophotometry using the molar extinction coefficient of delphinidin (23,700 at 521 nm). The results were expressed as mg/100 g of DM.

2.6. PPO activity

Polyphenol oxidase (PPO) activity was assayed as described by Fujita and Tono (1988), using 30 mg of lyophilised 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 (Concellón, Añón, & Chaves, 2004).

2.7. Thiols

For the analysis of the total content of the non-protein –SH groups, 50 mg of lyophilised powder were extracted with 1 mL of a 1:1 mixture of EtOH and 0.02 N HCl. The total content of the non-protein –SH groups was determined by spectrophotometry according to Hawrylak and Szymanska (2004), with some modifications. An aliquot of 0.25 mL of sample was treated with 0.5 mL

of a mixture of 4% 5-sulphosalicylic acid, 2 mM Na₂-EDTA and 0.3% of sodium ascorbate. Then, 1 mL of 1 M sodium phosphate buffer (pH 8.0) and 0.25 mL of 10 mM DTNB (dithionitrobenzoic acid) were added. The absorbance was recorded at 415 nm. The number of non-protein -SH groups was calculated from a standard curve made for L-cysteine, and the data are given as mg/100 g DM.

2.8. Biogenic amines

The main two free biogenic amines in eggplant tissue are putrescine and spermidine. Their measurement has been performed by means of HPLC according to Rodríguez, López, and Chaves (1999), working on an extract from lyophilized eggplant tissue in the same amount as described in the reference work. The data were expressed as the sum of the two main biogenic amines of eggplant by mg/100 g of DM.

2.9. Glycoalkaloids

The glycoalkaloids, solamargine and solasonine, were extracted from 0.5 g of lyophilized tissue using 95% ethanol as described by Birner (1969) with some modifications, and analyzed by means of RP-HPLC according to Kuronen, Väänänen, and Pehu (1999) with minor modifications, using purified solasonine and solamargine as the external standards. The data were expressed as the sum of the two main glycoalkaloids (SGA) of eggplant by mg/100 g of DM.

2.10. Total polyphenols

The total polyphenol index (TP) was assayed in an extract (see Section 2.2) of lyophilized tissue by means of spectrophotometric analysis, using a modified Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1974). The results were expressed as mg per 100 g of DM of chlorogenic acid equivalent. Chlorogenic acid was used because it is the main monomeric phenolic compound in eggplant fruit.

2.11. Free radical scavenging capacity

Antiradical activity was assayed by measuring the scavenging of eggplant extract (see Section 2.2) against superoxide anions, hydroxyl and peroxy radicals. In all three assays, to calculate the scavenging index, the percent protection index (*I*) was obtained using the equation: $I = 100 - (I_x/I_0 \times 100)$, where I_x is the response in the presence of eggplant extract, and I_0 the response in its absence. The results were obtained by interpolating the data from eggplant extracts with the scavenging index of chlorogenic acid solutions at known concentrations and expressed as mg of chlorogenic acid equivalents per 100 g of DM.

2.11.1. Superoxide and hydroxyl radicals scavenging

The superoxide and hydroxyl scavenging assays were performed using electron spin resonance (ESR) spectrometry, as previously described by Lo Scalzo et al. (2010). Free radical generation for superoxide anions (12.8 mM KO₂-crown-ether-18-6 1:1 in dimethylsulfoxide, 200 µL) was followed by spin trapping with 50 mM 5,5-dimethyl-1-pyrrolin-N-oxide (DMPO), 200 µL. The sample was composed by 10 µL of eggplant extract or blank (HCl 1 mM). 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.

For hydroxyl radicals, a Fenton system (FeSO₄·7H₂O 10 mM, Na-EDTA 10 mM, followed by adding H₂O₂ 10 mM in 0.1 M phosphate buffer pH 7.4, totally 300 µL) was used, followed by spin trapping with 50 mM DMPO (100 µL). The sample was composed by 100 µL of eggplant extract or blank (HCl 1 mM).

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.

2.11.2. Peroxyl radicals scavenging

Peroxy radical was assayed in a system composed by linoleic acid/lipoxygenase/crocin following the rationale of Huang, Ou, and Prior (2005), with the modification of the peroxy radical generation, here obtained from the reaction between linoleic acid as substrate and soybean lipoxygenase (Chamulitrat & Mason, 1989; Grossman & Zakut, 1979). This method was validated and compared with others in a previous work (Lo Scalzo, Todaro, & Rapisarda, 2012). The assay for the activity against peroxy radical was carried out by enzymatic degradation of Na linoleate, yielding peroxy radicals and subsequent crocin bleaching. The percent protection of crocin bleaching was calculated by the inhibition of linear decrease in absorbance at 445 nm in presence and in absence of eggplant extract.

The reaction system was composed as follows:

2 mL Mc Ilvaine buffer, pH 7.0, 0.3 mL of Na linoleate 3 mM, prepared from linoleic acid (25 mg) dissolved in EtOH-Tween 20, 99:1 (2.5 mL), dissolved in Na₂HPO₄ 0.05 M taken at pH 9.2 with 0.5 M NaOH to a final volume of 25 mL, 0.2 mL of crocin ($\epsilon = 1.33 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ at 445 nm), 18 mg in 20 mL EtOH/H₂O 1.5:3.5 v/v, 0.1 mL of sample extract or blank and the reaction was started with 0.1 mL of soybean lipoxygenase (EC 1.13.11.12), 14.4 U/mg, (15 mg enzyme in 25 mL Mc Ilvaine buffer, pH 7.0). The linear decrease in absorbance at 445 nm was followed during the first 3 min of reaction at 25 °C.

2.12. FFC NMR relaxometry

All the samples were analyzed in the solid state as reported in Conte et al. (2009). The ¹H NMRD profiles (i.e. relaxation rates R_1 or $1/T_1$ vs. proton Larmor frequencies) were acquired on a Stellar Spinmaster-FFC-2000 Fast-Field-Cycling Relaxometer (Stellar s.r.l., Mede, PV – Italy) at a constant temperature of 25 °C. The proton spins were polarized at a polarization field (B_{POL}) corresponding to a proton Larmor frequency (ω_L) of 25 MHz for a period of polarization (T_{POL}) of 1 s. The longitudinal magnetization evolution were recorded at values of a relaxation magnetic field (B_{RLX}) corresponding to ω_L comprised in the range 0.01–20 MHz for a period of time (τ) arrayed with 128 values, chosen in an exponential progression from 2.3 to 233 ms. The exponential progression ensured the covering of the entire relaxation curve of interest. Finally, a ¹H 90° pulse was used at the starting of the acquisition period at the same time of an acquisition magnetic field (B_{ACQ}) corresponding to a ω_L of 16.2 MHz. The observable magnetization was revealed as free induction decay (FID) with a time domain of 100 µs sampled with 512 points. Two scans were accumulated. Data elaboration applied in this study has been already reported in Maccotta et al. (2013).

2.13. Data analysis

All the determinations were carried out on three independent extracts. Data were subjected to ANOVA performed by JMP (SAS Institute, Cary, NC, USA) according to a completely randomized design. Means $\pm$ standard deviation (SD) were compared by using Tukey HSD test ($p < 0.05$).

3. Results

3.1. Chemical composition

In all the assayed genotypes, the fresh weight after grilling, in comparison to the RAW one, showed a decrease due to a water loss

Table 2

Composition of three eggplant genotypes for monomeric antioxidants before (RAW) and after two cooking treatments (GRILL and BOIL): CA, chlorogenic acid; HMF, 5-hydroxymethyl-furfural; ATH, total anthocyanin content. Different letters stand for statistically different values within each genotype ($p < 0.05$).

Genotype/ thermal treatment	CA (mg/100 g DM)	HMF (mg/100 g DM)	ATH (mg/100 g DM)	Thiols (mg/100 g DM)	Biogenic amines (mg/100 g DM)	Sum of antioxidants (mg/100 g DM)	Sum of antioxidants (mg/100 mg FW)
Tunisina RAW	1717 ± 87 b	0.0 b	41.3 ± 16.5 a	34.9 ± 2.5 a	8.7 ± 0.5 c	1801 ± 86 b	138.2 ± 6.6 b
GRILL	1894 ± 51 a	62.2 ± 6.9 a	15.1 ± 8.9 b	35.9 ± 0.6 a	11.4 ± 1.1 b	2019 ± 64 a	248.8 ± 7.9 a
BOIL	1134 ± 10 c	0.0 b	27.5 ± 18.8 ab	24.1 ± 0.3 b	13.4 ± 0.3 a	1199 ± 18 c	67.1 ± 1.0 c
Ratio GRILL/RAW	1.10	–	0.36	1.03	1.32	1.12	1.80
Ratio BOIL/RAW	0.66	–	0.67	0.69	1.55	0.67	0.49
Buia RAW	1557 ± 55 b	0.0 b	155.3 ± 3.2 a	35.8 ± 0.1 a	14.6 ± 1.6 a	1763 ± 54 a	161.2 ± 4.9 b
GRILL	1679 ± 25 a	22.2 ± 1.9 a	31.1 ± 3.8 c	35.7 ± 0.1 a	12.9 ± 0.1 a	1781 ± 26 a	256.0 ± 3.7 a
BOIL	983 ± 36 c	0.0 b	51.8 ± 7.2 b	24.8 ± 1.3 b	14.3 ± 0.0 a	1074 ± 38 b	66.7 ± 2.4 c
Ratio GRILL/RAW	1.08	–	0.20	1.00	0.89	1.01	1.59
Ratio BOIL/RAW	0.63	–	0.33	0.69	0.98	0.61	0.41
L 305 RAW	1294 ± 15 a	0.0 b	96.3 ± 23.9 a	36.1 ± 0.4 a	14.0 ± 0.2 a	1441 ± 23 a	114.7 ± 1.8 a
GRILL	665 ± 45 b	12.2 ± 3.8 a	17.5 ± 11.1 b	30.9 ± 0.6 b	13.7 ± 1.3 a	740 ± 54 b	85.1 ± 6.2 b
BOIL	685 ± 14 b	0.0 b	42.3 ± 18.3 b	23.3 ± 0.4 c	11.8 ± 0.5 a	762 ± 24 b	39.8 ± 1.2 c
Ratio GRILL/RAW	0.51	–	0.18	0.86	0.98	0.51	0.74
Ratio BOIL/RAW	0.53	–	0.44	0.65	0.84	0.53	0.35

of the eggplant tissue. On the other hand, the boiling process induced a water intake on fruit slices, and therefore, an increase of weight in all samples except in “Tunisina”, which had a slight weight loss. Consequently, after lyophilization the DM was increased in GRILL fruits and decreased in BOIL fruits with respect to RAW ones (Table 1). The residue after EtOH extraction, that represents all the insoluble complex sugars such as cellulose fibers and pectins, had a slight decrease after grilling and a significant increase after boiling with respect to untreated samples (EIR, Table 1). This has been almost certainly due to the degradation of cell wall components after grilling and to the extraction from the fibrous matrix of the eggplant tissue, following the denaturation, after boiling. As for these aspects, negligible differences were detected among the three assayed eggplant genotypes.

The simple sugars changes after cooking showed higher retentions in “Tunisina” with respect to the other two genotypes (see ratios GRILL vs. RAW and BOIL vs. RAW, Table 1), in particular grilling did not significantly affect such a parameter with respect to RAW fruits. The amount of organic acids was generally less influenced by the cooking treatments with the difference of a significant increase in GRILL samples vs. RAW ones in “Tunisina”, not evidenced in the other two assayed genotypes (Table 1).

As expected, an almost total loss in PPO activity was detected in all the genotypes after cooking, with the common feature of a slight higher residual PPO index in BOIL than in GRILL samples (Table 1).

Glycoalkaloids content (Table 1) generally was quite stable after cooking, in accordance with previous data reported on potato (Friedman & Si Dao, 1992). This is demonstrated by the general trend of total glycoalkaloids (Table 1) that showed an increase after grilling and a decrease after boiling, caused, as previously stated, by a water loss (concentration) and by a leaching (dilution), respectively. However, a differential genotypic response was found. In “Tunisina”, SGA content resulted slightly increased (but not significantly) after grilling and strongly depleted after boiling. A different trend was found for “Buia” and “L 305”, with a significant increase of the total glycoalkaloids after grilling, and in a general stability or slight significant reduction as in the case of “L 305” after boiling.

As regards the phenolic profile, significant differences in the behavior after cooking have been found among the three assayed genotypes.

As shown in Table 2, the average amount of chlorogenic acid of RAW samples resulted of 1717, 1557 and 1294 mg/100 g DM, in “Tunisina”, “Buia” and “L 305”, respectively. It was slightly, but significantly, increased after grilling for “Tunisina” and “Buia”, while

it was significantly reduced in “L 305”. The boiling treatment resulted in a general significant decrease, with the highest retention in “Tunisina” and the lowest in “L 305” (see ratios GRILL vs. RAW and BOIL vs. RAW, Table 2). These data are generally in agreement with those presented by Zaro et al. (2015), who reported a differential chlorogenic acid retention in some eggplant genotypes after cooking and processing. On the other hand, Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, and López-García de la Serrana (2015) found a strong increase of chlorogenic acid after boiling, even if the phenol content of their sample appears to be very lower with respect to those here reported.

The other main important phenolic compounds in eggplant are the peel anthocyanins. It has to be stated that the analyzed genotypes have two different main anthocyanins, as previously reported by other authors (Azuma et al., 2008): “Tunisina”, derived from Asiatic genotypes, possesses the nasunin (coumaroyl ester of delphinidin-3-rutinoside-5-glucoside), while in “Buia” and “L 305”, of Indoeuropean origin, the main pigment is the delphinidin-3-rutinoside.

The evolution after cooking of eggplant's anthocyanins showed a decrease of the pigment amount in all samples, due to their low chemical stability, especially after pH changes. A higher anthocyanin stability was shown by “Tunisina” (ratio GRILL vs. RAW 0.36, Table 2), although containing a lower initial pigment amount than “Buia” (ratio 0.20) and “L 305” (ratio 0.18). The boiling process induces a less diminution than grilling in anthocyanin content in the three genotypes. “Buia” and “L 305” exhibiting D3R pigment had a higher depletion (ratios 0.33 and 0.44, respectively) than “Tunisina” (ratio 0.67, Table 2) exhibiting the nasunin. This different response is due to the lower water solubility of nasunin with respect to D3R, with a consequent less leaching from the matrix after boiling and a higher stability due to the protection of phenol OH with sugars and with *p*-coumaric acid.

Besides phenols, other compounds with valuable antioxidant properties were considered: (i) the thiols, whose cysteine and glutathione are the main representatives being present at 0.4 and 0.5 µmol/g DM, respectively (Mills, Stinson, Liu, & Lang, 1997); (ii) the biogenic amines putrescine and spermidine (Rodríguez et al., 1999); (iii) the 5-hydroxymethyl-furfural (HMF), a marker of thermal treatment in plant materials (Rufián-Henares, García-Villanova, & Guerra-Hernández, 2008), that we only found in the GRILL eggplant samples and not in the RAW and BOIL ones.

The average amount of total thiols, measured as cysteine equivalents, in RAW eggplant was 35.6 mg/100 g DM, with no great variation among the assayed varieties. The amounts of these compounds were not influenced by grilling process in “Tunisina”

and “Buia” whereas only a barely significant depletion was detected in “L 305”. After boiling, a similar thiols retention was displayed by the three genotypes (average ratio 0.68), due to their leaching in water (Table 2).

The biogenic amines level resulted in a different change for “Tunisina” when compared to the other two genotypes, where it showed a significant increase of 32% after grilling and 55% after boiling, while the amount of these compounds in “Buia” and “L 305” was unaffected by the two cooking types, this further evidenced a different behavior between the assayed eggplant types.

HMF was found only in GRILL samples, its level was higher in “Tunisina” and lower in “L 305” (Table 2), this is in accordance with the simple sugars content, that represent the precursors for the generation of this compound.

The sum of the antioxidants has been evaluated both on dry matter and on fresh weight (Table 2). The maximum increase both for dry and fresh weight was in GRILL “Tunisina” (ratios 1.12 and 1.80, respectively), and the minimum retention resulted in “L 305” (ratios 0.51 for GRILL DM and 0.35 for BOIL FW; Table 2).

3.2. Free radical scavenging capacity

The Folin index increased in all samples after cooking, with a different behavior between grilling and boiling, and among the genotypes (see TP, Table 1). Grilling induced a significant

increment in the three genotypes with a ratio varying from 1.50-fold in “L 305” to 1.91 in “Tunisina”. Boiling, instead, induced a less marked increase that was significant only for “L 305” and “Buia” (ratio 1.36 and 1.31, respectively).

The measurement of the antiradical capacity was carried out with three different methods, involving three of the most important oxygen radicals produced in potential oxidative stress phenomena: superoxide anion, hydroxyl and peroxy radicals.

In all three methods, the general information that can be deduced is that the cooked samples have higher antioxidant indexes than the untreated ones, moreover, a different genotypic response was highlighted (Fig. 1). The change with cooking was expressed by the ratio of the cooked value vs. raw one. Average data for the superoxide scavenging capacity (Fig. 1A) resulted in a ratio of 1.40 after GRILL and 1.24 after BOIL; the hydroxyl radical scavenging capacity (Fig. 1B) resulted in a significant increase after grilling (ratio 1.28) and in a stability after boiling (1.04). The data of peroxy radical scavenging capacity (Fig. 1C) stood out for the highest changes of ratios: the GRILL samples resulted 3.05-fold more active than RAW, and the boiling induced the highest increase (4.34-fold).

The specific response of the single genotypes resulted in the stronger increase in “Tunisina” GRILL samples: 1.90 and 1.37 times more active than RAW in the superoxide and hydroxyl scavenging capacities, respectively. The data from “Tunisina” also showed the

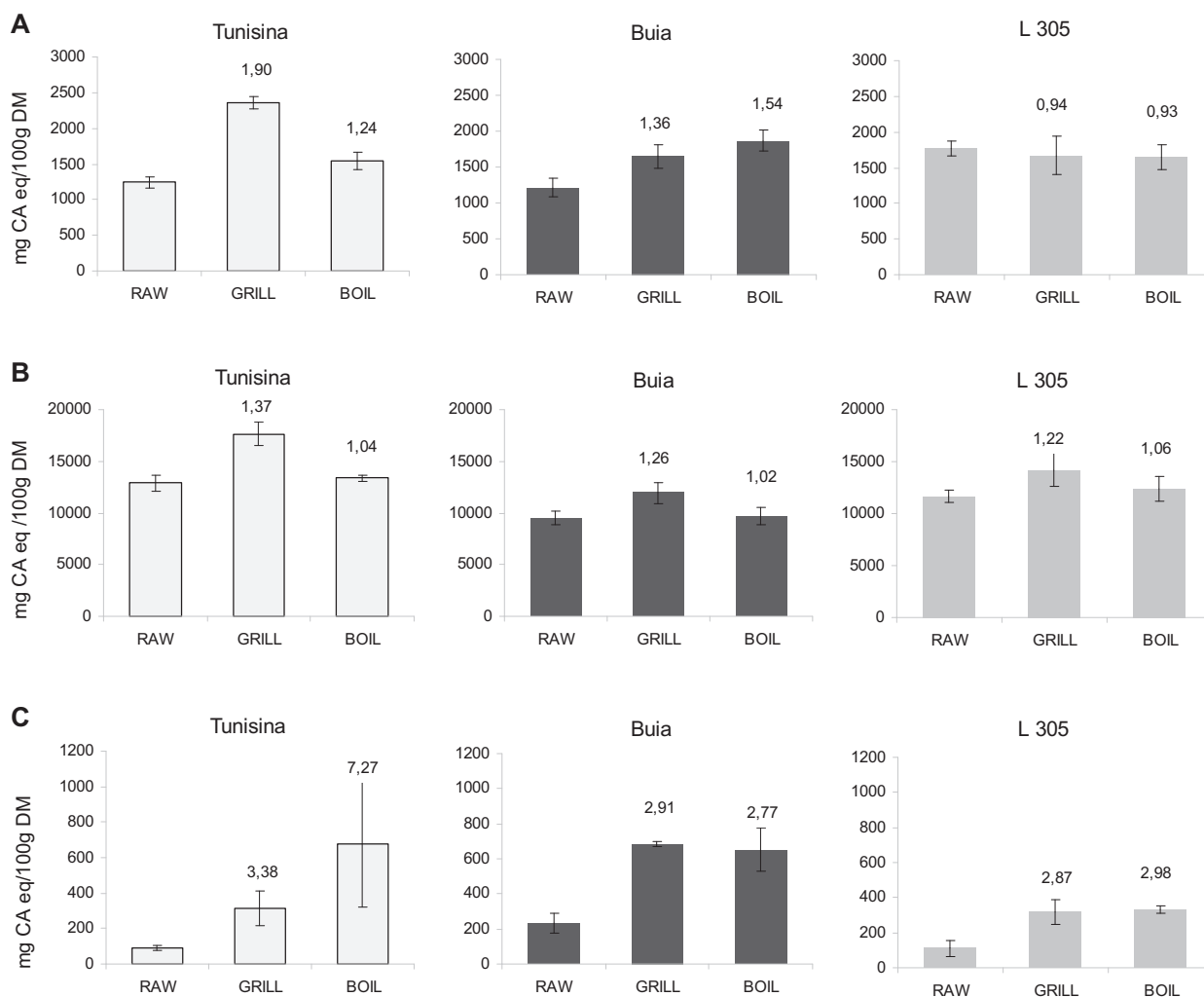


Fig. 1. Free radical scavenging capacity of three eggplant genotypes before (RAW) and after two cooking treatments (GRILL and BOIL) by three measuring methods, superoxide anion (A), hydroxyl (B) and peroxy (C) radicals. The amount of scavenging capacity has been expressed as chlorogenic acid equivalents on 100 g dry matter (CA eq/100 g DM). Ratios of the cooked values vs. RAW ones are reported on histograms.

highest ratio of the peroxyl scavenging (7.27) in the BOIL sample. “Buia” showed the highest ratio for superoxide scavenging (1.54) after boiling. On the other side, “L 305” had lower ratios for superoxide in both cooking ways and for hydroxyl radicals after GRILL with respect to the other genotypes. Similar data was found by Zaro et al. (2015), using the ABTS assay on raw, grilled and boiled eggplant samples. Moreover, also the data by Ramírez-Anaya et al. (2015), using DPPH, FRAP and ABTS assays, are in accordance with ours.

Similar responses among the assayed genotypes have been found in the hydroxyl and the peroxyl scavenging after boiling and grilling, respectively.

3.3. NMR relaxometry

NMR relaxometry response to the cooking in the three eggplant genotypes gave different patterns. Table 3 reports the NMR relaxometry parameters found by applying the model free analysis to the Bloembergen, Purcell and Pound (BPP) model (Conte & Alonzo, 2013) where α represents the high-field relaxation rate and β is a constant related to the dipolar interactions. τ_c is the correlation time, which describes the random molecular motions of molecular systems either in solution or in porous media. Namely, correlation time is the time taken for a molecule to rotate one radian or to move a distance of the order of its own dimension. The longer the τ_c value, the slower the molecular motions, thereby revealing restrictions in the motional freedom degrees of spatially restrained molecular systems. Conversely, as a molecule encompasses faster motions due to higher degrees of freedom in larger spaces, shorter correlation time values are expected.

As for the α index, “Tunisina” and “Buia” showed an increase in both cooked samples with respect to raw ones, with the strongest increase in “Buia”. The same fact occurred in “L 305” only for GRILL, while the RAW and BOILED eggplant samples showed practically the same values. The β index showed low ratio values in “Tunisina” with respect to the other two genotypes, meaning a decrease in the dipolar interactions for “Tunisina” in both cooking ways. The cooked-induced decomposition of the eggplant structure causes a formation of little molecules that tend to a reciprocal estrangement, so decreasing the β values in cooked samples with respect to the raw ones. This fact is evident in “Tunisina”, showing the main decreases, in “Buia” and only in the grilled samples of “L 305”, thereby confirming the trend of the α indexes. The τ_c values confirm the difference in the cooking changes for “Tunisina” with

respect to “Buia” and “L 305”. The increase in correlation times is exactly what happens in “Tunisina” and “Buia”, with a higher increase in “Tunisina” for both cooking ways. In the “L 305” genotype, only the boiled sample shows the expected τ_c value increase with respect to raw one, in a good accordance with the compared data of chemical composition changes in the three assayed genotypes.

4. Discussion and conclusion

It is well known that the most important antioxidant compounds in eggplant are represented by polyphenols. One important antioxidant compound is the ascorbic acid, but eggplant possesses a very low amount of it (Yamaguchi et al., 2001), so it was not considered in the present work, also for its certain thermal instability.

Our results indicate that the measured antioxidants show a general increase or stability after GRILL and a decrease after BOIL. As for the chlorogenic acid, the main antioxidant phenol in eggplant, its stability has been confirmed in accordance with previous studies (Ferracane et al., 2008; Lo Scalzo et al., 2010). In the present study, it was also outlined the occurrence of a significant differential genotypic effect with a reduced recovery of this compound in a particular genotype (i.e. “L 305”) also after grilling while the other two genotypes displayed an increment.

Interestingly, cooking induced a strong increase in the indexes of Folin reducing substances and free radical scavenging activities, markedly for GRILL samples. The increase of free radical scavenging in grilled samples can be due to the presence of Maillard reaction products, as demonstrated by the neo-formation of HMF, which are recognized as antioxidants (Manzocco, Calligaris, Mastrocola, Nicoli, & Leric, 2001).

The strong increase of free radical scavenging after boiling found by Ramírez-Anaya et al. (2015) resulted in a good accordance with the present data on peroxyl radical scavenging. With other assays, it resulted less prominent than in grilling. However, the boiling-induced increase, where present, is more difficult to explain, because of the decrease of most of the measured antioxidants, due to their leaching in boiling water and the concomitant absence of detectable Maillard reaction products. An alternative explanation could be the degradation of a pro-oxidant compound, after boiling thermal treatment, tentatively attributed to a peroxidase (Gazzani, Papetti, Massolini, & Daglia, 1998), a similar hypothesis was done for chicory by Papetti, Daglia, and Gazzani (2002). They used a peroxyl radical scavenging assay, and our results of peroxyl radical scavenging are in accordance with the data on chicory. Probably a similar phenomenon may happen after boiling of eggplant slices, with an evident increase of peroxyl radical scavenging activity in all genotypes (Fig. 1C).

To enforce this finding, the NMR relaxometry data give the chance to confirm that the cooking induces a process of decomposition of the eggplant tissue, resulting in an increase of potentially antioxidant compounds. The FFC NMR relaxation experiments were performed in solid phase. For this reason, the molecules present in the assayed tissue are mainly subjected to rotational movements, being the translational ones avoided by the physical status of the system. The cooking processes, yielding a significant number of molecules from the degradation of raw fruit tissues, gave a higher solvation with the residual water, causing a strengthening and a subsequent hindrance in their movement, hence longer correlation times. It's evident a different behavior mostly for “Tunisina” and “L 305”, also confirmed by the chemical and antioxidant data, with a higher retention of chemical composition in “Tunisina” and a lower one in “L 305”.

A general increase in the antioxidant activities of eggplant fruit after cooking was confirmed in the present work. Furthermore, we

Table 3
NMR relaxation properties of three eggplant genotypes before (RAW) and after two cooking treatments (GRILL and BOIL): α , high-field relaxation rate; β , constant related to the dipolar interactions; τ_c , correlation time (see Section 2).

Genotype/thermal treatment		α	β	$\tau_c \cdot 10^{-8}$ (s)
Tunisina	RAW	1.6	1463	0.395
	GRILL	3.4	801	1.000
	BOIL	4.4	788	1.070
	Ratio GRILL/RAW	2.1	0.5	2.5
Ratio BOIL/RAW		2.8	0.5	2.7
Buia	RAW	0.4	1400	0.513
	GRILL	14.9	904	0.881
	BOIL	4.0	1088	0.718
	Ratio GRILL/RAW	40.3	0.6	1.7
Ratio BOIL/RAW		10.8	0.8	1.4
L 305	RAW	4.3	773	0.604
	GRILL	14.8	587	0.333
	BOIL	4.4	788	1.060
	Ratio GRILL/RAW	3.4	0.8	0.6
Ratio BOIL/RAW		1.0	1.0	1.8

demonstrated as a considerable variation about the effect of thermal treatments (boiling and grilling) both on the retention of phytochemicals and on free radical scavenging capacity exists within the gene pool of *S. melongena*. This variation could be exploited to design genotypes with improved antioxidant characteristics to further enhance the beneficial effects on human health as most of the eggplants are consumed as cooked food.

The NMR relaxation experiments point out that cooking possibly causes a de-structuration of flesh tissue with the decomposition of large molecules into small ones, easily extractable from the eggplant fruit. This de-structuration was higher in “Tunisina” compared to the other two genotypes. This fact, together with the noted high softness of “Tunisina” flesh, confirmed by the low amount of DM and EIR (Table 1), might explain its general better retention of extractable phytochemicals with respect to “Buia” and “L305”.

However, further studies, for example through LC–MS techniques, are necessary with the aim to set up analytical methods for identifying other compounds, present or not in raw samples, putatively involved in the general increase of antioxidant capacity of eggplant after cooking.

References

- Azuma, K., Ohyama, A., Ippoushi, K., Ichiyanagi, T., Takeuchi, A., Saito, T., & Fukuoka, H. (2008). Structures and antioxidant activity of anthocyanins in many accessions of eggplant and its related species. *Journal of Agricultural and Food Chemistry*, 56, 10154–10159.
- Birner, J. (1969). A method for the determination of total steroid bases. *Journal of Pharmaceutical Sciences*, 58, 258–259.
- Chamulitrat, W., & Mason, R. P. (1989). Lipid peroxyl radical intermediates in the peroxidation of polyunsaturated fatty acids by lipoxygenase. *Journal of Biological Chemistry*, 264(35), 20968–20973.
- Concellón, A., Añón, M. C., & Chaves, A. R. (2004). Characterization and changes in polyphenol oxidase from eggplant fruit (*Solanum melongena* L.) during storage at low temperature. *Food Chemistry*, 88, 17–24.
- Conte, P., Bubici, S., Palazzolo, E., & Alonzo, G. (2009). Solid state ^1H -NMR relaxation properties of the fruit of a wild relative of eggplant at different proton Larmor frequencies. *Spectroscopy Letters*, 42, 235–239.
- Conte, P., & Alonzo, G. (2013). Environmental NMR: Fast-field cycling relaxometry. *eMagRes*, 2, 389–398.
- Ferracane, R., Pellegrini, N., Visconti, A., Graziani, G., Chiavaro, E., Miglio, C., & Fogliano, V. (2008). Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. *Journal of Agricultural and Food Chemistry*, 56, 8601–8608.
- Friedman, M., & Si Dao, L. (1992). Distribution of glycoalkaloids in potato plants and commercial potato products. *Journal of Agricultural and Food Chemistry*, 40, 409–413.
- Fujita, S., & Tono, T. (1988). Purification and some properties of polyphenoloxidase in eggplant (*Solanum melongena* L.). *Journal of the Science of Food and Agriculture*, 46, 115–123.
- Gazzani, G., Papetti, A., Massolini, G., & Daglia, M. (1998). Anti and prooxidant activity of water soluble components of some common diet vegetables and effect of thermal treatment. *Journal of Agricultural and Food Chemistry*, 46, 4118–4122.
- Grossman, S., & Zakut, R. (1979). Determination of the activity of lipoxygenase. In D. Glick (Ed.), *Method of biochemical analysis* (pp. 313–316). New York: Wiley Interscience.
- Hanson, P. M., Yang, R.-Y., Tsou, S. C. S., Ledesma, D., Engle, L., & Lee, T. C. (2006). Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolics and ascorbic acid. *Journal of Food Composition and Analysis*, 19, 594–600.
- Hawrylak, B., & Szymanska, M. (2004). Selenium as a sulphydryl group inductor in plant. *Cellular and Molecular Biology Letters*, 9(2), 329–336.
- Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53, 1841–1856.
- Ichiyanagi, T., Kashiwada, Y., Shida, Y., Ikeshiro, Y., Kaneyuki, T., & Konishi, T. (2005). Nasunin from eggplant consists of cis-trans isomers of delphinidin 3-[4-(p-coumaroyl)-L-rhamnosyl] (1 → 6) glucopyranoside-5-glucopyranoside. *Journal of Agricultural and Food Chemistry*, 53, 9472–9477.
- Jiménez-Monreal, A. M., García-Diz, L., Martínez-Tomé, M., Mariscal, M., & Murcia, M. A. (2009). Influence of cooking methods on antioxidant activity of vegetables. *Journal of Food Science*, 74(3), H97–H103.
- Kaneyuchi, T., Noda, Y., Traber, M. G., Mori, A., & Packer, L. (1999). Superoxide anion and hydroxyl radical scavenging activities of vegetable extracts measured using electron spin resonance. *Biochemistry and Molecular Biology International*, 47, 979–989.
- Kuronen, P., Väänänen, T., & Pehu, E. (1999). Reversed-phase liquid chromatographic separation and simultaneous profiling of steroidal glycoalkaloids and their aglycones. *Journal of Chromatography A*, 863, 25–35.
- Lafay, S., & Gil-Izquierdo, A. (2008). Bioavailability of phenolics acids. *Phytochemical Reviews*, 7, 301–311.
- Lo Scalzo, R., Fibiani, M., Mennella, G., Dal Rotino, G. L., Sasso, M., Culici, M., ... Braga, P. C. (2010). Thermal treatments of eggplant (*Solanum melongena* L.) increases the antioxidant content and the inhibitory effect on human neutrophil burst. *Journal of Agricultural and Food Chemistry*, 58, 3371–3379.
- Lo Scalzo, R., Todaro, A., & Rapisarda, P. (2012). Methods used to evaluate the peroxyl (ROO·) radical scavenging capacities of four common antioxidants. *European Food Research and Technology*, 235, 1141–1148.
- Maccotta, A., De Pasquale, C., Caruso, A., Cosentino, C., Alonzo, G., & Conte, P. (2013). Reconstruction of the environmental evolution of a sicilian salmtrash (Italy). *Environmental Science and Pollution Research*, 20(7), 4847–4858.
- Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., & Lerici, C. R. (2001). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science and Technology*, 11, 340–346.
- Mennella, G., Rotino, G. L., Fibiani, M., D'Alessandro, A., Francese, G., Toppino, L., ... Lo Scalzo, R. (2010). Characterization of health-related compounds in eggplant (*Solanum melongena* L.) lines derived from introgression of allied species. *Journal of Agricultural and Food Chemistry*, 58, 7598–7603.
- Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrini, N. (2008). Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *Journal of Agricultural and Food Chemistry*, 56, 139–147.
- Mills, B. J., Stinson, C. T., Liu, M. C., & Lang, C. A. (1997). Glutathione and cyst(e)ine profiles of vegetables using high performance liquid chromatography with dual electrochemical detection. *Journal of Food Composition and Analysis*, 10(2), 90–101.
- Papetti, A., Daglia, M., & Gazzani, G. (2002). Anti- and pro-oxidant water soluble activity of *Cichorium* genus vegetables and effect of thermal treatment. *Journal of Agricultural and Food Chemistry*, 50, 4696–4704.
- Pellegrini, N., Miglio, C., Del Rio, D., Salvatore, S., Serafini, M., & Brighenti, F. (2009). Effect of domestic cooking methods on the total antioxidant capacity of vegetables. *International Journal of Food Science and Nutrition*, 60(S2), 12–22.
- Plazas, M., López-Gresa, M. P., Vilanova, S., Torres, C., Hurtado, M., Gramazio, P., ... Prohens, J. (2013). Diversity and relationships in key traits for functional and apparent quality in a collection of eggplant: Fruit phenolics content, antioxidant activity, polyphenol oxidase activity, and browning. *Journal of Agricultural and Food Chemistry*, 61, 8871–8879.
- Prohens, J., Rodríguez-Burruezo, A., Raigon, M. D., & Nuez, F. (2007). Total phenolic concentration and browning susceptibility in a collection of different varietal types and hybrids of eggplant: Implication for breeding for higher nutritional quality and reduced browning. *Journal of American Society of Horticulture Science*, 132, 1–9.
- Prohens, J., Whitaker, B. D., Plazas, M., Vilanova, S., Hurtado, M., Blasco, M., ... Stommel, J. R. (2013). Genetic diversity in morphological characters and phenolic acids content resulting from an interspecific cross between eggplant, *Solanum melongena*, and its wild ancestor (*S. incanum*). *Annals of Applied Biology*, 162, 242–257.
- Ramírez-Anaya, J. P., Samaniego-Sánchez, C., Castañeda-Saucedo, M. C., Villalón-Mir, M., & López-García de la Serrana, H. (2015). Phenols and the antioxidant capacity of Mediterranean vegetables prepared with extra virgin olive oil using different domestic cooking techniques. *Food Chemistry*, 188, 430–438.
- Rodríguez, S. C., López, B., & Chaves, A. R. (1999). Changes in polyamines and ethylene during the development and ripening of eggplant fruits (*Solanum melongena* L.). *Journal of Agricultural and Food Chemistry*, 47, 1431–1434.
- Rufián-Henares, J. A., García-Villanova, B., & Guerra-Hernández, E. (2008). Occurrence of furosin and hydroxymethylfurfural as markers of thermal damage in dehydrated vegetables. *European Food Research and Technology*, 228, 249–256.
- Ruiz-Rodríguez, A., Marín, F. R., Ocaña, A., & Soler-Rivas, C. (2008). Effect of domestic processing on bioactive compounds. *Phytochemical Reviews*, 7, 345–384.
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1974). Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178.
- Whitaker, B. D., & Stommel, J. R. (2003). Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. *Journal of Agricultural and Food Chemistry*, 51, 3448–3454.
- Yamaguchi, T., Mizobuchi, T., Kajikawa, R., Kawashima, H., Miyabe, F., Terao, J., ... Matoba, T. (2001). Radical-scavenging activity of vegetables and the effect of cooking on their activity. *Food Science and Technology Research*, 7, 250–257.
- Zaro, M. J., Ortiz, L. C., Keunchkarian, S., Chaves, A. R., Vicente, A. R., & Concellon, A. (2015). Chlorogenic acid retention in white and purple eggplant after processing and cooking. *LWT – Food Science and Technology*, 64, 802–808.