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Pigment variation by colorimetric analysis in raw, grilled and boiled eggplant *(Solanum melongena L.)* fruits

1. INTRODUCTION

Eggplant fruit is known for its high content in phenolic compounds, which are plant secondary metabolites thought to be related to its antioxidant capacity and beneficial effects on human health [1] [2] [3] [4]. Phenolic compounds in pulp are mainly represented by chlorogenic acid and other caffeoyl-esters [1], while anthocyanins are the pigments conferring the purple colour of the peel.
In the plants, anthocyanins display a wide range of structures, due to different hydroxyl and methoxyl groups, glycosylation and acylation of the aglycone moieties. Thus, the chemical composition along with the medium conditions differently affected the colour and stability of anthocyanins. Eggplant anthocyanins have simple profiles generally characterized by a single delphinidin-glycoside [5] [6]. Delphinidin is the most hydroxylated and one of the most unstable, as the increasing hydroxylation on the B-ring shifts to longer wavelenghts its maximum visible absorption, nevertheless destabilizing the molecule [5] [7].
In particular, the ortodiphenolic structure increases the total antioxidant activity [8] [9] [10], but makes the molecule a better substrate for polyphenol oxidase [11] [12] [13].
Anthocyanins are also known to be, unlike other phenolic compounds, deeply affected by heating [14] [15] [16]. It is also reported that techniques involving a partial/mild heating can increase the yield during anthocyanins recovery, due to a protective effect during extraction or a better solubilization [16] or to the inactivation of degradative enzymes [17].
Processing and cooking practices can deeply modify physicochemical characteristics of raw vegetables, thus affecting their nutritional quality and antioxidant activity. Generally, eggplant fruits have to be cooked before eating, and although total antioxidant capacity and flesh polyphenols can be unaltered or even increased by cooking [18] [19] [20], peel anthocyanin pigments can be reduced [20].
This study reports about the global fate of eggplant pigments after thermal treatment. The modifications on methanolic extracts of raw and cooked fruits of three genotypes assayed with colorimetric and HPLC analyses are presented and discussed.

2. MATERIALS AND METHODS

Eggplant fruits of "Tunisina", "Buia" and "L 305" genotypes belonging, respectively, to the *"Round, Violetta pale-purple"*, *"Oval, deep purple-black"* and *"Long, deep purple"* typologies were harvested at the commercial ripening stage in the experimental field of CRA-ORL in Montanaso Lombardo (Lodi, Italy).
The assayed genotypes differentiate for the peel anthocyanins composition, being the deep purple non-Japanese types *"Buia"* and *"L 305"* characterized by delphinidin-3-rutinoside (D3R; mw 611), while the lighter purple Japanese type *"Tunisina"* is characterized by nasunin, a more complex delphinidin-3-(p-coumaroylrutinoside)-5-glucoside (mw 920), occurring in cis and trans configuration [6] [21].
Selected fruits were randomly divided into three portions and sliced: one portion left untreated (raw), the others employed in grilling and boiling processing, as described by Lo Scalzo et al. [20]. Raw, grilled and boiled samples were lyophilised. The shaken and subsequently centrifuged extracts of 800 mg of eggplant powder with 30 ml of 3% trifluoroacetic acid in MeOH were obtained according to Ichiyanagi et al. [21] with modifications.
CIE L*a*b*, Chroma* and hue colorimetric coordinates were recorded with a reflectance spectrophotometer (Konica Minolta Spectrophotometer CM-2600d), adapted for a liquid sample. The reflectance spectra of the extracts between 360 and 740 nm with an interval of 10 nm were obtained; the absorbance spectra were calculated according to the following equation:

$$\text{absorbance} = \log (100/\text{reflectance})$$

A blank consisting of the solvent was also measured, and the corresponding values were subtracted to samples values.
Moreover, the absorbance spectra in the visible region, the total anthocyanin content and total monomeric anthocyanins were evaluated through the traditional spectrophotometry, with an UNICAM UV/Vis spectrophotometer (1-cm pathlenght cuvette).
The whole spectra of extracts, 5-fold diluted with 1% HCl in MeOH, were plotted, then the total anthocyanin content (TA) was measured according to the Beer-Lambert equation,

using the maximum absorbance (541-543 nm) deducted with the absorbance at 700 nm for haze correction, and using the delphinidin-3-glucoside molar absorptivity (ε = 29000) reported in Giusti and Wrolstad [22].
Monomeric anthocyanins (MA) were estimated by a pH-differential method [22], diluting 4-fold the methanolic extracts in aqueous buffers pH 1 and 4.5 and using the cyanidin-3-glucoside absorptivity (ε = 26900).
Single anthocyanin pigments (SA) present in the extracts, 10-fold diluted with acetic acid, were also assayed by HPLC separation, in a Jasco system equipped with a Inertsil ODS-3 column (4.6 x 250 mm). The mobile phase consisted of 5% acetonitrile in MeOH (solvent A) and 5% acetonitrile in H2O (solvent B). Elution was performed at a flow rate of 0.7 mL/min at 40°C by the following linear gradient steps: start condition 10%A-90%B, kept for 5 min, then 75%A-25%B in 25 min, then 10%A-90%B in 5min, kept for 10 min. Cis- and trans-nasunin (536 nm, RT 16.5 and 17.0 min respectively) and D3R (526 nm, RT 7.8 min) were measured and quantified by comparison to a calibration curve of external standards of purified nasunin and D3R, as described by Lo Scalzo et al. [20].
Anthocyanin contents were expressed in mg/100 g fruit dw.

3. RESULTS

Extracts obtained from the different samples are shown in figure 1.
The extracts of non-Japanese types *"Buia"* and *"L 305"*, containing D3R, revealed a much deeper colour both in raw and cooked samples with respect to the Japanese type *"Tunisina"*, containing nasunin. As expected, cooked samples differed from raw ones not only for lower intensity but also in hue, being the grilled ones browner.
The visual observations well matched with colour coordinates (table 1).
In fact, in the raw samples the red component (a*) and colour saturation (C*) values were in the non-Japaneses on average twice as high as *"Tunisina"* (2-fold for a*, and 1.8-fold for C*, respectively), while the lighter purple of *"Tunisina"* peel fitted with the slight higher value of extract lightness (L*) with respect to non-Japaneses (39.2 vs 35.3 on average). After cooking, the red component and the saturation were depleted, while L* and b* values increased.
As summarized by the magnitude of the total colour difference (ΔE_{00}) between cooked and raw samples, calculated according to CIEDE2000 equation [23] [24], the boiled samples always had minor variations than grilled ones with respect to raw samples (on average 3.3 vs 7.2), with boiled "*Tunisina*" showing almost no variations (table1).
The higher colour intensity in non-Japanese types with respect to *"Tunisina"* was kept also in all cooked samples, even if with different proportions. In fact, in grilled samples, a* values of non-Japaneses were further increased with respect to *"Tunisina"* (on average 2.7-fold) while C* (including the b* values) was almost the same (on average 1.8-fold), and in boiled samples, due to the little variation of *"Tunisina"*, a* and C* of non-Japaneses were less different with respect to *"Tunisina"* (1.5- and 1.3-fold, respectively).

Even if different molar absorptivities in different anthocyanins may occur, the colour intensity exactly reflected the pigment amounts revealed by spectrophotometric and HPLC measurements (table 2), thus the data gathered using the three methods were in good agreement with each other.

In fact, in raw eggplants the average content of anthocyanin considering the different assays (TA, MA and SA) was 105 mg/100g for "Tunisina", 201 for "Buia" and 185 for "L 305".
After grilling, an overall average of only 35% of anthocyanins was recovered, while after boiling the retention was greater (55%). Furthermore, *"Tunisina"* anthocyanins always showed a higher stability with respect to non-Japanese ones, in agreement with the lower ΔE00 values obtained (table1).
The values of L*, a* and C* colorimetric coordinates were significantly correlated with anthocyanin contents, being a* and C* the highest positively correlated, and L* negatively correlated (table 3).

Figure 1 - Raw, grilled and boiled eggplant extracts (3% TFA in MeOH)

Table 1 - Colorimetric coordinates of eggplant extracts. ΔE00 = total colour difference of cooked vs raw samples.

		L*	a*	b*	C*	h°	ΔE_{00}
Tunisina	**raw**	39.2	5.2	-2.3	5.7	336.1	.
	grilled	40.1	1.4	1.9	2.4	54.9	6.1
	boiled	39.6	4.2	-2.0	4.7	334.9	1.2
Buia	**raw**	35.4	10.7	-2.9	11.1	345.0	.
	grilled	38.1	4.1	1.6	4.4	21.8	7.8
	boiled	37.8	6.0	-1.2	6.1	349.1	5.0
L 305	**raw**	35.2	9.5	-2.2	9.8	347.0	.
	grilled	38.2	3.5	1.9	4.0	27.7	7.6
	boiled	37.2	6.2	-0.7	6.2	353.4	3.8

Table 2 - Total (TA) and monomeric (MA) anthocyanin content measured by spectrophotometric assays, and single anthocyanins (SA) by HPLC assay (see text); anthocyanin retention percentage of cooked vs raw samples.
[1] nasunin; [2] D3R

		TA (spectr)		MA (spectr)		SA (HPLC)	
		mg/100g dw	**% ret. vs raw**	**mg/100g dw**	**% ret. vs raw**	**mg/100g dw**	**% ret. vs raw**
Tunisina [1]	**raw**	121	.	88	.	107	.
	grilled	51	42	41	47	37	35
	boiled	87	72	72	81	70	66
Buia [2]	**raw**	231	.	158	.	214	.
	grilled	80	35	60	38	52	24
	boiled	100	43	73	46	79	37
L 305 [2]	**raw**	207	.	146	.	202	.
	grilled	77	37	52	36	46	23
	boiled	120	58	76	52	91	45

Table 3 - Correlation indices between colorimetric coordinates and anthocyanin contents.
* : p<0.0025; ** : p< 0.001; *** : p< 0.0005

	L*	a*	b*	C*	h°
TA	-0.884 **	0.974 ***	-0.749	0.987 ***	0.615
MA	-0.846 *	0.961 ***	-0.782	0.978 ***	0.629
SA	-0.848 *	0.954 ***	-0.769	0.973 ***	0.630

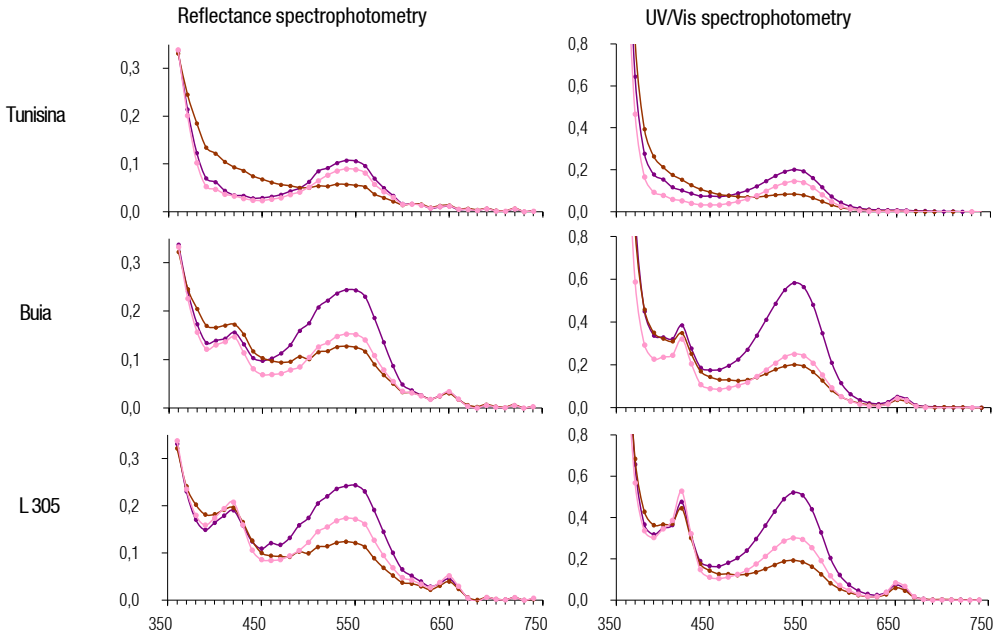

Figure 2 – Comparison of absorbance spectra of eggplant extracts obtained by reflectance spectrophotometry and UV/Vis spectrophotometry (see text). In the x-axis the wavelenght (350-750nm); in the y-axis the absorbance arbitrary units. Purple line: raw eggplant; brown line: grilled eggplant; pink line: boiled eggplant.

The variations in visible spectrum were not limited to the anthocyanins region (around 540 nm) but involved all the other regions as well (figure 2).

Reflectance and absorbance spectra generally well matched all over the visible region, describing the same trends in raw and cooked eggplants extracts.
The raw non-Japanese types showed two distinct additional peaks, at 420 and 650 nm, identifiable in both spectra types, while a third minor peak or shoulder, nearly at 400 nm, was just evident only in absorbance spectra (original scans, not shown).
These additional peaks were less influenced by cooking with respect to those of the anthocyanins, being little the optical density variations at 400 and 420 nm both in reflectance and in absorbance spectroscopy (on average 104 % for grilled samples, and 96 % for boiled ones).
A further extraction with n-hexane revealed, in absorbance spectroscopy, the almost complete disappearance of these peaks in the hydrophylic fraction (data not shown), giving the chance to state that these compounds have a lipophylic nature, hence related to other pigments instead of phenolics.
In reflectance spectrophotometry the Japanese type "Tunisina", showing no additional peaks, nevertheless showed a marked increase in optical density at 370-490 nm after grilling with respect to raw and boiled samples.

4. DISCUSSION AND CONCLUSIONS

Anthocyanin quantifications carried out by spectrophotometry and by HPLC separation were in good agreement.
Unlike other phenolic compounds such as caffeic acid derivatives, eggplant anthocyanins were strongly depleted after cooking, being boiling (a mild heating process) more preservative than grilling.
Nasunin, the anthocyanin detected in *"Tunisina"*, was in percentage more retained than non-Japanese anthocyanin D3R, particularly in boiling process.
Total anthocyanin content (TA) of methanolic extracts gave an overestimation with respect to monomeric anthocyanins (MA) and single anthocyanins (SA) by HPLC analysis. These differences were greater in cooked samples (tab.2), because copigmentation and formation of new compounds may occur after cooking [5].
Colorimetric coordinates well monitored colour modifications due to anthocyanins depletion, showing a decrease of a* value and an increase of b* value after cooking: this is in agreement with the loss of the delphinidin-related red-blue colour. In fact, the total colour difference $\Delta E00$ was highly correlated with the retention of anthocyanins after cooking ($r = -0.982$, $p<0.0005$).
Thermal treatments caused a strong depletion of the eggplant anthocyanins. It is known that the stability of anthocyanins is influenced by its total amount and the simultaneous presence of different types of anthocyanins. In particular, Hayashi et al. [14], investigating a number of vegetable and fruit, evidenced the positive relationship between the colour stability of anthocyanins subjected to heating and the number and acylation of anthocyanins. Eggplant, having a very simple anthocyanin profile characterized by a single anthocyanin, was one of the most unstable. Our results were in accordance to Hayashi et al. [14] findings, also with regards to the acylated anthocyanin nasunin, which was more stable than D3R, its non-acylated derivative.
Lipophylic compounds observed at 400-420 nm in non-Japanese types showed a better resistance to thermal treatments than anthocyanins, this indicates that they could belong to carotenoids and other pigments, whose presence in eggplant fruits has been previously reported [25] [26] [27].
Grilled *"Tunisina"* had the lowest anthocyanin loss with respect to grilled *"Buia"* and *"L 305"*, but the highest percentage loss of a* value with a marked increase in optical density at 370-490 nm. Probably, a* value is positively correlated to anthocyanin and also to other pigments such as melanoidins which are affected by heating.
Therefore, the browning process consequent to grilling seems different from enzymatic or non-enzimatic browning of pulp and juices, that is generally associated to an increase of a* value and to a loss of lightness [28].
In conclusion, colorimetric and HPLC analysis of methanolic eggplant extracts were equally suitable to describe the global fate of fruit eggplant pigments after thermal treatments.

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