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# CAROTENOIDS AND CHLOROPHYLLS IN BRONTE'S PISTACHIO (Pistacia vera, L.) AND PISTACHIO PROCESSED PRODUCTS

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The study provides data on carotenoids (β-carotene, lutein, violaxanthin) and chlorophylls (chlorophyll *a*, chlorophyll *b*) content in Bronte's pistachio (Pistacia vera, L.) and on their qualitative and quantitative changes occurring during processing. Raw, in-house roasted and four pistachio processed products (Flour, Finely chopped pistachio, Ice-cream paste, Oil) were studied. Lutein was the most abundant xanthophyll in raw pistachio (2.51 mg/100 g oil), followed by β-carotene (0.32 mg/100 g oil). Neolutein b was the most represented isoform in pistachio, it was mostly detected in pistachio processed products. Little chlorophyll a amount was detected in Bronte's pistachio (0.3 mg/100 g oil). A high sensitivity of chlorophylls to be converted into derivatives was highlighted: pheophytins and phyropheophytins coexisted in pistachio processed products, whereas only pyropheophytins were present in in-house roasted pistachio at temperature higher than 150 °C; at lower temperatures (120 °C) chlorophylls and derivatives coexisted. Isomerization of lutein and chlorophyll degradation occurring during processing support the hypothesis that lutein isomers (neolutein a and b) and pheophytins and pyropheophytins could be suitable marker for pistachio processed. The level of individual pigments formation could be also indicative of the temperature at which the pistachios were exposed.

***Keywords:*** Pistachio, Lutein isomers, Chlorophylls, Pheophytins, Phyropheophytins

## INTRODUCTION

Tree nuts are currently considered an important component of a healthy diet, a regular consumption of nuts provides several bioactive compounds that could protect against the risk of cardiovascular diseases (Kelly and Sabaté, 2006; Kris-Etherton and Others, 2008; Ros, 2009; Kendall and Others, 2010; and O'Neil and Others, 2010). The potential health benefit related to pistachio consumption is mainly associated to the protein content and the favourable lipid composition, mostly unsaturated fatty acids. Nuts provide also fiber, inositol phosphate, folate, minerals and a rich phytochemicals profile (carotenoids, phytosterols, tocopherols) (Giuffrida and Others, 2006; Bellomo and Fallico, 2007; Bullò and Others, 2015; and D'Evoli and Others, 2015). These phytochemicals, however, can be sensitive to thermal and technological stress during processing of the nut, so their content and biological activity may be impaired. This can spoil the healthy properties of pistachio.

Italy has a large production of pistachio (*Pistacia vera*, L.) mainly located in Sicily (Bronte area). Pistachio in Italy has a number of gastronomic uses so, other than as a snack (raw, roasted) or as recipe ingredient, it is also utilized for the production of traditional commercial pistachio processed products. Thermal and technological processes applied to pistachios may have a negative influence on their naturally occurring phytochemicals; therefore, to preserve their

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biological activity, it is useful to monitor their traceability along the production chain, to optimize technological processes. Chlorophylls and lutein are the main pigments in pistachios so their degradation, resulting from processing, can affect colour stability that is one of the main attributes of pistachio products thus deeply influencing consumer's quality perception.

This study was addressed to study the carotenoids (β-carotene, lutein, violaxanthin) and chlorophylls (chlorophyll *a*, chlorophyll *b*) content in raw Bronte's pistachio. Furthermore, to evaluate the impact of food processing on carotenoids and chlorophylls profile, roasted pistachio (at 60, 90, 120, 150 and 180 °C), and four commercial pistachio processed products (*Flour, Finely chopped pistachio, Ice-cream paste, Oil*) submitted to different technological processes including milling, mild roasting, centrifuging, cold pressure extraction, were also analyzed. Lutein isomerization to neolutein isoforms and chlorophyll isoforms (chlorophyll *a'*, chlorophyll *b'*) and dertivatives (pheophytins, pyropheophytins) were investigated in both roasted pistachio and commercial pistachio processed products as potential molecular markers of processing damages.

## MATERIALS

Commercial pistachio processed products (Sicily, Italy) were delivered to laboratories, raw and unshelled, packed in vacuum sealed storage bags. Pistachios were portioned in 50 gr. aliquots in aluminium bags, sealed under vacuum, and stored in refrigerator at +4 °C until analysis. Pistachios were analysed in triplicate.

*In-house roasted Bronte pistachios:* two batches of pistachio were submitted to roasting experiments at five different temperatures (60, 90, 120, 150, 180 °C), for 30 minutes. For each thermal experiment fifty grams sample, laid on a pyrex glass plate, were heated in a ventilated electric oven. After heating, pistachios were cooled in a desiccator, grounded by an electrical mixer and stored in a refrigerator at +4 °C in plastic jars until analysis.

Italian Bronte commercial pistachio processed products:

Commercial pistachio processed products were from the Bronte area:

**Flour:** Fine grounded 100% Sicilian pistachios, preservatives free.

**Finely Chopped Pistachio:** 100% Sicilian pistachio, slightly roasted, ground, preservatives free.

**Ice-Cream Paste:** Bronte pistachio, semi-manufactured, additives free.

**Oil:** Pure Sicilian pistachio oil, cold pressured and filtered, preservatives free.

## METHODS

### Oil Fraction

The oil fraction extracted from raw pistachio, *in-house* roasted pistachio and commercial pistachio processed products (*flour, finely chopped pistachio, ice-cream paste*), was obtained by a cold solvent extraction to preserve bioactive molecules from degradation according to the method described by Ballistreri and Others (2010). The commercial "*Oil*" sample was used as it is. The data reported for carotenoids and chlorophylls content in all pistachio samples studied were expressed as mg/100 g pistachio oil.

### Analysis of Carotenoids and Chlorophylls

Carotenoids and chlorophylls were determined in the oil fraction of raw pistachio, in-house roasted pistachio and commercial pistachio processed products. The commercial sample of pistachio *Oil* was analyzed as such. Carotenoids (β-carotene, lutein, violaxanthin, neolutein *a*, neolutein *b*) and chlorophylls (chlorophyll *a* and *a'* and chlorophyll *b* and *b'*, pheophytins and pyropheophitins) were determined following the method by Pilar Cano (1991). In brief, an aliquot of pistachio oil was dissolved in acetone/$CH_3OH$ (75:25 v/v), filtered and injected into HPLC/PAD system (Waters 500 series coupled with a PDA detector Waters 996 Series). Separation of pigments was carried out on a $C_{18}$ Inerstil ODS column (4.6 x 250 mm, GL Sciences Inc.), through gradient elution using a mobile phase composed by $CH_3OH$/$H_20$ (75:25, v/v) and ethyl-acetate. Quantification of pigments was performed using calibration curves or correspondent external analytical standards. Lutein isomers and chlorophylls derivatives were quantified using the calibration curve respectively of lutein, chlorophyll *a* and chlorophyll *b*.

### Quality Assurance

The quality assurance of the different analyses was performed assessing response linearity ($R^2$ = 0.99) of analytical standards in the range used for the calibration curves (0.01-3.58 μg/ml for carotenoids; 0.06-0.40 μg/ml for chlorophylls). Reproducibility was also tested, by duplicate analyses in different days (RSD 2.23-8.98). The intra-day instrumental Repeatability was evaluated by triplicate injections (RSD 1.50-2.62).

### Data Analysis

All experimental data are presented as Mean ± Standard Deviation (SD) of triplicate analyses. Statistical differences were assessed by the non parametric Wilcoxon test. In all the analyses P values lower than 0.05 were considered statistically different. The linearity of responses was determined using Pearson's correlation coefficient (r). Data were statistically processed by XL-STAT software.

## RESULTS AND DISCUSSION

### Carotenoids

The effect of processing on carotenoids content in both raw and commercial pistachio processed products is described in Table 1. Lutein was the most abundant xanthophyll found in raw Bronte's pistachio (2.51 mg/100 g oil), followed by β-carotene (0.32 mg/100 g oil). Little amounts of violaxanthin (0.07 mg/100 g oil) and of lutein isomers, neolutein *a* and neolutein *b* (0.05 and 0.02 mg/100 g oil, respectively) were also detected (Table 1). Among commercial pistachio processed products, *Finely chopped pistachio* showed the highest concentration of lutein (4.3 mg/100 g oil). *Pistachio Flour* had a lutein content (2.69 mg/100 g oil), *Ice-cream paste* and *Oil* showed the lowest amount of lutein (both 2.2 mg/100 g oil) (Table 1). *Flour* was the highest in β-carotene content (0.64 mg/100 g oil), 1.7 times higher than in raw pistachio. Lutein isoforms (neolutein *a* and neolutein *b*) were also detected. Neolutein *b* was mostly present in *Ice-cream paste* and *Finely chopped pistachio* (0.32 and 0.26 mg/100 g oil, respectively) while neolutein *a* was detected in lower amounts (Table 1). Violaxanthin was detected only in *raw* pistachio and not in pistachio processed products because of its well known thermal instability (Khachik and Others, 1992; and Aparicio-Ruiz and Gandul-Rojas, 2012) (Table 1). The effect of heating on carotenoids profile was investigated by roasting pistachios in laboratory (*in-house* roasted pistachios) at different temperatures (from 60 to 180 °C) for 30 min (Figures 1a and 1b). Lutein content trend was not linear: it showed a slight decrease when heating at 60 °C and a rapid and great increase up to 90 °C, followed by a gradual decrement up to 180 °C, when the lutein content was 2 mg/100 g oil, almost 20% less with respect to the initial content of the not-heated samples (2.5 mg/100 g oil) (Figure 1a). Durmaz and Gokmen (2011) in *P. terebinthus* oil found significant losses in both lutein and β-carotene content with the roasting duration (180 °C). By contrast, Pumilia and others (2014) reported an increase in pistachio lutein content after 5 minutes of roasting at 138 °C, and a stable content until 60 minutes. Our findings highlighed also that during heating lutein isoforms formation occurred (Figure 1b). The neolutein *b* isomer, the most represented isomer in pistachio, increased the initial content in the not-heated sample following an exponential like trend (Figure 1b). By contrast, the neolutein *a* isomer was formed in rilevant amounts at 90° and than declined steadily until 180 °C. Beta-carotene was also affected by heating process, at 180° its concentration fell gradually down up to 58% of the initial content (Figure 1b). Violaxanthin was destroyed already at 120 °C, reaching a maximum of chemical extractability at 90 °C (Figure 1b).

**Chlorophylls and Pheophytins:** In *raw* Bronte's pistachio only a little chlorophyll *a* was detected (0.3 mg/100 g oil) (Figure 2). In commercial pistachio processed products, chlorophylls were detected mainly in *Finely chopped pistachio* (except chlorophyll *a'*) and in *Flour* where only chlorophyll *a* and *a'* were found (Figure 2). Pheophytins *a* and *a'* were mainly detected in *Finely chopped pistachio* (2.45 mg/100 g oil and 0.65 mg/100 g oil, respectively) and in *Flour* (0.34 mg/100 g oil and 0.15 mg/100 g oil, respectively); pheophytin *b* was found in both *Finely chopped pistachio*

**Table 1: Carotenoids Content (mg/100 g Oil) in Raw Pistachio, in-House Roasted and Pistachio Processed Products**

| | violaxanthin | lutein | neolutein *b* | neolutein *a* | β-carotene |
|---|---|---|---|---|---|
| Raw | 0.07 ± 0.02 | 2.51 ± 0.36 | 0.02± 0.01 | 0.05 ± 0.02 | 0.32 ± 0.10 |
| Flour | nd | 2.69 ± 0.24 | 0.11 ± 0.00 | 0.09 ± 0.01 | 0.64 ± 0.06 |
| Finely Chopped | nd | 4.29 ± 0.73 | 0.26 ± 0.05 | 0.15 ± 0.01 | 0.51 ± 0.05 |
| Ice-cream paste | nd | 2.23 ± 0.39 | 0.32 ± 0.08 | 0.06 ± 0.01 | 0.46 ± 0.03 |
| Oil | nd | 2.24 ± 0.51 | 0.20 ± 0.04 | 0.06 ± 0.01 | 0.38 ± 0.04 |

**Note:** Data are the Mean ± SD of three determinations. nd = not detected.

**Figure 1: (a and b) Carotenoids Profile of in-House Roasted Pistachio at Different Heating Temperatures**

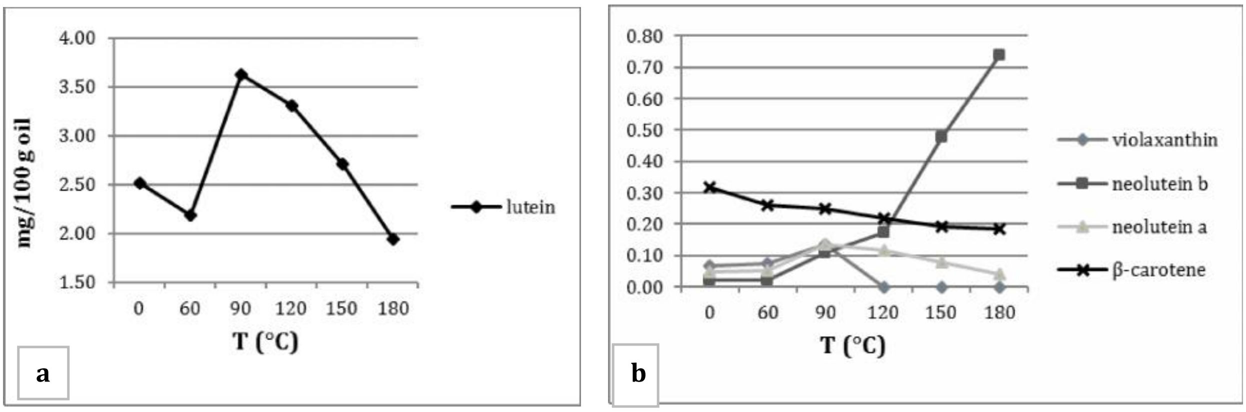
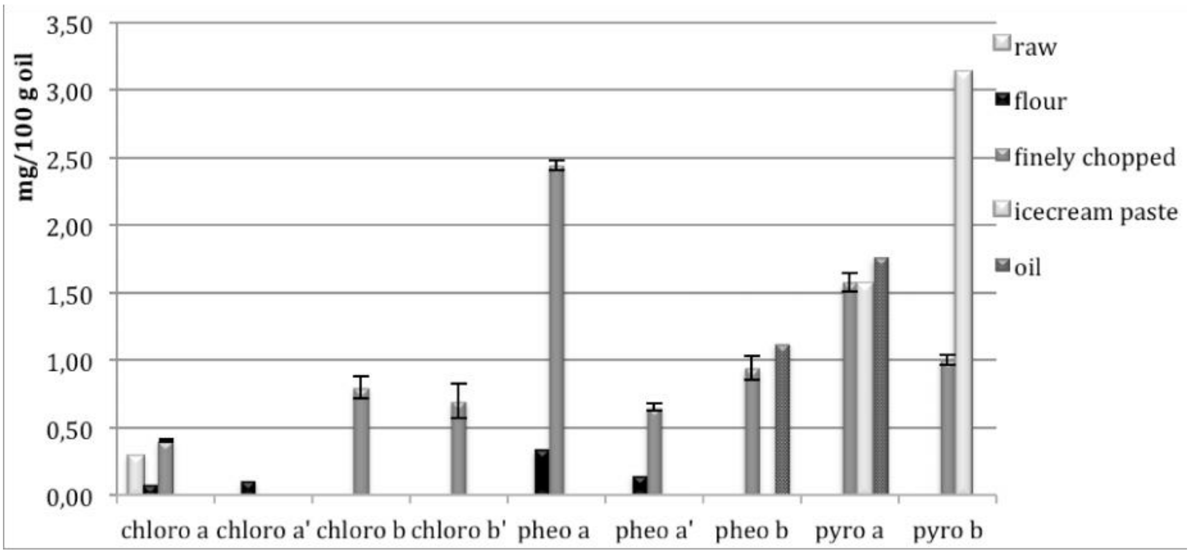
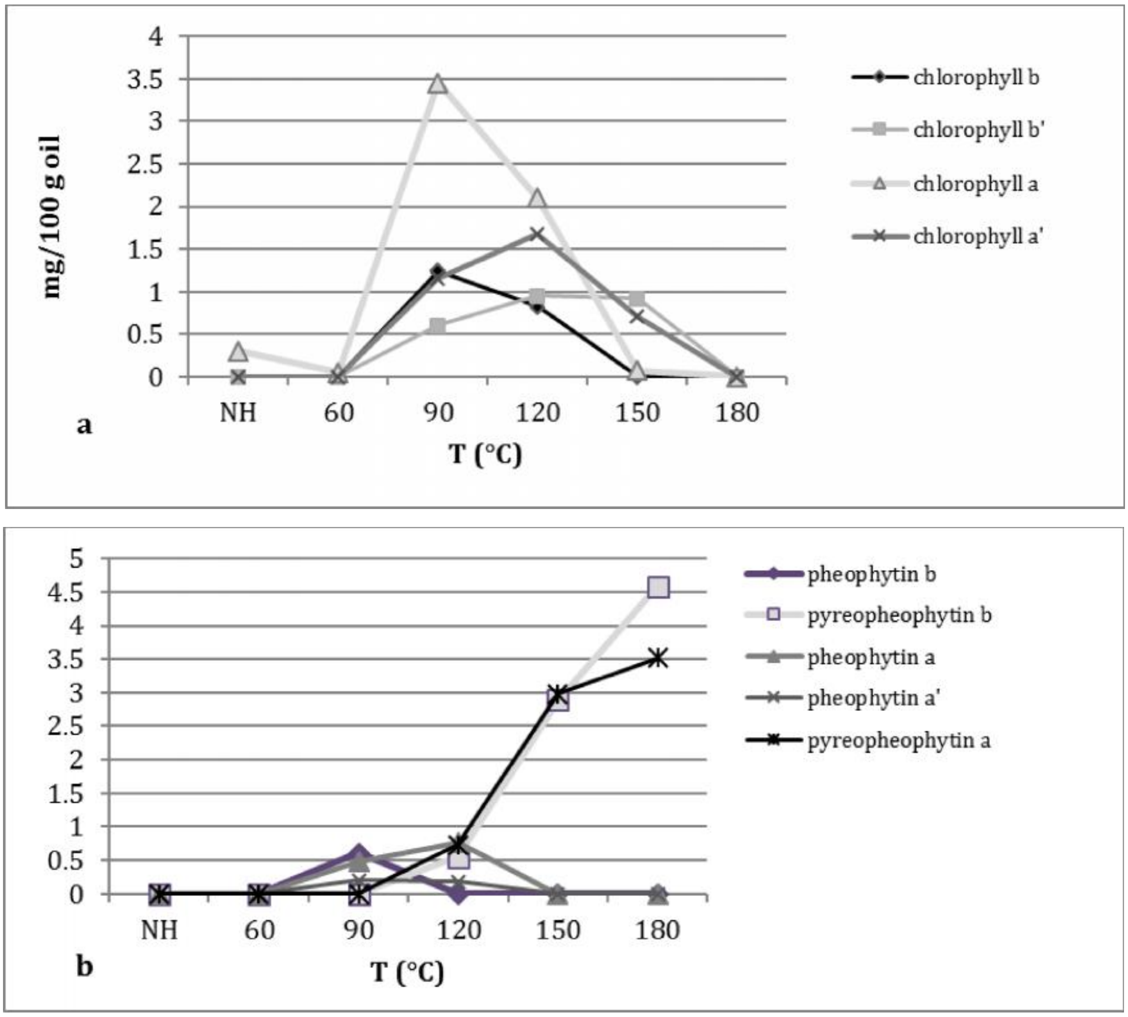



**Figure 2: Chlorophylls and Chlorophyll Derivatives Content of Raw Pistachio and Pistachio Processed Products**




(0.95 mg/100 g oil) and *Oil* (1.12 mg/100 g oil) (Figure 2). The pyropheophytins were mostly present in *Ice-cream paste* (pyropheophytin *a* 1.57 mg/100 g oil; pyropheophytin *b* 3.13 mg/100 g oil) and *Finely chopped pistachio* (pyropheophytin *a* 1.58 mg/100 g oil; pyropheophytin *b* 1.01 mg/100 g oil), pyropheophytin *a* was found also in *Oil* (1.76 mg/100 g oil). Chlorophylls and pheophytins content of *in-house* roasted pistachios is showed in Figures 3a and 3b. Except for the chlorophyll *a*, that was already detected in the raw pistachio (Figure 2), chlorophyll *b* and both chlorophyll isoforms *a'* and *b'* begin to be detectable at 90 °C (Figure 3a); at this temperature the most abundant pigment was chlorophyll *a* (3.5 mg/100 g oil), the chlorophyll isoform *a'* was about 1.2 mg/100 g oil followed by the isoform chlorophyll *b'* (0.5 mg/100 g oil) (Figure 3a). At 120 °C chlorophylls *a* and *b* content was strongly reduced, in favor of their respective isoform *a'* and *b'* (Figure 3a). At 150 °C both chlorophyll *a* and chlorophyll *b* were no more detectable, while the isomeric forms *a'* decreased and *b'* was nearly steady. At the highest heating temperature (180 °C) no chlorophyll isoforms were detectable (Figure 3a). Pheophytins and isoforms also started to be formed at 90 °C (Figure 3b): pheophytin *b* was detectable only at this temperature (0.6 mg/100 g oil), while pheophytin *a* and pheophytin *a'* were formed up to 120 °C following a different trend, the former gradually increasing, the latter remaining

**Figure 3: (a and b. Chrorophylls Profile (a) and Chlorophyll Derivatives Formation (b) of in-House Roasted Pistachios NH = Non-Heated Sample**




nearly steady. Over 120 °C only the pyropheophytins were present, strongly increasing their concentration up to 180 °C, when the most abundant pigment was pyropheophytin *b* (4.6 mg/100 g oil), followed by pyropheophytin *a* (3.5 mg/100 g oil). Thus, the sensitivity of chlorophylls to be converted into derivatives during heating process was highlighted in both commercial pistachio processed products (Figure 2) and *in-house* roasted pistachio (Figures 3a and 3b). Pumilia and others (2014) also detected pheophytins *a* and *b* in raw pistachio, roasting at 138 °C up to 60 min decreased their content by 85% and increased pyropheophytins content. Conversion of pheophytins into pyropheophytins was already observed in heat processed spinach (Schwartz and Von Elbe, 1983; and Schwartz and Lorenzo, 1991). The conversion of pheophytin *a* into pyropheophytin *a* was found slower than conversion of pheophytin *b* into pyropheophytin *b*, as it occurred through the formation of the isomeric form pheophytin *a*' (Schwartz and Von Elbe, 1983). Carotenoids and chlorophylls profile in commercial pistachio processed products was quite different compared to that found in raw pistachios. Lutein and its *cis* isomers content varied greatly among products, the type of processing (mechanical treatment, centrifuging and temperature) may improve molecule extraction in commercial pistachio processed products with respect to the raw nut. This could be explained by a lutein enhanced extractability from plant cells due to the protein-carotenoid complexes breakdown during processing (Pumilia and Others, 2014). Generally, carotenoids in foods are mostly present in the more thermodynamically stable *all-trans* form. *Cis* isomers are usually formed during food processing and storage (Oliver and Palou, 2000), also in this study a marked increment in neoluteins formation were detected in both commercial pistachio processed products and *in-house* roasted pistachios (Table 1 and Figure 1b). The *in-house* roasting experiments highlighted that lutein was more extractable at 90 °C (Figure 1a), in fact its content strongly increased compared to the initial content in the raw pistachio. However, with the rise of temperature the pigment degradation prevailed leading to *cis* isomers formation (Figure 1b), in particular neolutein *b* formation gradually increased up to 180 °C. Taking into account that the formation of *cis* isomers depends on both temperature and duration of heating treatment, the lutein isomers content could be used for discriminating among processed products, resulting as suitable marker for processing.

Technological processes influenced also chlorophylls profile of pistachio processed products. The *Ice-cream paste* resulted the most affected product as high content of pyropheophytins (*a*, *b*) were found, confirmed by the color of this product, darker than the raw pistachio. Also *Finely chopped pistachio* was affected by technological processes, although still containing all the chlorophylls (except chlorophyll *a'*) together with chlorophyll derivatives (Figure 2). As expected, *Flour* was the lowest in chlorophyll derivatives, even though the lowest in chlorophylls too, probably due to the fact that the applied technological treatments (milling) were minimal. The degradation pattern of chlorophylls and their conversion to pheophythins and pyropheophytins were followed in the *in-house* roasted pistachios, where the effect of temperature was directly associated to pigment changes. Pheophytins and phyropheophytins coexisted in commercial pistachio processed products, whereas in *in-house* roasted pistachio at lower temperatures (about 120 °C) different amounts of chlorophylls and derivatives coexisted, while at temperature higher than 150 °C only pyropheophytins were present, showing to be negative for nut processing. In the *in-house* roasted pistachio, the highest content of both chlorophylls a and b was found at 90 °C. Chlorophylls *a* and *b* are magnesium-containing pigments non-covalently bound to specific proteins to form chlorophyll-protein complexes which are inserted in the thylakoid membrane of the chloroplast (Light-Harvesting Complexes, LHC) (Croce and van Amerongen, 2011). These complexes are probably broken at the temperature of 90 °C and chlorophylls can be extracted from the food matrix; this explains why in raw pistachio only little chlorophyll *a* was detectable. More energetic heat treatment results in the loss of the Mg2+ and the formation of pyropheophytins, altering the color from bright green to an olive brown.

## CONCLUSION

The data provided by this study on the carotenoids and chlorophylls profile in Bronte's pistachio, allows to highlights both qualitative and quantitative changes occurring during pistachio processing. The understanding of the impact of food processing on naturally occurring phytochemicals in pistachios can help improve the technological conditions mostly preserving their content and biological activity. Our findings showed a correlation between lutein isomerization products formation, chlorophylls derivatives profile and thermal/technological treatments. Therefore, the data reported support the hypothesis that the traceability of lutein (mainly *cis* isomers) and chlorophylls derivatives (pheophytins and pyropheophytins) is suitable to monitor product quality and processing. Furthermore, the level of individual pigments formation, with respect to their presence in the raw sample, could be indicative of the temperature at which pistachios were exposed. These preliminary findings should lead to further studies for future applications towards the development of safer and higher quality processes.

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