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# Detection of not allowed food-coloring additives (copper chlorophyllin, copper-sulphate) in green table olives sold on the Italian market

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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**Abstract: Table olives are a common and well-known food in the whole Mediterranean area, produced and consumed in great quantities. Many deep-green olives can be found on sale in the South of Italy. Sometimes a deep color could be the result of the fraudulent addition of a coloring agent (E141ii, copper chlorophyllins) during the pickling process, in spite of the European Union legislation that does not allow the addition of any colorant to fruits included table olives. The objectives of this study were to use a relatively simple method of detection of E141ii added to table olives, to verify the presence on the Italian market of artificially colored table olives, and to show that also $CuSO_4$ can be employed for table olive re-greening. Compounds with chromatographic and spectral characteristics similar to the ones from the E141ii (Cu chlorin $e_6$, Cu isochlorin $e_4$, Cu pyropheophorbide a) were found in 8 samples out of 16. These results show that the fraudulent addition of colorant to table olives is a quite common practice. More pressing controls and analysis are required to ensure the complete food safety and the compliance with the current law.**

## 1. Introduction

Table olives are a common and well-known food in the whole Mediterranean area, produced and consumed in great quantities especially in Southern Italy. Manufacturing techniques have been refined along the years, in order to optimize the quality and attractiveness of the final product and to cover different market niches. Table olives found on market's shelves exhibit different colors and shapes, depending on cultivar type, ripening stage or processing method. There are green olives, spotted and fully ripened ones. In Southern Italy the Greek method and the Spanish method are commonly used to produce green table olives, while the Castelvetrano method covers a smaller but increasing market.

With the Greek method, olives are washed with water and then stored in brine (5-8%). The addition of salt promotes the product preservation

and the development of fermentation-capable microorganisms. During this stage of treatment, olives slowly lose their bitterness (due to oleuropein's enzymatic degradation) and acquires their final taste and properties. The development of lactic fermentation is favoured (olives average final pH of 5.2), in order to improve both the food safety and its organoleptic characteristics (Piga *et al*., 2001).

The Spanish method, the most used worldwide, requires the olives to be treated with a solution of sodium-hydroxide and water in order to hydrolyze most of the oleuropeinic glucosides. Afterwards, olives are washed repeatedly with water and then stored in brine (5-6%) in which they naturally undergo a complete lactic fermentation, reaching an average pH value of 4 (Garrido-Fernandez *et al*., 1997).

Olives treated with the Castelvetrano method are plunged for 10 to 15 days in a sodium-hydroxide solution which concentration depends on olive caliber and their ripening stage. Later on, marine salt (NaCl) will be slowly added to the solution. After such treatment drupes will undergo several washings with water (Salvo *et al*., 1995) so that, in absence of a lactic fermentation, pH never goes below 6.5 (Fodale *et al*., 2007). Anyway, Castelvetrano type olives appears brilliant-green and with more compact pulp (Owen *et al*., 2003), so that they are preferred to green table olives prepared with the Greek or Spanish methods. As a matter of fact, treatment and storage in acid solutions are known to be the main responsables of chlorophylls degradation in table olives (Minguez Mosquera *et al*., 1989). The loss of the magnesium from the chlorophylls causes to turn into the corresponding pheophytins, shifting their color from green to brown (Scotter *et al*., 2005). At the end of the various pickling processes, therefore, olives had lost most part of their original chlorophylls (and of their original dye) becoming yellowish-green; in spite of that, many deep-green olives can be found on sale in the South of Italy, especially in the Bari Province.

Their sometimes unnatural colour could be the result of the fraudulent addition of a colouring agent during the pickling process, in spite of the European Union legislation does not allow the addition of any colorant to fruit and table olives (EU, Reg CE 94/36/CE, 1994).

Among the most used green food colorants, there are the chlorophyll-derived ones; Copper complexes of chlorophyillins, particularly, play a major role within this group. Copper chlorophyllins are manufactured by chlorophyll saponification which, in turn, is extracted by means of organic solvents from edible plant, such as alfalfa (*Medicago sativa* L.) and nettle (*Urtica dioica* L.) (Mortensen, 2006). The resulting chlorophyll-based salts are marketed as E141 colorant and are available in two forms: E141i (liposoluble) and E141ii (hydrophilic). These salts are widely used as food colorants (e.g. ice creams, snacks, food decorations) but their addition to table olives and other fruits is expressly forbidden. In spite of that, E141ii (due to its hydrophilic properties and color stability) is sometimes fraudulently added to table olives during the pickling process as re-greening agent.

The objectives of this study were to confirm the presence on the market of artificially colored table olives through the detect fraudulent color adulteration with E141ii, and to verify if the addition of Copper-sulphate during the pickling process could allow a re-greening of table olives as result of its interaction with Chlorophylls, leading to the formation of Cu-chlorophyllins. Nowadays, Copper-sulphate is not allowed and not even mentioned in food legislation, but it is known that it has been widely utilized by food manufacturers because of its re-greening properties on preserved vegetables (Cerutti, 2006) but its use is very dangerous because copper is toxic to the liver. Continuous consumption of olives treated with this compound could result in damage to the consumer due to its accumulation in the body (Stern, 2010). All this to generate a warning for consumers and public authorities.

## 2. Materials and Methods

*Chemicals*

All reagents were analytical or HPLC grade: Hexane and Water were supplied from Romil Ltd. (Cambrdge, UK); Methanol from Carlo Erba Reagents (Rodano, Italy); Acetic acid and *tert*-butyl metyl ether from J.T. Baker (Deventer, Netherlands). Chlorophyll a and b, pheophorbide a and copper sulfate were purchased from Sigma-Aldrich Co. (Saint Louis, USA). Sample of E-141ii colorant were supplied by Chimica D'agostino (Bari, Italy).

*Plant material*

Obviously deep-green table olives may have been fraudulently colored, while table olives appearing from pale-green to mustard-yellow are to be considered not colored. Therefore, 16 table olives samples were purchased from local markets (Bari, Brindisi and Lecce): 9 olives were with a brilliant-green or deep-

green color, while drupes from the remaining 7 samples were pale-green or mustard-yellow. pH values of the brine were recorded with a PC 650 probe (Eutech instruments, Singapore). Due to the lack of information reported on the labels, we could not assess the cultivar and origin of each olive sample (Table 1).

Table 1 - Main characteristics of the olives samples analyzed

| Sample | pH | Colour | Cultivar | Origin |
|---|---|---|---|---|
| S1 | 5.4 | G | n.r. | n.r. |
| S2 | 5.9 | G | n.r. | Greece |
| S3 | 7.1 | G | n.r. | Greece |
| S4 | 5.7 | G | Nocellara del Belice | Italy (Sicily) |
| S5 | 6.1 | G | n.r. | n.r. |
| S6 | 5.9 | G | n.r. | Italy |
| S7 | 5.1 | G | n.r. | Italy |
| S8 | 5.7 | Y | n.r. | n.r. |
| S9 | 6.4 | G | n.r. | n.r. |
| S10 | 4.2 | Y | Bella di Cerignola | n.r. |
| S11 | 3.8 | Y | n.r. | n.r. |
| S12 | 4.1 | Y | n.r. | n.r. |
| S13 | 5.0 | Y | n.r. | n.r. |
| S14 | 3.3 | Y | n.r. | n.r. |
| S15 | 4.0 | Y | n.r. | n.r. |
| S16 | 4.4 | G | n.r. | n.r. |

G= green; Y= pale-green or mustard-yellow.
n.r.= not reported.

*Pigment extraction*

All procedures were performed under dimmed green light to avoid any photo-oxidation of chlorophylls. 40 g of olive pulp were collected and homogenized with 25 ml of Methanol-water solution (80/20, v/v) using an Ultra-Turrax T25 (Janke e Kunkle, IKA-Labortechnik, Germany); the resulting paste was filtered by means of a Buckner's funnel with a paper filter (Perfecte 2 extra rapida, Superfiltro Milano, Italy) and a suction flask connected to a vacuum pump. The solid residue was collected, added to 50 ml of Methanol and stirred for 1 hour; the resulting solution underwent a second filtration step with the same procedure previously used. For same samples appearing still green, 50 ml of Methanol were added and a third stirring-filtering cycle has been performed. The filtrate was then mixed with an identical amount of hexane in a separating funnel in order to separate the lipophilic substances from the hydrophilic ones. The latter phase was recovered and evaporated under vacuum in a RE 111 rotavapor (Büchi, Flawil, Switzerland) at room temperature. The evaporation remnant was finally diluted up to 2 ml with Methanol and utilized for the HPLC/DAD analysis. This procedure was made up for extraction hydrophilic pigments preferably.

*$CuSO_4$ addition tests*

In order to verify the possibility of re-greening using copper sulphate during the olive production process or the marketing, samples of olives were treated with $CuSO_4$. It is known, in fact, that the addition of copper stabilizes the tetrapyrrolic ring of pheophytin, resulting a re-greening of olive drupes (NIIR, 2004).

A local food company provided us a sample of olives processed with the Castelvetrano method and stored in a Sodium-hydroxide/salt/water solution (pH 11.5). Four groups of 10 olives were taken from the sample: the first one was washed with water for 24 hours, stored in brine (6% NaCl, 0.6% Citric acid, 0.05% Ascorbic acid, pH: 2.3) for 48 hours (pH stabilized at 7.3) and then analyzed; the remaining 3 sub-samples were placed in beakers with 150 ml of their original packing solution, 1.5 g (1%), 7.5 g (5%) and 30 g (20%) of $CuSO_4$ were then added. After 3 hours of stirring, the coloring solution was discarded and the samples were washed for 24 hours with water. The samples were then stored in brine solution for 48 hours until pH stabilization (pH 5.4). After the brine storage, all the sub-samples went through the before mentioned pigments extraction procedure. Another trial was run to test the effect of $CuSO_4$ addition on olives treated with the classical Spanish method. 10 olives were selected from a sample which analysis already excluded any fraudulent colouration (S16). Drupes were first put in an alkaline solution (150 ml of 1% NaOH-water solution, pH: 12.8) until pH stabilization was reached (pH: 8.3), 30 g (20%) of $CuSO_4$ were then added to this medium. The drupes were then immersed in brine solution; after 48 hours the pH reached a stable value of 4.0. The sample was then treated until pigment extraction following the already cited procedures.

*Analysis of chlorophylls compounds by HPLC/DAD*

The determination of pigment products were carried out by HPLC using a Agilent 1100 liquid chromatograph fitted with a manual injector. A stainless steel column, Alltech Prontosil C30, 200 Å, 5 µ, 250× 4.6 mm I.D. was used. The column was protected by precolumn packed with the same material. Separation was performed using an elution gradient (flow rate 1 ml $min^{-1}$) with the mobile phases (A) Methanol: distilled water: Acetic acid (90:10:0.5 v/v/v) and (B) *tert*-butyl metyl ether: Methanol: Acetic acid (100:10:0.5 v/v). The gradient scheme was 0-50% B in 30 minutes, 50-100% B in 10 minutes, 100% B for 5 minutes and 100-0% B in 5 minutes.

Sequential detection was performed with a photodiode array detector (DAD) at 430 nm and 650 nm also the online UV-vis spectra were recorded from 250 to 800 nm. 100 µl of 1 mg/ml E141ii in methanol or 100 µl of extract was utilized for the analysis. To analyze the samples treated with $CuSO_4$ the flow rate was lowered at 0.7 ml/min and the elution of B was set to 0-45% in 30 minutes, 45-100% in 10 minutes, 100% for 5 minutes and 100-0% in 5 minutes. Data were collected and processed with a LC Agilent ChemStation (revision software B.04.02). Pigments were identified by co-chromatography with authentic samples and/or by comparison their spectral characteristics with literature or compound standard when available. All analysis were made in duplicate.

## 3. Results and Discussion

In order to identify and recognize the E141ii components likely to be present in olive samples, industrial copper chlorophyllin was analyzed by HPLC/DAD, injected and monitored at 650 nm. The resulting chromatogram shows 8 major peaks indicates with **a** to **h** in figure 1. The analysis of the elution times and absorption spectra of the peaks (Fig. 2) compared with literature (Inoue *et al*., 1994; Chernomorsky *et al*., 1997; Mortensen and Geppel, 2007; Roca *et al*., 2010; Aparicio-Ruiz *et al*., 2011; Gandul-Rojas *et al*., 2012) allowed the tentative identification of 7 of the 8 peaks: (**a**) Cu rhodin g7, (**b**) Cu chlorin $e_6$, (**c**) Cu chlorin p6, (**d**) Cu isochlorin $e_4$, (**e**) Cu 15$^1$-OH-lactone-pheophytin a (**g**) Cu rhodochlorin, (**h**) Cu pyropheophorbide a. We were unable to identify without any doubt peak **f**. The main components of our E141ii standard eluted according to Montensen and Geppel (2007) procedure which utilize a chromatographic method similar, were: Cu Rhodin g7 < Cu Chlorin $e_6$ < Cu Isochlorin $e_4$ < Cu Pyropheophorbide a.

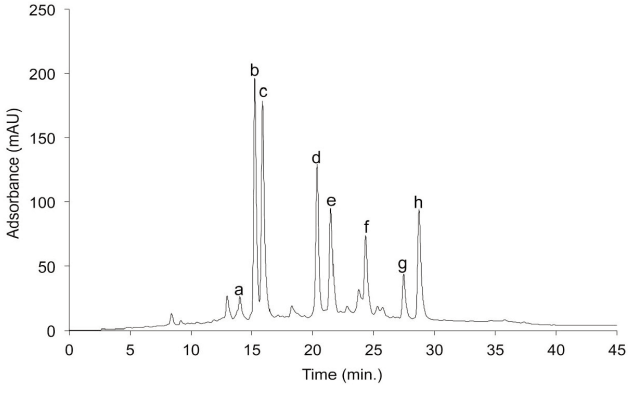


Fig. 1 - HPLC/DAD analysis of food colorant E141ii, recorded at 650 nm, showing the peaks of the main identified components: a) Cu rhodin g7; b) Cu chlorin e6; c) Cu chlorin p6; d) Cu isochlorin e4; e) 151 OH lactone pheophytin a; f) unknown; g) Cu rhodochlorin; h) Cu pyropheophorbide a.

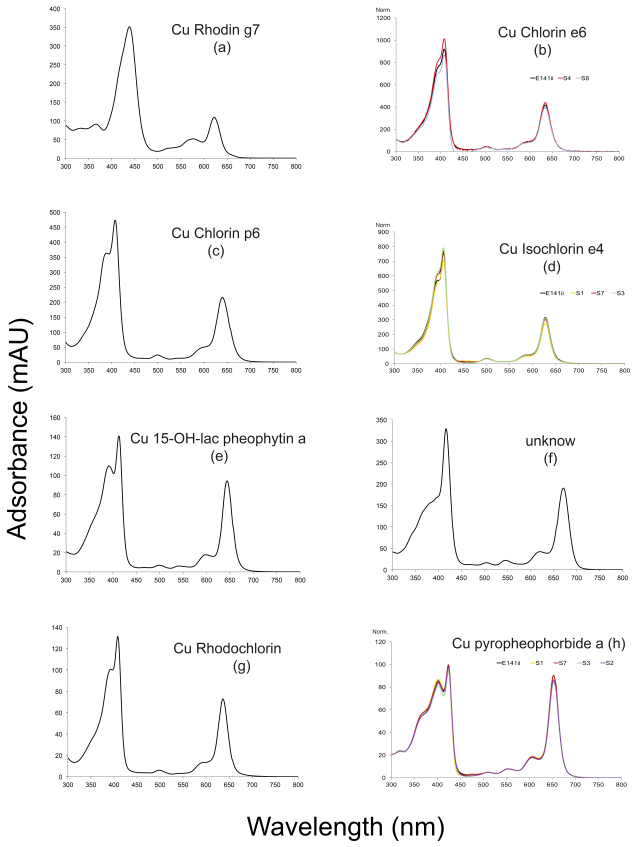


Fig. 2 - Spectra UV/Vis of the Copper chlorophyllins pigments found in the colorant E141ii and in some samples of green table olives.

Figures 3 and 4 show chromatograms resulting from the HPLC/DAD analysis of the studied samples; they have been recorded at 650 nm to facilitate the detection of chlorophylls and their derivatives, avoiding in the same time interferences from carotenoids. In 7 samples out of 16 (Fig. 3) we assessed the presence of compounds with chromatographic characteristics similar to the ones from the industrial chlorophyllin sample: Cu isochlorin $e_4$ (peak **d**) and Cu pyropheophorbide a (peak **h**). This finding agrees with bibliographical data, since Cu isochlorin $e_4$ is referred as one of the main component of commercial copper chlorophyllins (Inoue *et al*., 1994; Chernomorsky *et al*., 1997; Ferruzzi *et al*., 2002). Cu chlorin $e_6$ (peak **b**) were found in 2 samples. Finally, the presence of Cu rhodin g7, Cu chlorin $p_6$, Cu 15$^1$-OH-lactone-pheophytin a and Cu rhodochlorin was never observed. This result is supported by the work of Gandul-Rojas *et al*. (2012) which demonstrates

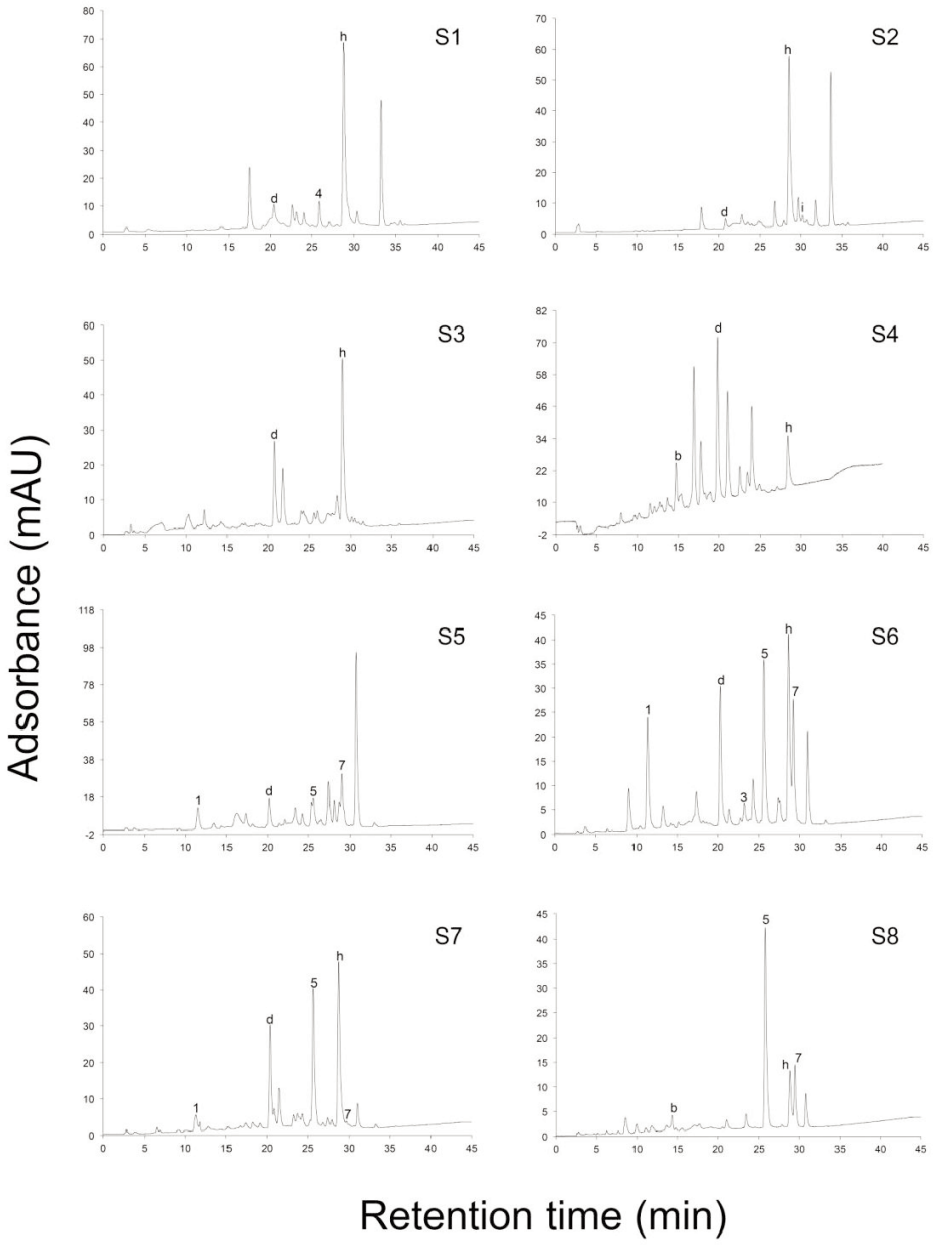


Fig. 3 - HPLC/DAD chromatograms recorded at 650 nm of the samples (S1-S8) containing Cu-components related to ones from E141ii colorant.
Peaks described in Table 2.

that the addition of E141ii to table olives led to the chromatographic identification of Cu-chlorin type compounds (mainly Cu chlorine $e_6$ and Cu isochlorin $e_4$) very different from chlorophyll derivatives usually found in green table olives. Moreover, Minguez-Mosquera *et al*. (1995) showed that under certain (still unexplained) circumstances, small amounts of Cu-chlorophyll compounds can be spontaneously synthesized within the olives, leading to localized pigment alteration on their surface (*green staining* alteration). The visual analysis of our samples led us to notice no traces of *green staining* on the drupes: Cu-compounds identified in our samples must therefore be the result of a colorant addition.

In those samples we were also able to identify several other pigments, marked with numbers, such as chlorophyll b (peak **4**; Fig. 3-S1); pheophitin a (peak **5**; Fig. 3-S6 to 3-S8) and isochlorin $e_4$ (peak **7**; Fig. 3-S5 to 3-S8).

Cu-compounds were never found in 8 of the analyzed samples (Fig. 4). On the other hand, those samples contained pigments identified as chlorophylls (e.g chlorophyll a; peak **6**; Fig. 4-S9, 4S11, 4-S13, 4S16) and chlorophyll derivatives (e.g. pheophorbide a; peak **2**; Fig. 4-S13, 4-S15, 4-S16, pheophytin a; peak **5**; Fig. 4-S10 to 4-S16).

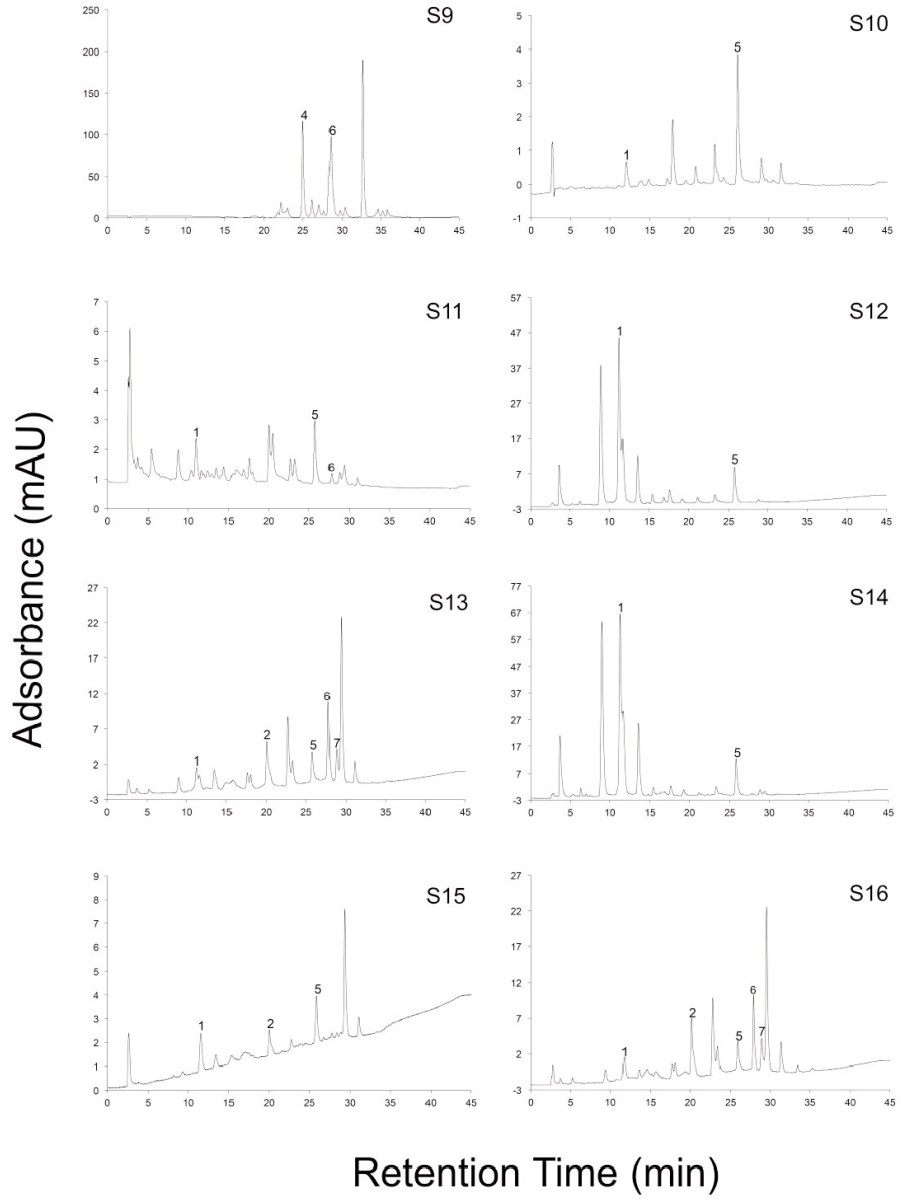


Fig. 4 - HPLC/DAD chromatograms recorded at 650 nm of the samples (S9-S16) do not contain Cu-components related to ones from E141ii colorant. Peaks described in Table 2.

Gandul-Rojas *et al*. (2012) show that chromatograms of olives treated with the Spanish method exhibit a series of major peaks belonging to Mg-free chlorophyll derivatives (mainly phaeophytins), while chromatograms of samples treated with the Castelvetrano method show peaks from chlorophylls or degraded chlorophylls with Mg (e.g. Glycoxylic acid chlorophylls; Formyl chlorophylls) in addition to the first ones.

Table 2 indicates the chromatographic, spectral characteristics and origin of the chlorophyll derivates pigments identified in the commercial E141ii and in the olive samples. The results obtained are in agreement with the literature (Hynninen 1973; Inoue *et al*., 1994; Chernomorsky *et al*., 1997; Mortensen and Geppel, 2007; Roca *et al*., 2010; Aparicio-Ruiz *et al*., 2011; Gandul-Rojas *et al*., 2012). The pigments identified are Cu chlorophyllins (peaks **a**-**h**), chlorophylls and derivatives (peaks **1**-**7**).

The analysis of the 9 samples initially suspected of have been fraudulently colored (indicated as G in Table 1), largely confirmed our hypothesys: 8 samples contains Cu compounds related to the E141ii colorant (Fig. 3). On the other hand, 6 out of the 7 samples chosen accordingly to their pale-green or mustad-yellow dyes, do not contain compounds related to the food coloring agent, underlining that the addition of E141ii during the pickling process could be the

Table 2 - The chromatographic and spectroscopic characteristics of the chlorophyll derivates pigments present in the E141ii dye and in the analyzed green table olives

| Pigment | Peak [z] | Kc | Soret | Spectral data in HPLC eluent | | | | | | | | | | | | Sample (and/or E141ii dye) where the pigment is present | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | I | | II | | III | | IV | | V | | VI | | | |
| | | | | M | R | M | R | M | R | M | R | M | R | M | R | | |
| Chlorine e6 | 1 | 8.83 | 402 | - | - | - | - | 500 | 18.02 | 528 | 25.66 | (642) | 9.45 | 662 | 5.47 | S5 to S7, S10, S11, S13 to S16 | [4] |
| Cu rhodin g7 | a | 11.21 | 436 | 366 | 3.27 | - | - | - | - | 577 | 6.85 | - | - | 623 | 3.11 | E141ii | [3] |
| Cu chlorin e6 | b | 12.42 | 408 | (395) | 1.21 | - | - | - | - | 502 | 8.02 | (588) | 4.80 | 634 | 2.19 | E141ii, S4, S8, S18 to S21 | [2] [3] [4] [6] |
| Cu chlorin p6 | c | 13.48 | 407 | 388 | 1.29 | - | - | - | - | 500 | 19.52 | (597) | 9.76 | 640 | 2.22 | E141ii | [4] |
| Pheophorbide a | 2 | 17.77 | 408 | (380) | 1.53 | (400) | 1.10 | 507 | 9.33 | 537 | 9.34 | 609 | 9.76 | 666 | 2.01 | S13, S15, S16 | [2] [7] std |
| Cu isochlorin e4 | d | 17.96 | 406 | (394) | 1.16 | - | - | - | - | 501 | 18.71 | (585) | 11.65 | 627 | 2.20 | E141ii, S1 to S7, S18 to S20 | [2] [4] |
| Cu 15[1]-OH-lactone-pheophytin a | e | 19.07 | 412 | 392 | 1.27 | - | - | 498 | 19.33 | 540 | 23.22 | 598 | 7.96 | 644 | 1.53 | E141ii | [5] |
| Pheophytin b | 3 | 20.75 | 436 | - | - | 416 | 2.58 | 526 | 8.03 | 560 | 19.84 | 600 | 16.01 | 656 | 4.43 | S6 | [2] |
| unknown | f | 21.93 | 417 | (397) | 1.86 | - | - | 505 | 19.50 | 545 | 14.31 | 623 | 6.78 | 671 | 1.67 | E141ii | - |
| Chlorophyll b | 4 | 22.62 | 466 | - | - | - | - | - | - | (550) | 17.46 | 600 | 8.45 | 650 | 2.49 | S1, S9 | [2] std |
| Pheophytin a | 5 | 23.31 | 410 | (380) | 1.52 | (400) | 1.11 | 507 | 9.44 | 538 | 10.11 | 610 | 10.62 | 667 | 2.12 | S5 to S7, S11, S13 to S16 | [2] |
| Cu rhodochlorin | g | 25.04 | 408 | 393 | 1.29 | - | - | - | - | 498 | 21.50 | (593) | 9.67 | 636 | 1.83 | E141ii | [4] |
| Chlorophyll a | 6 | 26.16 | 430 | (386) | 1.70 | (416) | 1.12 | - | - | (580) | 9.80 | 618 | 5.10 | 665 | 1.13 | S9, S11, S16 | [2] std |
| Cu pyropheophorbide a | h | 26.31 | 424 | (366) | 1.78 | 403 | 1.15 | 510 | 19.60 | 554 | | 606 | 5.44 | 652 | 1.13 | E141ii, S1 to S4, S6, to S8, S18 to S20 | [1] [4] [5] |
| Isochlorin e4 | 7 | 28.55 | 400 | - | - | 500 | 11.87 | 530 | 31.62 | 560 | 74.80 | 609 | 31.60 | 665 | 3.06 | S5 to S8, S13, S16 | [1] |

Compounds derived from copper-free chlorophylls are indicated by numbers; the other, containing copper, present in the E141ii dye and in some samples, with letters.
Retention factor. Kc = (tR - tM)/tM where tR is the retention time of the pigment peak and tM is the retention time of an unretained component.
M = maximun absorbance (nm); R = quotient of absorbance at Soret band divided by absorbance at wavelength indicated.
The values in parentheses indicate inflection points in the absorption spectrum.
[1] Aparicio *et al.,* 2011, [2] Gangul-Rojas *et al.*, 2012; [3] Inoue *et al*., 1994; [4] Mortensen *et al.,* 2007; [5] Roca *et al.,* 2010, [6] Chernomorsky *et al*., 1997, [7] Hynninen, 1973, std = chemical standard.

main responsible of the bright or deep-green dye of the analyzed olives.

Concerning the addition of Copper-sulphate, the re-greening made at alkaline pH was more pronounced in samples treated with the addition of 5 and 20% $CuSO_4$ in comparison respect to olives treated with 1% $CuSO_4$. In fact, untreated samples do not show peaks referable to Cu-chlorophyll compounds (Fig. 5-S17), while samples treated with 1, 5 and 20% of $CuSO_4$ show well defined peaks in their chromatograms: 2 of them were identified as Cu chlorin $e_6$, (peak **b**) and Cu pyropheophorbide a (peak **h**) (Fig. 5-S18; 5-S19; 5-S20).

The sample previously processed with Spanish method (therefore at acid pH) and treated with $CuSO_4$, shows a lighter colour change with respect to the other three samples. This is supported by the very low adsorbance values of Cu chlorin $e_6$ (peak **b**) pointed out in its chromatogram (Fig. 5-S21).

The Copper of $CuSO_4$ seems therefore to react only at alkaline pH (before the final acidification) with the degraded chlorophylls within the olives the same way it reacts with saponificated chlorophylls during industrial colorant production, leading to the synthesis of Cu-chlorophyll derivatives similar to the ones identified in the E141ii reference sample.

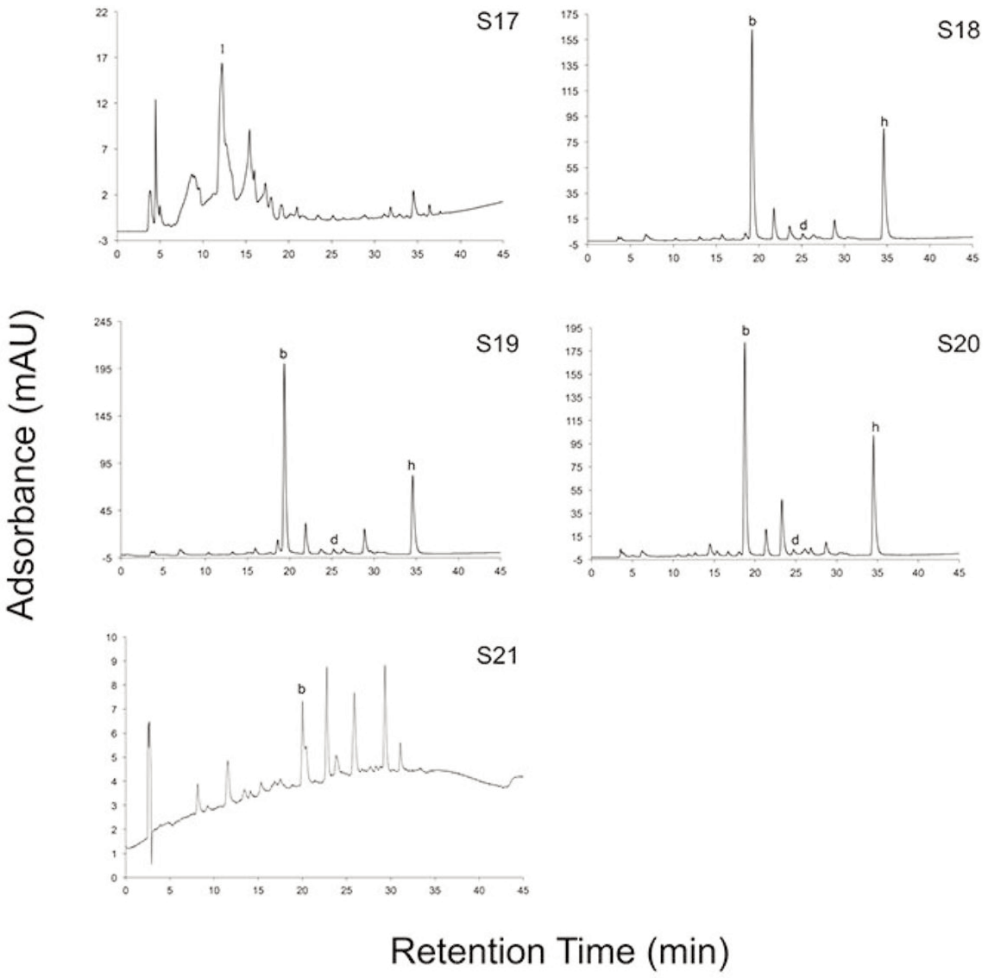


Fig. 5 - HPLC/DAD chromatograms, recorded at 650 nm, of samples treated with various percentages of CuSO4: (S17) without CuSO4 addition; (S18) CuSO4 1%; (S19) CuSO4 5%; (S20) CuSO4 20%. (S21) classical Spanish method and CuSO4 20%. Peaks described in Table 2.

## 4. Conclusions

The use of a relatively simple method of detection of E141ii added to table olives shows that the fraudulent addition of colorant (or copper sulfate) to table olives is a quite common practice. In fact, the 50% of the analyzed samples possess copper chlorophyll pigments, most likely due to the addition of colorant E141ii, or, worse still, of copper sulphate during the production process. Our work has shown that in these samples there is often the presence of Cu chlorin $e_6$, Cu isochlorin $e_4$ and Cu pyropheophorbide to which can therefore be considered markers for these adulterations. More controls and analysis are required to ensure the complete food safety and the compliance with the current law. The finding that even Copper-sulphate can be used to modify olive dye before marketing them, makes this need even more pressing, especially in relation to the severe liver damages it may cause.

## References

APARICIO-RUIZ R., RIEDL K.M., SCHWARTZ S.J., 2011 - *Identification and quantification of metallo-chlorophyll complexes in bright green table olives by High-Performance Liquid Chromatography-Mass spectrometry Quadrupole/time-of-flight*. - J. Agr. Food Chem., 59: 11100-11108.

CERUTTI G., 2006 - *Residui, additivi e contaminanti degli alimenti*. - Tecniche Nuove, Milano, Italy, pp. 128-129.

CHERNOMORSKY S., RANCOURT R., SAHAI D., PORETZ R., 1997 - *Evaluation of commercial chlorophyllin copper complex preparations by liquid chromatography with Photodiode Array Detection*. - Journal of Association of Analytical Communities International, 80: 433-435.

EUROPEAN UNION, 1994 - *Official Journal of the Commission of the European Communities*. - EU Regulation No. 94/36/CE, L237.

FERRUZZI M.G., FAILLA M.L., SCHWARTZ J., 2002 - *Sodium copper chlorophyllin:* in vitro *digestive stability and accumulation by Caco-2 human intestinal cells*. - J. Agr. Food Chem., 50: 2173-2179.

FODALE A.S., MULE R., TAGLIAVIA M., TUCCI A., 2007 - *The processing of green olives using the "Castelvetrano" method*. - Industrie alimentari, 46(742): 895-900.

GANDUL-ROJAS B., ROCA M., GALLARDO-GUERRERO L., 2012 - *Detection of the colour adulteration of green table olive with copper chlorophyllin complexes (E141ii colorant).* - LWT-Food Sci. Technol., 46: 311-318.

GARRIDO-FERNANDEZ A., FERNANDEZ-DIEZ M.J., ADAMS M.R., 1997 - *Table olives. Production and processing*. - Chapman & Hall, London, UK, pp. 496

HYNNINEN P., 1973 - *Preparation and purification of some derivatives of chlorophylls a and b*. - Acta Chemica Scandinavica, 5: 1771-1780.

INOUE H., YAMASHITA H., FURUYA K., NONOMURA Y., YOSHIOKA N., LI S., 1994 - *Determination of copper (II) chlorophyllin by reversed-phase high-performance liquid chromatography*. - J. Chromatogr. A, 679: 99-104.

MINGUEZ-MOSQUERA M.I., GALLARDO-GUERRERO L., HORNERO-MENDEZ D., GARRIDO-FERNANDEZ J., 1995 - *Involvement of Copper and Zinc ions in Green Staining of table olives of the variety Gordal*. - J. Food Protect., 58: 564-569.

MINGUEZ-MOSQUERA M.I., GARRIDO-FERNANDEZ J., GANDUL-ROJAS B., 1989 - *Pigment changes in olives during fermentation and brine storage.* - J. Agr. Food Chem., 37: 8-11.

MORTENSEN A., 2006 - *Carotenoids and other pigments as natural colorants*. - Pure Appl. Chem., 78: 1477-1491.

MORTENSEN A., GEPPEL A., 2007 - *HPLC-MS analysis of the green food colorant sodium copper chlorophyllin*. - Innov. Food Sci. Emerg., 8: 419-425.

NIIR, 2004 - *Food colours, Flavours and additives technology handbook. Chapter 9 Natural colours in food*. - NIIR National Institute of Industrial Research, pp. 626.

OWEN R.W., HAUBNER R., MIER W., GIACOSA A., HULL W.E., SPIEGELHALDER B., BARTSCH, H., 2003 - *Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes*. - Food Chem. Toxicol., 41: 703-717.

PIGA A., GAMBELLA F., VACCA V., AGABBIO M., 2001 - *Response of three Sardinian olive cultivars to Greek-style processing*. - Ital. J. Food Sci., 1: 29-40.

ROCA M., GALLARDO-GUERRERO L., MINGUEZ-MOSQUERA M.I., GANDUL ROJAS B., 2010 - *Control of olive oil adulteration with copper-chlorophyll derivates*. - J. Agr. Food Chem., 58: 51-56.

SALVO F., CAPPELLO A., GIACALONE L., 1995 - *Destinazione della produzione olivicola*. - L'olivicoltura nella valle del Belice, Istituto Nazionale di Economia Agraria, Italy, pp. 155-187.

SCOTTER M.J., CASTLE L., ROBERTS D., 2005 - *Method development and HPLC analysis of retail foods and beverages for copper chlorophyll (E141[i]) and chlorophyllin (E141[ii]) food colouring materials*. - Food Addit. Contam., 22: 1163-1175.

STERN B.R., 2010 - *Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations.* - J. Toxicol. Env. Health, Part A, 73: 114-127.