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Polyphenol oxidase, total phenolics and ascorbic acid changes during storage of minimally processed 'California Wonder' and 'Quadrato d'Asti' sweet peppers

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ABSTRACT

A growing sector of 'minimally processed' vegetables market is represented by sweet peppers, whose quality may be affected by enzymatic activities. Among these, polyphenol oxidase leads to browning reactions, which is a major cause of quality loss. This research aimed at assessing the changes in PPO activity, total phenolics and ascorbic acid throughout a 30-days cold storage in minimally processed green (cv. 'California Wonder'), yellow and red (cv. 'Quadrato d'Asti') sweet peppers. At day 0 PPO was active in red and yellow fruits but not in green ones, where it started to show relevant activity from the 3rd week of storage. At the end of the storage period (day 30), PPO activity was 1.36, 0.94 and 0.61 U/g d.m. in yellow, red and green peppers, respectively. Total phenols content was highest in green peppers, followed by red and yellow ones. In green fruits it increased up to the 3rd week of storage, decreasing afterwards, whilst in red and yellow fruits phenols content progressively declined after the 2nd week. Yellow fruits showed the highest ascorbic acid content, followed by red and green ones.

Results confirm that green peppers 'California Wonder' are more suitable to minimal processing than yellow and red fruits.

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1. Introduction

Pepper is the fruit produced by a *Solanaceae* belonging to *Capsicum annuum* (L.) species, which is extensively consumed worldwide, due to the numerous available types of colours (from green to yellow and red), forms and tastes (Conforti, Statti, & Manichini, 2007). In recent years, peppers have grown in popularity, and a wide number of varieties are now available in the grocery stores. This taxon includes both sweet cultivars eaten mainly as vegetables and hot ones, often used as a spice (Guil-Guerrero, Martínez-Guirado, Rebolloso-Fuentes, & Carrique-Pérez, 2006).

In Sicily (South Italy), sweet peppers and eggplants are the main ingredients in traditional dishes, prepared with cut, mixed and cooked vegetables, with additional ingredients and olive oil. Therefore, fresh-cut peppers differently consumed represent a growing sector of 'minimally processed' vegetables market. Unfortunately, their quality tends to decline by endogenous enzymatic activities whose reactions are promoted under certain technological conditions of production. The various processes of chopping or slicing cut through cells and release cell contents at the sites of wounding. Subcellular compartmentalization is disrupted at the cut surfaces, and the mixing of substrates and enzymes (mainly, oxidases and pectinases), which are normally separated can initiate reactions that do not occur otherwise.

Among these activities, the polyphenol oxidase (PPO, EC 1.14.18.1) catalyzes the hydroxylation of monophenols to *o*-diphenols (cresolase activity) and the oxidation of *o*-diphenols to *o*-quinones (catecholase activity), which represent its main reaction products. Subsequent reactions of the quinones lead to melanin accumulation, which is the brown or black pigment associated with 'browning' in plant tissues (Barbagallo, Chisari, & Patané, 2012). The specific reaction sequence which results in brown or black-coloured products depends on the specific structure of the polyphenolic substrate. PPO is located in cell organelles such as chloroplasts, mitochondria and peroxisomes where it is firmly bound to the membrane and may even be found in the soluble fraction of the cell membrane (Barbagallo, Chisari, & Spagna, 2009). In young and unripe fruits, it is mostly present under conjugated form, while in the ripe fruits (Conforti et al., 2007). Van Lelyveld, Gerrish, and Dixon (1984) found increased PPO activity in response to mechanical shock.

Peppers are a remarkable source of antioxidants including flavonoids, phenolic acids, ascorbic acid and carotenoids (Castro

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et al., 2008; Howard, Talcott, Brenes, & Villalon, 2000; Yahia, Contreras-Padilla, & Gonzalez-Aguilar, 2001). In fresh products these endogenous substances can play a role in contrasting the browning process.

Among these, ascorbic acid reduces the quinones back to the phenolic acids, but during this reduction it is irreversibly oxidized to dehydroascorbic acid, thus allowing browning to occur upon its depletion. More stable forms of ascorbic acid derivatives, such as erythrobic acid 2- and 3-phosphate derivatives of ascorbic acid, phosphinate esters of ascorbic acid, and ascorbyl-6-fatty acid esters of ascorbic acid, have however been developed to overcome these problems (Sapers & Hicks, 1989).

Qualitative and quantitative level of phenolic content in pepper fruits is at the base of inhibition of PPO. Nevertheless, the phenolics composition determined in pepper fruits is incomplete. The presence of derivatives of cinnamic acid and flavonoids has been found in pepper fruit by Sukrasno and Yeoman (1993). They determined the contents of *p*-coumaryl, caffeoyl, and 3,4-dimethoxycinnamoyl glucoside and of four flavonoid compounds, but identification was given for only two: 3-O-rhamnosylquercetin and 7-O-glucosylluteolin. A more detailed analysis of pepper phenolics was made by Iorizzi et al. (2001), who identified 10 compounds in pepper fruit, 3 of which were based on a new structure; they were capsioside A, capsioside B, and capsianoside VII. Main phenolic compounds were isolated by Materska and Perucka (2005) in hot pepper: *trans-p*-feruloyl-β-D-glucopyranoside, *trans-p*-sinapoyl-β-D-glucopyranoside, quercetin 3-*O*-α-L-rhamnopyranoside-7-O-β-D-glucopyranoside, *trans-p*-feruloyl alcohol-4-*O*-[6-(2-methyl-3-hydroxypropionyl)] glucopyranoside, luteolin 6-C-β-D-glucopyranoside-8-C-α-L-arabinopyranoside, apigenin 6-*C*-β-D-glucopyranoside-8-C-α-L-arabinopyranoside, luteolin 7-*O*-[2-(β-D-apiofuranosyl)-β-D-glucopyranoside], quercetin 3-*O*-α-L-rhamnopyranoside, and luteolin 7-*O*-[2-(β-D-apiofuranosyl)-4-(β-D-glucopyranosyl)-6-malonyl]-β-D-glucopyranoside. If the composition of PPO inhibitors is mostly represented by coumaroyl derivatives in higher concentrations than caffeoyl derivatives, this could explain the relation between PPO and browning in peppers, in comparison with fruits more susceptible to the enzyme activity and with high flavanols content, such as apple.

Certainly, some additional agronomic, cultivating, technological, microbiological, physico-chemical and enzymatic factors (e.g. role of pectinases in product softening) could have a role in keeping the quality of fresh-cut sweet pepper.

This research aimed exclusively to evaluate the variation in PPO activity, total phenols and ascorbic acid, measured weekly throughout a 30-day storage period (at day 0, 7, 14, 21 and 30), in 'California Wonder' and 'Quadrato d'Asti' minimally processed sweet peppers.

2. Materials and methods

2.1. Sample preparation

Peppers used for the experiment belonged to the square type (*C. annuum* L., var. grossum) and were the following: 'California Wonder' green, 'Quadrato d'Asti' red and yellow.

The plants were produced under greenhouse conditions in Pachino site (South Sicily, Italy). The 'California Wonder' bell is a robust pepper that has been designed to thrive in cooler climates, giving northern gardeners a chance to grow their own tropical produce. The stocky, vigorous plants reach about 70 cm in height and produce an abundance of medium–large, blocky, deep green peppers that turn red when fully ripe as well as stable-green peppers. They matures 75 days after transplanting.

The 'Quadrato d'Asti' is the most is the most famous Italian sweet bell pepper. The fruit is huge (length ca. 15 cm; ratio length–width 1.0–1.2; weight ca. 400 g). Blocky, typically four-lobed peppers have a sweet flavour, and change from a brilliant green to red or golden-yellow when ripe. Thick walls make these ideal for salads, roasting, or stuffing. These fruits ripen 75 days after transplanting.

At marketable ripening (100% of fruits fully coloured), 25 fruits for each cultivar were hand harvested, selected for freshness, uniform shape and lack of mechanical damages. Pepper fruits were randomly distributed in groups of 5. Within 10 h of harvest the samples were washed to remove dirt and left to dry, then cut in a 2.5 × 2.5 cm square form and packaged under normal atmosphere inside polyethylene terephthalate (PET) trays, covered with a 25 μm double barrier 'anti-fog' film (Melinex 850, Monteveglio, Bologna, Italy – O_2 permeability: 35 cc/m^2/24 h, CO_2 permeability: 135.8 cc/m^2/24 h; H_2O permeability: 15 g/m^2/24 h), and finally stored at 4.0 ± 0.5 °C, 95% R.H.

In order to make the physico-chemical and enzymatic measurements, peppers were weekly homogenized in Ultraturrax T25 (Janke & Kunkel, Staufen, Germany) for 3 min in an iced bath, to avoid oxidative processes. Results are referred to the dry matter (g), in order to make comparable the data of fruits with a different water content. For that purpose a 5 g of homogenized sample was dried at 105 °C until constant weight (approximately 9 h).

2.2. Polyphenol oxidase activity

The catecholase activity of polyphenol oxidase (PPO) was determined spectrophotometrically (Cary IE-100 UV–VIS, Varian, Palo Alto, CA, USA) using a modified version of the method proposed by Espìn, Morales, Varon, Tudela, and Garcìa-Canovas (1996). Other tested methodologies did not give the same reproducibility. Twenty grams of homogenate were added to 40 mL cold acetone (−20 °C) and continuously stirred for 10 min. The homogenate was filtered through Whatman No. 42 paper (Milan, Italy) under vacuum on Buchner funnel and the obtained acetone powder, collected and suspended in 30 mL 0.1 mol/L citrate phosphate buffer pH 7.5, was kept over night at 4.0 °C, before being again filtered through Whatman No. 42 paper under vacuum on Buchner funnel. The clear solution was then ultrafiltered in a cell equipped with a 10 kDa membrane (Millipore 8050, Milan, Italy). The enzymatic activity was assayed spectrophotometrically at 505 nm using 3,4-dihydroxyphenyl acetic acid (DOPAC) as specific phenolic substrate according to earlier work (Spagna, Barbagallo, Chisari, & Branca, 2005). The standard reaction mixture contained 0.9 mL of 0.04 mol/L phenolic substrate, 0.1 mL of 0.093 mol/L MBTH (3-methyl-2-benzothiazolinone hydrazone) in methanol, 0.05 mL of DMF (N,N-dimethylformamide), 1.5 mL of 0.05 mol/L sodium acetate buffer pH 7.0 and 0.5 mL of enzymatic extract. Reaction was stopped at different times with 0.5 mL of 0.9 mol/L H_2SO_4. Blank was prepared by inverting the order between the enzymatic extract and H_2SO_4. One unit of PPO activity was defined as the amount of enzyme which produces an increase in absorbance of 0.001 per min at 25 ± 0.5 °C under the conditions above described.

2.3. Total phenols measurement

Total phenols (TP) content was extracted from the tissues with a solution of 0.1 mL/100 mL HCl in methanol and assayed using the Folin–Ciocalteu method as proposed by Singleton, Orthofer, and Lamuela-Raventos (1999). Since ascorbic acid and other substances may interfere to the results of Folin–Ciocalteu assay (Georgé, Brat, Alter, & Amiot, 2005), methanolic extracts were settled on a C-18 end-capped cartridge, Phenomenex-Strata (Castel Maggiore, Bologna, Italy), 1.0 g/6 mL, according to following method: cartridge was washed with 2 mL methanol and conditioned with 5 mL H_2SO_4 0.01 mol/L. One mL sample was adsorbed and then 2 mL H_2SO_4 0.01 mol/L was added. The cartridge was linked to a 20 mL flask and eluted with 2 mL methanol and 5 mL

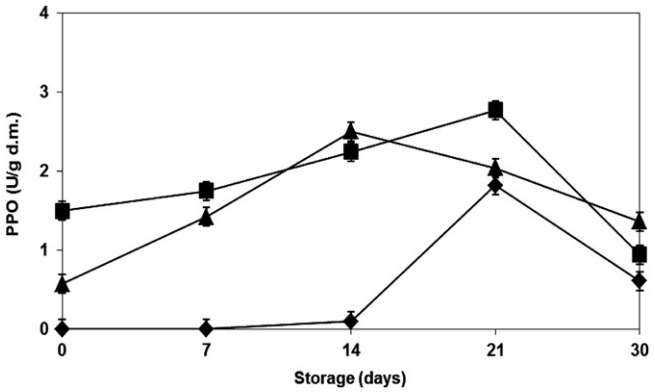

Fig. 1. PPO activity in green (-◆-), red (-■-) and yellow (-▲-) 'minimally processed' peppers. All measurements were conducted in triplicate ($n = 3$).

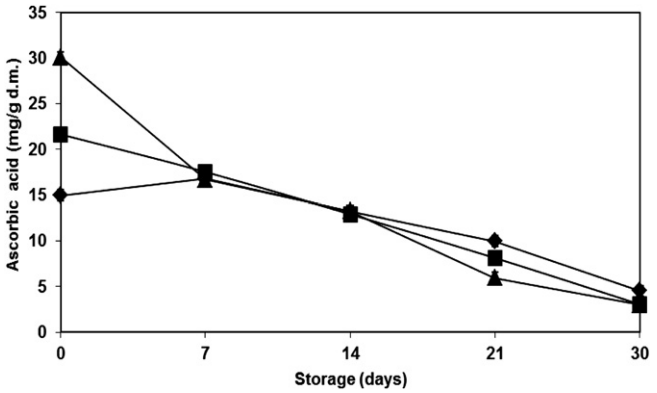

Fig. 3. Ascorbic acid content in green (-◆-), red (-■-) and yellow (-▲-) 'minimally processed' peppers. All measurements were conducted in triplicate ($n = 3$).

deionized water. To the flask content 1 mL Folin–Ciocalteau solution was added and, after 5 min, 4 mL Na_2CO_3 20 g/100 g carrying to volume with deionized water. The mixture was incubated at 20 °C for 2 h and after centrifugation of the sample in order to separate the carbonate fraction, the absorbance at 765 nm was spectrophotometrically measured (Cary IE-100 UV–VIS Spectrophotometer system, Varian, Palo Alto, CA, USA), against a blank prepared in a flask containing all reagents and 2 mL methanol without phenols. Total phenols content was expressed as gallic acid equivalent (GAE).

2.4. Ascorbic acid measurement

The ascorbic acid concentration was measured spectrophotometrically adopting the enzymatic kit specific for the ascorbic acid (Cat. No. 10409677035, Boehringer-Mannheim, Monza, Italy), based upon the method as proposed by Beutler (1984).

2.5. Reagents

When not specified otherwise, all chemicals used were of analytical grade and were supplied by Sigma–Aldrich Chemicals Co. (Milan, Italy).

2.6. Total Visual Quality

For a simple sensory evaluation of vegetables, a panel of five judges assessed the Total Visual Quality (TVQ) of minimally processed sweet pepper, evaluating colour, texture and overall acceptance (including absence of off-flavours) as quality attributes, by comparing results at the beginning and at the end of storage period. Visual quality parameters was scored on a scale from 1 to 9, where 9 = excellent, fresh appearance, 7 = good, 5 = fair, limit of marketability, 3 = fair, useable but not saleable, 1 = unusable. Intermediate numbers were assigned as appropriate (Baur, Klaiber, Hammes, & Carle, 2004).

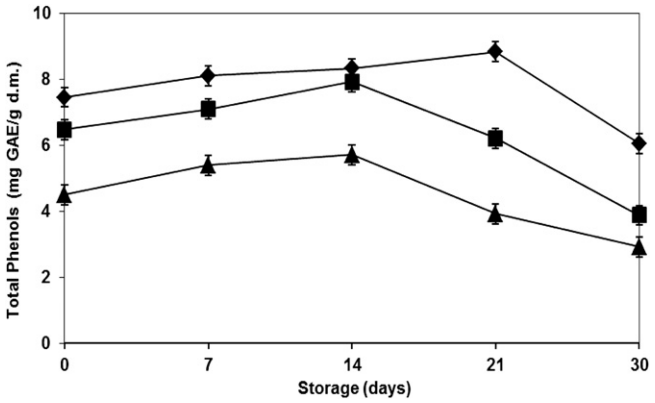

Fig. 2. Total phenols content in green (-◆-), red (-■-) and yellow (-▲-) 'minimally processed' peppers. All measurements were conducted in triplicate ($n = 3$).

2.7. Statistical analysis

All measurements were conducted in triplicate. Data were statistically evaluated by the analysis of variance (ANOVA) using the Statistical Analysis System (SAS Ver. 9.0). Differences among means were evaluated for significance using the Duncan Multiple Range Test (DMRT) at $p \leq 0.05$.

3. Results and discussion

At the time of initial packaging (day 0) the catecholase activity of polyphenol oxidase (PPO) was observed (Fig. 1) in red and yellow fruits, while in the green fruits PPO activity became evident at the 3rd week of storage.

In particular, yellow peppers exhibited the maximum oxidative activity earliest (after 14 days), then the red and green ones (after 21 days). According to ANOVA statistical analysis, carried out by comparing three pepper types in each storage day, it was possible to evidence that data were statistically different ($p \geq 0.05$), with enzyme activity values decreasing from red to yellow to green fruits, except in correspondence of day 14 in which no significant differences ($p \leq 0.05$) were noticed between PPO activity of red and yellow fruits.

The role of phenols as antioxidant is supported by several researches and the recovery methods have a great importance for industrial use. Velioglu, Mazza, Gao, and Oomah (1998) reported a strong relationship between total phenolic content and antioxidant activity in fresh fruits, vegetables and grain products, but in fresh-cut products their availability as substrates of PPO can makes them perishable. On the other hand, washing of green pepper bell slices after cutting improves firmness retention, probably due to the removal from the cut surfaces of solutes and stress-related signalling compounds such as phenolics (Toivonen & Stan, 2004).

According to statistical analysis, it was possible to evidence that total phenols content of the three pepper typologies was statistically different ($p \geq 0.05$) in each storage day, with decreasing values from green to red to yellow fruits (Fig. 2). In green fruits total phenols increased up to day 21, decreasing afterwards, whilst in red and yellow fruits phenols content started to decline progressively until day 14. Phenols content of red and green types was higher

Table 1
Total Visual Quality (TVQ) of minimally processed sweet pepper during cold storage assessed by five judges. Scores range on a scale from 1 to 9, where 9 = excellent, fresh appearance, 7 = good, 5 = fair, limit of marketability, 3 = fair, useable but not saleable, 1 = unusable.

Sensory parameters	Green pepper					Red pepper					Yellow pepper				
	Day 0	Day 7	Day 14	Day 21	Day 30	Day 0	Day 7	Day 14	Day 21	Day 30	Day 0	Day 7	Day 14	Day 21	Day 30
Colour	9.0 ± 0.00	9.0 ± 0.00	8.5 ± 0.25	8.0 ± 0.25	7.5 ± 0.50	9.0 ± 0.00	8.5 ± 0.25	8.0 ± 0.50	6.5 ± 0.25	6.5 ± 0.25	8.5 ± 0.00	7.5 ± 0.25	7.5 ± 0.25	6.5 ± 0.50	5.5 ± 0.50
Texture	9.0 ± 0.00	8.5 ± 0.25	8.5 ± 0.25	7.5 ± 0.25	7.5 ± 0.50	8.5 ± 0.25	8.5 ± 0.25	8.0 ± 0.50	7.0 ± 0.25	7.0 ± 0.25	8.0 ± 0.25	7.5 ± 0.00	7.5 ± 0.50	6.0 ± 0.50	5.0 ± 0.25
Overall acceptance	9.0 ± 0.00	8.5 ± 0.25	8.0 ± 0.00	8.0 ± 0.25	7.0 ± 0.50	9.0 ± 0.00	8.0 ± 0.00	8.0 ± 0.50	7.5 ± 0.00	6.5 ± 0.25	8.5 ± 0.25	8.0 ± 0.00	7.0 ± 0.00	6.0 ± 0.25	6.0 ± 0.75

than yellow fruits and with no significant differences between them ($p \leq 0.05$).

The fresh sweet peppers analyzed were an important source of ascorbic acid for human consumption, presenting at the time of initial packaging (day 0) value of 124 mg/100 g (green), 139 mg/100 g (red) and 175 mg/100 g, higher than those of navel orange (74 mg/100), mango Rosa (84 mg/100 g) and guava (90 mg/100 g) (Mangels et al., 1993; Mélo, Lima, Maciel, Caetano, & Leal, 2006). Ascorbic acid concentrations, although initially (day 0) statistically different among the three pepper typologies tested ($p \geq 0.05$), starting from day 7 up to day 14 showed similar values, with no significant difference ($p \leq 0.05$), following then a different trend with highest residual concentrations in green fruits, although yellow fruits showed the highest ascorbic acid content at the beginning of storage period (Fig. 3).

When considering PPO degradative activity, yellow peppers are less suitable to 'minimal processing' due to the earlier oxidative enzyme expression as compared to green and red fruits. Green peppers exhibited the lowest maximum PPO activity and a delayed expression of it, as red fruits. PPO in red fruits was higher than in green and yellow ones up to day 21, although at day 14 no significant differences were noticed between red and yellow fruits. This greater PPO activity in red peppers, already during first days of storage, is probably due to a greater enzyme affinity for the specific phenols (substrates) present in red fruits.

The sensory evaluation assessed by Total Visual Quality (TVQ) of minimally processed sweet pepper through the 30-days storage period, showed a good correspondence with changes of tested parameters (Table 1). In addition to texture modifications, due to synergistic action of pectinase enzymes (mainly PME and PG) which also influenced the overall acceptance, the colour changes on a weekly basis confirmed a higher potential suitability to storage of green peppers, followed by red and yellow fruits.

4. Conclusions

Green peppers as the most suitable to be "minimally processed", followed by red fruits. Yellow fruits, due to a greater sensitivity to oxidation, seemed to be less suitable to this use, at least in packaging under normal atmosphere.

Moreover, some additional agronomic, cultivating, technological, microbiological, physico-chemical and enzymatic factors could have a role in keeping the quality of fresh-cut sweet pepper and should be considered in overall quality evaluation of the commercial product.

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