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Effect of type of fertilization and maturity on quality of fresh-cut red and yellow peppers (*Capsicum annuum* L.)

F. Piazzolla*, M.L. Amodio*, R. Rinaldi*, F. Raimo, G. Colelli***

* *Dipartimento di Scienze delle Produzioni e dell'Innovazione dei Sistemi Agroalimentari Mediterranei, Università degli Studi di Foggia, Via Napoli, 25, 71121 Foggia, Italy.*

** *Consiglio per la Ricerca e Sperimentazione in Agricoltura, Unità di Ricerca per le Colture Alternative al Tabacco, CRA-CAT, Via P. Vitiello, 108, 84018 Scafati (SA), Italy.*

Key words: appearance, organic fertilization, phenolics, Vitamin C.

Abstract: The aim of this work was to evaluate the effect of the type of fertilization (mineral and combined fertilization with compost in pre-transplant plus mineral addition during cultivation) and stage of maturity at harvest (mature-green and full-colored) on post-cutting quality of red and yellow 'Cazzone' peppers. Peppers were cut into strips, and air-stored for 8 days at 5°C. During storage, color, appearance score, firmness, respiration rate, soluble solids, acidity, pH, vitamin C, total phenols, and antioxidant activity were measured. The maturity stage influenced color parameters and soluble solids, acidity and pH for both yellow and red types. Full-colored peppers showed a lower respiration rate, and higher SSC than mature-green peppers; for the yellow type, a lower firmness value was observed for full-colored fruits compared to the mature-green ones. A lower antioxidant activity was also observed in the yellow type fertilized with the combined treatment, while phenol content in full-colored peppers was higher than in mature-green ones. Fresh-cut yellow peppers showed higher susceptibility to decay compared to red types: after 8 days of storage, the appearance score in mineral fertilized full-colored yellow peppers dramatically decreased below the limit of marketability. The results of this experiment show that the type of fertilization and maturity stage can have varying impact on the quality of yellow and red peppers.

1. Introduction

Due to consumer demand for high-convenience foods, fresh-cut peppers may represent an interesting product to add to the existing fresh-cut products. The quality of a fresh-cut product is generally affected by pre-harvest and post-harvest factors, including processing. Genotype, growing conditions, cultural practices, and maturity stage at harvest, from a pre-harvest point of view, may greatly influence initial quality of the product to be processed; on the other hand, postharvest handling and storage of raw materials, and processing conditions, including finished product fate throughout the distribution chain, markedly determine its final quality. Maturity stage is an important factor conditioning final quality and processability of fresh-cut products, particularly fruits. Less mature fruits, in fact, are more suitable for processing due to their greater firmness, compared to more mature fruits, but this can result in lower sensorial quality as observed in melons (Watada and Qi, 1999) and mangoes (Bender *et al.*, 2000). In peppers, the maturity stage was found to have an impact on flavonoids, carotenoids, and ascorbic acid concentration (Marin *et al.*, 2004; Fox *et al.*, 2005), with carotenoids and ascorbic acid increasing, and flavonoids decreasing, as maturity proceeds, while Deepa *et al.* (2007) observed an increase in the main antioxidant compounds, including phenols ascorbic acid, capsicin, and carotenoids for 10 different genotypes. Moreover, in 'Domino' bell peppers, an increase in firmness was observed with the increasing of fruit size, occurring with ripening, most probably due to the increase of pericarp thickness (Tadesse *et al.*, 2002). The same authors reported an increase in soluble solids, and a decrease in respiration rate and ethylene production and suggested as maturity index at harvest for this cultivar firmness values of 35 N and a minimum of 6°Brix. Molinari *et al.* (1999) reported greater titrable acidity on full-ripe peppers ripened on the plant compared to those harvested at the color-break stage and ripened in storage.

In addition, agricultural practices, soil, and climate conditions may differently affect quality attributes of fresh produce, including external attributes (Kays, 1999), firmness (Sams, 1999), and nutritional composition (Lee and Kader, 2000). In particular, nitrogen fertilization seems to decrease the concentration of vitamin C in many fruits and vegetables (Lisiewska and Kmiecik, 1996). All these factors can consequently affect quality of fresh-cut produce

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and their storability, but very few works have directly investigated the impact of pre-harvest factors on the quality of fresh-cut products, and none of them regard bell peppers. More works are available on the impact of processing, and among them Artés-Hernández *et al.* (2010) studied the effect of the cut type and of the modified atmosphere packaging on quality of bell peppers (cv. Requena) observing that in general peppers cut in a 'ring' suffer greater weight loss than peppers cut in strips or dice, although respiration was not affected by the cutting mode and was not significantly different from the whole product.

The objective of the present work was to evaluate the effect of the type of fertilization (mineral and combined fertilization with compost in pre-transplant plus mineral addition during cultivation) and stage of maturity at harvest (mature-green and full-colored) on post-cutting quality of red and yellow *'*Cazzone*'* peppers.

2. Materials and Methods

Plant material

The experiment was carried out in Scafati (SA, Italy, coordinates 40° 44'N, 14° 30'E, 10 m a.s.l.), on soil of sandy loam texture, basic pH and with organic and mineral content as reported in Table 1.

Table 1 - Soil parameters

Soil parameters	
pH in water (1:2.5)	8.4
EC 25°C (1:2) (dS/m)	0.6
C (g/kg)	15.4
Organic matter (g/kg)	26.6
N (g/kg)	1.3
C/N	11.6
Assimilated P_2O_5 (mg/kg)	85.0
Exchangeable K (ppm)	917.1
Exchangeable Na (ppm)	251.8
Exchangeable Ca (ppm)	2090.9
Exchangeable Mg (ppm)	497

Ecotypes of red and yellow 'Cazzone' pepper (*Capsicum annuum* L.) (density of 3.3 plants m^{-2}) were subjected to two different tecniques of fertilization: mineral and combined fertilization with compost in pre-transplant plus mineral addition during cultivation. Mineral fertilization was in compliance with the Campania Region guidelines: 100 kg ha^{-1} of nitrogen were applied for 1/3 in pre-transplant and 2/3 during plant growth. For the combined fertilization, 20 t ha^{-1} of dry organic compost obtained from urban organic waste (characteristics reported in Table 2) were applied in pre-transplant, and integrated with 50 kg ha^{-1} of mineral nitrogen during plant growth. Transplanting was carried out on 25 May. Pepper fruits were harvested on 15 September at two stages of maturity (mature-green and full-colored), and transported to the Laboratory of Postharvest Technology at the University of Foggia (Italy).

Table 2 - Compost parameters

Compost parameters	
Humidity (%)	31
pH	6-8
C (% DM)	28
Humic and fulvic carbon	8
N (% DM)	2
C/N	14
Cu (mg/kg DM)	110
Zn (mg/kg DM)	250
Salinity (meq/100 g)	21

Experimental design and protocol

For each ecotype/fertilization/stage of maturity combination, six lots (two replicates x three storage sampling) of 15 strips were individually placed in plastic trays closed in PET macroperforated bags, together with wet paper (to maintain high level of RH), and stored at 5°C for eight days. Initially, and after four and eight days of storage at 5°C quality attributes (including color, appearance score, firmness, respiration rate, weight loss, soluble solids, titrable acidity, pH, vitamin C, phenols content, and antioxidant activity) were monitored.

Respiration rate and weight loss

Respiration rate (ml CO_2 kg^{-1} hr^{-1}) was measured using the static system, measuring the amount of CO_2 accumulated in the headspace of sealed PVC containers (5 l). CO_2 concentration, determined by a Shimadzu gas chromatograph (model 17A) equipped with a TCD detector, was then referred to the weight of the sample, to the volume of the headspace, and to the elapsed time. Samples were individually weighed and the weight loss was calculated as % of the initial fresh weight.

Physical analysis

The colour of the strips was measured in two different points of the mesocarp, randomly selected, using a spectrophotometer (CM 2600d Konica Minolta, Osaka, Japan) in the reflectance mode using the CIE L*a*b* colour scale. Hue angle and saturation were then calculated.

Appearance score evaluation was subjectively assessed using a scale of 5 to 1 where 5= excellent, no defects; 4= very good, few defects; 3= good, moderate defects, limit of marketability; 2= poor, many defects; and 1= inedible. A score of 3 was considered as the limit of marketability and a score of 2 as the limit of edibility (Amodio *et al.*, 2007).

Firmness was measured on two points of the mesocarp, as resistance of the strips to a 2-mm penetration by a probe

of 6-mm diameter, using a digital penetometer (Tierre S.r.l., Torino).

Chemical analysis

Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by Zapata and Dufour (1992), with some modifications. Samples of 20 µL were analysed with an Agilent 1200 Series HPLC (Waldbronn, Germany) equipped with a binary pump, an autosampler, and a photodiode array detector. Separations of DHAA and AA were achieved on a Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm; 5 µm of particle size; Agilent Technologies, Santa Clara, CA, USA). AA and DHA contents were expressed as milligrams of ascorbic or dehydroascorbic acid per kilogram of fresh weight (mg kg^{-1}).

Total phenols were determined according to the method of Singleton and Rossi (1965). The absorbance was read at 725 nm against a blank using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China). The content of total phenols was calculated on the basis of the calibration curve of gallic acid, and was expressed as grams of gallic acid per kilogram of fresh weight (g GA kg^{-1}). Antioxidant assay was performed following the procedure described by Brand-Williams *et al.* (1995), with minor modifications. Trolox was used as a standard and the antioxidant activity was reported in milligrams of Trolox equivalents per kilogram of fresh weight (g TE kg^{-1}).

Four grams of fresh juice were used to determined total soluble solids (TSS) (measured with a digital refractometer, ATAGO PR32), pH and titratable acidity (TA). Titratable acidity was determined with an automatic titrator (TitroMatic CRISON 1S), using the juice samples and titrating with 0.1 N NaOH up to pH 8.1, and the value was expressed as percentage of citric acid.

Data analysis

For mean data at harvest of peppers of both ecotypes, a two-way ANOVA for stage of maturity and fertilization effects was run, while on the whole data set, a three-way ANOVA was performed with stage of maturity, fertilization treatment and time of storage as factors. When interactions among factors were not significant, main effects were analyzed. Mean separation among treatments at each time of storage was performed with the Tukey test. ($P< 0.05$, N=10).

3. Results and Discussion

Most of the quality attributes were not affected by the type of fertilization, except respiration rate, a* value and pH, whereas, as expected, most of them were affected by the stage of maturity. The interaction between the type of fertilization and the stage of maturity was statistically significant for respiration rate and pH (Table 3).

Table 3 - Effect of type of fertilization (mineral and combined) and stage of maturity (mature-green and full-colored), and their interaction on quality attributes of fresh-cut yellow 'Cazzone' peppers at harvest

Quality attributes	Fertilization (F)	Stage of maturity (S)	FxS
Firmness (N)	NS	NS	NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	*	***	***
L*	NS	****	NS
a*	*	****	NS
b*	NS	****	NS
Hue angle (*)	NS	****	NS
Chroma	NS	****	NS
Soluble solids (°Brix)	NS	***	NS
Titrable acidity (% citric acid)	NS	NS	NS
pH	**	****	***
Vitamin C (mg/100 g FW)	NS	NS	NS
Ascorbic acid (mg/100 g FW)	NS	NS	NS
L-dehydroascorbic acid (mg/100 g)	NS	NS	NS
Antioxidant activity (mg Trolox/100 g FW)	NS	NS	NS
Total phenol content (mg gallic acid/100 g FW)	NS	NS	NS

* when P≤ 0.05; ** when P≤ 0.01; *** when P≤ 0.001; **** when P≤ 0.0001; NS when not significant; N= 10.

Pepper fruits treated with mineral fertilization showed a higher respiration rate (9.6 ml CO_2 kg^{-1} hr^{-1}) than fruits treated with the combined fertilization (8.8 ml CO_2 kg^{-1} hr^{-1}), a higher a* value (0.6 vs. -1.1) and pH (5.44 vs. 5.35) although the absolute pH difference was very little (Table 4). Table 4 illustrates the effect of stage of maturity on quality attributes of fresh-cut yellow peppers at harvest. In particular, the stage of maturity affected the respiration rate, L*, a*, and b* values, hue angle, chroma, soluble solids, and pH. Full-colored peppers showed lower respiration rate than mature-green peppers (7.6 and 10.7 ml CO_2 kg^{-1} hr^{-1} respectively), higher soluble solids (6.1 vs. 4.7°Brix) and lower pH (5.75 vs. 5.04). These findings confirmed that reported by Tadesse *et al.* (2002) for 'Domino' bell peppers, and mainly that soluble solids, respiration rate and firmness (other than color) evolve during fruit ripening, and may be used as maturity index at harvest. The increase of titratable acidity and decrease of pH during bell pepper growth and ripening has also been observed in several studies (Fox *et al*., 2005), during plant ripening (Molinari *et al.*, 1999; Serrano *et al.*, 2010) and, in particular Serrano *et al.* (2010) observed an increase of citric acid during ripening.

For red 'Cazzone' peppers, no differences were observed for the measured attributes according to the type of fertilization and interaction between the type of fertilization and the stage of maturity, whereas the stage of matu-

Table 4 - Main effect of type of fertilization (mineral and combined) and stage of maturity (mature-green and full-colored) on mean values of quality attributes of fresh-cut yellow 'Cazzone' peppers at harvest

Quality attributes	Type of Fertilization		Stage of maturity	
	Mineral	Combined	Mature green	Full-colored
Firmness (N)	19.0 NS	18.3 NS	19.2 NS	18.1 NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	9.6 a	8.8 b	10.7 a	7.6 b
L*	45.2 NS	46.1 NS	39.3 b	52.1 a
a*	0.6 a	-1.1 b	-13.2 b	12.7 a
b*	37.7 NS	38.3 NS	26.4 b	49.6 a
Hue angle (*)	95.0 NS	97.3 NS	116.6 a	75.6 a
Chroma	40.3 NS	40.5 NS	29.5 b	51.3 a
Soluble solids (°Brix)	5.4 NS	5.5 NS	4.7 b	61.0 a
Titrable acidity (% citric acid)	0.16 NS	0.14 NS	0.14 NS	0.15 NS
pH	5.44 a	5.36 b	5.75 a	5.04 b
Vitamin C (mg/100 g FW)	59.4 NS	58.8 NS	61.4 NS	56.8 NS
Ascorbic acid (mg/100 g FW)	58.6 NS	58.0 NS	60.4 NS	56.2 NS
L-dehydroascorbic acid (mg/100 g)	0.8 NS	0.8 NS	1.0 NS	0.7 NS
Antioxidant activity (mg Trolox/100 g FW)	169.8 NS	155.0 NS	153.7 NS	171.1 NS
Total phenol content (mg gallic acid/100 g FW)	114.1 NS	119.5 NS	108.0 NS	125.5 NS

For each row, mean values followed by a different letter are significantly different (N=10 and P≤0.05).

rity affected several quality attributes (Table 5). The respiration rate of full-colored peppers was about half that of peppers at a mature green stage; soluble solids increased by about 2°Brix with ripening. In addition, an increase of acidity from 0.1% to 0.15% and a decrease of pH were also observed (5.93 and 4.93, respectively) (Table 6).

Most of the differences observed at harvest were maintained during storage for yellow peppers. Some differences among chemical constituents were found for yellow

Table 5 - Effect of type of fertilization (mineral and combined) and stage of maturity (mature-green and full-colored), and their interaction on quality attributes of fresh-cut red 'Cazzone' peppers at harvest

Quality attributes	Fertilization (F)	Stage of maturity (S)	FxS
Firmness (N)	NS	NS	NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	NS	**	NS
L*	NS	****	NS
a*	NS	****	NS
b*	NS	***	NS
Hue angle (*)	NS	****	NS
Chroma	NS	**	NS
Soluble solids (°Brix)	NS	***	NS
Titrable acidity (% citric acid)	NS	***	NS
pH	NS	***	NS
Vitamin C (mg/100 g FW)	NS	NS	NS
Ascorbic acid (mg/100 g FW)	NS	NS	NS
L-dehydroascorbic acid (mg/100 g)	NS	*	NS
Antioxidant activity (mg Trolox/100 g FW)	NS	NS	NS
Total phenol content (mg gallic acid/100 g FW)	NS	NS	NS

* when P≤ 0.05; ** when P≤ 0.01; *** when P≤ 0.001; **** when P≤ 0.0001; NS when not significant; N= 10.

Table 6 - Main effect of stage of maturity (mature-green and full-colored) on mean values of quality attributes of fresh-cut red 'Cazzone' peppers at harvest

Quality attributes	Mature green	Full-colored
Firmness (N)	18.9 NS	18.9 NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	9.8 a	4.9 b
L*	40.2 b	31.8 b
a*	-13.1 b	32.4 a
b*	27.0 b	16.6 b
Hue angle (*)	115.9 a	27.2 b
Chroma	30.0 b	36.4 a
Soluble solids (°Brix)	4.1 b	6.3 a
Titrable acidity (% citric acid)	0.10 b	0.16 a
pH	5.93 a	4.93 b
Vitamin C (mg/100 g FW)	55.0 NS	52.0 NS
Ascorbic acid (mg/100 g FW)	53.6 NS	51.4 NS
L-dehydroascorbic acid (mg/100 g)	1.4 a	0.6 b
Antioxidant activity (mg Trolox/100 g FW)	149.1 NS	163.9 NS
Total phenol content (mg gallic acid/100 g FW)	107.2 NS	123.2 NS

For each row mean values followed by a different letter are significantly different (N=10; P≤0.05).

'Cazzone' peppers, depending on the fertilization treatment. For this last ecotype, Table 7, the main effect of the fertilization treatment over storage on quality attributes is shown. In particular, a lower antioxidant activity was observed for peppers fertilized with the combined treatment (139 mg Trolox/100g FW) compared to mineral fertilization (157 mg Trolox/100g FW), whereas phenol content was positively affected by the stage of maturity, showing a value 10% higher for full-colored peppers compared to mature green (Table 7). The higher phenol content of full-coloured peppers compared to mature green ones confirms that phenols and antioxidant compounds increase during ripening, as found by Marin *et al.* (2004) and Deepa *et al.* (2007). In addition, a higher susceptibility to decay of fresh-cut yellow peppers compared to red type was observed. As shown in figure 1, after eight days of storage the appearance score in mineral-fertilized full-colored yellow peppers dramatically decreased below the limit of marketability (score 3) due to the presence of decay and most likely to the high respiration rate, even if it was found not significant among treatments (Fig. 1).

For red peppers, no differences related to the fertilization treatment were observed during storage; on the other hand, the stage of maturity had effects on many attributes of fresh-cut red peppers after storage (Table 8). In particular, the mature-green peppers showed a higher respiration rate (8.6 ml CO_2/kg/h) than full-colored peppers (5.9 ml CO_2/kg/h), probably causing the greater weight loss (0.9% and 0.5%, respectively). Full-colored red peppers accumulated 2.4°Brix with respect to the mature green fruits, also showing an increase of titrable acidity and lower pH (0.20% citric acid vs. 0.11%) which resulted in a lower pH (4.72 vs. 5.80).

Table 8 - Main effect of type stage of maturity (mature-green and full-colored) on mean values of quality attributes of fresh-cut yellow 'Cazzone' peppers during storage

Quality attributes	Mature green	Full-colored
Firmness (N)	16.5 NS	17.2 NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	8.6 a	5.9 b
L*	39.7 a	30.9 a
a*	-12.6 b	31.4 a
b*	26.6 a	16.3 b
Hue angle (*)	115.3 a	27.4 b
Chroma	29.5 b	35.4 a
Appearance score	4.2 NS	4.2 NS
Soluble solids (°Brix)	4.4 b	6.8 a
Titrable acidity (% citric acid)	0.11 b	0.20 a
pH	5.80 a	4.72 b
Weight loss (%)	0.9 a	0.5 b
Vitamin C (mg/100 g FW)	54.5 NS	58.8 NS
Ascorbic acid (mg/100 g FW)	51.8 NS	56.3 NS
L-dehydroascorbic acid (mg/100 g)	2.7 NS	2.5 NS
Antioxidant activity (mg Trolox/100 g FW)	136.6 NS	147.9 NS
Total phenol content (mg gallic acid/100 g FW)	96.1 NS	107.2 NS

For each row mean values followed by a different letter are significantly different (N=10; P ≤0.05).

Table 7 - Main effect of type of fertilization (mineral and combined) and stage of maturity (mature-green and full-colored) on mean values of quality attributes of fresh-cut yellow 'Cazzone' peppers during storage

Quality attributes	Type of Fertilization		Stage of maturity	
	Mineral	Combined	Mature - green	Full-colored
Firmness (N)	16.5 NS	17.0 NS	15.9 NS	17.5 NS
Respiration rate (ml CO_2 Kg^{-1} hr^{-1})	9.7 NS	7.8 NS	9.2 NS	8.3 NS
L*	44.6 NS	45.0 NS	39.0 b	50.6 a
a*	0.4 a	-0.8 b	-12.6 b	12.2 a
b*	37.5 NS	37.7 NS	26.4 b	48.8 a
Hue angle (*)	95.0 NS	96.6 a	115.6 a	76.0 b
Chroma	39.8 NS	39.8 NS	29.3 b	50.3 a
Appearence score	4.0 b	4.2 a	4.1 NS	4.0 NS
Soluble solids (°Brix)	5.9 NS	5.8 NS	4.8 b	6.9 a
Titrable acidity (% citric acid)	0.15 NS	0.14 NS	0.11 b	0.18 a
pH	5.26 NS	5.26 NS	5.66 a	4.86 b
Weight loss (%)	0.8 NS	0.7 NS	0.9 NS	0.6 NS
Vitamin C (mg/100 g FW)	60.9 NS	61.8 NS	58.5 NS	64.2 NS
Ascorbic acid (mg/100 g FW)	58.6 NS	59.3 NS	55.9 NS	62.1 NS
L-dehydroascorbic acid (mg/100 g)	2.3 NS	2.5 NS	2.6 NS	2.2 NS
Antioxidant activity (mg Trolox/100 g FW)	157.0 a	139.0 b	141.7 NS	154.3 NS
Total phenol content (mg gallic acid/100 g FW)	111.4 NS	106.8 NS	103.6 b	114.6 a

For each row mean values followed by a different letter are significantly different (N=10; P ≤0.05).

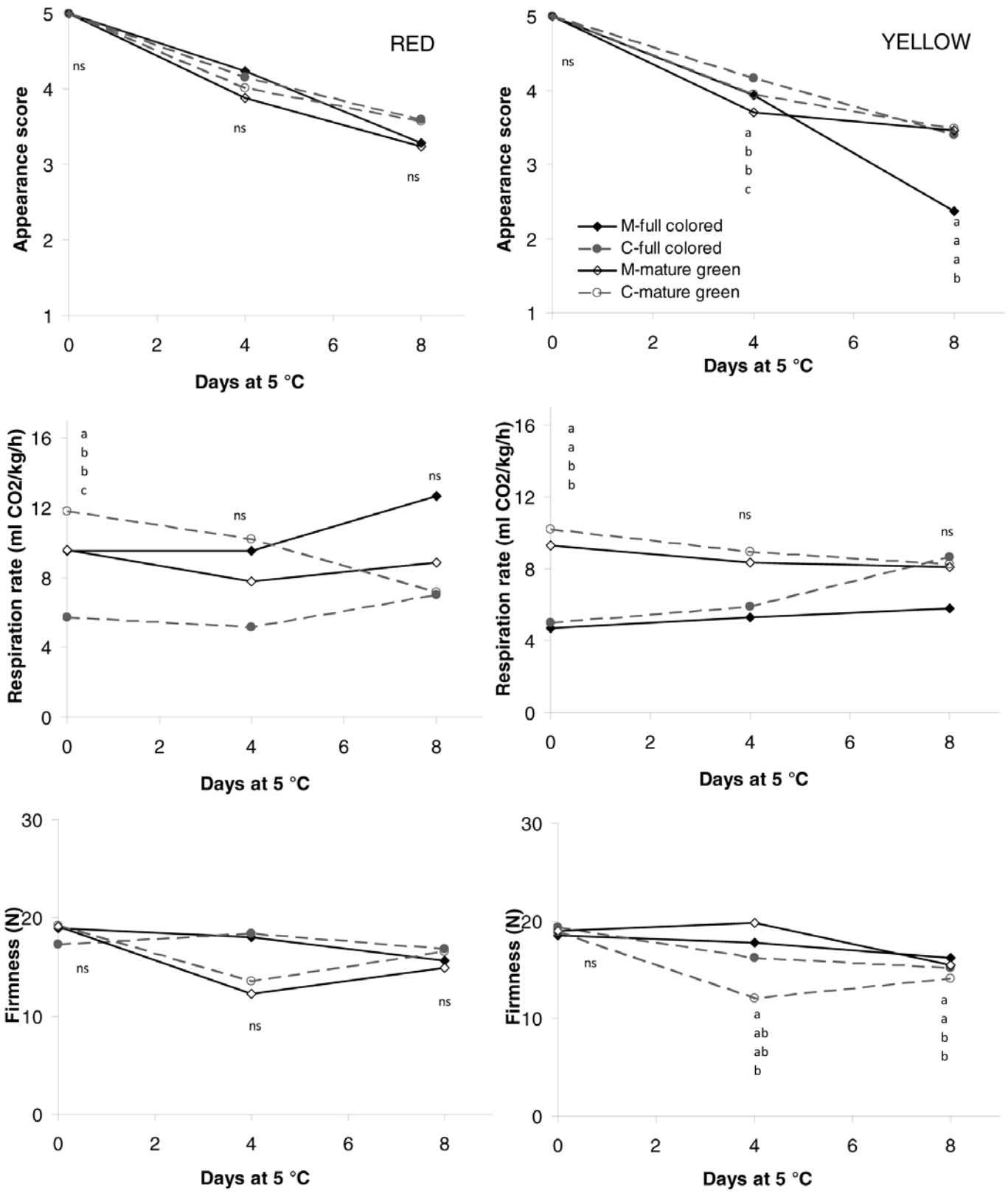

Fig. 1 - Effect of storage at 5°C on appearance score, respiration rate and firmness of fresh-cut yellow and red *'Cazzone'* peppers (M=mineral fertilization; C=combined fertilization). At each storage evaluation different letters indicate significant differences among treatments (N=10; P≤0.05).

All red peppers received a score higher than 3 at the end of storage, without differences among treatments, whereas a lower firmness was observed for peppers fertilized with the combined treatment (Fig. 1). Particularly, after four days of storage, the mature-green peppers fertilized with the mineral system showed greater firmness (19.80 N) than mature-green peppers fertilized with the combined system (12.09 N), while intermediate results were observed for full-colored peppers. At eight days of storage, full-colored and mature-green peppers treated with mineral fertilization showed greater firmness than peppers fertilized with the combined treatment. For yellow peppers, no significant differences in firmness were observed.

In conclusion, the results of this experiment show that the type of fertilization and the maturity stage had a different impact on quality of yellow and red peppers. In particular, for yellow full-colored peppers, the combined fertilization treatment allowed a longer shelf-life than the mineral treatment, and this should be considered when processing fresh-cut peppers. These results may also encourage further study of the feasibility of using 'environmentally friendly' fertilization techniques on bell peppers and eventually to extend these trials to other species.

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