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Genotype influence on shelf life behaviour of minimal processed loquat (*Eriobotrya japonica* (Thunb.) Lindl.) fruit: the role of sugar, acid organics and phenolic compounds

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Abstract

Background: Loquat cultivars cultivated in Southern Italy are very appreciated by consumers for their sensorial characteristics, such as persistent aroma and taste. Apposite maturity indexes for peeling and processing loquat fruit were investigated to increase diffusion of minimally processed loquat. The genotype's effect on the minimally processed loquat fruit shelf life and quality harvested at commercial maturity (80% yellow color) was investigated on peeled fruit stored at 5 °C for 10 days. The role of sugars, organic acids and phenols composition was observed through in depth qualitative analysis. In addition, several qualitative analyses were carried out to determine the quality of minimal processed fruit.

Results: Loquat fruits harvested at commercial ripening stage performed very good palatability and flesh color persistency. Late ripening fruits genotypes shown a low rate of pulp oxidation and quality decay, while early ripening fruits were not suitable for fresh-cut. Genotype had a great influence on weight loss, β -carotene content, fruit respiration, ascorbic acid and total phenols content during the shelf life.

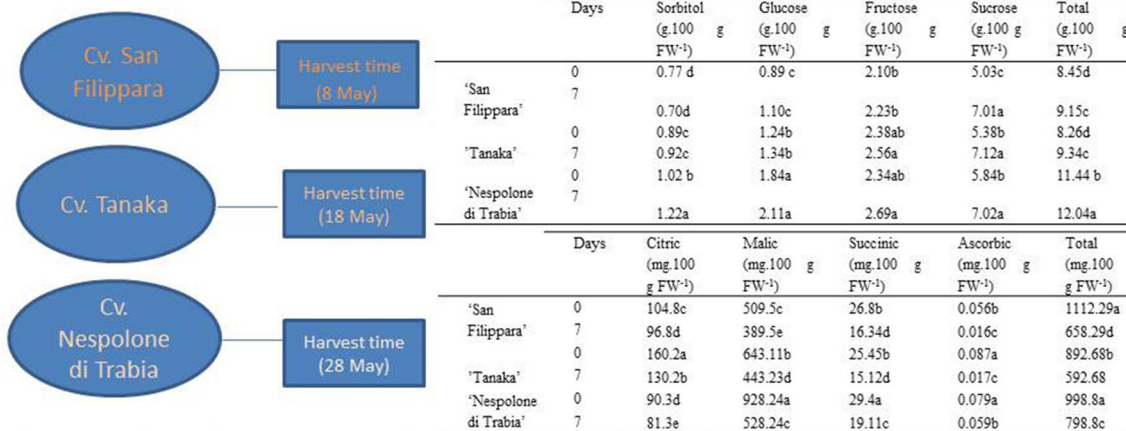
Conclusions: This work shows how the amount of the composition of sugars and organic acids as an intrinsic characteristic of genotype influences the quality of loquat fruits minimal processed. The higher values of glucose, sorbitol and ascorbic acid accumulated in the cv 'Nespolone Trabia' contributed to a reduction in chilling injury and oxidative stress after cutting.

Keywords: *Eriobotrya japonica*, Microbial spoilage, Fresh-cut, Flavor score, Sucrose, Succinic acid

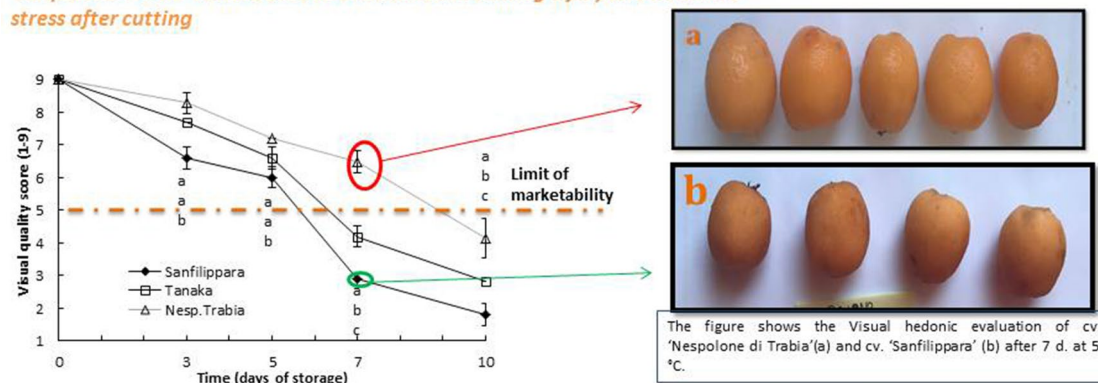
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Graphical Abstract



The higher values of glucose, sorbitol and ascorbic acid accumulated in the cv 'Nespalone Trabia' contributed to a reduction in chilling injury and oxidative stress after cutting



The figure shows the Visual hedonic evaluation of cv. 'Nespalone di Trabia' (a) and cv. 'Sanfilippara' (b) after 7 d. at 5 °C.

Introduction

Loquat (*Eriobotrya japonica* (Thunb.) Lindl.) cultivars grown in the Mediterranean area are local and international genotypes characterized by succulent, white, yellow or orange flesh and a sweet to sub-acid or acid taste [1]. A total of 536 metabolites were identified on two loquat cultivars white and yellow-fleshed, 193 of which include 7 carbohydrates, 12 organic acids and 8 amino acids [2]. Local genotypes are largely diffused in Italy, but their marketable quality is not properly defined [3]. Loquat fruit is classified as non-climacteric fruit [4, 5] and requires about 120–150 days from full bloom in December [6] to fruit ripening in April–May. Determination of ripeness can be divided into five stages [7], and it is important, because unripen fruits are excessively acid.

Due to scalar on-tree ripening, harvest of loquat fruit lasts for several weeks, depending on genotype [8, 9]. Loquat fruit is susceptible to chilling injury and to scab (*Fusicladium eriobotryae*), which symptoms

appear throughout flower and fruit development, with the most serious damage occurring in leaves and fruits during wet seasons, when control measures are inadequate [10]. To the best of our knowledge, so far there were very few attempts to test minimal processing on loquat fruit. So far, studies about shelf life of minimally processed fruit focused largely on appearance, neglecting taste, texture and nutraceutical compounds. Flesh of loquat fruit contains carotenoids that are responsible for the color of loquat fruit, the most abundant ones being β -carotene, β -cryptoxanthin, lutein, violaxanthin, α -carotene and γ -carotene [11]. During postharvest, loquat fruit may have severe problems of flesh browning caused by enzymatic oxidation [12]. Some authors show that loquat fruits retained their initial quality and chemical components for 30 days during storage in micro-perforated polyethylene (PE) film packaging at 1 and 5 °C [12, 40].

However, consumer acceptance of flavor, aroma and taste is crucial to ensure a stable and continuous

commercialization in the market. In fact, these sensory attributes are very important indicators of shelf life even for non-expert consumers [13]. Unfortunately, in preserved fresh fruit, flavor tend to deteriorate faster than appearance according to consumers [14]. Therefore, the maintenance of satisfactory gustatory sensory characteristics of the processed fruit, together with a good level of nutraceutical compounds, may allow a brand to consolidate its position on the market, creating loyalty in consumers who appreciate them. In loquat, sucrose and sorbitol are the main photosynthetic products and the main form of translocated carbon [15], those of which can be used as fuel during the ripening process [16] and be converted to glucose and fructose with specific enzymes. Soluble sugars also exert positive effects to protect plant cells from damage caused by cold stress [17]. Glucose [18] and fructose [19] play a very important role in protecting plants and fruits from cold but not always involved in this process. These sugars are used as osmoregulators, cryoprotectants, signaling molecules, and scavengers of reactive oxygen species [20] to protect plants from chilling stress. Furthermore, we believe that high sugar content and higher activities of hexokinase, were also beneficial to sugar signal generation, enhancing the chilling resistance of loquat fruit [21].

In loquat fruit there are 15 volatile compounds as: phenylethanol, 3-hydroxy-2-butanone, phenylacetaldehyde, and isomeric hexen-1-ols, ethylacetate, methyl cinnamate, β -ionone [22], hexanal, (E)-2-hexenal and benzaldehyde [23]. Large variability in fruit components occur in relation to loquat genotype. In cv. 'Tanaka' hexanal, (E)-2-hexenal, hexanoic acid and β -ionone significantly contribute to fruit aroma, while phenylacetaldehyde is the most important aroma component [24]. In Akko XIII, 'Champagne de Grasse', 'Guzelyurt 6', 'Hafif Cukurgobek', 'KKTC 3', 'KKTC 4' and 'Sayda' cultivars, positive correlations occurred between antioxidant capacity and total phenolic content [25]. The activity of the enzyme peroxidase increased from the green phase to the color change, the enzymes such as pectin methyl esterase, cellulose and polygalacturonase showed a constant reduction from the green to the orange phase, and polyphenol oxidase showed no change during ripening in the 'Golden Nugget' cultivar [26]. Amoros [27], in addition, reported that flesh firmness decreased in different loquat fruit cultivars during the last 4–5 weeks of development. This decrease was more pronounced and occurred first for cvs. 'Cardona' and 'Magdall'. Firmness values for mature fruits were similar for the cultivars 'Algerie', 'Magdall' and 'Peluche', while in 'Cardona' fruit they were significantly lower. No information exists on shelf life behavior of minimally processed loquat fruit. We investigated the effect of an early and late ripeness

stage and the role of sugar and acid organic, phenolic compound on shelf life behavior of minimal processing loquat fruit (*Eriobotrya japonica* (Thunb.) Lindl).

Materials and methods

Plant material and orchard descriptions

This research was conducted in 2019, on yellow-flesh loquat (*Eriobotrya japonica* (Thunb.) Lindl) cultivars 'Tanaka', 'Sanfilippara', 'Nespolone di Trabia', grafted onto loquat seedling rootstock, grown in a commercial orchard, located in Castelvetro (Trapani, Italy) (Latitude 37° 40' 50" N; Longitude: 12° 47' 30" E, 80 m a.s.l.), planted in 2009. This orchard is managed according to the organic farming protocol. Trees are grown to full globe, spaced at 5 × 4 m apart, branched about 1.2 m above the ground and reach 3 to 3.5 m in height. The soil was managed by leaving a permanent cover of grass, mowed once or twice in spring. This practice allows trees to intercept solar radiations and supply nutrients, improving the efficiency of the ecosystem. An efficient ecosystem implies the use of fewer inputs to produce, because it is able to maximize its potential, and consequently, a reduction in cultivation costs. The trees were pruned after harvesting in late spring to renew the fruiting wood and encourage light penetration into the canopy. The average annual rainfall for the district in which the orchard is located amounts to about 500 mm and average day/night temperatures during the last 4 weeks of fruit ripening were 28/23 °C.

Harvesting method and sample preparations

The proper maturity stage for peeling and processing was evaluated on 30 fruits for each cultivar at each maturity stage (commercial ripe 'CM' = 80% yellow, 20% orange ground color; full ripe 'FR' = 100% orange color [4, 26, 28]. Regarding to peel color, fruit total soluble solid content (TSS), fruit peelability Cefola et al. 2013 [29] and flesh color after peeling, were evaluated using a 9-pt hedonic scale (9 = very high and 1 = very low) but all qualitative and biochemical analyses were performed at harvest for both ripening stages (CM and FR). Only fruit harvested at commercial maturity (CM) stage were used for further analysis.

Three hundred and thirty fruits of each cultivar were hand-picked at commercial harvest time that was on 8 May for cv. 'Sanfilippara'; 18 May for cv 'Tanaka'; 28 May for cv. 'Nespolone di Trabia', and transferred to the post-harvest laboratory. The sampled fruits of the three cultivars were sanitized by immersion in 200 mg kg⁻¹ of sodium hypochlorite for 5 min and then dried at room temperature (16 ± 2 °C). Peeling was performed manually in a refrigerated room at 5 ± 1 °C and the fruits were placed in polyethylene terephthalate (PET)

boxes (500cc) in a passive atmosphere and sealed with a composite film (PP-PET, 64 μm , O_2 permeability = $5.30 \times 10^{-8} \mu\text{L m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$). The boxes were stored at $5 \pm 0.5 \text{ }^\circ\text{C}$ and 90% relative humidity (RH) for 10 days (T0, T3, T5, T7, T10). Chemical and physical parameters were analyzed at the beginning of the experiment (T0) and then after 3, 5, 7 and 10 days of storage. Each box was used as single replicate for each cultivar (3 cultivars \times 5 time of storage \times 9 replicates (T0, T3, T5, T7, T10) = 135 box). Each box contained 6 fruits (135 box \times 6 fruits = 810 fruits).

Physicochemical analyses

Ethylene production at harvest

Ethylene production and fruit respiration rate were measured immediately after harvest both at $20 \text{ }^\circ\text{C}$ and after storage at $4 \text{ }^\circ\text{C}$ for 72 h. Ten fruit were weighed with a digital scale and placed individually in 705-mL sealed glass containers, according to Crisosto [30]. In intact fruit following harvest, ethylene production ($\mu\text{L kg}^{-1} \text{h}^{-1}$) was measured in an acclimatized chamber at $20 \text{ }^\circ\text{C}$. According to previous literature, gas samples (1 mL) were taken of effluent air from respiration jars, using a 1 mL syringe and injected into a gas chromatograph (GC, Agilent Technologies 6890, Wilmington, Germany) and fitted with a FID detector and an alumina column F1 80/100 (2 $\text{m}\text{\AA} \sim 1/8 \text{ }\text{\AA} \sim 2.1$, Tecknokroma, Barcelona, Spain). The oven temperature was $140 \text{ }^\circ\text{C}$, while the injector and detector were kept at 180 and $280 \text{ }^\circ\text{C}$, respectively.

Respiration rate during storage

Six fruits per treatments per time were enclosed in 1 L glass jars at $5 \text{ }^\circ\text{C}$. 10 mL of headspace gas was taken from each jar after 2 h. CO_2 was measured with GC (Agilent Technologies 6890, Wilmington, Germany). Respiration rate was expressed in $\text{mL CO}_2 \text{ kg}^{-1}$ fresh weight (FW) h^{-1} .

Weight loss

The weight (g) of each tray of 6 fruits was recorded using a 0.01 g precision balance (Gibertini, Italy), immediately after harvest at T0 (day 0) and subsequently at 3, 5, 7 and 10 days of storage. Weight loss was estimated as a percentage (%) reduction from the initial time, using the following equation

$$\text{Weight loss \%} : \left[\frac{(\text{initial weight of fruit bags} - \text{final weight of fruit bags}) \times 100}{\text{initial weight of fruit bags}} \right]$$

Soluble sugar and organics acid

Soluble sugar and organics acid were extracted from fruits and analyzed by HPLC, according to a method described by Amoros [27]. Five grams of loquat mesocarp from each

sub-sample were homogenized with 10 mL of deionised water using a polytron homogenizer (IKA Labortechnik) and centrifuged at $10,000g$ for 10 min. An aliquot of 10 mL of the supernatant was used to quantify sugars and organic acids by HPLC (Hewlett-Packard, series 1100, Waldbrom, Germany) chromatograph equipped with a SUPELCO-GEL C-610H (30 cm 7.8 mm) column (at $30 \text{ }^\circ\text{C}$), a refractive index detector (for sugar analysis) and an UV-V detector (210 nm, for acid analysis). The elution system consisted of 0.1% H_3PO_4 , running isocratically at a flow rate of 0.5 mL min^{-1} . Two separate extractions were made from each sub-sample of 5 fruits and for each extraction, sugars and organic acids were determined in duplicate.

Color

The color of each fruit (six replicates for each cultivar and sampling date) was measured with a portable colorimeter (Minolta Chroma Meter CM400R, Minolta, Osaka, Japan), equipped with an 8-mm measuring head and a C illuminant (6774K). The instrument was calibrated using the manufacturer's standard white plate.

Within the CIELAB uniform space, a psychometric index of lightness, L^* (ranging from 0, black, to 100, white) and two-color coordinates a^* (which takes positive values for reddish colors and negative values for greenish ones) and b^* (positive for yellowish colors and negative for the bluish ones) are defined. Total color difference (ΔE^*) expressed the magnitude of difference between the initial non-aged color pulp (zero time) and storage-aged samples. Total color difference (ΔE^*) was calculated according to the following:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

where $\Delta a^* = a^* - a0^*$, $\Delta b^* = b^* - b0^*$, $\Delta L^* = L^* - L0^*$; $a0^*$, $b0^*$ and $L0^*$ are the corresponding blank values of control sample, and $a0^* = -2.00$, $b0^* = 4.00$ and $L0^* = 80.00$, respectively. All data were reported as mean \pm standard error (SE, $n = 6$).

Visual appearance, flavor score, peeling, change color and crunchiness score

To measure the effect of storage on fruit visual traits (color, visible structural integrity and visual appearance) at each storage period, six replicates for each cultivar and

time of storage were scored by six scientific judges, using a subjective 9–1 acceptance scale from 9 = very good (just peeled), to 1 = very poor (inedible). To measure the effect of cold storage on fruit flavor traits at each storage

time, six fruit, used as six replicates for each cultivar and time sampling date were scored by six scientific judges, using a subjective 9–1 acceptance scale from 9 = very high to 1 = none.

The crunchiness of each loquat was determined by six judges, based on the resistance of fruit to chewing, according to a 9–1 scale, from 9 = very crunchy to 1 = crummy (inedible). Each judge analyzed a sample of six fruit used as six replicates for each cultivar at each sampling date.

The peelability score of each loquat fruit was determined by six judges, based on the resistance of fruit to peeling, according to a scale from 9 = high peelability to 1 = no peelability.

Flesh color change score after peeling was determined by six judges according to a scale from 9 = very high to 1 = imperceptible.

Sensory evaluation

Fruits were tested to evaluate the organoleptic characteristics through a semi-trained panel tests.

The sensory profile was defined by a panel of 10 judges (5 females and 5 males, between 22 and 45 years of age) with extensive experience in the sensory evaluation of other types of fruit Gentile [31]. Before each evaluation, trained panelists signed consent forms for voluntary participation in the present study. Training was conducted in three orientation sessions of 2 h each, wherein panelists developed scales to score fruit sensory descriptors [32]. For training purposes and familiarization, to panelists were given fruit pieces of various level of quality. Loquat sensory evaluations, on post-processing, were conducted under white fluorescent light. 16 attributes were chosen by the judges in a preliminary meeting on the basis of frequency of citation (> 60%): appearance (APP), pulp color (PC), Consistency (C), fruity odour (FO), herbaceous odour (GO), off-odour (OFO), sweet (S), acid (A), juiciness (J), astringent (AST), pungent (P), fruity flavor (FFL), fermented flavor (FFE), flavour alcohol (ALF), off-flavour (OFF), overall evaluation (OVE). Panelists performed “difference from control” discrimination sensory test for the degree of sensory descriptors on a 1–9 scoring scale; 1 and 9, respectively, correspond to “no difference” and “extremely different” from the control, respectively. Each sample (at harvest, and after 3, 5, 7 and 10 days of storage), was presented in a white plastic plate with a 3-digit code on the side and tasted 1 h after they were taken out of the 5 °C cold room. Sparkling water for rinsing between samples and unsalted crackers to neutralize mouth feel were provided to the panelists.

Package O₂ and CO₂ analysis

CO₂ and O₂ levels (%) were measured on each box at the beginning of each experiment and after 3, 5, 7 and 10 days of storage, using a PBI Dansensor Checkpoint O₂ and CO₂ analyzer (Topac, Hingham, MS, USA) with zirconium and infrared detectors, respectively.

Carotenoids extraction

Carotenoids were extracted from fruits and analyzed by HPLC, according to a method previously described by Xiong [33]. Component's identification and quantitative analysis were done using Agilent 1200 HPLC-DAD analysis system, 5 µm C18 m reverse phase column (250 mm × 4.6 mm, Japan) and 20 mm × 4.6 mm C18 pre column maintained at 35 °C. Using external standard method. The compounds β-carotenoid, and lutein were detected at different stages. The standard samples of β-carotenoid and lutein were purchased from Sigma-Aldrich Company (Ge). The concentration of β-carotenoid and lutein were calculated from the experimental peak area by analytical interpolation in a standard calibration curve and was expressed as mg 100 g⁻¹ of fresh weight.

Extraction and separation of phenolic compounds

Twenty grams of freshly prepared pulp was homogenized in 80 mL of cold methanol (95%) for 1 min, shaken for 10 min, and filtered. The pellet was extracted twice again with cold methanol (80%). The combined extracts were evaporated under vacuum at 35 °C until the methanol was removed. The concentrate was then extracted with hexane three times to remove lipids, carotenoids, and chlorophyll. The aqueous phase was evaporated again to remove the hexane. Finally, water was added to the extract to constitute a total of 50 mL. Total phenolics were determined with the Folin–Ciocalteu phenol reagent using this extract solution. For identification and quantification, with HPLC column was a fused-core Poroshell 120, SB-C18 (3.0 × 100 mm, 2.7 µm) from Agilent Technologies (Agilent Technologies, Palo Alto, CA, USA) Separation was carried out with different gradient dilution programs depending on the phenolic classes (0 min, 5% B; 2 min, 7% B; 4 min, 9% B; 6 min, 12% B; 8 min, 15% B; 9 min, 16% B; 10 min, 17% B; 11 min, 17.5% B; 12 min, 18% B; 14 min, 20% B; 16 min, 28% B; 18 min, 100% B; 22 min, 100% B; 23 min, 5% B).

Acid ascorbic content

The ascorbic acid concentration was determined according to Rapisarda and Intelisano [34] using liquid chromatography HPLC (Perkin Elmer, Australia), with an

injector (Rheodyne with 20 μL loop), a photodiode detector, and a Knauer Eurospher II 100-5 C18 column 250 mm 4.6 mm I.D. (Berlin, Germany), and a similarly packed precolumn. The dilution was performed with a buffer solution consisting of $\text{KH}_2\text{PO}_4/\text{H}_3\text{PO}_4$ at pH 2.3, at a flow rate of 1 mL min^{-1} , at a wavelength of 260 nm

Statistical analysis

The experimental design was made with three cultivars: ‘Tanaka’, ‘Sanfilippa’ and ‘Nespolone di Trabia’. Observations were made at T_0 and after 3, 5, 7 and 10 days of storage at $5 \pm 0.5 \text{ }^\circ\text{C}$ and with 90% relative humidity. ANOVA (SYSTAT™ statistical software v.13.0, USA, for Windows was used) was performed and mean values were compared with Tukey’s test during storage, for each storage time and for each cultivar. Significant differences were calculated with $p \leq 0.05$.

Results and discussion

Loquat fruits during the last days of ripeness involve physical–chemical changes that lead the fruit to the mature stage for commercial distributions, after 24, 48 or 36 h, depending on temperature, the ripeness stage at this stage, the fruit will have reached a stage of development change in physiological ripeness in which its quality will be at least the minimum acceptable for consumers [35]. All the values of chemical and physical composition of loquat fruit evaluated over time were influenced by the ripeness stage. The maturity stages of peel color yellow (CM) and orange (FR) present significant different ($p \leq 0.05$) characteristics in terms of fruit quality (Table 1). Besada [36] reported that some volatile

compounds show a rapid increase with full ripening of loquat fruits. A large group of volatile, mostly alcohols (aliphatic, methyl and ethyl alcohols) and, to a lesser extent, aldehydes, ketones and esters compounds, did not have showed dramatic changes during last stages of ripeness. Fruit of ‘Sanfilippa’, ‘Tanaka’ and ‘Nespolone di Trabia’ harvested at commercial maturity (CM) had a higher peelability, a higher ethylene production and a higher tritatable acidity, crunchiness score, lutein and β -carotene content than those harvested at fully ripe stage (FR). The genotype had a significant ($p \leq 0.05$) influence on ethylene production at $20 \text{ }^\circ\text{C}$. Moreover, CM fruit showed a significantly lower change in color after peeling than FR ones (Table 1). Similar results are reported in loquat fruit of cvs. ‘Karantoki’ and ‘Morphitiki’ cultivated in Cyprus [8].

Respiration rate, weight loss, headspace gas and color

Loquat fruit is a non-climacteric fruit that exhibits a decrease in respiration rate. In our experiment, loquat fruit values show some significant differences (T7 and T10) among cultivars for each time of storage, the respiration remains stable for 10 days, during storage at $5 \text{ }^\circ\text{C}$ (Fig. 1).

Weight loss sharply increased 7 days after storage and only at this stage significant differences among genotypes occurred (Fig. 2), while TSS and TA did not significantly change during shelf life (data not shown). Loquat fruit, after peeling and packaging in passive atmosphere, showed a progressive increase of CO_2 and a decrease in O_2 as in fresh cut peach with or without chemicals treatment [30, 36]. Samples of cv

Table 1 Quality parameters of ‘Sanfilippa’, ‘Tanaka’ and ‘Nespolone di Trabia’ loquat fruit harvested at commercial ripeness (CM = 10% green ground-color 70% yellow and 20% orange color) and fully ripe (FR = 60% yellow and 40% orange color)

	‘Sanfilippa’		‘Tanaka’		‘Nespolone di Trabia’	
	(CM)	(FR)	(CM)	(FR)	(CM)	(FR)
Peelability score ^a	7.0a	5.2b	8.4a	5.1b	7.4a	5.4b
Ethylene production at $20 \text{ }^\circ\text{C}$ ($\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$)	9.5a	2.3b	8.7a	1.9b	6.3a	1.7b
Crunchiness score (1–9)	9a	5.3b	9a	6.1b	9a	6.6b
Lutein content $\mu\text{g g}^{-1}$ (FW)	10.2a	5.1b	9.11a	4.2b	8.44a	3.6b
β -Carotene content mg g^{-1} (FW)	3.0a	5.2b	2.4a	4.6b	1.5a	4.3b
Flesh color change after peeling (20 min at $25 \text{ }^\circ\text{C}$)						
L^*	58.4a	53.3b	59.4a	53.6b	58.6a	53.2b
a^*	14.2a	11.2b	15.0 a	11.1b	14.7a	11.5b
b^*	44.6b	59.2a	50.2b	58.5a	49.6b	56.6a
Flesh color change after peeling ^b	1.5b	5.1a	1.6b	4.6a	1.4b	3.8a

^a Peelability score: to 9 = high peelability a 1 = no peelability. Mean values of 30 fruit for each cultivar

^b Flesh color change after peeling (9–1) score: from 9 = very high to 1 = imperceptible. Mean values of 10 fruits for each cultivar and maturity stage (commercial ripeness CM and full ripeness FR). Different letters indicate significant differences between ripening stages and within genotype, at $p \leq 0.05$

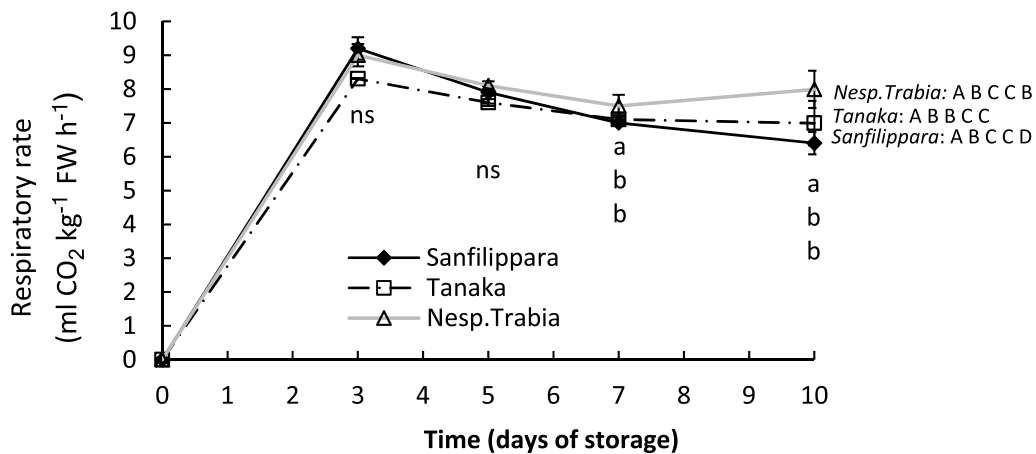


Fig. 1 Respiration rate of loquat fruit cv. ‘Sanfilippara,’ ‘Tanaka’ and ‘Nespolone di Trabia’ immediately after peeling (0) and stored for 3, 5, 7 and 10 days at 5 °C ($n = 6$ replicates per cultivar and sampling date). For each cultivar, different capital letters indicate significant differences between sampling dates, and different lowercase letters indicate significant differences (Tukey’s test at $p \leq 0.05$) between different cultivar at the same sampling date (0, 3, 5, 7 and 10 days). Vertical bars represent standard error (SE)

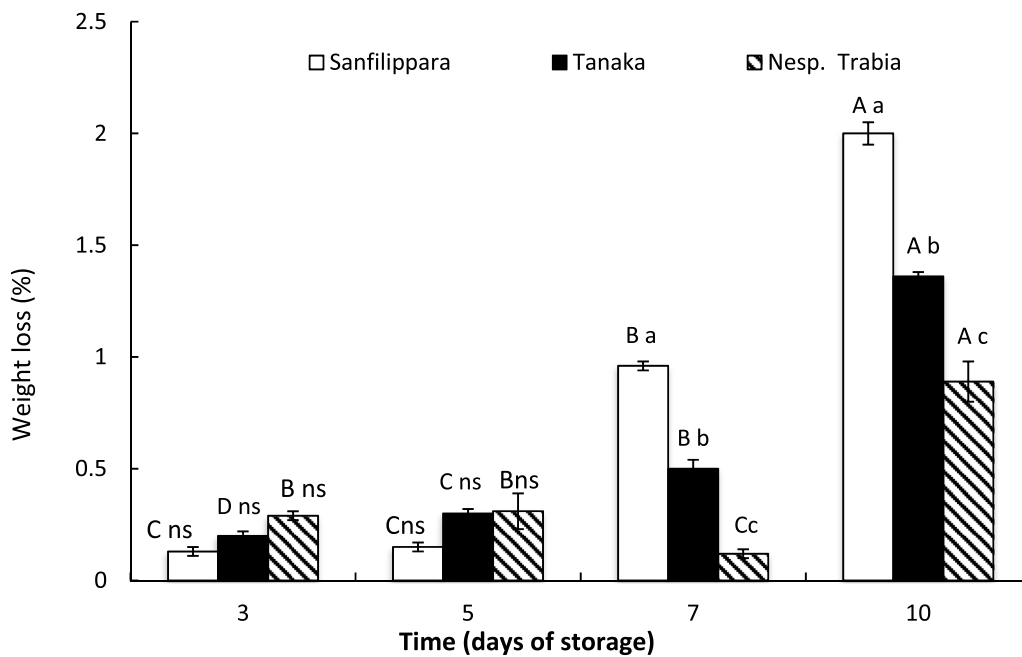
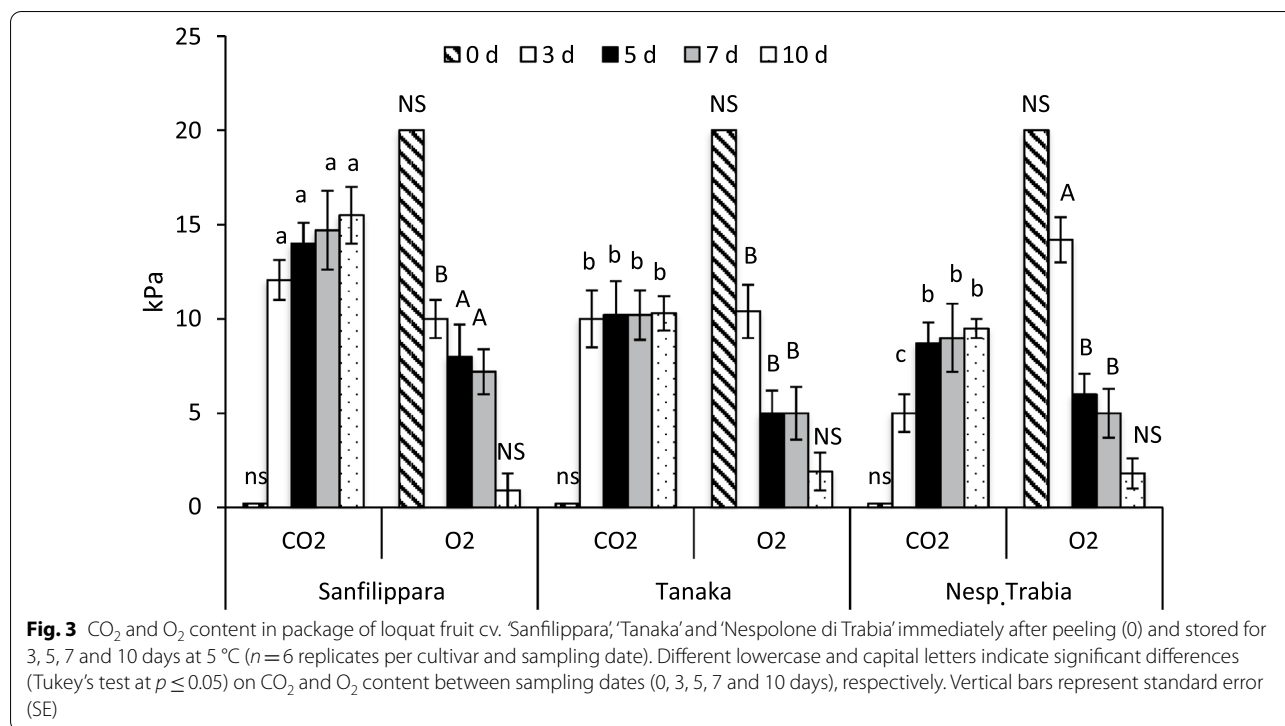


Fig. 2 Weight loss (%) of loquat fruit cv. ‘Sanfilippara,’ ‘Tanaka’ and ‘Nespolone di Trabia’ immediately after peeling (0) and stored for 3, 5, 7 and 10 days at 5 °C ($n = 6$ replicates per cultivar and sampling date). For each cultivar, different capital letters indicate significant differences between sampling dates, and different lowercase letters indicate significant differences (Tukey’s test at $p \leq 0.05$) between different cultivar at the same sampling date (0, 3, 5, 7 and 10 days). Vertical bars represent standard error (SE)

‘Sanfilippara’ showed a significant difference in terms of CO₂ Kpa in all times of storage than other genotypes. Regarding to oxygen concentration at 3th day of storage cv ‘Nespolone di Trabia’ shown higher values than cvs ‘Sanfilippara’ and ‘Tanaka,’ while after 3 days and

until the 7th day cv ‘Sanfilippara’ showed significant differences between loquat fruit cultivars. No differences occurred between genotypes during the last days of storage (Fig. 3).



For instance, fruit of cvs. 'Nespolone di Trabia' and 'Tanaka' kept CO₂ values under 10% but cv. 'Sanfilippara' ones exceed 15% since 5 days after storage. Low O₂ atmospheres have been shown to inhibit respiration through a shift to anaerobic respiration [37], with a development of ethanol [38]. Gao et al. [39] reported that fresh loquat fruit stored for 49 days at 1.1–1.4 kPaO₂ and 2–4 °C had lower decay, reduced respiration and ethylene production rates and inhibited activities of PPO and POD. According to other authors, the development of fermentative substance begins at high concentrations of CO₂ and levels lower than 1% of O₂ [37]. In our experiment, these conditions occurred only after 10 days of storage when O₂ content was lower than 1% (Fig. 3). The color change of each cultivar during shelf life was measured as color change during time (ΔE) showed significant behaviors between cultivars and during storage (Fig. 4).

Flesh color of loquat fruit sharply changed during the first 3 days after storage. It is clear that the higher respiration rates are related to the change in color, as shown by 'Sanfilippara' fruit (Figs. 3, 4) 5 days after storage onwards. The color change between genotypes of the same population, have also been detected on different species [40] or using different treatments [41] for evaluate oxidation and freshness condition of fresh-cut fruit. Note that a ΔE value > 4 is considered perceptible to human eyes [40].

Soluble sugars and organic acids

All loquat fruits ('Nespolone di Trabia' 'Tanaka' and 'San Filippara') significantly increase content in total sugars after 7 days of storage (Table 2). The cultivar 'Nespolone di Trabia' showed significantly higher values than cultivars 'Tanaka' and 'San Filippara'. Among the sugars, sucrose is the one that increases by about 19.8% in all cultivars, while sorbitol increases by about 15% in the later-ripening cv. On the other hand, in the early ripening cvs there is no significant increase.

Regarding to organic acids, malic acid is predominant both at harvest and after 7 days of storage. Malic acid was followed by citric, succinic and ascorbic acid (Table 3). After 7 days, malic acid decreases significantly in all cultivars by 45–60%, while citric acid decrease by about 7.63–18.7%. Loquat fruit is characterized by a low vitamin C content [40] and the fruit shows a height variability between 0.46 and 13.80 mg FW of ascorbic content within nine loquat cultivars, including five international affirmed cultivars: 'Algerie', 'Golden Nugget', 'Peluche', 'Bueno', 'El Buenet' and four local cultivars: 'Sanfilippara', 'Nespolone di Trabia', 'BRT20' and 'Claudia' [31]. In other cvs ascorbic acid content was low and ranged from 4.17 to 8.33 mg per 100 g indicated cultivar specific differences [22].

The resistance of change colour gives to 'Nespolone di Trabia' (Fig. 4) an optimal quality as a fresh cut product. Loquat fruit also contains ascorbic acid; however, when stored as a fresh cut fruit, the ascorbic acid content

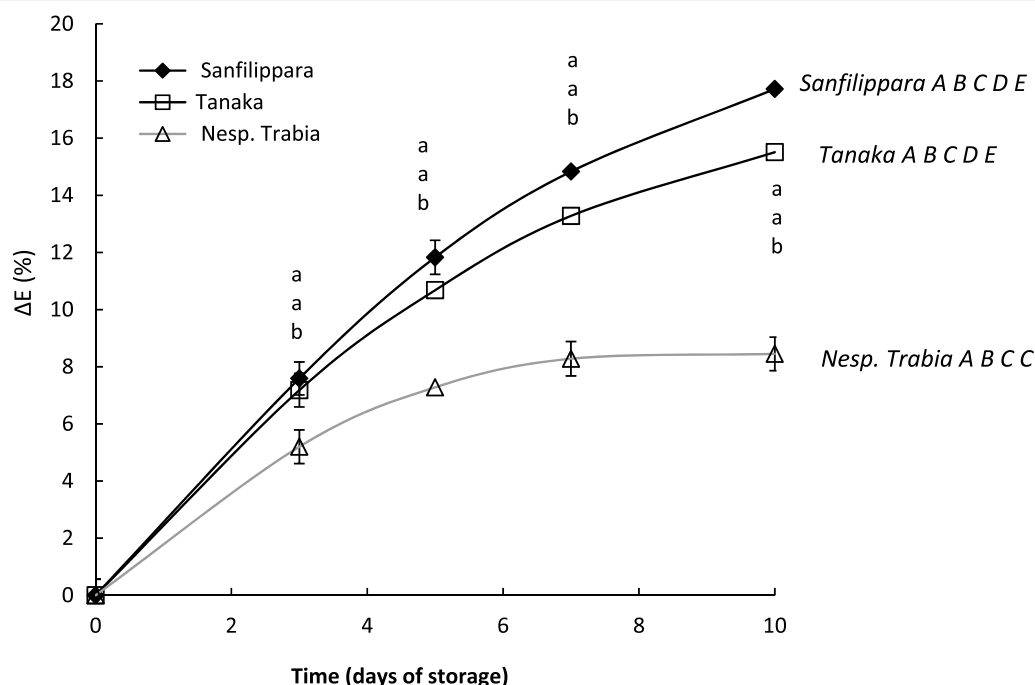


Fig. 4 ΔE in loquat fruit cv. ‘Sanfilippara’, ‘Tanaka’ and ‘Nespolone di Trabia’ immediately after peeling (0) and stored for 3, 5, 7 and 10 days at 5 °C ($n = 6$ replicates per cultivar and sampling date). For each cultivar, different capital letters indicate significant differences between sampling dates, and different lowercase letters indicate significant differences (Tukey’s test at $p \leq 0.05$) between different cultivar at the same sampling date (0, 3, 5, 7 and 10 days). Vertical bars represent standard error (SE)

Table 2 Total sugars, glucose, fructose, sorbitol, in minimal processed loquat fruit (cvs. ‘San Filippara’, ‘Tanaka’, ‘Nespolone di Trabia’) immediately after harvest

	Days	Sorbitol (g 100 g FW ⁻¹)	Glucose (g 100 g FW ⁻¹)	Fructose (g 100 g FW ⁻¹)	Sucrose (g 100 g FW ⁻¹)	Total (g 100 g FW ⁻¹)
San Filippara	0	0.77d	0.89c	2.10b	5.03c	8.45d
	7	0.70d	1.10c	2.23b	7.01a	9.15c
Tanaka	0	0.89c	1.24b	2.38ab	5.38b	8.26d
	7	0.92c	1.34b	2.56a	7.12a	9.34c
Nespolone di Trabia	0	1.02b	1.84a	2.34ab	5.84b	11.44b
	7	1.22a	2.11a	2.69a	7.02a	12.04a

Values with different letters are significantly different at $p < 0.05$

decreases significantly during storage. The decrease in ascorbic acid during storage and processing in fruits and vegetables is a common phenomenon [41]. In fresh cut pineapple, the ascorbic acid content decreases during storage but the antioxidant capacity does not change, indicating that it is not only this organic compound that contributes to total antioxidant capacity [42]. Table 3 shows that the amount of ascorbic acid was higher in the cv. ‘Nespolone di Trabia’, while it is lower in the other

two cultivars; while citric acid compound is higher in cvs ‘Tanaka’ and ‘Sanfilippara’ than in cv ‘Nespolone di Trabia’.

Our data indicate that ascorbic acid content decreased during storage with the same rate pattern in all genotypes, and until 7 days after storage in 2 out of 3 genotypes (Table 3). These results suggest that in loquat fruits, polyphenols contributed to antioxidant capacity much more than ascorbic acid and carotenoids content [18] when in other fruit predominated ascorbic [43] or citric acid [44].

Table 3 Organic acids, citric, malic, succinic and ascorbic acids in minimal processed loquat fruit (cvs. 'San Filippara', 'Tanaka', 'Nespolone di Trabia') immediately after harvest

	Days	Citric (mg 100 g FW ⁻¹)	Malic (mg 100 g FW ⁻¹)	Succinic (mg 100 g FW ⁻¹)	Ascorbic (mg 100 g FW ⁻¹)	Total (mg 100 g FW ⁻¹)
San Filippara	0	104.8c	509.5c	26.8b	0.056b	1112.29a
	7	96.8d	389.5e	16.34d	0.016c	658.29d
Tanaka	0	160.2a	643.11b	25.45b	0.087a	892.68b
	7	130.2b	443.23d	15.12d	0.017c	592.68
Nespolone di Trabia	0	90.3d	928.24a	29.4a	0.079a	998.8a
	7	81.3e	528.24c	19.11c	0.059b	798.8c

Values with different letters are significantly different at $p < 0.05$

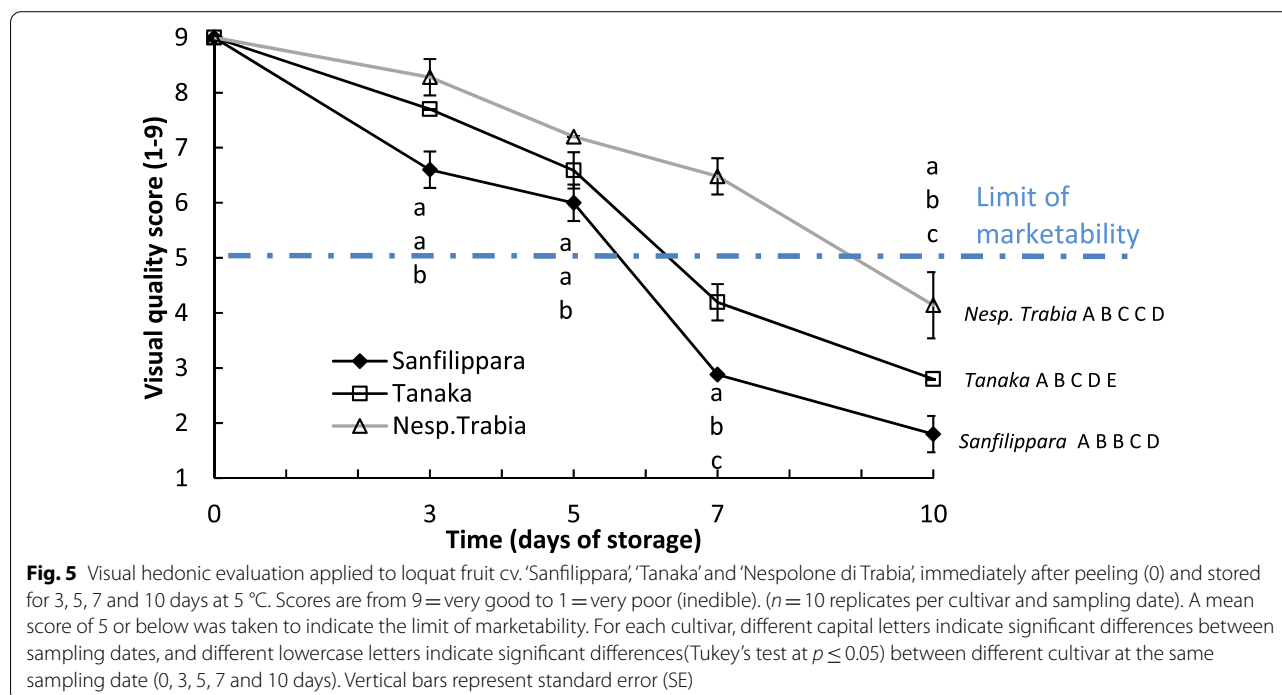
Visual quality, flavor score and crunchiness score

Loquat fruit quality is influenced by several factors, but during postharvest the one that regulates quality maintenance is the production of ethylene, even if the quantity after harvest is low. The use of 1-MCP (*1-methylcyclopropene*), in fact, inhibited the formation of lignin on fruits [45].

The amount of ethylene produced is different among different cultivars and storage temperature. After 8 days of storage at 20 °C on loquat fruits, flesh firmness increases and production respiration is reduced [21]. Amoròs et al., [27] show that different firmness values occurred between 'Algerie', 'Cardona', 'Golden', 'Magdall'

and 'Peluche' during ripeness; Kahramanoglu [46] uses a value of flesh firmness at harvest of 0.4 kg⁻¹ for his experiments on postharvest of loquat fruits. In this study visual appearance, flavor and crunchiness score (Figs. 5, 6, 7, 8) showed the same rate pattern with significant genotype differences related to their metabolic activity (Fig. 3).

Most significant changes and genotype differences occurred after 7 days of storage, when all scores declined rapidly, and only one genotype ('Nespolone di Trabia') kept its flavor, and visual and crunchiness score above the threshold marketability. Similar results were shown by Ding [47] on loquats in 20 µm thick PE film at 5 °C with



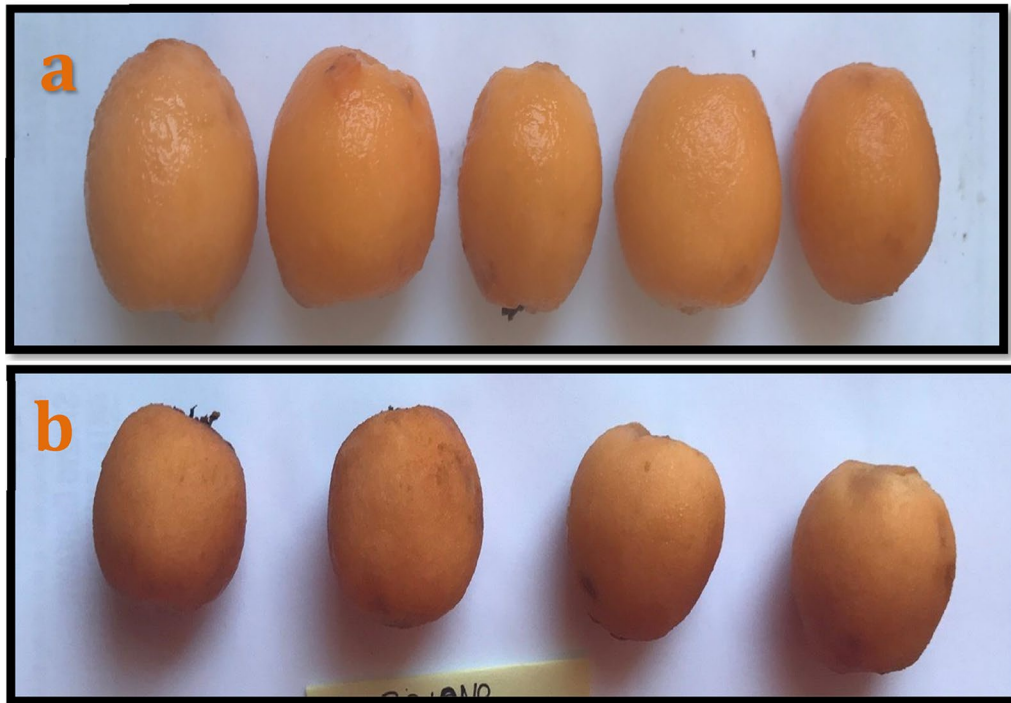


Fig. 6 Visual hedonic evaluation of cv. 'Nespolone di Trabia' (a) and cv. 'Sanfilippara' (b) after 7 days at 5 °C

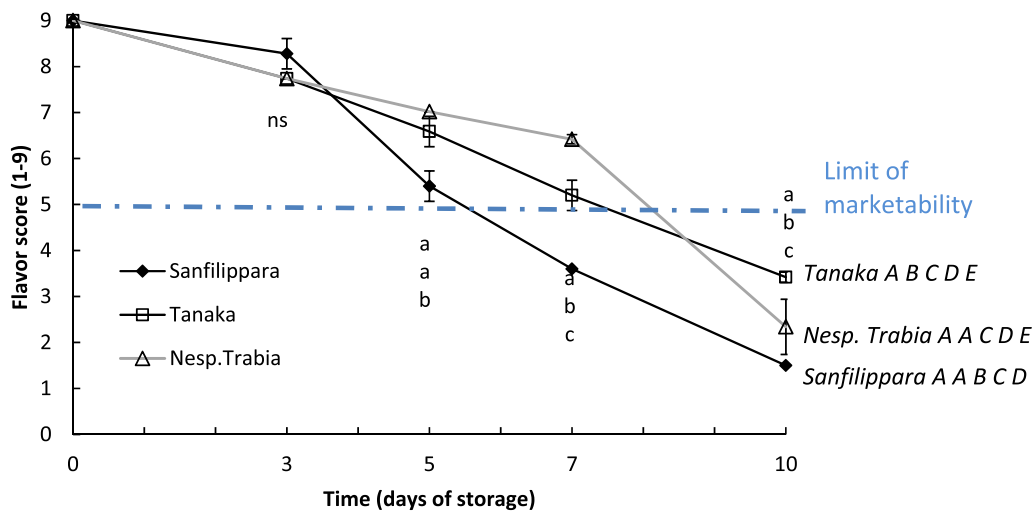
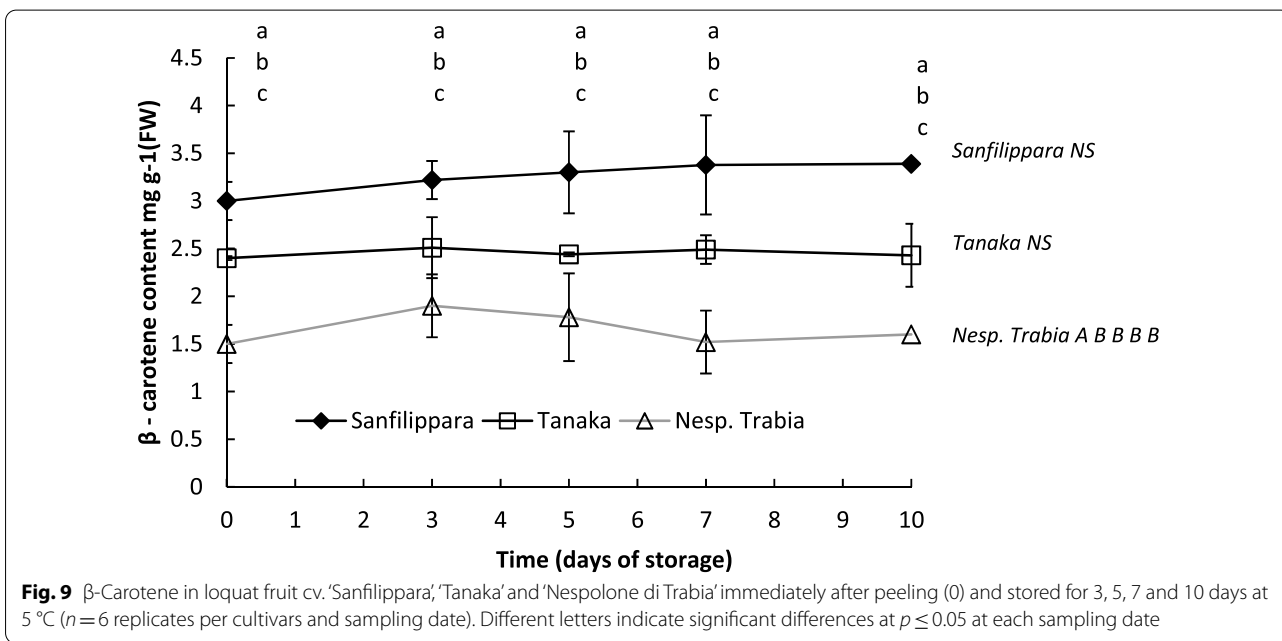
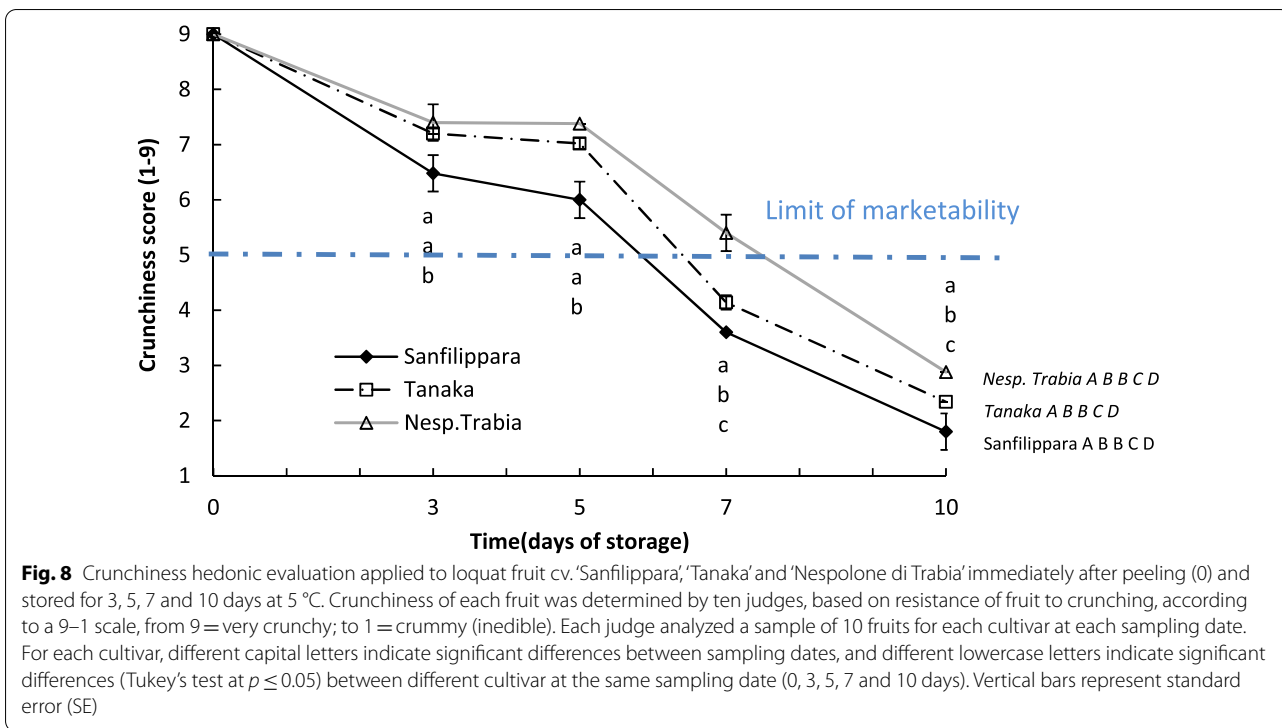


Fig. 7 Flavor hedonic evaluation applied to loquat fruit cv. 'Sanfilippara', 'Tanaka' and 'Nespolone di Trabia' immediately after peeling (0) and stored for 0, 3, 5, 7 and 10 days at 5 °C. Data are from 9 = very high, to no flavor = 1 (n = 10 replicates per cultivars and sampling date). A mean score of 5 or below was taken to indicate the limit of marketability. For each cultivar, different capital letters indicate significant differences between sampling dates, and different lowercase letters indicate significant differences (Tukey's test at $p \leq 0.05$) between different cultivar at the same sampling date (0, 3, 5, 7 and 10 days). Vertical bars represent standard error (SE)

an in-bag atmosphere of approximately 4 kPa O_2 and 5 kPa CO_2 resulting in the highest scores for appearance and chemical compounds.

Carotenoids and phenols total

According to Lu [48] carotenoids are synthesized and accumulated in plastid during fruit ripening. In loquat fruit β -carotene and total carotenoids are more abundant



in the peel than in the flesh [48] as fig fruit [49]. However, Fu [50] reported that carotenoids are deposited mainly in the lipid globules in the chromoplasts of the peel, while in 'Luoyangqing' cultivar, they were also present in its flesh though in crystalline form. Genotype-related significant differences appeared immediately after storage, to

be kept during the whole shelf life period (Fig. 9). Eventually, β -carotene content did not change significantly in any genotype during shelf life.

Another difference concerns the proportion of lutein in relation to genotype and total carotenoid content at

harvest. De Faria [51] reported that the proportion of lutein in relation to total carotenoid content, was 3.3% in cv. 'Nectar de Cristal' and from 0.2 to 0.6% in the other loquat cultivars, while [52], in cv 'Toi', have occurred that b-cryptoxanthin content was higher than that of

β -carotene and lutein proportion about 4.2%. During storage, a significant degradation of Lutein in all cultivars was observed only after 7 days of storage; at the end of the storage period Lutein content decreased by 40–60%, depending on genotype. Eventually, genotype-related

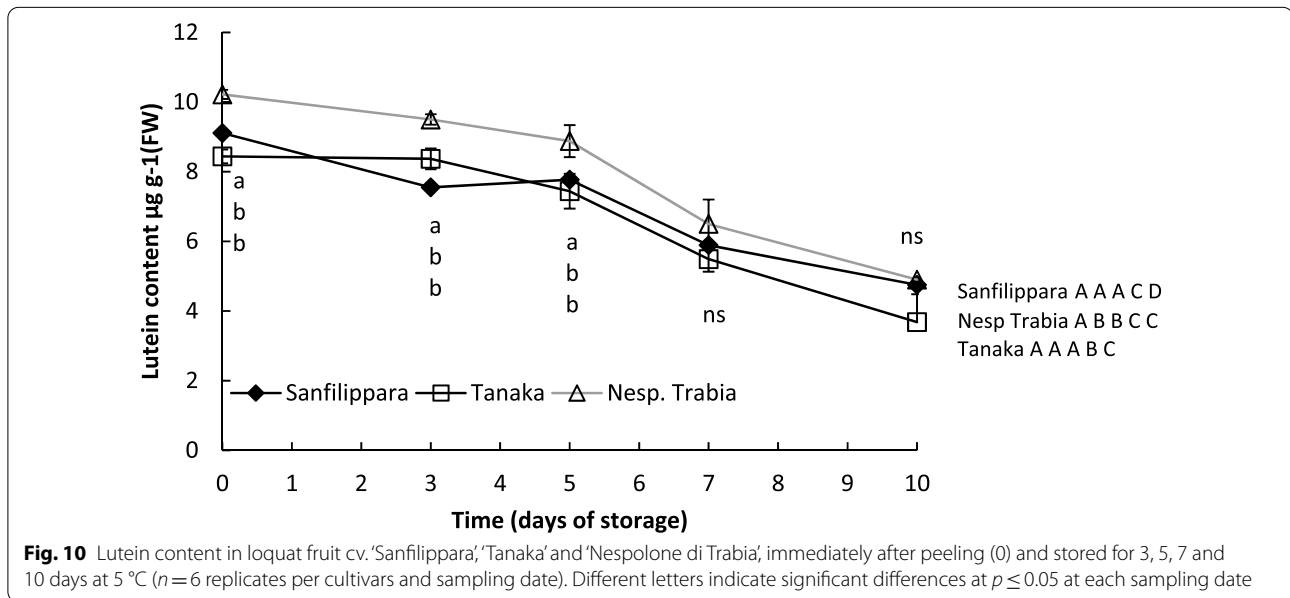


Table 4 Qualitative phenols content in loquat cv. 'San Filippara' (milligrams per 100 g of fresh weight) fruit during storage time 0, 3, 5, 7 and 10 days)

'San Filippara'						
Day of storage	Total	5-Caffeoylquinic acid	3-Caffeoylquinic acid	4-Caffeoylquinic acid	5-Feruloylquinic acid	Hydroxybenzoic acid
0	110.77a	52.22a	17.70ab	4.33a	2.81a	3.64a
3	111.22a	54.33b	16.2a	3.22ab	3.10a	3.80a
5	114.54b	57.62c	15.98ab	3.19b	3.97b	3.23a
7	110.45a	56.22c	18.88c	3.43b	2.99a	1.97b
10	105.44a	57.13c	15.64a	2.15c	1.23c	2.23b

Values with different letters are significantly different at $p < 0.05$

Table 5 Qualitative phenols content in loquat fruit cv. 'Tanaka', (milligrams per 100 g of fresh weight) during storage time 0, 3, 5, 7 and 10 days

'Tanaka'						
Day of storage	Total	5-Caffeoylquinic acid	3-Caffeoylquinic acid	4-Caffeoylquinic acid	5-Feruloylquinic acid	Hydroxybenzoic acid
0	115.91a	61.18a	19.34a	3.89a	4.21a	4.09a
3	121.02b	59.35b	20.39a	4.79b	5.33b	5.01ab
5	119.89a	89.32c	34.09b	3.98a	4.38a	4.89a
7	118.32a	83.11c	35.43b	3.74a	3.98a	3.32b
10	113.45a	82.75c	31.39b	2.98c	4.22a	3.43b

Values with different letters are significantly different at $p < 0.05$

Table 6 Qualitative phenols content in loquatfruit cv ‘Nespolone di Trabia’ (milligrams per 100 g of fresh weight) during storage time 0, 3, 5, 7 and 10 days

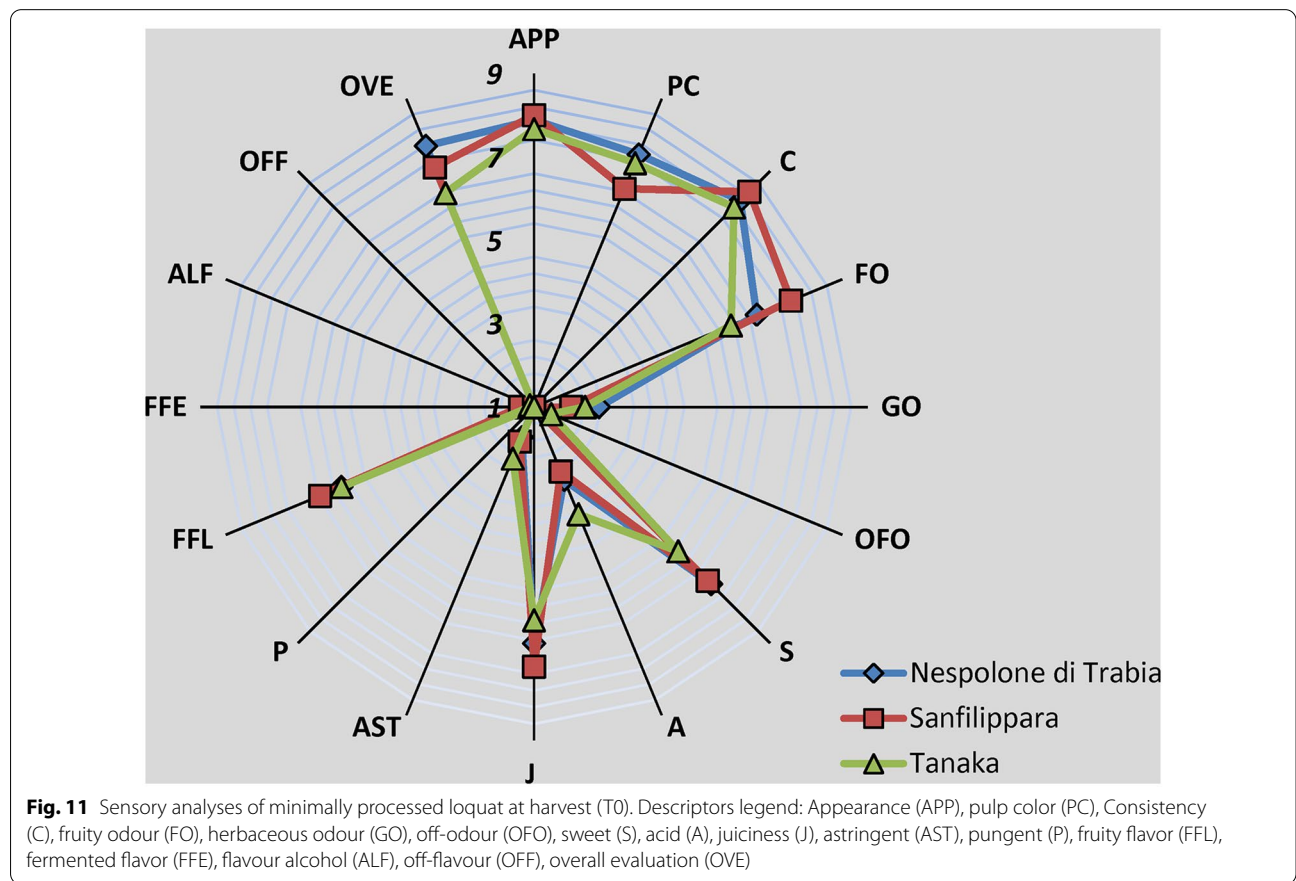
‘Nespolone di Trabia’						
Day of storage	Total	5-Caffeoylquinic acid	3-Caffeoylquinic acid	4-Caffeoylquinic acid	5-Feruloylquinic acid	Hydroxybenzoic acid
0	150.46a	80.32a	26.94a	6.89a	7.56a	7.65a
3	145.41b	75.96b	32.33b	5.23b	8.45b	7.98b
5	146.12b	77.43b	33.42b	6.02a	7.42a	7.44a
7	130.78c	65.40c	26.90a	4.32c	6.32a	6.78a
10	127.44c	61.32c	27.11c	4.76c	6.23a	5.23c

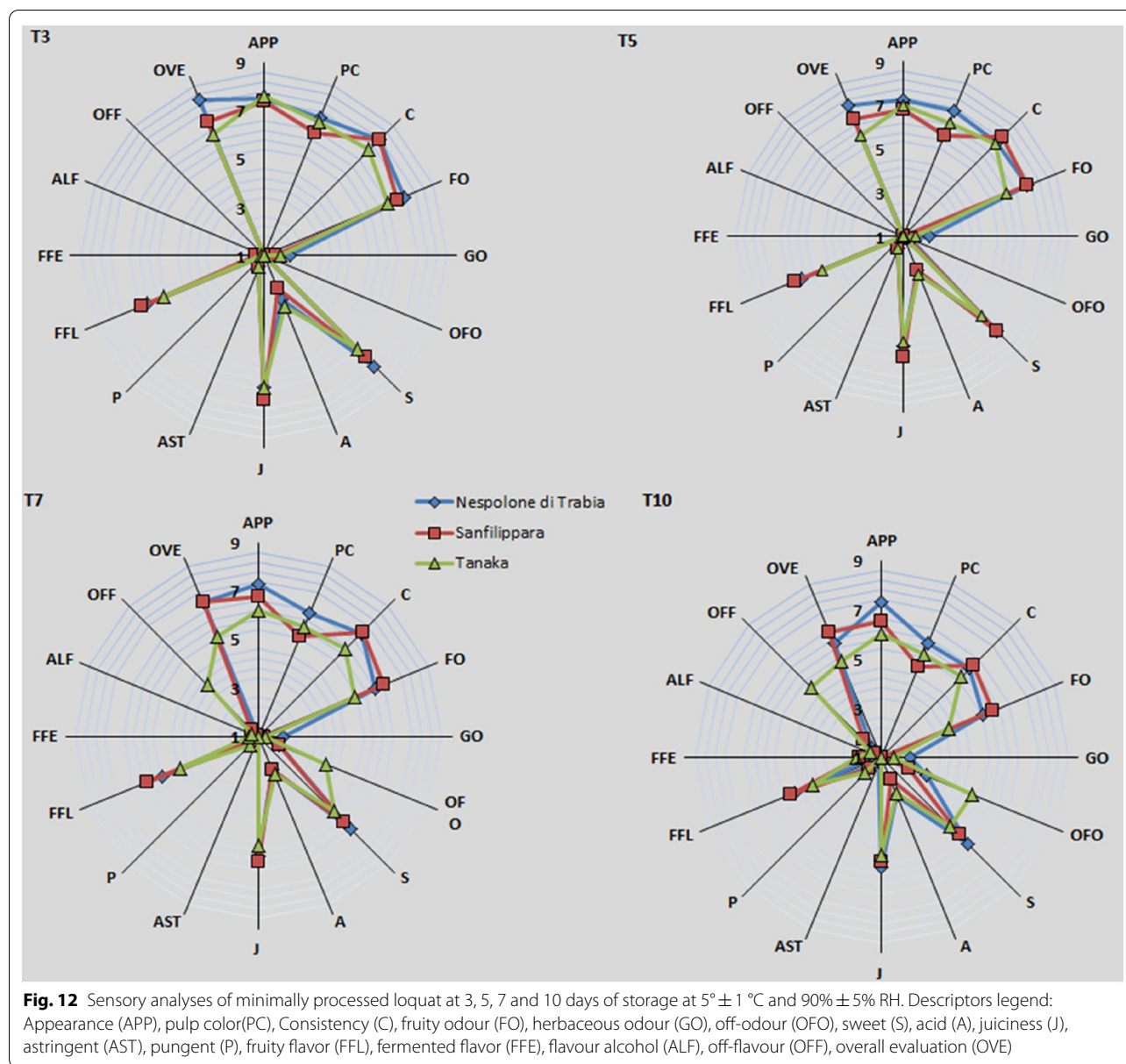
Values with different letters are significantly different at $p < 0.05$

differences appeared immediately after storage to become not significant along with the decrease of lutein content in the fruit (Fig. 10).

During fruit ripening, the total phenols contents of loquats, first decreased sharply and then increased dramatically [7]. In our work (Tables 4, 5), phenols total content remained stable ($p < 0.05$) throughout the storage period and, obviously, the same occurs for genotype-related differences that occurred at time 0.

This trend is observed on nectarine fruit [53] and on fig fruit [54] during cold storage. Different trend occurred for loquat fruit with a late ripeness stage (Table 6), the values shown a decrease of 15% during storage at 5 °C. In detail, 5-caffeoylquinic acid, significant increased (Tables 3, 4), while decreased in cv ‘Nespolone di Trabia’ (Tables 4, 5 and 6). 3-Caffeoylquinic acid in loquat fruit shoed a different trend among cultivar ‘Tanaka’ cvs (Table 5) occurred a significant increase of value, while no significant change occurred in cv ‘Nespolone di





Trabia". 4-Caffeoylquinic acid, decreased in all cultivars during cold storage. 5-Feruloylquinic acid, remained stable and did not change significantly in the cvs. 'Tanaka' and 'Nespalone di Trabia', while it decreased significantly in the cv 'San Filippara', while hydroxybenzoic acid in loquat fruit decreased significant in all cultivars (Tables 5 and 6).

Sensory analysis

The sensory analysis indicated high value of overall evaluation, appearance, pulp color, consistency, fruity odor, sweet and juiciness at harvest time, with no significant influence of the genotype (Fig. 11) at harvest.

Few differences occurred between cultivars, after sensory analysis. At time 0 the genotype had poor significant effects on sensory descriptors, except for fruity odor (FO) sweetness (S) and acidity (A). These differences were smaller in T3 and T5 when overall values kept closer to T0 (Fig. 12). At T7 and T10 there was a decrease of the values of most of descriptors and the genotype effect was significant for some of them (OFF, OVE, OFO, S, C. and FFL).

At T10 the values of most of sensory descriptors were much lower than at T0 and T5 and the effect of genotype was the same as at T7. Zou [2] reported that taste differences between the cultivars can be

explained by variations in composition and abundance of carbohydrates, organic acids, amino acids, and phenolics.

Browning, dehydration, off flavor, acetaldehyde and ethanol production could be phenomena that typically develop during storage. Severe symptoms of dehydration and browning occurred after 5 and 7 days of storage at 5 °C on early genotype ('San Filippa') followed by a significant reduction of juiciness (J). Therefore, the cold storage at 5 °C along with the genetic basis of some loquat fruit, limited the loss of sensory quality during storage.

Conclusions

This study is the first contribution that describes minimally processed loquats behavior. This research provides information about loquat cultivars suitability to be minimally processed based on consumer and sensory data as well as several biochemical parameters. This study highlights the effect of genotype and harvest time for shelf life prolong. Results highlighted that the later was the maturity stage the lower was the oxidation of the pulp and its qualitative decay. The higher values of glucose, sorbitol and ascorbic acid accumulated in the cv 'Nespolone Trabia' might have contributed to a reduction in chilling injury and oxidative stress after cutting. The same results were shown on peaches of same genotypic population of white and yellow flesh [33] and on summer and late cactus pear fruits [55]. These results show that later harvest fruit have propensity to lower oxidation of pulp and to qualitative decay. Low-temperature storage extends the shelf life of loquat fruit, and its flavor tend to deteriorate at a quicker rate than appearance does. These data are reflected in the sensory analysis. In addition, the polyphenols found in peeled fruits place loquat's cv as an high-value fruit if we consider it combined with sugars and organic acids data. The results showed that storage at 5 °C could be a good market opportunity, because it is relatively inexpensive. It can be a solution in case of high costs of storage techniques at low temperatures and, last but not least, in case of high quantities of not marketable fruit because of large spots of the epicarp (as it happens often in organic production). However, there is a clear indication of a strong genotype effect that must be taken into consideration to improve loquat minimal processing. Further studies could highlight if temperature and photoperiod conditions affect carotenoid content and if this response can be linked to a more efficient antioxidative system based on increased ascorbic acid and total phenol biosynthesis during third and fourth phases of fruit maturation. This information will help to formulate new strategies

for postharvest management development and seize new market opportunities toward the improvement of loquat fruits quality.

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Authors' contributions

Conceptualization, AA and GS; methodology, AA and GS; software, AA, VF; validation AA and GS and VF; formal analysis, AA, VF and MLVDC; investigation, AA and GS; resources, AA and GS; data curation, AA and GS; writing—original draft preparation, AA and GS; writing—review and editing, AA, GS and PI; visualization, AA and GS; supervision, AA, GS and PI; funding acquisition, AA, VF and GS. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study does not involve any human, animal or endangered species.

Consent for publication

Not applicable.

Competing interests

G.S. is an associate editor of Chemical and Biological Technologies in Agriculture. The rest of the authors have no conflicts of interest to declare.

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