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Sensory perception of citrate and malate and their impact on the overall taste in apricot (*Prunus armeniaca* L.) fruits

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ABSTRACT

Understanding the relationship between composition and taste is of pivotal importance for fruit quality improvement. This study aims to shed a light on the complex relationship between apricot fruit composition (both flesh and skin) and sensory perception. For this purpose, a total of 23 between apricot cultivars and breeding selections were characterized for a range of fruit-quality-related traits (maturity date, fresh weight, flesh firmness, soluble solids content, titratable acidity and total polyphenols content) and ten organic acids pattern in both flesh and skin. Fruit analytical data were correlated to sensory intensity through a detailed evaluation performed by ten trained panelists', with a particular focus on the impact of malate and citrate content on taste and acidity perception. Malate and citrate account for the 95% of the whole organic acids, although their content and ratio widely varied across the evaluated accessions (range of 0.96 – 14.05 and 0.33 – 20.08, respectively). Sweet-sour taste perception was greatly predicted by soluble solids content in apricots flesh (correlation of 0.60), but even more affected by titratable acidity (correlation between -0.84 and -0.97). In turn, titratable acidity was the main responsible of sour taste (correlation of 0.76) where citrate taste intensity (correlation up to 0.42) was stronger than malate in both fruit flesh and skin, with a negative effect on eating pleasantness. Results confirmed the importance of combining objective and sensory analyses to adequately comprehend apricot fruit quality. Moreover, the results corroborated the complexity of features defining the apricot fruit taste. This study provides novel insights into fruit quality- criteria to be considered in both breeding purposes and consumer's satisfaction-driven selection procedures.

1. Introduction

Among the most popular fleshy fruits, apricots (*Prunus armeniaca* L.) are notably consumed around the world. Recently, apricot is receiving a fervent interest in breeding programmes not only for introducing *Plum Pox Virus* (PPV) resistance, self-compatibility and extending the harvesting calendar, but also for improving the overall fresh fruit quality (FQ) and nutritional content (Drogoudi et al., 2008; Tricon et al., 2010; Fideghelli and Della Strada, 2010; Piagnani et al., 2013; De Mori et al., 2019; Bassi and Foschi, 2020).

Fresh FQ results from complex and mutually connected biological processes occurring throughout the growth and ripening stages, finally resulting in the increase of palatability (Genard et al., 2006). Several features shape apricot FQ, including exterior parameters (i.e. fruit size, peel color and absence of defects), flesh texture and, mostly, the flavor. In addition to aromatic volatiles (e.g. β-ionone and γ-decalactone), taste effects on apricot flavor are strictly related to water-soluble compounds such as sugars (estimated in terms of soluble solids content, SSC), organic acids (OAs) and their blend (Bartolozzi et al., 1997; Harker et al., 2002; Crisosto et al., 2004; Colaric et al., 2005; Guillot et al., 2006; Hormaza et al., 2007; Fan et al., 2017; Zhang et al., 2019). Taste perception refers to different in-mouth sensations (quality, intensity and pleasantness or unpleasantness) and is a pivotal determinant of consumer's preference (Egea et al., 2006; Farina et al., 2010; Louro et al., 2021). A bland apricot taste has been frequently reported by consumers, where flavor-related attributes seem to affect an 83% of the total acceptability (Xi et al., 2016). However, on-market price reductions often encourage consumers to purchase apricots with poor quality, even though consumer seems to be will to pay more when superior organoleptic properties are ensured (Harker et al., 2003; Helmert et al., 2017).

An enhanced fruit taste is perceived with higher SSC and moderate acid level at full fruit maturity stage rather than SSC alone, making SSC/

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acid ratio (*BrimA* index) an important FQ index of consumers' satisfaction (Bassi and Selli, 1990; Harker et al., 2002; Crisosto et al., 2004; Colaric et al., 2005; Crisosto and Crisosto, 2005; Xi et al., 2016; Fan et al., 2017). Hence, both sweetness and sourness perception depend on OAs pattern and acidity level seeming positively correlated to aroma and taste in peach (*Prunus persica* L. Batsch.) and apple (*Malus* x *domestica* Borkh.) (Esti et al., 1997; Harker et al., 2002; Colaric et al., 2005). Sensory characteristics of sugars (mainly sucrose, glucose and fructose) have been widely investigated in apricot (Bassi et al., 1996; Dolenc-Šturm et al. 1999; Mratinić et al., 2011; Xi et al., 2016; Fan et al., 2017). In contrast, the acidity perception of the most abundant OAs is still almost unexplored.

Apricot germplasm encloses a large variability for OA content and profile. OA decreases during ripening, probably because OAs supply the substrates for respiration, regulation of cells osmotic pressure and production of other flavor-related compounds (e.g. polyphenols) (Harker et al., 2002; Baldicchi et al., 2015; Etienne et al., 2013; Xi et al., 2016; Huang et al., 2021). Total OAs content provides the protons pool inside fruit cells and, thus, greatly predicts the acidity of both flesh and skin in apricot (Famiani et al., 2020). Apricot accessions displayed a consistent and selective accumulation of tricarboxylate citrate (CIT) and dicarboxylate malate (MAL) over years in both fruit flesh and skin, with a profile scarcely affected by non-genotypic effect, and corroborating a strong genetic basis behind these traits (Bassi et al., 1996; Bartolozzi et al., 1997; Moing et al., 1998; Gurrieri et al., 2001; Bureau et al., 20069; Chen et al., 2006; Martonoia et al., 2007; Akin et al., 2008; Ruiz and Egea, 2008; Ruiz et al., 2010; Salazar et al., 2013; Baldicchi et al., 2015; Xi et al., 2016; Fan et al., 2017; Zhang et al., 2019; García et al., 2019 and 2020; Baccichet et al., 2022; Dondini et al., 2022). Most of the OAs are Krebs cycle intermediates – although other metabolic pathways also contribute to their metabolism- and their vacuolar storage seems largely accountable to 'acid trap' facilitated diffusion mechanism driven by several proton pumps such as H^+-PPase (V-PPase) and P-ATPase (i.e. PH1 and PH5) (Etienne et al., 2013; Cohen et al., 2014; Ma et al., 2019). MAL is synthetized in cytosol by the phosphoenolpyruvate carboxylase (PEPC) activity while CIT is consumed and regenerated by the tricarboxylic acid cycle (TCA) reactions in the mitochondria producing energy through the CO_2 release. Proton transport mediated by V-PPase and P-ATPase generates an electrochemical gradient responsible for vacuolar acidification that facilitates CIT and MAL uptake guaranteeing the homeostasis maintenance. Balance among metabolism, catabolism and intracellular transport of CIT and MAL affects the relative OA pattern and fruit acidity, hence impacting the taste (Etienne et al., 2013; Zhang et al., 2020).

The selective accumulation of MAL and/or CIT into vacuoles is an intriguing topic to be elucidated in apricot but may occur for a competitive inhibition at the same tonoplastic carriers of CIT on MAL and *viceversa* (Rentsch and Martinoia, 1991; Moing et al., 1998). Apricot accessions showed a MAL/CIT ratio markedly diversified, mostly occurring between 0.12 and 1.25 and with a value of about 0.8 associated to an optimal response on taste (Dolenc-Šturm et al., 1999; Gurrieri et al., 2001). In turn, CIT and MAL seemed to add a smooth and sharp effect in mouth respectively, leading to consider chemical analyses as unpredictable and often inadequate for the final taste assessment (Harker et al., 2002; Aprea et al., 2017).

Understanding the contribution of CIT and MAL on apricot taste is of primary importance for fruit quality improvement as it can exacerbate or mitigate the sourness and/or sweetness perception. The major aims of this work were to investigate the presence of CIT and MAL in both apricot fruit flesh and skin of 23 accessions and to decipher the overall taste and flavor perception by ten trained panelists. The chemical characterization of the analyzed apricots panel supported by the clarification of citrate and malate contribution can provide valuable information for driving the ongoing apricot selection strategies toward specific consumers' preference.

2. Materials and methods

2.1. Plant material and fruit collection

The tested apricot accessions and advanced breeding selections were maintained at 'Ri.Nova – Ricerca e Sviluppo' (www.crpv.it) located near Imola in Northern Italy (44° 33' 66''N, 11° 75' 57'' E) at 53 m from near sea level. Trees were 4 to 8 years old, grafted onto 'Mirabolan 29C' rootstock, trained according to a free open-vase system and spaced at 4 m × 2.5 m (between and within rows, respectively). A total of 3 replicated trees (arranged in a non-randomized plot) per each of the 23 apricot accessions (Supplementary Table 1) were analyzed during the harvesting season 2021 (from the 31st of May to the 21st of July) that was characterized by a low monthly rainfall rate (mm) with an average temperature range (°C) consistent with previous years (Supplementary Fig. 1; ARPAE-SIMC, www.simc.arpae.it). Manual thinning was carried out in early Spring; a medium-to-low fruit load was carefully set in order to ensure an optimum fruit quality potential, considering the number of fruits per trunk cross-sectional-area and harvesting period. Forty uniform fruits (ten for quality-related analyses and thirty for panel test) were randomly picked at full maturity stage ("ready-to-eat") preferring the ones more exposed to sunlight. All fruits were harvested in the same day of sensory evaluation.

2.2. Instrumental-based analyses for fruit quality-related parameters

Harvest day (maturity date, MD) was expressed as Julian days (JD). The proper physiological fruit ripening degree was defined as the index of chlorophyll absorbance difference (IAD) estimated as the average value read for each fruit cheeks by a DA-meter portable spectrometer (Sintéleia S.r.l., Bologna, Italy). Fresh weight (FW) of each fruit was measured in grams using a precision scale. Individual fruit flesh firmness (F) was expressed in Newton (N) and tested using a constant rate (5 mm s^{-1} of speed) digital penetrometer (Andilog Centor AC TEXT08) with a flat metal tip (6 mm) after removing a round area (1.5 cm of size) of apricot skin from the middle of the sun-exposed cheek, by a slicer. Three biological replicates of flesh juices and skin juices (dilution 1:10 w v^{-1} of fruit tissue in 18 MΩ H_2O) were separately prepared and held at - 20°C. After centrifugation at 5000 rpm for 20 min at 4°C, clarified flesh and skin juices were used for analyses of soluble solid content (SSC), titratable acidity (TA), total polyphenols content (PP) and organic acids (OAs) profiles. SSC measurement was performed with a digital refractometer (Atago, Co., Tokyo, Japan) and values were expressed as °Brix. Determination of TA (expressed as g L^{-1} of malic acid) and ten OAs (cis-aconitate, citrate, fumarate, galacturonate, malate, oxalate, quinate, shikimate, succinate and tartrate) patterns in flesh and skin, separately, were carried out adopting a previously developed protocol (Baccichet et al., 2022). Quantification of PP in skin juice was assayed by a modified Folin-Ciocalteu method (Folin and Ciocalteu, 1927). Calibration curve was built using gallic acid (GAE; Sigma-Aldrich, USA) as reference standard and PP data were expressed as mg (GAE) in 100 mL^{-1} of fruit extract. Two milliliters of 18 MΩ H_2O, 100 μL of diluted (1:10) skin sample and 250 μL of Folin-Ciocalteu reagent (Merck, Germany) were placed in a test tube and incubated for 5 min. The reaction was neutralized with 1 mL of Na_2CO_3 (10% w v^{-1}) and 1.65 mL of 18 MΩ H_2O. After 90 min, absorbance of the sample solutions was measured at 700 nm using a UV/VIS spectrophotometer (GENESYS 180; Thermo Fisher Scientific, USA).

2.3. Sensory analysis

Sensory analysis was carried out by 'ASTRA Innovation and Development Agency' in Faenza (Ravenna, Northern Italy) by the quantitative descriptive analysis method (QDA). Ten trained sensory panelists examined thirty fruits from each accessions (each of them identified as three digit codes) for several fruit quality (FQ)-related attributes: skin colour, flesh firmness, texture and mealiness, juiciness, apricot fruity flavor and aroma, bitterness, sweetness, sourness and their balance. A particular focus was played on the intensity of citric (CIT) and malic (MAL) acid taste. Each FQ parameter was evaluated for the overall satisfaction on an intensity perception scale ranging from 1 (lowest score) to 9 (highest score) with the minimum threshold set at 5. Sensory analysis included a pleasantness score (from 1 to 9 with 5 as the minimum threshold) for each panelist's based on visual, olfactory, taste and flesh textural properties with an overall degree of liking. Panelists' taste sensitivity was trained using standard solutions of CIT and MAL with added sucrose (10° Brix) at three concentrations: 7, 18 and 29 g L^{-1}. Odorless tap water was used to prepare the solutions. Each fruit sample was cut into fifths immediately prior the sensory evaluations to avoid oxidative browning that could influence the overall quality assessment. Samples were tested in different random sequences and the whole analysis was held at room temperature in individual testing booths under normal indoor lighting.

2.4. Statistical analysis

All statistical analysis was carried out using *RStudio* (version 1.4.1717) tool in the *R* statistics software(version 4.1.3). Descriptive statistics (minimum, maximum, mean values standard deviation and distribution) was calculated for each FQ-related attribute and OA in both flesh and skin among the dataset and within each accession (Table 1; Figs. 1 and 2; Supplementary Fig. 2; Supplementary Fig. 3; Supplementary Fig. 4; Supplementary Fig. 5; Supplementary Fig. 6 and Supplementary Fig. 7). Quantitative sensory profiles of each parameter were standardized (*Z*-scores with mean 0 and standard deviation 1) on the overall apricots set mean for a better visibility. Absolute hedonic scores (i.e. overall degree of liking and pleasantness) and sensory fingerprint were averaged over panelists' and data were reported as barplot and spider-plot using *ggplot2* (version 3.4.0) and *fsmb* (version 0.7.3) package, respectively (Fig. 4; Supplementary Fig. 12). All analytical (excluding SSC, TA and CIT both in flesh and skin) and sensory were not normally distributed according to Shapiro-Wilk's test ($p \leq 0.05$). Thus, homoscedasticity of panel dataset variance was assessed through Levene's test (Levene, 1960) at *p-value* higher than 0.05. Yeo-Johnson's data transformation of fruit flesh bitterness and doughy (i.e. fruits with very soft texture, easily mashed to flesh and following viscous behavior when squashed) data was applied in *BestNormalize* package (version 1.8.3) as normality and homoscedasticity assumptions were violated. Flesh doughy attribute was removed from the final sensory dataset because no improvements were achieved after data transformation, allowing to consider it a complex fruit parameter to evaluate. Organoleptic differences between samples were investigated by Kruskal-Wallis's test ($p \leq 0.05$; Supplementary Fig. 9, Supplementary Figs. 11 and 3) and means were compared by Dunn's multiple pairwise *post-hoc* test under Bonferroni's correction (Supplementary File 1). To investigate the relationships among the FQ parameters (Supplementary Table 2) and, then, between attributes and sensory ratings (Fig. 5), Spearman's correlation coefficients were estimated in *corrplot* package (version 0.92). Furthermore, principal component analysis (PCA) was carried out including apricot accessions groupings, in order to find the main variation trends in the fruit parameters and in the four most abundant OAs (CIT, MAL, QUI and SUC; Table 1), both in flesh and skin, (Supplementary Fig. 8).

3. Results

3.1. Titratable acidity, SSC and other fruit quality-related parameters in apricot accessions panel

Fruit-quality (FQ) related attributes showed an extensive variation among the 23 apricot accessions for both flesh and skin (Table 1 and Supplementary Table 1). The ripening calendar (MD) covered the early (151 JD for 'Pricia' and 'Tsunami') to the medium-late (202 JD for 'BO04639076' and 'BO04639319') season, enabling to test an extremely diversified range of fruit types. In flesh, both soluble solids content (SSC) and titratable acidity (TA) differed considerably ranging from a minimum of 10.3 (in 'Pricia') to a maximum of 20.7°Brix (in 'BO03608119') and from 6.1 (in 'Portici') to 26.3 g L^{-1} of malic acid (in 'Pricia'), respectively. Therefore, SSC/TA ratio (*BrimA* index) revealed a wide diversity in the range between 3.90 in 'Pricia' and 28.90 in 'BO03608119' (Supplementary Fig. 2). Skin TA content level (minimum of 5.1 and maximum of 33.1 g L^{-1} of malic acid in 'Portici' and 'Pricia', respectively) and mean value (17.4 ± 6.5 g L^{-1} of malic acid) was similar to flesh (14.6 ± 5.4 g L^{-1} of malic acid; Table 1), suggesting a consistent acid accumulation pattern between these tissues at full-physiological maturity (ρ of 0.86; Supplementary Table 2). However, a tendency toward larger SSC (ρ = 0.82) and lower TA levels in flesh (ρ = -0.61) and skin (ρ = 0.67) occurred within MD (Supplementary Table 2

Table 1

Minimum, maximum, mean and standard deviation values of fruit quality-related attributes and ten organic acids (OAs) detected through HPLC analysis in the apricot accessions panel. Minimum, maximum, mean and standard deviation (SD) values were estimated for each fruit quality-related attribute and OA found in flesh and skin, separately. Abbreviations: *SSC*, soluble solids content.

FRUIT QUALITY-RELATED ATTRIBUTES

Parameter	Unit of measure	Min – Max range	Mean	SD (±)
Firmness	N	0.7 – 31.6	11.5	9.2
Fresh weight	g	39.5 – 87.8	65.1	16.4
Maturity date	Julian days (JD)	151 - 202	171	14

COMPOUNDS DETECTED

		Flesh			Skin		
Parameter	Unit of measure	Min – Max range	Mean	SD (±)	Min – Max range	Mean	SD (±)
Titratable acidity	g L^{-1} of malic acid	6.1 – 26.3	14.6	5.4	5.1 – 33.1	17.4	6.5
Total polyphenols	mg (GAE) 100 mL^{-1}	–	–	–	71 - 224	145	48
SSC	°Brix	10.3 – 20.7	15.4	2.4	–	–	–
Cis-aconitic acid	ng μL^{-1}	0.31 – 5.46	2	1	0 – 4.08	1.06	1.09
Citric acid	mg mL^{-1}	0.56 – 17.94	8.74	4.46	0.33 – 20.08	10.48	5.48
Fumaric acid	ng μL^{-1}	12.60 – 32.80	20.23	5.53	3.23 – 21.92	11.19	5.18
Galacturonic acid	mg mL^{-1}	0 - 0.15	0.03	0.04	0 – 1.07	0.38	0.31
Malic acid	mg mL^{-1}	1.65 – 14.05	4.92	3.19	0.96 – 12.79	4.95	3.34
Oxalic acid	ng μL^{-1}	0 – 0.63	0.23	0.21	0 -3	0.16	0.63
Quinic acid	mg mL^{-1}	0 – 0.24	0.07	0.09	0 – 2.71	0.18	0.56
Shikimic acid	ng μL^{-1}	0 – 8.94	1.44	2.23	0.14 – 18.50	5.82	4.65
Succinic acid	mg mL^{-1}	0.11 – 1.39	0.62	0.35	0.01 – 0.96	0.27	0.25
Tartaric acid	ng μL^{-1}	0 – 14.80	3.09	3.91	0 – 684	85.65	149.2

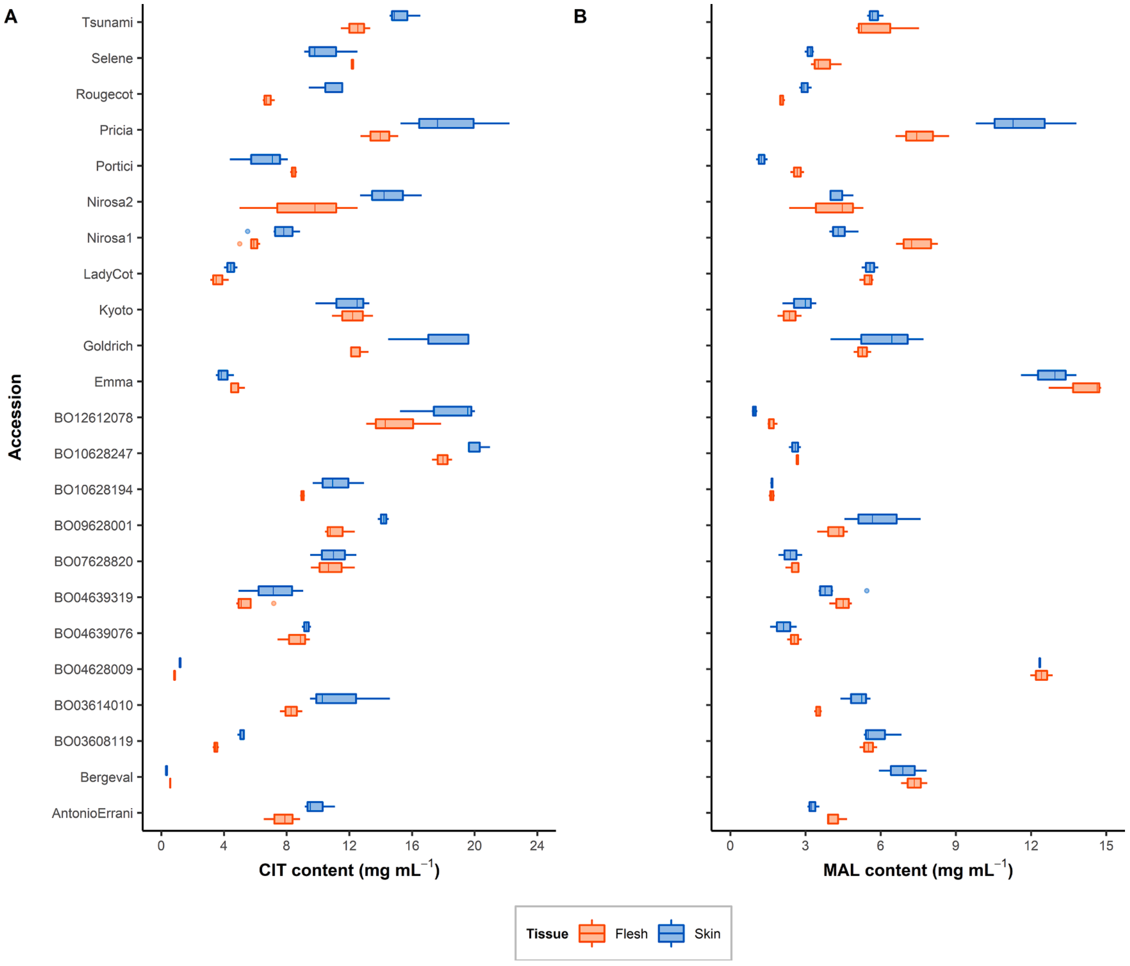


Fig. 1. Distribution of citrate and malate content (mg mL^{-1}) between fruit flesh and skin in the 23 apricot accessions tested. Citrate (*A*) and malate (*B*) concentration widely occurred in the apricot accessions panel and were separately evaluated in fruit flesh (orange colour) and skin (blue colour).

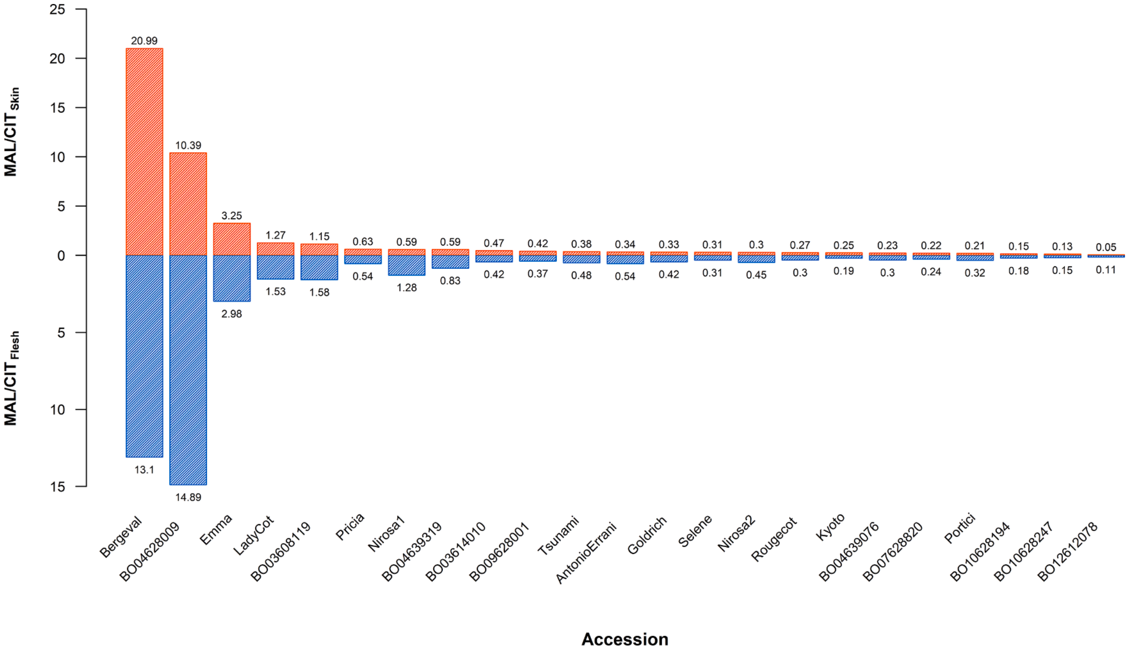


Fig. 2. Ratio between malate and citrate content (MAL/CIT ratio) in apricot flesh and skin. Apricot accession displayed a variegated MAL/CIT ratio ranging from 0.11 (in ‘BO12612078’) to 14.89 (in ‘BO04628009’) in fruit flesh and from 0.05 (in ‘BO12612078’) to almost 21 (in ‘Bergeval’) in skin.

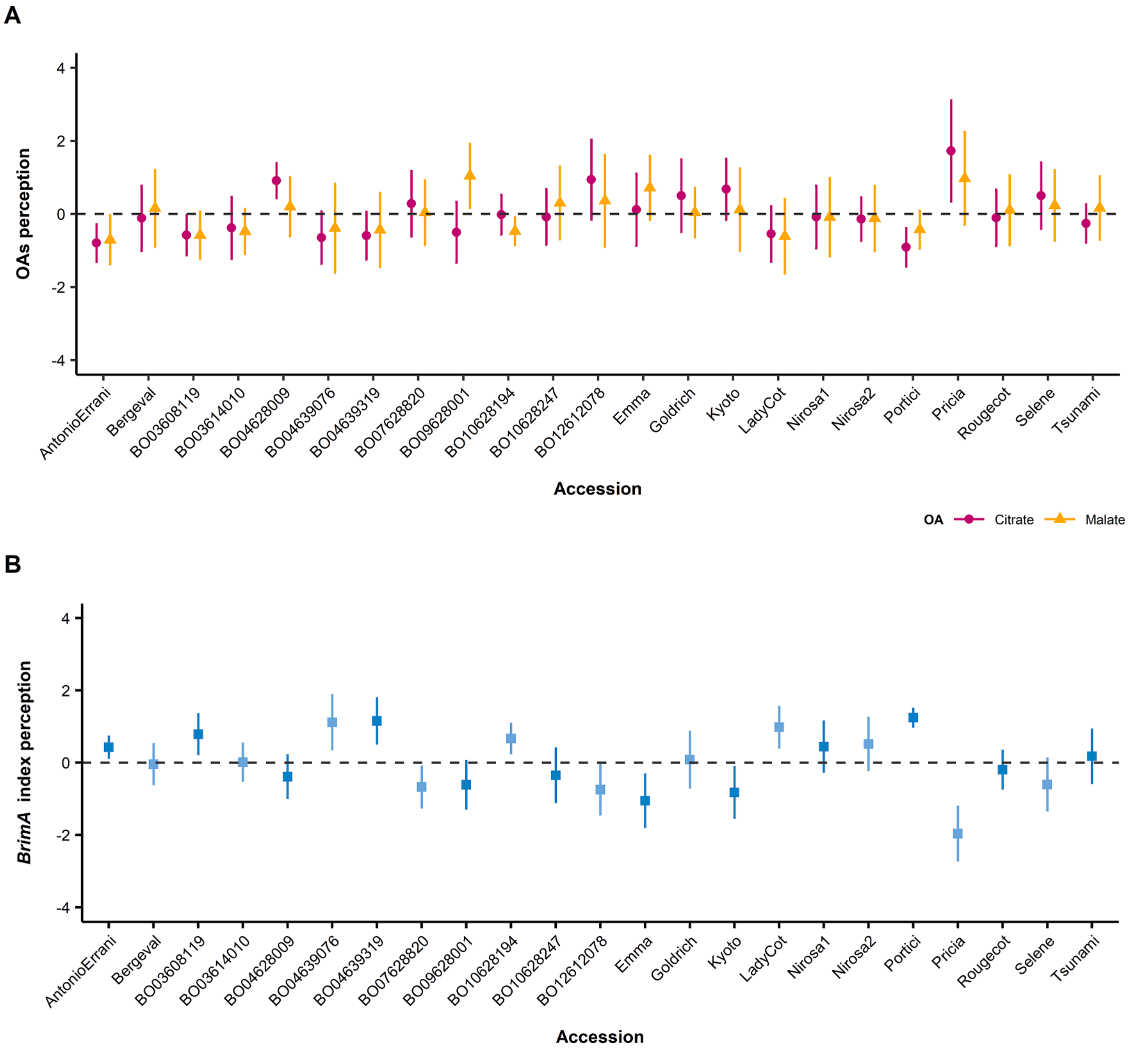


Fig. 3. Normalized distribution of sensory intensity for citrate and malate (A) and *BrimA* index (B). Panel test scores for citrate (in purple; CIT), malate (in yellow; MAL) and *BrimA index* (in blue) were normalized. A greater perception of CIT on MAL taste was observed in 'Pricia' (value of 1.73 *vs.* 1.04) and 'BO12612078' (value of 0.94 *vs.* 0.36) while the opposite occurred in other accessions such as 'BO09628001' (value of 1.04 *vs.* -0.50). *BrimA* index sensory response reached the lowest and the highest score in 'Pricia' (-1.96) and 'Portici' (1.24), respectively.

and Supplementary Fig. 8). Mid-season ripening accessions (around 171 JD such as 'BO07628820') showed a higher pressure-resistance of fruit explaining the slight MD-dependence of firmness (ρ = 0.53; Supplementary Table 2). However, no significant correlation was observed between fresh fruit weight (FW) and the other fruit-quality features, although they ranged widely (from 39.50 g in 'Tsunami' to 87.80 g in 'BO03614010'). Total polyphenols content (PP) in fruit skin differed among samples (from 71 to 224 mg GAE in 100 mL^{-1} in 'Nirosa2' and 'BO09628001', respectively) revealing a poor relationship with other FQ features (Supplementary Table 2).

3.2. Organic acid content characterization

Among the ten OAs patterns investigated in drupes flesh and skin (Supplementary Fig. 3 and Supplementary Fig. 4), only citrate (CIT), malate (MAL), quinate (QUI) and succinate (SUC) occurred more abundantly (means range of 0.07 – 10.48 mg mL^{-1}; Table 1) and, thus, considered as informative descriptors of the overall taste in panel test analysis. For almost all the OAs (i.e. CIT, MAL, FUM, GAL, QUI, SUC and SHIK), each accession displayed consistent qualitative and quantitative profile between flesh and skin (ρ from 0.48 for QUI to 0.93 for CIT; Supplementary Table 2). CIT and MAL were the most abundant OAs in this study and accounted for about 95% of the OAs with a maximum concentration of 20.08 mg mL^{-1} in 'BO10628247' skin and 14.05 mg mL^{-1} in 'Emma' flesh, respectively (Table 1 and Fig. 1). MAL and CIT were negatively correlated (ρ = -0.43; Supplementary Table 2) and they showed a balance content (MAL/CIT ratio) highly variable among samples (Fig. 2) but consistent between skin and flesh of each accession (ρ of 0.95; Supplementary Table 2). MAL was more abundantly accumulated in 'BO03608119' (ratio of 1.15 in flesh and 1.58 in skin), 'LadyCot' (1.27 in flesh and 1.53 in skin), 'Emma' (ratio of 3.25 in + flesh), 'BO04628009' (ratio of 10.39 in flesh and 14.89 in skin) and 'Bergeval' (highest value: 20.99), while larger CIT concentrations mostly occurred in early-ripening accessions skin (ρ of -0.47; Supplementary Table 2). Interestingly, CIT seemed the greatest contributors of the overall TA detected in both fruit flesh (ρ = 0.70; Supplementary Table 2) and skin (ρ = 0.82; Supplementary Table 2) while accessions with higher SSC often occurred with reduced MAL level (ρ = -0.42; Supplementary Table 2). QUI was mostly abundant in 'Selene' flesh (0.24 mg mL^{-1}; Table 1) and 'Goldrich' skin (2.71 mg mL^{-1}; Table 1). 'Pricia' flesh showed the lowest concentration of SUC (0.11 mg mL^{-1}; Table 1) and FUM (12.60 ng μL^{-1}; Table 1); nevertheless, both occurred in all tissues (ρ = -0.49; Supplementary Table 2) showing a reduced content variation among the all accessions (Table 1; Supplementary Fig. 4B). GAL and TRT occurred mostly in fruit skin with the largest concentration in 'Tsunami' (1.07 mg mL^{-1}) and 'Rougecot' (0.68 mg mL^{-1}), respectively (Table 1; Supplementary Fig. 4C and 4F). FUM and TRT occurred at reduced levels in high-acid content samples (ρ = -0.60; Supplementary Table 2) and seemed more MD-dependent (ρ = 0.60 and 0.80, respectively; Supplementary Table 2). CIS and SHIK were mostly present in traces (Table 1; Supplementary Figs. 4A and 3E) while OX was almost undetected and, thus, removed from the sensory analysis. Accumulation of three OAs linked with secondary metabolism (i.e. SHIK, QUI and TRT) abundantly occurred in accessions with larger total PP levels in apricot skin (ρ in the range of 0.34 – 0.56; Supplementary Table 2). Inclusion of the four major OAs (Table 1) in two Principal Component Analysis (PCA) with the FQ attributes (i.e. FW, F, MD, SSC, TA and PP) explained 53.9% of the overall variance observed, clearly separating the accessions into two main groups in terms of CIT and MAL content (Supplementary Fig. 8).

3.3. Sensory analysis of apricot fruit taste and relationship with other sensory attributes

Fruit acidity score was extremely wide, occurring between -1.32 in ‘BO03608119’ and 1.70 in ‘Pricia’, (Supplementary Fig. 11D) where only ‘Rougecot’ dwelled almost on the general mean value. The intensity of CIT and MAL in fruit varied from – 0.91 in ‘Portici’ to 1.73 in ‘Pricia’ and from – 0.71 in ‘Antonio Errani’ to 1.04 in ‘BO09628001’, respectively (Fig. 3A). Targeted-perception of CIT and MAL taste seemed specific to each accession (Fig. 3A). For instance, a greater sensory impact of CIT on MAL was observed in ‘Pricia’ (1.73 vs. 1.04) and ‘BO12612078’ (0.94 *vs.* 0.36) whereas the opposite occurred in ‘BO09628001’ (1.04 *vs.* – 0.50). In some cases, intensity of CIT and MAL taste (Fig. 3A) was equally perceived as pointed out in ‘BO03608119’ (-0.58), ‘LadyCot’ (-0.55 and -0.61 for CIT and MAL, respectively), ‘Nirosa1’ (about -0.85) and ‘Nirosa2’ (about -0.13). Differences among the accessions were observed in astringency profiles (Supplementary Fig. 11F) that probably enhanced sour taste such as in ‘Pricia’ and ‘Emma’ (1.05 for astringency and 0.72 for MAL). Sweetness perception varied from a minimum score of – 1.28 in ‘BO07628820’ to a 0.96 in ‘BO04639076’ where only ‘BO04628009’ (0.02) and ‘Tsunami’ (0.07) were almost on the mean threshold (Supplementary Fig. 9C). Sweet-sour taste balance (also defined as *BrimA* index perception; Fig. 3B) seemed driven by the overall sourness perception rather than sweetness alone. The highest averaged score was reached in ‘Portici’ (1.24), that was also judged very poor in CIT taste (averaged score of –0.91; Fig. 3A) and with a low roughening and puckering power in mouth (- 0.75; Supplementary Fig. 11F). Instead, the most significant unbalanced *BrimA* index was detected in ‘Pricia’ (-1.96 with a Z-score of 6.63 in ‘Portici’; Fig. 3 and

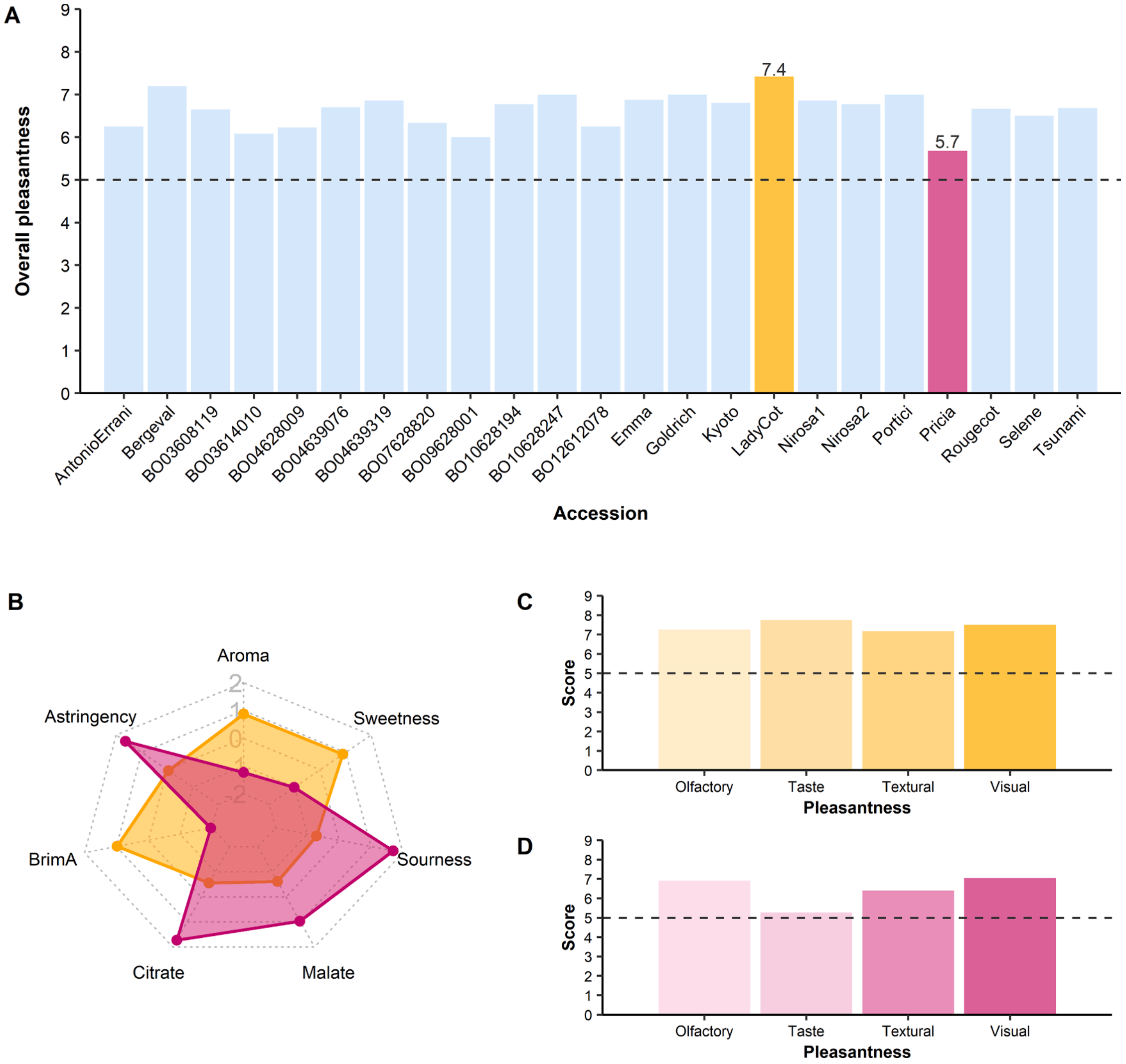
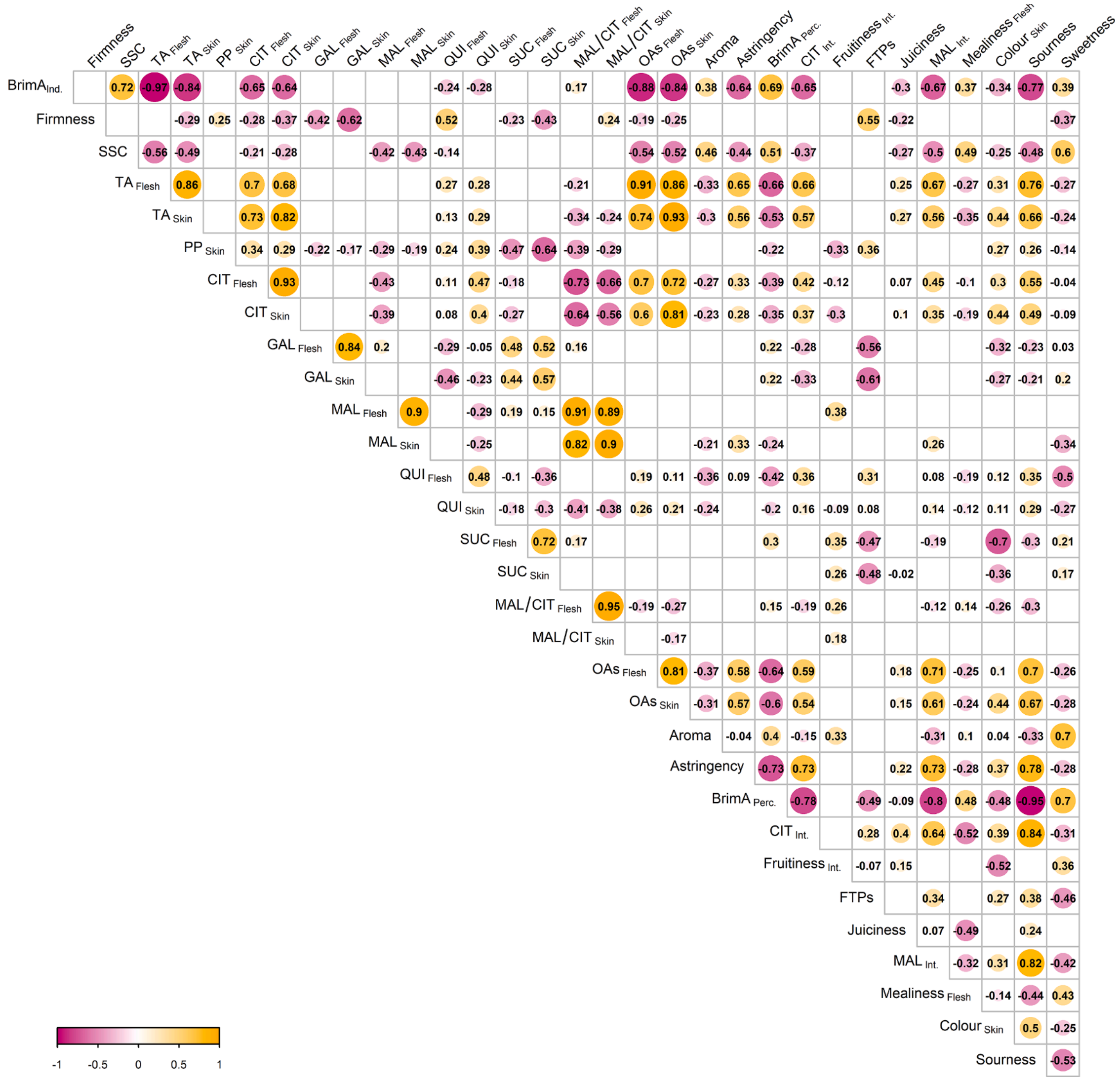


Fig. 4. Apricot fruit panel test results. Pleasantness and sensory fingerprint scores were averaged across the ten expert trained panelists. Among the 23 apricot accessions analyzed, ‘LadyCot’ (score of 7.4) and ‘Pricia’ (score of 5.7) were the most and the less appreciated, respectively. In overall pleasantness graph (A) and hedonic evaluations plot for ‘LadyCot’ (C) and ‘Pricia’ (D), the grey horizontal line at 5 marks the minimum threshold set in sensory analysis. Sensory fingerprint (B) includes the seven contributors of apricot taste perception.

Supplementary File 1). Sensory analysis did not report significant ($p < 0.05$) differences among the bitterness perception (Supplementary Fig. 11E) leading to consider this fruit attribute as not important in panelists' preference. Nonetheless, the effects of sourness and sweetness perception on other sensory attributes were also investigated, particularly on the olfactory profile (fruitiness and aroma) and overall pleasantness. Regarding overall pleasantness, this attribute was always above the minimum acceptable threshold with 'Pricia' and 'Ladycot' ranked as the lowest (5.7) and the highest (7.4), respectively (Fig. 4A). Notably, both cultivars were also characterized by the highest and lowest scores for sweetness and sourness. In general, sweetness perception appeared positively related with 'apricot' aroma ($\rho = 0.70$; Fig. 5) as evident particularly in 'LadyCot' that achieved the most pleasant flavor (0.88; Supplementary Fig. 11B) and the second highest score in sweetness perception (0.89; Supplementary Fig. 11C). The effect of sourness on olfactory perception was much smaller and systematic within the 23 accessions assayed in this study (Fig. 5). Fruitiness perception widely varied from the excellent intensity of 'Emma' (0.99), rated as acidic (0.78) and with a strong MAL taste (0.72), to the worst of 'Rougecot (-1.25) and 'Antonio Errani' (- 0.90) both characterized by a balanced sweet/sour and MAL/CIT taste (Supplementary Figs. 11A and 3).

Focusing on the other fruit quality features, panelists were able to distinguish different shades of apricot skin color (Supplementary Fig. 9A) pointing out 'Rougecot' and 'Antonio Errani' as the most different accessions in the whole set (*Z-score* of – 6.34; Supplementary File 1; Supplementary 10). Fruit skin discoloration of 'Antonio Errani', 'Selene' and 'Portici' was not perceived positively by the panelists who attributed the typical "apricot-orange" tonality to 'Tsunami' (Supplementary Fig. 9A and Supplementary 10). Higher flesh textural property (FTP) of 'BO07628820' and 'Selene' (both with a mean equal to 0.77) were significantly preferred to 'Nirosa2' (- 1.10), 'Tsunami' (- 0.93), 'BO03614010' (- 0.76), 'Nirosa1' (- 0.68) and 'BO04639319' (- 0.64). All the comparisons had a *Z-score* in the range of 3 – 4 (Supplementary File 1), although panelists rated most of the accessions close to the mean average (Supplementary Fig. 9B). Panel test results showed a slight inverse relationship between flesh mealiness and juiciness, especially highlighted in 'BO04639076, 'BO04639319' and 'Portici' (Supplementary Fig. 9C and D). Among the whole apricot set, panelists positively



	Firmness	SSC	TA Flesh	TA Skin	PP Skin	CIT Flesh	CIT Skin	GAL Flesh	GAL Skin	MAL Flesh	MAL Skin	QUI Flesh	QUI Skin	SUC Flesh	SUC Skin	MAL/CIT Flesh	MAL/CIT Skin	OAs Flesh	OAs Skin	Aroma	Astringency	BrimA Perc.	CIT Int.	Fruitiness Int.	FTPs	Juiciness	MAL Int.	Mealiness Flesh	Colour Skin	Sourness	Sweetness
BrimA Ind.		0.72	-0.97	-0.84		-0.65	-0.64					-0.24	-0.28			0.17		-0.88	-0.84	0.38	-0.64	0.69	-0.65			-0.3	-0.67	0.37	-0.34	-0.77	0.39
Firmness				-0.29	0.25	-0.28	-0.37	-0.42	-0.62			0.52		-0.23	-0.43		0.24	-0.19	-0.25						0.55	-0.22					-0.37
SSC			-0.56	-0.49		-0.21	-0.28			-0.42	-0.43	-0.14						-0.54	-0.52	0.46	-0.44	0.51	-0.37			-0.27	-0.5	0.49	-0.25	-0.48	0.6
TA Flesh				0.86		0.7	0.68					0.27	0.28			-0.21		0.91	0.86	-0.33	0.65	-0.66	0.66			0.25	0.67	-0.27	0.31	0.76	-0.27
TA Skin						0.73	0.82					0.13	0.29			-0.34	-0.24	0.74	0.93	-0.3	0.56	-0.53	0.57			0.27	0.56	-0.35	0.44	0.66	-0.24
PP Skin						0.34	0.29	-0.22	-0.17	-0.29	-0.19	0.24	0.39	-0.47	-0.64	-0.39	-0.29					-0.22		-0.33	0.36				0.27	0.26	-0.14
CIT Flesh							0.93			-0.43		0.11	0.47	-0.18		-0.73	-0.66	0.7	0.72	-0.27	0.33	-0.39	0.42	-0.12		0.07	0.45	-0.1	0.3	0.55	-0.04
CIT Skin										-0.39		0.08	0.4	-0.27		-0.64	-0.56	0.6	0.81	-0.23	0.28	-0.35	0.37	-0.3		0.1	0.35	-0.19	0.44	0.49	-0.09
GAL Flesh									0.84	0.2		-0.29	-0.05	0.48	0.52	0.16						0.22	-0.28		-0.56				-0.32	-0.23	0.03
GAL Skin												-0.46	-0.23	0.44	0.57							0.22	-0.33		-0.61				-0.27	-0.21	0.2
MAL Flesh											0.9		-0.29	0.19	0.15	0.91	0.89							0.38							
MAL Skin													-0.25			0.82	0.9			-0.21	0.33	-0.24					0.26				-0.34
QUI Flesh													0.48	-0.1	-0.36			0.19	0.11	-0.36	0.09	-0.42	0.36		0.31		0.08	-0.19	0.12	0.35	-0.5
QUI Skin														-0.18	-0.3	-0.41	-0.38	0.26	0.21	-0.24		-0.2	0.16	-0.09	0.08		0.14	-0.12	0.11	0.29	-0.27
SUC Flesh															0.72	0.17						0.3		0.35	-0.47		-0.19		-0.7	-0.3	0.21
SUC Skin																								0.26	-0.48	-0.02			-0.36		0.17
MAL/CIT Flesh																	0.95	-0.19	-0.27			0.15	-0.19	0.26			-0.12	0.14	-0.26	-0.3	
MAL/CIT Skin																			-0.17					0.18							
OAs Flesh																			0.81	-0.37	0.58	-0.64	0.59			0.18	0.71	-0.25	0.1	0.7	-0.26
OAs Skin																				-0.31	0.57	-0.6	0.54			0.15	0.61	-0.24	0.44	0.67	-0.28
Aroma																					-0.04	0.4	-0.15	0.33			-0.31	0.1	0.04	-0.33	0.7
Astringency																						-0.73	0.73			0.22	0.73	-0.28	0.37	0.78	-0.28
BrimA Perc.																							-0.78		-0.49	-0.09	-0.8	0.48	-0.48	-0.95	0.7
CIT Int.																									0.28	0.4	0.64	-0.52	0.39	0.84	-0.31
Fruitiness Int.																									-0.07	0.15			-0.52		0.36
FTPs																											0.34		0.27	0.38	-0.46
Juiciness																											0.07	-0.49		0.24	
MAL Int.																												-0.32	0.31	0.82	-0.42
Mealiness Flesh																													-0.14	-0.44	0.43
Colour Skin																														0.5	-0.25
Sourness																															-0.53

Fig. 5. Correlation analysis among fruit quality objective measurements and panel test scores to decipher apricot taste in the 23 accessions. A total of 23 apricot accessions were tested for fruit quality-related parameters (firmness, SSC, TA, BrimA index and PP) and the five most abundant organic acids (CIT, MAL, QUI and SUC) detected in apricot fruit flesh and skin, separately. Replicated objective measurements and sensory scores for each accession were averaged. Correlations were significant p-value < 0.05. Abbreviations: *PP*, total polyphenols content (mg GAE in 100 mL of apricot skin extract); *SSC*, soluble solids content (°Brix); *TA*, titratable acidity ($g\ L^{-1}$ of malic acid) for flesh and skin, separately; *OAs*, total sum of organic acid content in flesh and skin, separately; *CIT*, citrate; *GAL*, galacturonate; *MAL*, malate; *MAL/CIT*, balance between MAL and CIT content in flesh and skin, separately; *QUI*, quinate; *SUC*, succinate.

judged 'BO04628009', 'BO07628820', 'BO12612078' and 'Emma' for their good FTP, juiciness and absence of mealiness (Supplementary Fig. 9B, C and D).

3.4. Correlation between instrumental measurement and sensory perception

Sour taste was strongly correlated to TA and total OAs content in both flesh and skin (ρ from 0.66 to 0.76; Fig. 5), corroborating the association between the total OAs amount and the apricots sourness. Among the most abundant OAs detected (Table 1), MAL was a poor predictor of the overall sour taste (*p*-value $\leq$ 0.05) which seemed enhanced by QUI and CIT flavor (ρ up to 0.35 and 0.55, respectively) and slightly mitigated by SUC content in flesh (ρ – 0.30). CIT intensity was identified (ρ = 0.42 in flesh and 0.37 in skin) but the estimation of MAL effect on sensory response was weaker (ρ = 0.26 in skin). Accumulation of MAL and SUC in flesh seems to slightly improve the fruitiness intensity (ρ of about 0.38) that, instead, was slightly toned down by CIT and QUI content in skin (p= -0.30 and -0.26, respectively). High acid concentration (ρ between 0.56 and 0.65) as well as the high level of CIT (ρ of about 0.30 for both fruit tissues) and MAL in skin (0.33) increased the astringent feeling, although the interaction between polyphenols and salivary proteins in mouth is the main elicitor of astringency (Pires et al., 2020). On the other hand, polyphenols accumulation in apricot skin slightly enhanced the overall sourness (ρ = 0.26) with an impact also on the *BrimA* index and fruitiness perception (ρ between -0.33 and -0.22). A positive association of juiciness to the intensity of CIT flavor (ρ of 0.40) and sourness (0.24) was found. During the apricot consumption, the drastic breakdown of apricot fruit cells mainly released inorganic and organic acids from vacuoles (ρ up to 0.27) into the oral cavity rather than sugars (ρ of -0.27) which were abundantly perceived in mealy fruits (ρ = 0.43). Total SSC was excellent good predictor of sweetness (ρ = 0.60) improving the aromatic perception (ρ = 0.46), although acid level (ρ = 0.60) had the greatest impact on the overall sweet-sour taste balance (ρ = - 0.66). However, QUI negatively affected sweet taste (ρ = - 0.50) and aroma (ρ up to - 0.36) in both tissues. Fruit firmness was greatly associated to sensory evaluation (ρ = 0.55). Another interesting relationship was observed between red-skinned apricots and sensory perception of sourness (ρ = 0.50) and astringent flavor (ρ = 0.37).

4. Discussion

Several previous studies emphasized the necessity of associating phenotypic data to sensory evaluation to fulfill a more reliable assessment of fruit-eating quality (FQ) (Harker et al., 2002; Piagnani et al., 2013; Xi et al., 2016; Aprea et al., 2017; Fan et al., 2017; Ayour et al., 2020). FQ is evaluated on several physicochemical features, even though mostly reflects sensory perception (i.e. aroma, astringency, sweetness, sourness and their blend) and consumers' preference that encourages the repeated purchase and partially drive the selection of novel accessions (Souty et al., 1990; Ruiz and Egea, 2008; Harker et al., 2002). Malate (MAL) and citrate (CIT) are the two most abundant organic acids (OAs) found in several apricot germplasm collections worldwide, accounting for 95% of the whole OA pool (Table 1 and Fig. 1; Bassi et al., 1996; Chen et al., 2006; Akin et al., 2008; Ruiz and Egea, 2008; Bureau et al., 2006; Ruiz et al., 2010; Xi et al., 2016; Fan et al., 2017; Zhang et al., 2019; García et al., 2019 and 2020). Moreover, MAL and CIT content and ratios are accession-dependent characteristics, with a strong genetic bases and almost unaffected by the environment and/or management conditions (Souty, 1976; Bartolozzi et al., 1997; Etienne et al., 2013; Gurrieri et al., 2001, Baccichet et al., 2022). Nevertheless, apart from titratable acidity (TA), a target selection of these OA-related traits has not systematically introduced in breeding programmes, also due to the poor knowledge on their impact on fruit taste and sensory attributes.

This study aimed to elucidate the specific contribution of organic acids (particularly CIT and MAL) on overall apricot fruit taste perception as well other sensory attributes. Consistent with previous studies, sensory fingerprint confirmed that taste was the pivotal FQ parameters in defining the overall degree of liking and largely driven by the balance (*BrimA* index) between soluble-solids content (SSC) and TA (Bartolozzi et al., 1997; Xi et al., 2016; Aprea et al., 2017; Fan et al., 2017). In our panel, trees were managed in order to explain the maximum quality potential of the fruits, as reflected by the SSC values (with an average of 15 °Brix) and by sensory tests where almost all the analyzed apricot accessions scored sufficient or higher. Also, TA levels were highly representative of the acidity variation in apricot germplasms, determining a wide range of *BrimA* index (Audergon et al. 1991; Bassi et al., 1996; Gurrieri et al. 2001; Ruiz and Egea, 2008; Lo Bianco et al., 2010; Piagnani et al., 2013). Results confirmed the multiplicity of features affecting the final apricot fruit taste, suggesting a greater impact of sourness over sweetness. Sourness attribute was almost perfectly explained by *BrimA* index, although TA, total OAs content, CIT and/or MAL abundance highly enhanced sour perception. Interestingly, sour taste was somewhat correlated with astringency, although this last was scarcely related with total phenol content. In contrast, the MAL/CIT balance seems not to play a pivotal role in sourness perception, not showing a clear rank for the range of high/low ratios. However, variations of MAL/CIT ratio tend to be perceived by panelists and associated to different sensory properties specific of each compound. This aspect was in part supposed by Dolenc-Šturm et al. (1999), assuming that cultivars with higher CIT content were more appreciated compared to those with higher MAL. However, in our panel, sour taste prediction tend to be positively correlated to CIT content and very slight to MAL, with panelists able to perceive the different intensity in cases where MAL was more abundant than CIT. The different solubility reported for CIT (960 g L^{-1} in water at 25°C) and MAL (845 g L^{-1} in water at 25°C), the greater ionic pK_a strength of CIT in water (*pKa*1 =3.12, *pKa*2= 4.76 and *pKa*3= 6.40; Goldberg et al., 2002) and a dissimilar acid perception threshold at the same pH might confound the sensory response in mouth, supporting the larger contribution of CIT on TA level and sour taste as previously described in peach, grape and *Citrus* (Moing and Svanella, 1998; Colaric et al., 2005; Albertini et al., 2006; Xi et al., 2016; Gancel et al., 2022). At taste level, CIT presence seems associated to a bright and tart flavor with a scarce persistency in mouth while MAL produced a smooth and lingering sourness (Ayour et al., 2020). In our panel, a reduced content of CIT seemed more appreciated by panelists, as clearly demonstrated by the higher pleasantness score of 'LadyCot' compared to 'Pricia'. Among the other most abundant OAs detected, QUI had a great impact on taste, mostly contributing to sour sensation and, thus, contrasting aroma and sweetness in fruit and skin as seen in other fruit such as blueberry (Bett-Garber et al. 2015). QUI seemed involved in aromatic amino acids biosynthesis and chlorogenic acids – such as 5-caffeoylquinic acid (5-CQA) largely detected in coffee beans and apples-that globally confer a bitter sensation (Leuschner et al. 1995; Kahle et al., 2005; Farah et al., 2006; Perrone et al., 2010). Succinate (SUC) in apricot flesh enhanced fruitiness and sweetness intensity confirming the low acidifying power in mouthfeel sensation (Chimirri et al., 2010).

Several other fruit internal physic parameters affected taste perception (and overall pleasantness), as evidenced by the correlation between mealiness and sweetness (ρ = 0.43) and between juiciness and CIT intensity (ρ = 0.40). This study assessed only flesh firmness among fruit texture-related properties, although apricot accessions were greatly diversified (minimum- maximum range of 0.7 – 31.6 N; Table 1). Nevertheless, this topic remains almost unexplored in apricot (Souty et al., 1990; Piagnani et al., 2013) compared to peach (Peace et al. 2005; Ciacciulli et al., 2018) and apple (Harker et al. 2002; Gatti et al., 2011; Di Guardo et al., 2017).

Clearly, this study only allows to draw preliminary conclusion. The involvement of a single-stand and country-specific trained tasters might limit our results to a restricted apricot germplasm material. Although the evaluated apricot accessions derived from different programmes

(France, Spain, Italy and US) and embrace a wide fruit type variation, cross-cultural habits can hamper the standard FQ assessment criteria, being strictly related to the different sensory characteristics experienced in fresh fruit consumption. In apricot, a preference for low-acid fruit types have been found although without testing a “country-of-origin” effect in sensorial appreciation (Valentini et al., 2006; Tricon et al., 2010; Piagnani et al., 2013). These preferences for sweetness by panelists can be also exacerbated by the poor quality of apricot currently on the market, often characterized by an unbalanced *BrimA* index (e.g. due to low sugar amounts caused by premature harvesting and/or over-cropping) that merely increases the sour perception (as particularly evident in early ripening cultivars) (Ruiz and Egea, 2008). Thus, the role of CIT and MAL ratio on overall apricot fruit taste perception as well other sensory attributes need to be further confirmed. Although out of the scope of the present work, a second limitation was the lack of instrumental measurement for fruit aroma, which was only evaluated in sensory panel assay. The phenotypic characterization of olfactory profile would separate the mutual effect of volatile organic compounds (VOCs) and OAs (mostly MAL and CIT) on both fruit taste and flavor. Certainly, VOCs level tend to be much variables and lower than SSC and OAs in fruit but, not for that, they have a negligible effect in determining the overall eating pleasantness. A systematic characterization of VOCs, CIT and MAL with their contribution on apricot flavor might avoid the selection of individual with a scarce olfactory intensity and a high taste profile, a challenging aspect in breeding programs oriented toward an improved fruit quality. Furthermore, the identification of aromatic profiles that enhanced the taste perception might pursue the nuanced variations expected by consumers of fresh apricots.

5. Conclusions

This study attempted to clarify the specific contribution of citrate and malate to apricot acidic taste, providing evidences about a larger contribution of citrate rather than malate to sour perception, with a different impact on overall apricots pleasantness. Fine tuning of citric and malate acid content and their balance should be considered a valuable breeding objective to achieve an improved apricot flavor in order to meet consumer’s satisfaction and bridge the gap between excellent appearance and bland taste.

CRediT authorship contribution statement

Irina Baccichet: Data curation, Formal analysis, Investigation, Writing – original draft. **Giulio Alessandro Tagliabue:** Data curation, Formal analysis. **Cassia da Silva Linge:** Data curation, Formal analysis. **Debora Tura:** Data curation. **Remo Chiozzotto:** Data curation. **Daniele Bassi:** Funding acquisition, Project administration, Resources. **Marco Cirilli:** Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112266.

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