Scientia Horticulturae 233 (2018) 29–37

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

# Relatively high acidity is an important breeding objective for fresh juice-specific apple cultivars

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ARTICLE INFO

*Keywords:*
Acid
Juicing cultivar
Quantitative trait loci
*M. asiatica*
*Malus domestica*

ABSTRACT

Minimally processed fresh apple juice is a popular processed food product as it preserves the fruit's flavor and nutrients. To better understand the genetics of traits related to the quality of freshly squeezed apple juice (FS) in apple cultivars, we phenotyped hybrids derived from five biparental crosses of *Malus asiatica* 'Zisai Pearl', and *M. domestica* 'Red Fuji', 'Golden Delicious' and 'Jonathan' for a total of individuals 2575 in the year 2015 and 2023 in the year 2016. We also studied the inheritance of these traits with a quantitative trait loci (QTLs) analysis. Our results indicate that fruit juice yield, total soluble solid content (TSSC), pH value, light transmittance (TRA), and color traits (CIE coordinates, $L^*$, $a^*$, and $b^*$) segregated quantitatively. We measured the average broad sense heritability and coefficient of variance to be 32.1–68.9% and 8.11–132.22%, respectively. Results from an untrained sensory evaluation panel indicated that FS with sour-sweet flavor, golden yellow color, moderate clarity and green apple aroma were most preferred. The overall sensory scores were closely related to TSSC, titratable acids (TA) and $L^*$ value. FS acidity (TA or pH) correlated with all physiochemical parameters except for polyphenol contents. We detected thirteen QTLs for juice yield, pH, TRA and $b^*$, while QTLs for pH value co-localized with TRA and $b^*$ on linkage group 16 of both 'Zisai Pearl' and 'Red Fuji'. In summary, juice acidity, affecting juice flavor, color, clarity and the overall sensory scores, is an important breeding objective for FS specific apple cultivars.

## 1. Background

World apple production mounted up to 84.6 million tonnes in year 2014 and exhibited an ever-growing trend (FAOSTAT, 2015). Approximately 35% of the total apple annual production is used for processed food, of which apple juice and cider represent up to 16% (Nicklas et al., 2015). Apple juice is the second most commonly consumed fruit juice on the world market, just following orange juice (Abel and Aidoo, 2016). Apple products and whole apple consumers are respectively 25% and 30% less likely to be obese than non-consumers (Nicklas et al., 2015; O'Neil et al., 2015). Two liters of cloudy apple juice may cover the consumers' daily requirements of inorganic cations such as K+, Mg2+ and Ca2+ (Sager and Gössinger 2015).

The end-products of 100% apple juices include juices reconstituted from concentrate (FC), naturally pasteurized juices not from concentrate (NFC), and freshly squeezed non-pasteurized juice (FS) (Włodarska et al., 2017). In the last few years, a fast-growing trend in juice market is the preference of minimally processed products, characterized by their sensory and physicochemical profiles, and health-related properties very similar to those of fresh fruit. Consumers favor FS or high hydrostatic pressure processed juice than for thermal pasteurized apple juice, especially when the origin and pressing methodology of juice were present on the label (Lee et al., 2016; Rihn and Yue 2016). The apples used for juice are often non-commercial apples or off-grade dessert apples, thus, consumers rate these mainstream juices as having low palatability when compared with single-cultivar FS and blend FS. In contrast, consumers and panelists rate FS juice higher than NFC cloudy juices and clear FC juice (Stolzenbach et al., 2016; Włodarska et al., 2016). In summary, single-cultivar FS apple juice should be the paramount occupant of the juice market in the near future, to meet the future consumer's demands, apple juice producers should focus on single-cultivar FS apple juices.

Due to the high ranked sensory attributes and health-related properties of single-cultivar FS apple juices, apple cultivars destined for

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https://doi.org/10.1016/j.scienta.2018.01.026
Received 11 October 2017; Received in revised form 11 January 2018; Accepted 14 January 2018

juicing must be of good edible quality, full flavored, and able to ensure good appearance of the juice. Sugar content is the most prominent factor affecting taste, but the dominant driving force for consumers' sensory preference is the sugar/acid ratio. Apple juice of very good quality should meet a °Brix/acid ratio of 21:1–53:1 (Jaros et al., 2009; Stolzenbach et al., 2016). European consumers generally favor apple juice with sugar/acid ratio between 15 and 18, however, a fraction of people does prefer apple juice with higher sugar/acid ratio, i.e. from 'Golden Delicious' (28) and 'Jonagold' (27) (Jaros et al., 2009). An assessment of ninety apple juice samples available on the Polish market, with a sugar/acid ratio, ranging from 15 to 38, indicate that this is the key characteristic determining the flavor of fruit products (Włodarska et al., 2017). In short, to obtain an optimal flavor of single-cultivar apple juice, sugar content plays the fundamental role while acidity is the indispensable regulatory factor.

Apart from sweet and sour taste, polyphenolic composition gives a significant contribution to the flavor of these products and to their antioxidant activity. Apple juice has strong antioxidant activity, dependent on polyphenolics and ascorbic acid (Schilling et al., 2008). The total antioxidant activity of quercetin, epicatechin, and procyanidin B2 in apples is much higher than vitamin C (Lee et al., 2003). Apples and processed apple products provide 22% of the fruit phenols per capita for the US population and are the third largest resources of flavonoids in the diet in the Netherlands (Hertog et al., 1993; Scalbert and Williamson, 2000). Apples contain around 2.0 g of phenols per kilogram of fresh weight, whose antioxidant activity is equivalent to 3.0 g of pure vitamin C (Eberhardt et al., 2000; Lotito and Frei, 2006).

Both sensory profiles and biochemical properties of apple juices are highly dependent on the cultivars. The apple cultivar influences the characteristics of the deriving juice more than fruit ripening stage, year of harvest, orchard management system or centrifugation of the crude juice (Van Der Sluis et al., 2001a, 2001b; Guyot et al., 2003; Bourvellec et al., 2015; Alberti et al., 2016). Soluble solid content varies between 10.3°Brix and 16.2°Brix in FS or pasteurized NFC juices derived from 28 apple cultivars (Ai-Turki et al., 2008). Previous studies indicated the traditional apple cultivars, such as 'Kronprinz Rudolf', are most suitable for single-cultivar FS juices, as they are characterized by high sugar, relatively high acidity, proper sugar to acid ratio, and high total phenolic content (Persic et al., 2017). Moreover, wild *Malus* species contain higher concentrations of phenols than domesticated apple cultivars (Farneti et al., 2015; Volz and Mcghie, 2011; Zhao et al., 2015).

French commercial juicing cultivars 'Chanteline', 'Frequin Rouge', 'Judaine', and 'Judeline' have a much higher antioxidant activity and lower total soluble solid content/titratable acid ratio (TSSC/TA) or total sugar/acid ratio (Ai-Turki et al., 2008; Markowski et al., 2015). For dessert apple cultivars, several cultivars such as 'Crimes Golden', 'Red Delicious', 'Szampion', and some Chinese domestic cultivars as 'Baishaguo' (*Malus asiatica*) have a total phenolic content higher than the other cultivars as 'Royal Gala' and 'Pink Lady' (Girschik et al., 2017; Zhao et al., 2015). These indicate the possibility to select juice-specific apple cultivars from *M. domestica* or its relative species.

To better understand the role of acidity in relation to sensory attributes and physicochemical characteristics of fresh apple juice for breeding purposes, we analyzed the segregation, inheritance, and quantitative trait loci (QTLs) of juice quality traits in five hybrid populations in year 2015 and 2016.

## 2. Materials and methods

### 2.1. Plant material

Fruits of F1 hybrids derived from five crosses ('Zisai Pearl' (Z) × 'Red Fuji' (F), 'Zisai Pearl' × 'Golden Delicious' (G) and their reciprocal crosses, and 'Jonathan' (J) × 'Golden Delicious') were used as raw materials for fresh juice (Table 1). Z is a Chinese domestic apple cultivar which belongs to *Malus asiatica* taxa, from which we expected to introduce disease resistance and small fruit trait into domesticated apple cultivars (Abe et al., 2007; Zhang et al., 2010). The other three parents are commercial dessert apple cultivars belonging to *M. domestica*. J × G cross was made in 2002 and other four crosses were made in 2007. The hybrids on their own roots were planted in a density of 0.5 × 2.5 m, and maintained under conventional management and pest control. In year 2015 and 2016, ripe apples (300–600 g of flesh per individual hybrid) with consistent maturity determined by peel color, flesh taste, and seed color, were harvested from around the canopy and juiced using a FSL-7100 kitchen juicer (Fissler, Germany).

**Table 1**
Number of hybrids of five apple cross populations phenotyped in year 2015 and 2016.

| Hybrid crosses | Total number of hybrids | Number of hybrids bearing enough fruits | |
|---|---|---|---|
| | | Year 2015 | Year 2016 |
| Zisai Pearl × Red Fuji | 2637 | 752 | 631 |
| Red Fuji × Zisai Pearl | 1372 | 354 | 250 |
| Golden Delicious × Zisai Pearl | 276 | 70 | 40 |
| Zisai Pearl × Golden Delicious | 3569 | 1151 | 737 |
| Jonathan × Golden Delicious | 1800 | 164 | 255 |
| Total | 9654 | 2575 | 2023 |

### 2.2. Juice yield

Juice yield was calculated using the following equation:

$$\text{Juice yield} = \frac{m_{juice}}{m_{fruit}} * 100\%.$$

Where $m_{fruit}$ and $m_{juice}$ symbolize the weight of fruit used for juice squeezing and the weight of juice obtained, respectively. After weighing, the juice was filtered with 200-mesh gauze and stored at 4 °C for further measurements.

### 2.3. Sensory evaluation

The sensory profiles of thirty apple juices were evaluated by an untrained panel, consisting of twenty-three assessors recruited from the campus. The appropriate descriptive sensory attributes included flavor (sweet, sour, sour-sweet, sweet-sour, and strong sweet), color (golden yellow, light yellow, creamy yellow, amber, and light brown), aroma (green apple, green grassy, fruity, and caramel) and clarity attributes (clear, moderate, light cloudy, and cloudy) (Cliff et al., 2000). Before testing, the panelists were invested for the importance of flavor, color, aroma and clarity of apple juice (0 = not interested; 1 = not very interested; 2 = sometimes interested; 3 = fairly important; 4 = very important; 5 = extremely important). The panelists were then asked to taste the samples and to evaluate their overall liking using a five-point hedonic scale (0 = dislike extremely; 1 = dislike; 2 = neither like nor dislike; 3 = like; 4 = like extremely) (Włodarska et al., 2016).

### 2.4. Measurement of physicochemical parameters

TSSC was measured using a MASTER-S10 M refractometer (ATAGO, Japan) after calibrating with distilled water.

pH value was measured using a PH3000 acidometer (STEPS, Germany) after calibrating with two standard samples (pH = 4.0 and 7.0).

Light transmittance was detected under UV wavelength 625 nm by the UV-1800 ultraviolet spectrophotometer (Shimadzu, Japan), and was calculated using the following equation (Nie et al., 2012; General Administration of Quality Supervision, 2012):

$$TRA = 10^{\wedge\,(2-A)}.$$