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Impact on chemical profile in apple juice and cider made from unripe, ripe and senescent dessert varieties

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ABSTRACT

The chemical profile of juices and ciders prepared with three varieties of dessert apples (Gala, Lis Gala and Fuji Suprema) at three different ripening stages (unripe, ripe and senescent) were established. Sixty-five analytical parameters were determined and the data processed using two-way ANOVA and principal component analysis (PCA). The phenolic content and antioxidant capacity in the juices were different between the cultivars. However, after fermentation phenol content reduced (15%) only in the unripe ciders. The juices prepared with unripe fruit contained 55% less volatile compounds than the juices from ripe and senescent fruit, while the cider made with senescent apples contained 24–52% more volatile (Lis Gala and Gala, respectively) than the cider from unripe apples. Variety, ripening stage and their interaction showed significant effect ($p < 0.05$) for most of the variables analysed. PCA analysis was used to classify juices and ciders in relationship with the variables analysed. Variety had a higher impact than ripening stage. The choice of variety and the knowledge of the cultivar composition, which increase or decrease along the maturation, allows at the producer to establish the sensory quality of the apple juice.
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1. Introduction

In 2012–2013, apples (*Malus domestica* Borkh) were the fifth most cultivated fruit in the world (4,842,822 ha) and the second most produced fruit (76,335,000 tonnes). Apples are widely cultivated in temperate climate zones (more than 90 countries) and recently apple production has been expanding into subtropical and tropical climate zones (Brown, 2012; FAOSTAT, 2014).

The majority of world apple production (more than 60%) is sold and consumed as fresh fruit. Gala and Fuji and their clones are among the five most produced varieties due to their high productivity, high nutritive value, and good sensory quality (Shao, Tu, Tu, Su, & Zhao, 2010; Ye, Yue, & Yuan, 2014). Most of apples discarded in the commercial classification process (around 25–35% of the total of production), as well as industrial apple varieties, are used as raw material in the processing of apple juice, apple wine (usually above 7.0 GL and not carbonated) and cider or hard cider (usually below 7.0 GL and may or may not be carbonated) (Brown, 2012; Lea & Drilleau, 2003). In addition, these fruit can be used to obtain vinegar (Natera, Castro, De Valme García-Moreno, Hernández, & García-Barroso, 2003), distilled beverages (Madrera, Valles, Hevia, Fernández, Tascón, & Alonso, 2006), applesauce (Le Bourvellec, Bouzerzour, Ginies, Regis, Plé, & Renard, 2011) and dehydrated apple slice (Lavelli & Vantaggi, 2009).

Fruit can be harvested ripe or unripe for storage in cold rooms or in cold rooms with controlled atmosphere (Paganini, Nogueira, Denardi, & Wosiacki, 2004). Immature fruit are often harvested in order to increase storage performance, lengthen availability in the market, and to minimise physical damage (Song & Forney, 2008). The fruit declassified in this process, or which have problems during storage (cold rooms or cold rooms with controlled atmosphere), may be destined for processing in juices or ciders. Ripe fruits that remain exposed at room temperature (>20 °C) for periods longer than 3 days waiting processing can reach the stage of senescence quickly (Wijewardane & Guleria, 2013). In addition, immature apples without phytopathological defects from fruit

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thinning (around 30 days after full bloom) and selection to storage in a cold room (around 2.0 of iodine value) can be used in blends to increase the acidity of the juice; since these fruits have a high acidity and low sugar content, and therefore enhances the commercial value of product which is established based on the acid content (Reid, Padfield, Watkins, & Harman, 1982; Way & McLellan, 1989).

Thus, the processing of both apple juice and cider can include fruit at different ripening stages, which may change the composition, quality and compromise international commercialisation of the products. The effect of the degree of ripening in apple products has been studied mainly with majority compounds (Zielinski, Braga, Demiate, Beltrame, Nogueira, & Wosiacki, 2014; Etienne, Génard, Lobit, Mbeguié-A-Mbéguié, & Bugaud, 2013; Li, Feng, & Cheng, 2012 and Kader, 1999). However, in order to establish a high quality standard of these beverages is important to evaluate the impact of the variety at different ripening stages on the majority and minority compounds. Therefore, this study aims to generate new information about the chemical composition of juices and ciders elaborated with dessert apples in different stages of maturation.

2. Materials and methods

2.1. Samples

Apples of the 'Fuji Suprema' and 'Lis Gala' varieties (2011–2012 harvest), were collected at the Experimental Station of the Agricultural and Rural Extension Company, (Caçador, Santa Catarina, Brazil). The 'Gala' apple variety (2011–2012 harvest) was collected at Boutin Agricola in Porto Amazonas, Paraná, Brazil. The apples (25 kg) from the same variety were collected at different cardinal points (at the top, centre and bottom) from six trees, at three ripeness stages (unripe, ripe and senescent). The ripening index was determined by using the starch-iodine test (Blanpied & Silsby, 1992). The iodine values for the fruits of all the cultivars were 1 for unripe, 4–5 for ripe, and 8 for senescent.

2.2. Instruments

The HPLC system used was a Waters 2695 Alliance (Milford, MA, USA), composed of a quaternary pump, degasser, and an auto injector. The detectors (Waters; Milford, MA, USA) used in the HPLC system were a Waters RI 2414 refraction index detector, a PDA 2998 photodiode array detector, and an FLD 2475 fluorescence detector. The atomic absorption spectrophotometer used was a Varian Techtron AA-240FS with multi element hollow cathode lamps. The GC system was Young Lin Instrument (YL 6100 GC, Anyang, Korea) equipped with FID, capillary column (30 m × 25 mm, 0.25 μm thick ZB-WAX film; Phenomenex).

2.3. Chemicals

The standards and chemicals used in this study were ethanal, ethyl propanoate, ethyl 3-methyl butanoate, propyl ethanoate, 2-methylpropyl ethanoate, ethyl butanoate, hexanone, 2-heptanone, 2-methyl-1-butanol, 2-hexanol, 2-octanone, ethyl hexanoate purchased from Interchim (Montluçon, France). Ethyl ethanoate, butyl ethanoate, 3-methylbutyl ethanoate, hexyl ethanoate, 2-hydroxy ethyl propanoate, 1-hexanol, ethyl octanoate, ethyl decanoate, butanoic acid, diethyl butanedioate, 2-phenyl-ethanol, ethyl dodecanoate, octanoic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid, gallic acid, phloretin, phloridzin, (+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2, quercetin-3-D-galactoside, quercetin-3-β-D-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-rutinoside, myricetin, kaempferol, sucrose, D-glucose, D-sorbitol, ethanol, Folin–Ciocalteau reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine), and DPPH (2,2-diphenyl-1-picrylhydrazyl) were all acquired from Sigma–Aldrich (Steinheim, Germany). D-Fructose, 3-methyl-1-butanol, nitric acid and perchloric acid were purchased from Merck (Darmstadt, Germany). Acetonitrile and acetic acid, HPLC grade were purchased from J. T. Baker (Phillipsburg, NJ, USA). All other solvents were of analytical grade. The aqueous solutions were prepared using ultra-pure water (Millipore, São Paulo, Brazil).

2.4. Processing of apple beverages

2.4.1. Processing of apple juice

The fruits (25 kg for each variety and ripening stage totalling 225 kg) were selected, cleaned and fragmented in a microprocessor (Metvisa, Brusque, SC, Brazil). The crushed apples were conditioned and stacked in polyethylene screen packages and were subjected to a pressure of 294 kPa for 5 min (Hoppe Ind. Ltda., Gravataí, RS, Brazil). The apple juice yields ranged from 50 to 60%. The juice was treated with pectinase (Pectinex® Ultra Clear, LNF Bento Gonçalvez – Novozymes Latin America, RS, Brazil) at 3.0 mL/hL (25 °C, 2 h) and one part was stored at −18 °C until further analysis. The apple juice processing was performed in duplicate.

2.4.2. Processing of cider

The other part of the depectinised apple juice or apple must was placed in fermenters (500 mL) and inoculated (10^6 cells/mL) with *Saccharomyces cerevisiae* Bouquet (Fermol Bouquet, ref. PB 2004, AEB Group, Italy). Alcoholic fermentation occurred during 12 days in anaerobiosis at 20 °C (the time required for complete exhaustion of fermentable sugars in the used conditions). The cider was centrifuged at 10,200 *g* at 5 °C (Hitachi Himac CR21GII centrifuge, Tokyo, Japan) for 20 min and then was racked, bottled and frozen (−18 °C) until further analysis. The cider processing was performed in duplicate.

2.5. Instrumental and physicochemical analysis

2.5.1. Chromatographic analysis of sugar and ethanol

For the quantification of sugars, the method described by Zielinski et al. (2014) was employed. The samples of apple juice and cider at different stages of ripeness were diluted 1:10 (mL/mL) with ultrapure water and then filtered through a membrane prior to analysis. The injection volume was 10 μL and flow was 0.5 mL/min in isocratic condition with ultrapure water (Milli-Q). The column used was a Waters Sugar Pak™ 1 (300 × 6.5 mm) and detection of the samples was established by comparing the retention times of the reference standards. The quantification was calculated for the calibration curves of sucrose, D-glucose, D-fructose, D-sorbitol and ethanol.

2.5.2. Mineral analysis

The samples (0.25 mL) were prepared by acid digestion with 4 mL of nitropercloric solution (nitric acid (65%) and perchloric acids (70%), 1:3 mL/mL) for 2 h at 50 °C. The calcium, copper, iron and magnesium content was analysed by atomic absorption spectrophotometry (AOAC, 2003). For the analysis of sodium and potassium, a drop of 5 g/L phenolphthalein and dripped NH_4OH 1:1 (mL/mL) was added to the samples until they reached a pink colour and the readings were then performed using a flame-emission spectrophotometer (FES) (Micronal model 262 B). The phosphorus content was determined using the colourimetric method with ammonium molybdate-vanadate solution, according to the

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