LWT - Food Science and Technology 57 (2014) 223–229

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Effect of mash maceration and ripening stage of apples on phenolic compounds and antioxidant power of cloudy juices: A study using chemometrics

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ARTICLE INFO

Article history:
Received 27 June 2013
Received in revised form
17 January 2014
Accepted 19 January 2014

Keywords:
Malus domestica Borkh
Polyphenol
Pectolytic enzyme

ABSTRACT

The effects of different enzymatic preparations on total phenolic content, phenolic profile (HPLC), and ferric reducing antioxidant power (FRAP) of cloudy juices from *Lis Gala* and *Fuji Suprema* apples varieties, at three ripening stages (unripe, ripe and senescent) were investigated using Principal Component Analysis and Hierarchical Cluster Analysis. The commercial preparations enzymatic (Ultrazym® AFPL; Pectinex® Ultra Clear; Pectinex® SMASH XXL; Panzym® YieldMASH) increased the total phenolic compounds and ferric reducing capacity of the cloudy juice from unripe and ripe *Lis Gala* (respectively by 67 and 49% for unripe apples, and 28 and 33% for ripe apples) and unripe *Fuji Suprema* apples (23 and 55%), while for the ripe *Fuji Suprema* apples only Pectinex® Ultra Clear and Panzym® YieldMASH had this effect. No significant ($p > 0.05$) was observed on senescent stage, whatever the enzymatic preparation. Enzymatic preparations could increase phenolic compounds concentration and antioxidant capacity of cloudy apple juice, but this effect depended on the maturity of the apples.

1. Introduction

The phenolic compounds in apple products, such as juice and cider, are considered to be important because they influence some important quality parameters, such as color, acidity and astringency, aromas and clarification (Mangas, Rodriguez, Suarez, Picinelli, & Dapena, 1999). There has been a growing interest in phenolic compounds present in many food preparations due to their antioxidant capacity, which contributes to protect human health from the deleterious effects of oxidative stress events (Oszmianski, Wojdylo, & Kolniak, 2009; Ribeiro, Henrique, Oliveira, Macedo, & Fleuri, 2010). The major classes of phenolic compounds found in apples are phenolic acids and flavonoids. The main representatives of phenolic acids are 5-caffeoylquinic acid (chlorogenic acid) and *p*-coumaroylquinic acid. The main flavonoids are flavan-3-ols (epicatechin, catechin and procyanidins), dihydrochalcones (phloretin as phloridzin, its glucoside, and xyloglucoside), flavonols (quercetin glycosides) and anthocyanins (cyanidin galactoside), the last two being as a general rule exclusively present in the peel (Awad, De Jager, & Van Westing, 2000; Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005; Vanzani et al., 2005).

The apple cultivar has a major effect on the juice phenolic composition since cultivars differ greatly in their phenolic compounds content (Will, Roth, Olk, Ludwig, & Dietrich, 2008). The application of pectinolytic enzymes in the production of apple juices results in a higher extraction of phenolic compounds, and a higher antioxidant capacity of the juice (Oszmianski, Wojdylo, & Kolniak, 2011; Oszmianski et al., 2009; Will, Ludwig, Dietrich, Schulz, & Otto, 2002). The ripening stage of the fruit to be processed also has some influence (Zhang, Li, & Cheng, 2010).

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0023-6438/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2014.01.029

Apples are harvested at different degrees of ripeness; fruits that are at an unripe stage (pre-maturation) are destined for cold-storage and ripe fruits are readily marketed (Harker & Hallett, 1992). Apples destined for the industrial sector are those that do not have acceptable physical characteristics (appearance, size and shape). In some countries, such as Brazil, apples that are rejected for marketing are processed during warm periods (25–35 °C) and fruits usually remain at the reception point for hours or even days, which speeds up the ripening process (Nogueira & Wosiacki, 2012). In this sense, there are three different ripening stages in apple processing: unripe (pre-maturation), ripe or full ripe, and senescent phase.

In apple juice processing, many different quality properties are usually studied, and this fact makes necessary the use of accurate and innovative classical mathematical and statistical approaches instead of only univariate comparisons among samples. Herein, the application of chemometric tools for the characterization, determination of origin, and quality control of food products has been increasingly used in food research. There are many applications of multivariate statistical techniques in order to explore and classify the antioxidant capacity and major phenolic compounds present in many foods, including apple-based products (Çam, Hisil, & Durmaz, 2009; Hossain, Patras, Barry-Ryan, Martin-Diana, & Brunton, 2011; Braga et al., 2013). Consequently, this study aimed to assess the effects of mash maceration with different commercial pectinases and ripening stage (unripe, ripe and senescent) of apples (*Lis Gala* and *Fuji Suprema*) on phenolic composition and ferric reducing antioxidant power of cloudy juices using multivariate statistical techniques.

2. Material and methods

2.1. Apple samples

Apples from *Lis Gala* and *Fuji Suprema* varieties, harvested in 2011–2012, were collected in Caçador, Santa Catarina, Brazil at the Experimental Station of the Agricultural and Rural Extension Company, Santa Catarina (EPAGRI) at three different ripening stages (unripe, ripe and senescent), with about 20 kg of samples for each ripening stage and each variety. Apples from the same cultivar were collected at different cardinal points from six different trees, and the top and the bottom of those trees, to homogenize the samples. The maturation index was determined by using the Starch-iodine test (Reid, Padfield, Watkins, & Harman, 1982). The fruits at different ripening stages were weighed (60 units for variety) and measured with a digital pachymeter (Insize, Waregem, Belgium) in relation to height and diameter (Table 1).

2.2. Chemicals and enzymes

Folin–Ciocalteau reagent; Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), TPTZ (2,4,6-Tri (2-pyridyl)-s-triazine), chlorogenic acid, phloridzin, (+)-catechin, procyanidin B1, procyanidin B2, quercetin-3-D-galactoside, quercetin-3-β-D-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-rutinoside were purchased from Sigma–Aldrich (Steinheim, Germany) and acetonitrile from Merck (Darmstadt, Germany). Commercial pectinolytic preparations: Ultrazym® AFPL; Pectinex® Ultra Clear; Pectinex® SMASH XXL were provided by LNF Bento Gonçalves – Novozymes Latin America and Panzym® YieldMASH was donated by Begerow (Germany).

Table 1
Physical characteristics of *Lis Gala* and *Fuji Suprema* at different ripening stages.

Variety	Ripening stage	[a]Maturation index	Weight (g)	Height (mm)	Diameter (mm)
Lis Gala	Unripe	1.0	110 ± 22	60 ± 4	56 ± 6
	Ripe	3.5	146 ± 31	68 ± 5	63 ± 6
	Senescent	4.5	165 ± 21	71 ± 55	66 ± 6
Fuji Suprema	Unripe	1.0	172 ± 42	75 ± 7	63 ± 7
	Ripe	3.5	183 ± 58	77 ± 9	64 ± 8
	Senescent	4.5	175 ± 44	76 ± 7	64 ± 7

Note: The physical measurements were performed on 60 fruit pieces and data are presented as mean ± standard deviation.

[a] The iodine regression test is an indicator of residual starch in fruits, lower values indicate less ripe. No standard deviation given as this was a visual comparison to the standard table of the maturity index for apples.

2.3. Enzymatic maceration in cloudy juice processing

The apples were selected, washed, sanitized (100 mg/L of sodium hypochlorite, 25 °C/15 min) and fragmented (ground) in an industrial blender LAR2 (Metvisa, Brusque, SC, Brazil). The apple pulp (100 g) was placed in each Erlenmeyer and conditioned at 35 °C in a shaker MA832 (Marconi, Piracicaba, SP, Brazil) under agitation (150 rpm/5 min). After temperature stabilization, enzymatic preparations were added to the apple pulp according to the manufacturer's recommendation and reaction was conducted for 1 h. Then, the Erlenmeyers were placed in a bath of boiling water (for 1 min) for the denaturation of the enzymes added. The supernatants, denominated laboratory juice in this study, were obtained by centrifugation (8000×*g*, 20 min) with a laboratory centrifuge HIMAC CR-GII (Hitachi, Ibaraki, Japan). Three replications were completed for each treatment. A triplicate control was performed under the same conditions but without enzyme addition. Juices were stored at −20 °C for further analysis.

2.4. HPLC analysis of phenolic compounds

The HPLC analysis of phenolic compounds (monomers and dimers) was based on the methodology described by Alberti et al. (2014). Apple juices (4 mL) were freeze-dried (model LD, Terroni, São Paulo, SP, Brazil) and reconstituted with a solution (2 mL) of 25 mL/L acetic acid and methanol. Then, samples were filtered through a syringe filter 0.2 μm (Nylon) prior to analysis. The HPLC apparatus was a 2695 Alliance (Waters, Milford, MA, USA), with photodiode array detector PDA 2998 (Waters, Milford, MA, USA), quaternary pump and auto-sampler. Separation was performed on a Symmetry C18 (4.6 × 150 mm, 3.5 μm) column (Waters, Milford, MA, USA) at 20 °C. The mobile phase was composed of solvent A (25 mL/L of acetic acid) and solvent B (acetonitrile). The following gradient was applied: 3–9% B (0–5 min), 9–16% B (5–15 min), 16–36.4% B (15–33 min), followed by washing and reconditioning of the column. The flow rate was 1.0 mL/min, and the runs were monitored at 280 nm (flavan-3-ols and dihydrochalcones), 320 nm (hydroxycinnamic acids), 350 nm (flavonols) and 520 nm (anthocyanins). Quantification was performed using calibration curves of standards (at least 5 concentrations were used to build the curves) described in chemicals (see Section 2.2). All determinations were performed in triplicate samples.

2.5. Total phenolic content

The total phenolic content was determined by colorimetric analysis using Folin–Ciocalteau reagent as described by Singleton and Rossi (1965). In a test tube, 8.4 mL of distilled water, 100 μL of sample or (+)-catechin (standard, 10–400 mg/L), and 500 μL of Folin–Ciocalteau reagent were added and the mixture was vortexed for 10 s. After 3 min, 1.0 mL of saturated sodium carbonate was added into each tube and the tube was agitated immediately in a vortex. After 1 h, the absorbance was measured using a spectrophotometer (model mini UV 1240, Shimadzu, Tokyo, Japan) at 720 nm. The total phenolic content was expressed as catechin