LWT - Food Science and Technology 98 (2018) 366–371

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Effect of pasteurization on *in vitro* α-glucosidase inhibitory activity of apple juice

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ARTICLE INFO

Keywords:
Apple juice
α-Glucosidase inhibition
Pasteurization
Type 2 diabetes
Acarbose

ABSTRACT

The in vitro α-glucosidase inhibitory activity of raw, mildly ($F^{5}_{71.7}$ = 0.4 min, 5 Log reductions of *Cryptosporidium parvum*) and intensively (F^{12}_{90} = 14.8 min, 2 Log reductions of *Alicyclobacillus acidoterrestris*) pasteurized apple juice was studied. Raw apple juice (23 mg_{dw}/mL) caused 90% α-glucosidase inhibition. Analogous results were obtained for the mildly treated sample. The most intense treatment reduced by 35% the α-glucosidase inhibition. However, such a decrease was associated with an increase in the phenolic content, suggesting that α-glucosidase inhibition might not rely on these compounds, but depend on more complex mechanisms. Apple juice was combined with acarbose to investigate their interaction towards α-glucosidase inhibition. A synergistic behavior was observed for concentrations < 2 mg/mL. Increasing the concentration of the combined system (up to 9 mg/mL) produced an antagonistic effect, while a further increase (< 9 mg/mL) allowed approaching an addictive behavior.

1. Introduction

Apple consumption is known to reduce the risk of chronic diseases, such as cancer, cardiovascular diseases and type 2 diabetes (Boyer & Liu, 2004; Guo, Yang, Tang, Jiang, & Li, 2017). The protective effect has mainly been attributed to polyphenols, and in particular to the chemical families of flavones (e.g. luteolin, apigenin), flavonols (e.g. quercetin, kaempferol), flavanols (e.g. catechin, epicatechin), hydroxycinnamic acids (e.g. chlorogenic acid) and anthocyanidins (Boyer & Liu, 2004; Hanhineva et al., 2010; Shoji et al., 2017). Apple phytochemicals affect carbohydrate metabolism and glucose homeostasis at different sites (Hanhineva et al., 2010). Individual phenolic compounds (e.g. catechol, catechin, chlorogenic, ferulic and caffeic acid) extracted from apple reduced intestinal glucose uptake through SGLT1 transporter inhibition (Schulze et al., 2014). Some phenolic compounds also inhibited the enzyme α-glucosidase, which plays a key role during carbohydrates digestion (Agustinah, Sarkar, Woods, & Shetty, 2016; Tadera, 2006). Bortolotto and Piangiolino (2013) reported that an apple extract inhibited the activity of α-amylase and α-glucosidase by 70% and 90%, respectively. Despite these studies showed the potential of apples in facing the risk of type 2 diabetes, the relationship between the whole fruit intake and the reduced diabetes risk has not been fully elucidated yet. Most effects have actually been demonstrated on simplified systems obtained upon extraction of bioactive compounds from the original matrix (Williamson, 2013).

Apple juice is the most consumed apple derivative since more than 20% of freshly harvested apples are consumed as juice (Schulze et al., 2014). Apple juice production implies several technological interventions, among which are skin and pomace removal, enzymatic depectinization, and pasteurization. These technological treatments, which are intended to improve the stability of fruit and vegetable derivatives, significantly affect the phenolic content of the final product (Schulze et al., 2014; Van Buren, De Vos, & Pilnik, 1976) and thus its potential health benefits. To our knowledge, no data regarding the effect of pasteurization on the ability of apple juice to inhibit α-glucosidase are available. Therefore, the aim of the present study was to investigate the effect of pasteurization on the *in vitro* inhibitory activity of apple juice against α-glucosidase. Apple juice was subjected to a conventional thermal treatment to obtain 5 Log reductions of *Cryptosporidium parvum* (FDA, 2004), or to a more intense pasteurization to achieve 2 Log reductions of *Alicyclobacillus acidoterrestris* (Silva & Gibbs, 2001). Further, since the drugs currently used to treat type 2 diabetes often carry undesired side effects (Kumar & Sinha, 2012), for the first time the interaction between apple juice and acarbose was studied. Acarbose was chosen because it is widely used as a therapy for type 2 diabetes. The purpose was to understand whether the combination of juice and acarbose might allow drug dosage reduction while keeping the efficacy against α-glucosidase.

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https://doi.org/10.1016/j.lwt.2018.08.065
Received 27 June 2018; Received in revised form 29 August 2018; Accepted 31 August 2018
Available online 31 August 2018

2. Materials and methods

2.1. Chemicals and materials

Methanol (MeOH), formic acid (HCOOH), fructose, glucose, sucrose, (+)-catechin, (−)-epicatechin, chlorogenic acid, phloridzin, phloretin, 3-hydroxycinnamic acid (internal standard; I.S.), α-glucosidase, 4-nitrophenyl-α-D-glucopyranoside, and acarbose were purchased from Sigma-Aldrich (Milan, Italy). Quercetin-3-*O*-galactoside, procyanidin B2, and epigallocatechin gallate were obtained from ExtraSynthese (Lyon, France). Quercetin-3-*O*-arabinoside and quercetin-3-*O*-rhamnoside were purchased from Carbosynth (Berkshire, UK). Milli-Q grade water was produced by Elgastat UHQ-PS system (ELGA, High Wycombe Bucks, UK).

Solid phase extraction (SPE) columns ISOLUTE C18, 1 g, 6 mL were from Biotage (Milan, Italy).

2.2. Sample preparation

A 10 kg batch of apples (Malus domestica Borkh., cv. Golden Delicious) were purchased at the local market and maintained at 7 °C until use. Apples were washed, wiped and the juice was extracted (Ariston Hotpoint Slow Juicer, Fabriano, Italy) at 4 °C to minimize enzymatic browning. The juice was centrifuged at 5000 g for 5 min at 4 °C (Beckman Avanti J-25 Beckman Instruments Inc., Palo Alto, CA, USA) and filtered through filter paper. Approximately 1.5 kg of apples were used for each replicate. Ten mL aliquots of apple juice were poured into 20 mL capacity glass vials (Vetrotecnica, Padova, Italy), which were closed with screw caps and kept refrigerated. Samples were subjected to technological treatments within 10 min after preparation.

2.3. Pasteurization

Thermal treatments were performed in a silicone oil bath (Haake Phoenix B5, Thermo Electron Co., Karlsruhe, Germany). Samples were pasteurized by applying two different time-temperature combinations. A copper-constantan thermocouple probe (Ellab, Denmark), whose tip (2.0 mm) was placed in the coldest point of the sample (i.e. at two-thirds of depth in glass vials), measured temperature changes of apple juice during pasteurization. The thermal effect F (min) was computed using Equation (1) (Ball, 1923):

$$F = \int_0^t 10^{(T-T_{ref})/z}\, dt \quad (1)$$

where T_{ref} is the reference temperature, T is the actual temperature of the treatment (°C), t is the time (min). The first treatment ($P_{71.7}$) provided a sterilizing equivalent to 0.4 min at 71.7 °C and aimed at achieving 5 Log reductions of *Cryptosporidium parvum* ($D_{71.7}$ = 3 s and z = 5 °C) (FDA, 2004). The second treatment (P_{90}) provided a sterilizing equivalent to 14.8 min at 90 °C and aimed at reducing by 2 Log *Alicyclobacillus acidoterrestris* (D_{90} = 7.4 min and z = 12 °C) (Silva & Gibbs, 2001). After treatment, the samples were rapidly cooled in a spray of water until they reached a temperature of approximately 30 °C. Apple juice not subjected to heat treatment was taken as a control.

2.4. Total solid content and pH

The total solid content was measured by a gravimetric method (AOAC Official Method 925.009 (1995). pH was measured by a pHmeter (HANNA Instruments, pH 301, Padova, Italy).

2.5. Sugar content

The method by Englyst, Englyst, Hudson, Cole, and Cummings (1999) was followed, upon slight changes. Apple juice was mixed with methanol (1:5, mL: mL), left at room temperature for 1 h and centrifuged at 4000 g for 10 min at 4 °C (Beckman Avanti tm J-25, Beckman Instruments Inc., Palo Alto, CA, USA). The supernatant was analyzed using an HPLC pump (LC-10AT VP, Shimadzu, Japan) equipped with a refractive index detector (RID-10A, Shimadzu, Japan). An inverse phase apolar C18 column (5 µm, 250 × 4.6 mm) was used (Grace Davison Discovery Sciences, Alltima, Lokeren Belgium). The injection valve (Rheodyne, Sigma-Aldrich, Milano, Italia) was equipped with a 20 µL plastic loop and samples were injected using a syringe (SGE LC, 100 µL, FN). The mobile phase was represented by acetonitrile and deionized water (70:30, mL: mL) and 1.3 mL/min flow rate was applied. Quantitative analysis of sugars was carried out by comparing the sugar peak area with the results of calibration lines obtained by injecting fructose, glucose, and sucrose standard solutions serially diluted. Calibration lines were linear ($R^2 > 0.995$) in the 1.0–250.0 g/L concentration interval.

2.6. Phenolic composition

2.6.1. SPE purification

Ten µL 3-hydroxycinnamic acid (50 µg/mL) methanolic solution as internal standard and 1 mL juice was diluted with 2 mL deionized water and loaded on a C18 SPE column previously conditioned with 5 mL of 2 mL/L formic acid in methanol and 5 mL of 20 mL/L formic acid in water.

After loading, the column was washed with 10 mL of 20 mL/L formic acid in water and the phenolic fraction was eluted with 5 mL methanol. The solvent was removed and the residue was properly diluted with H_2O/MeOH (9:1, mL: mL). The solution was transferred to an autosampler vial for the HPLC-DAD-ESI-MS/MS analysis.

2.6.2. HPLC-DAD-ESI-MS^n analysis

The method by Kahle, Kraus, and Richling (2005) was followed, with slight changes. Chromatographic analysis was performed with a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) equipped with a thermostated autosampler and a column oven. The UPLC system was coupled with a diode array detector and an electrospray ionization mass detector (HPLC-DAD-ESI-MS^n) in parallel by splitting the mobile phase 1:1.

Negative-ion ESI mass spectra were obtained with a Finnigan LXQ linear trap mass spectrometer (Thermo Scientific, San Jose, CA, USA). The typical ESI source conditions were transfer line capillary at 275 °C; ion spray voltage at 3.30 kV; sheath, auxiliary and sweep gas (N_2) flow rates at 10, 5 and 0 arbitrary units, respectively. Helium was used as the collision damping gas in the ion trap set at a pressure of 0.13 Pa. ESI-MS^n spectra were obtained by collision-induced dissociation (CID) experiments after isolation of the appropriate precursor ions in the ion trap (isolation width 1.2 m/z unit), and subjecting them to the following typical conditions: normalized collision energy between 20% and 30%, selected to preserve a signal of the precursor ion in the order of 5%; 0.25 activation Q and 30 ms activation time.

The chromatographic separation was performed with a column Synergi Hydro, 4 mm, 250 × 2.0 mm (Phenomenex, Italy), thermostated at 30 °C. Elution was carried out at 0.3 mL/min flow rate, using as mobile phase 2 mL/L formic acid in methanol (A) and 2 mL/L formic acid in water (B) with the following gradient: 0–6 min 10% A, 20 min 40% A, 40 min 40% A, 46 min 100% A, 52 min 100% A, 54 min 10% A, 54–60 min 10% A. The injection volume was 20 µL. The acquisition was carried out in full scan (*m/z* 50–1500) and in full scan MS^2 (*m/z* 50–600) selecting the precursor ion $[M\text{-}H]^-$ at *m/z* 289.1 for (+)-catechin and (−)-epicatechin, *m/z* 577.2 for procyanidin B2, *m/z* 457.1 for epigallocatechin gallate, *m/z* 353.1 for chlorogenic acid, *m/z* 163.0 for 3-hydroxycinnamic acid (I.S.), *m/z* 463.1 for quercetin galactoside, *m/z* 435.2 for phloridzin, *m/z* 433.1 for quercetin xyloside and quercetin arabinoside, *m/z* 447.1 for quercetin rhamnoside and *m/z* 273.1 for phloretin, respectively. Phloretin-xyloglucoside ($[M\text{-}H]^-$ *m/z* 567.2;