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Production of Bioavailable Flavonoid Glucosides in Fruit Juices and Green Tea by Use of Fungal α-L-Rhamnosidases

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Flavonoid glucosides have been reported to be more bioavailable than their rutinoside counterparts. The aim of this study is to describe a first step in the use of α-L-rhamnosidases (RhaA and RhaB) from *Aspergillus aculeatus* as a way to produce functional beverages based on their potentially increased flavonoid bioavailability. Blackcurrant juice (BCJ), orange juice (OJ), and green tea infusion (GT) were incubated with either RhaA or RhaB at 30 °C for 10 h. Aliquots of controls and enzyme-treated samples were taken at different time points and analyzed by high-performance liquid chromatography−photodiode-array detector−mass spectrometry of daughter fragments (HPLC−DAD−MS−MS). Both RhaA and RhaB selectively catalyze in situ the removal of terminal rhamnosyl groups in the three beverages despite the heterogeneity of assay conditions such as different rutinosides and pH. Incubation of the three beverages with the two rhamnosidases resulted in a hyperbolic decrease in the flavonoid rutinosides (anthocyanins in BCJ, flavanones in OJ, and flavonols in GT) and a concomitant increase in their flavonoid glucoside counterparts. The time required for conversion of 50% of the rutinoside into the corresponding flavonoid glucoside ranged from 30 min (RhaB−rutin in GT) to 6 h (RhaB−delphinidin 3-rutinoside in BCJ). The results presented in this paper are a step forward in the use of enzyme-treated beverages as a source of bioavailable flavonoid glucosides.

KEYWORDS: Rhamnosidase; *Aspergillus*; bioavailability; flavonoid; rutinoside; glucoside; functional beverage; green tea; blackcurrant juice; orange juice

INTRODUCTION

The health-promoting activity of polyphenols present in fruits, vegetables, and derived foodstuffs such as juices, tea, and wine has been extensively described in vitro, ex vivo, and in vivo (*1*−*3*). Flavonoids are the most abundant polyphenols in nature and have attracted great attention due to their beneficial properties. These beneficial properties included those related to the prevention of cardiovascular diseases and cancer (*4*−*6*). One important drawback is the limited bioavailability of many flavonoids. In fact, the sugar moiety has been proposed as the major determinant of the absorption of dietary flavonoids in humans (*7*, *8*). This is the case of flavonoid rutinosides that are very abundant in many plant foods and derived products. The rutinoside moiety of the flavonoid (glucose + rhamnose) has been described to hamper its absorption in the intestine (*8*). On the contrary, the removal of the rhamnose group to yield the corresponding counterpart flavonoid glucoside has been reported to increase its bioavailability (*8*, *9*). However, this is still under discussion in the case of anthocyanins (*10*, *11*) whose bioavailability is lower than most of the other flavonoids (*12*).

The enzymes α-L-rhamnosidases (Rhams) (EC 3.2.1.40) hydrolyze the terminal nonreducing α-L-rhamnose residues in α-L-rhamnosides. Rhams can be used to elucidate the structure of glycosides, polysaccharides, and glycolipids (*13*); to remove the bitterness from citrus juices, which is caused by the flavonoids naringin and neohesperidin (for example, by using active packaging with immobilized Rham in the inner surface of cartons (*14*); to hydrolyze hesperidin to yield rhamnose and hesperetin 7-glucoside, which is an important precursor in sweetener production (*15*); and also to enhance the aroma in grape juices and wines (*16*, *17*). Although, the Rhams purified from *Aspergillus aculeatus* have been shown to be effective in removal of the rhamnose moiety from various flavonoid

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rutinosides in model solutions to produce the corresponding flavonoid glucoside counterparts (*18*), the feasibility of these enzymes to catalyze the removal of α-L-rhamnosides in situ, in beverages with different flavonoid rutinoside content, pH, osmolarity, etc., has not been reported so far.

The aim of the present work is to describe a first, semiquantitative step in the transformation of flavonoid rutinosides from fruit juices (orange and blackcurrant) and green tea into their flavonoid glucoside counterparts upon rhamnosidase treatment purified from *A. aculeatus* under the specific conditions of each beverage. The results presented here could be useful in the production of functional fruit juices and green tea with potentially increased flavonoid bioavailability.

MATERIALS AND METHODS

Reagents. Hesperidin (HES, hesperetin 7-*O*-rutinoside; 4′-methoxy-3′,5,7-trihydroxyflavanone 7-*O*-rutinoside), and rutin (quercetin 3-*O*-rutinoside) were purchased from Sigma (St. Louis, MO) and cyanidin 3-*O*-rutinoside (cy-3-rut) from Polyphenols A.S. (Sandnes, Norway). Formic acid and methanol (MeOH) were of analytical grade and supplied by Merck (Darmstadt, Germany). Milli-Q system (Millipore Corp., Bedford, MA) ultrapure water was used in all experiments.

Beverages. Oranges were purchased in a local supermarket and the juice (OJ) was prepared by hand-squeezing the oranges immediately before the assays, as described in Gil-Izquierdo et al. (*19*). OJ was centrifuged at 5000*g* for 5 min at room temperature in a Centromix centrifuge (Selecta, Barcelona) to obtain the soluble fraction (supernatant) and the so-called cloud fraction (the pellet). This pellet was treated with 1 mL of dimethyl sulfoxide (DMSO) to extract polyphenols. Aliquots from both fractions (soluble and DMSO-treated cloud) were filtered through a 0.45 μm membrane filter, Millex-HV_{13} (Millipore Corp.), and analyzed by high-performance liquid chromatography−photodiode-array detector−mass spectrometry of daughter fragments (HPLC−DAD−MS−MS).

Concentrated blackcurrant juice (BCJ) was kindly provided by Kiantama Oy Ltd. (Suomussalmi, Finland), diluted (1:10 v/v), filtered through a Millex-HV_{13} filter, and analyzed by HPLC−DAD−MS−MS.

Green tea (GT) was obtained from a local supermarket. A 1.5 g portion of green tea was placed in 150 mL of boiling water for 5 min, filtered through a Millex-HV_{13} filter, and analyzed by HPLC−DAD−MS−MS.

Preparation of Rhamnosidases. Two α-L-rhamnosidases (RhaA and RhaB) were purified from *A. aculeatus* NW240 according to Manzanares et al. (*18*). These enzymes showed an optimal pH between 4.5 and 5.5 (*18*). Both enzymes were stable and retained more than 85% of their maximum activity in the pH ranges of 4−5.5 for RhaA and 3−5.5 for RhaB (*18*). In addition, the enzymes retained 80% (RhaA) and 95% (RhaB) of their initial activities after 20 h at 30 °C (*18*).

Enzymatic Treatments. The reaction medium contained 10 mL of OJ, GT, or BCJ (1:10 v/v) with 2 μg/mL either RhaA or RhaB for 10 h at 30 °C, unless otherwise stated. Aliquots of controls (nontreated samples) and enzyme-treated samples were taken at different time points and analyzed by HPLC−DAD−MS−MS. Aliquots from OJ were centrifuged and the polyphenol content was analyzed in both soluble and cloud fractions. The assays were performed in triplicate.

Identification and Quantification of Flavonoid Rutinosides. Flavonoid rutinosides were identified according to their UV spectra, retention times, ion mass (MS), and daughter fragments (MS-MS) by ion trap and comparison, when possible, with commercially available standards. The HPLC system equipped with both a photodiode-array detector (DAD) and mass detector in series consisted of a HPLC binary pump, autosampler, and degasser controlled by software from Agilent Technologies (Waldbronn, Germany). The mass detector was an ion-trap mass spectrometer (Agilent) equipped with an electrospray ionization (ESI) system (capillary voltage 4 kV, dry temperature 350 °C). Mass scan (MS) and MS−MS daughter spectra were measured from m/z 150 up to 800. Collision-induced fragmentation experiments were performed in the ion trap with helium as the collision gas and the collision energy set at 50%. Mass spectrometry data were acquired in the alternative negative/positive ionization mode.

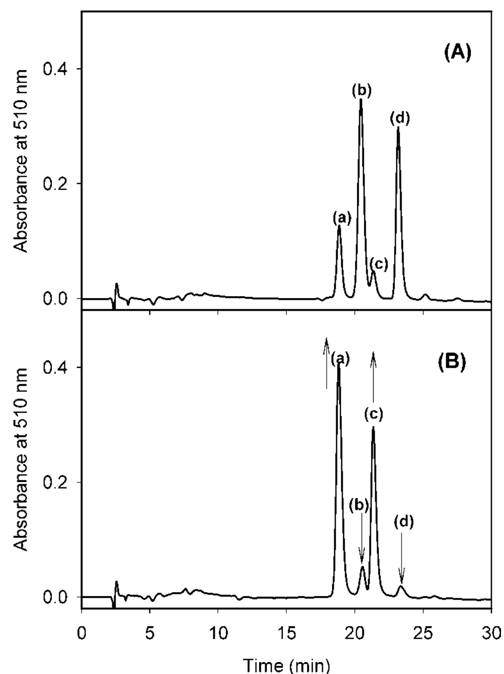

Figure 1. Anthocyanin profile of blackcurrant juice (BCJ) in the absence (**A**) and presence (**B**) of rhamnosidase. (**A**) Control juice; (**B**) BCJ incubated with 2 μg/mL RhaA after 8 h at 30 °C. (a) dp-3-glu; (b) dp-3-rut; (c)cy-3-glu; (d) cy-3-rut. The arrows designate the evolution of the peaks.

Chromatographic separations were carried out on a reverse-phase C_{18} LiChroCART column (25 × 0.4 cm, particle size 5 μm, Merck, Darmstadt, Germany) with water/formic acid (99.9/0.1 v:v) (A) and MeOH (B) as the mobile phases at a flow rate of 1 mL/min. The gradient for BCJ started with 2% B in A, to reach 32% B in A at 30 min and 95% B in A at 31 min. The gradient for OJ started with 15% B in A, to reach 35% B in A at 25 min and 95% B in A at 35 min. The gradient for GT started with 15% B in A, to reach 40% B in A at 25 min and 95% B in A at 33 min. UV chromatograms were recorded at 280, 320, 360, and 510 nm.

Delphinidin 3-rutinoside (dp-3-rut) and cy-3-rut from BCJ were quantified at 510 nm as cy-3-rut by use of the commercial standard. HES, narirutin (5,7,4′-trihydroxyflavanone 7-*O*-rutinoside), and didymin (isosakuranetin 7-*O*-rutinoside; 4′-methoxy-5,7-dihydroxyflavanone 7-*O*-rutinoside) from OJ were quantified as HES by extracting their ion mass (m/z^- at 609, 579 and 593, respectively). Rutin and kaempferol 3-*O*-rutinoside (k-3-rut) from GT were quantified as rutin by extracting their corresponding ion mass (m/z^- at 609 for rutin and m/z^- at 593 for k-3-rut). Quantification by extracting the ion mass was carried out because rutinosides and their corresponding glucoside counterparts coeluted in the same peak in the above chromatographic conditions.

Identification and Quantification of Flavonoid Glucosides. In BCJ, delphinidin 3-*O*-glucoside (dp-3-glu) and cyanidin 3-*O*-glucoside (cy-3-glu) showed approximately the same UV response as their rutinoside counterparts and were quantified as cy-3-rut because they eluted in different peaks under the above chromatographic conditions (*20*). In the case of OJ and GT, model solutions containing either 5 mg of HES or 5 mg of rutin in 100 mL of sodium acetate buffer (pH 4.5) were incubated with 4 μg/mL RhaB. Aliquots (100 μL) were taken at different times and analyzed by HPLC−DAD−MS−MS on a Nova-Pak C_{18} column (15 × 0.4 cm, particle size 4 μm, Waters, Milford, MA) with water/formic acid (99.9/0.1 v/v) (A) and MeOH (B) as the mobile phases at a flow rate of 1 mL/min. Chromatographic conditions used were those described in the previous section. With this column,

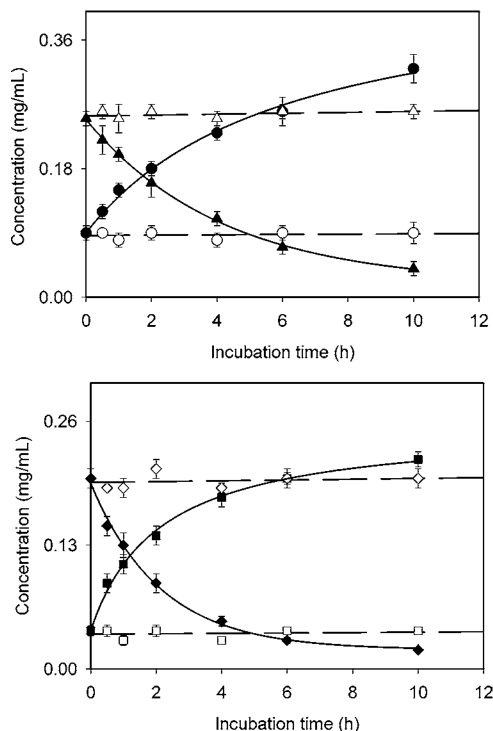

Figure 2. Time-course evolution of anthocyanin rutinosides and glucosides from BCJ, in the presence and absence of rhamnosidase. (Open symbols) Anthocyanins in the absence of Rham but incubated at 30 °C: (△) dp-3-rut; (○) dp-3-glu; (◇) cy-3-rut; (□) cy-3-glu. (Solid symbols) Anthocyanins in the presence of 2 μg/mL RhaA at 30 °C: (▲) dp-3-rut; (●) dp-3-glu; (◆) cy-3-rut; (■) cy-3-glu. (– – –) Linear regression fittings; (—) nonlinear regression fittings of experimental data to eq 1 (flavonoid glucosides) and eq 2 (flavonoid rutinosides).

both HES and rutin were separated from their glucoside counterparts upon RhaB incubation, yielding the flavonoids hesperetin 7-*O*-glucoside (h-7-glu) and quercetin 3-*O*-glucoside (q-3-glu), respectively. However, the column Nova-Pak could not be routinely used because the extremely good resolution needed to separate these compounds was lost after several analyses.

UV detector response of h-7-glu was 50-fold higher than that after ionization in the MS analysis. UV response of both q-3-glu and k-3-glu was 2-fold higher than that after ionization in the MS analysis. Therefore, quantification was achieved by extracting their ion mass (m/z^- at 463 for both q-3-glu and h-7-glu, and m/z^- at 447 for k-3-glu), by use of the corresponding standards HES and rutin, and taking into account the above response factors.

Anthocyanin Stability in BCJ. BCJ (control and enzyme-treated for 12 h) was pasteurized and stored for 40 days at room temperature (RT) and at 4 °C (protected from light in both conditions). Aliquots were taken every 7 days and analyzed by LC-MS/MS under the above chromatographic conditions for BCJ.

Graphs and Mathematical Fits. Experimental data on the time-course formation of flavonoid glucosides were fitted by nonlinear regression via the Marquardt−Levenberg algorithm (*21*) implemented in the SigmaPlot 6.0 program for Windows (SPSS Science, Chicago) to the hyperbola

$$A = \frac{[A]t}{t_{1/2} + t} \quad (1)$$

where A is the flavonoid glucoside concentration at any time, $[A]$ is the maximum flavonoid glucoside concentration reached, t is the incubation time with Rhams, and $t_{1/2}$ is the incubation time required to yield 50% flavonoid glucoside upon Rhams treatment.

Table 1. Representative Flavonoid Rutinosides and Their Glucoside Counterparts from GT, OJ, and BCJ upon Rhams Treatment[a]

rutinoside	beverage	$t_{1/2}$ (RhaA)	$t_{1/2}$ (RhaB)	glucoside
rutin (m/z^- at 609)	GT	2 h	30 min	Q-3-glu (m/z^- at 463)
K-3-rut (m/z^- at 594)	GT	2 h	1 h	K-3-glu (m/z^- at 447)
Q-glu-rham-glu (m/z^- at 771)	GT	NC	NC	
K-gal-rham-glu (m/z^- at 755)	GT	NC	NC	
K-glu-rham-glu (m/z^- at 755)	GT	NC	NC	
hesperidin (m/z^- at 609)	OJ	45 min	30 min	H-7-glu (m/z^- at 463)
narirutin (m/z^- at 609)	OJ	NE	NE	naringenin-7-*O*-glu (m/z^- at 463)
didymin (m/z^- at 593)	OJ	NE	NE	isosakuranetin-7-*O*-glu (m/z^- at 447)
Dp-3-rut (m/z^+ at 595)	BCJ	4 h	6 h	Dp-3-glu (m/z^+ at 465)
Cy-3-rut (m/z^+ at 611)	BCJ	2 h	4 h	Cy-3-glu (m/z^+ at 449)

[a] Assay conditions were 10 mL of beverage [dilution 1:10 (v/v) in the case of BCJ] and 2 μg/mL either Rha or RhaB for 10 h at 30 °C. NE, not evaluated. NC, not catalyzed by Rhams.

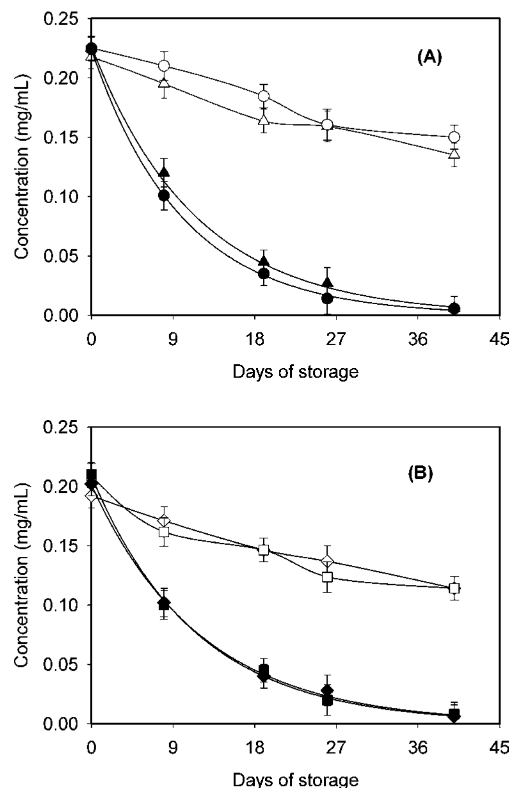

Figure 3. Stability of BCJ anthocyanins. (Open symbols) Storage at 4 °C; (solid symbols) storage at room temperature. Anthocyanin glucosides were produced upon incubation of BCJ with 2 μg/mL RhaA at 30 °C for 12 h before the stability assay. (A) Delphinidin: (△, ▲) dp-3-rut; (○, ●) dp-3-glu. (B) Cyanidin: (◇, ◆) cy-3-rut; (□, ■) cy-3-glu. Experimental data at room temperature (solid symbols) were fitted by nonlinear regression to eq 3.

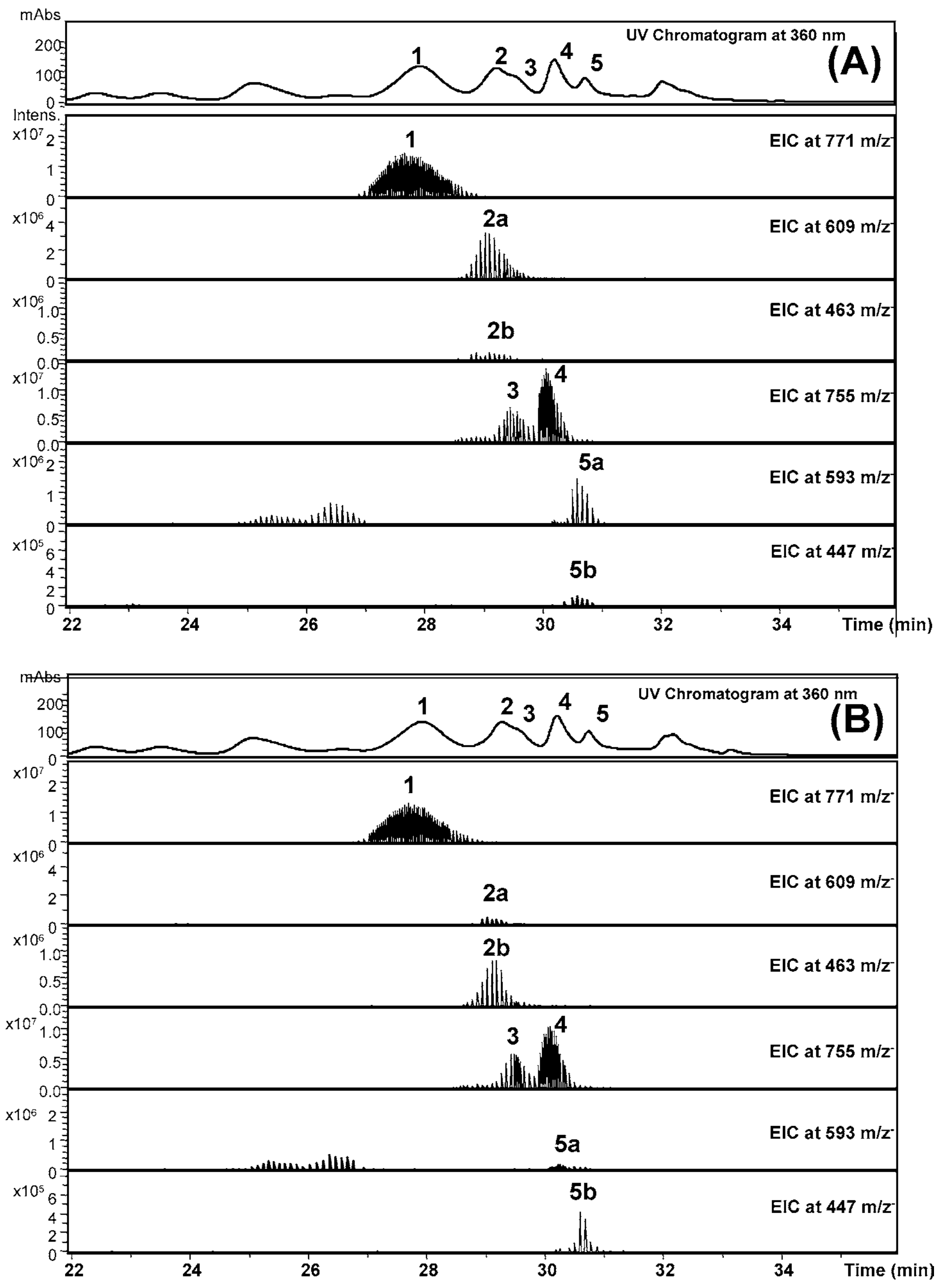

Figure 4. Representative flavonols from GT in the absence (**A**) and presence (**B**) of rhamnosidase. (**A**) UV chromatogram showing the main flavonols in the absence of Rham and their corresponding extracted ion chromatograms (EIC). (**B**) UV chromatogram after incubation of GT in the presence of 2 μg/mL RhaB at 30 °C for 6 h and their corresponding extracted ion chromatograms (EIC). (1) Q-glu-rham-glu; (2) rutin + q-3-glu (2a and 2b, respectively, in EIC); (3) k-gal-rham-glu; (4) k-glu-rham-glu; (5) k-rut + k-glu (5a and 5b, respectively, in EIC). Other identified flavonols not shown: my-3-rut, my-3-glu, and q-gal-rham-glu.

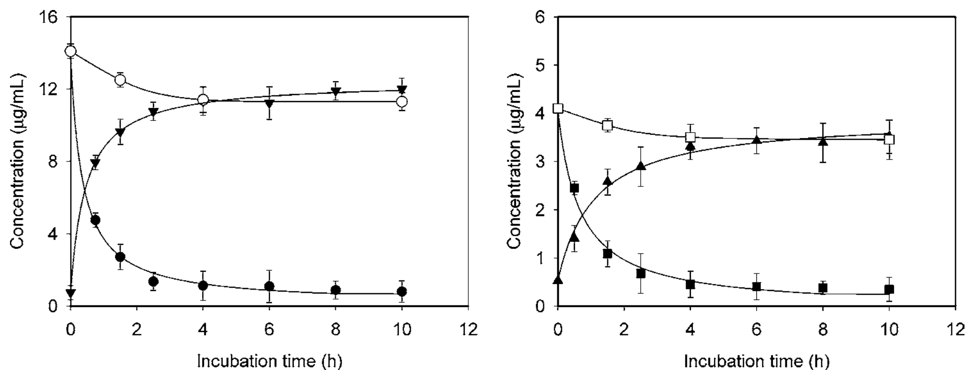

Figure 5. Time-course evolution of quercetin and kaempferol derivatives upon rhamnosidase treatment. (Open symbols) Flavonol rutinosides in the absence of Rham but incubated at 30 °C: (○) rutin; (□) k-3-rut. (Solid symbols) Flavonol rutinosides in the presence of 2 μg/mL RhaB at 30 °C: (●) rutin; (▼) q-3-glu; (■) k-3-rut; (▲) k-3-glu. Experimental data were fitted by nonlinear regression to eq 1 (flavonol glucosides) and eq 2 (flavonol rutinosides).

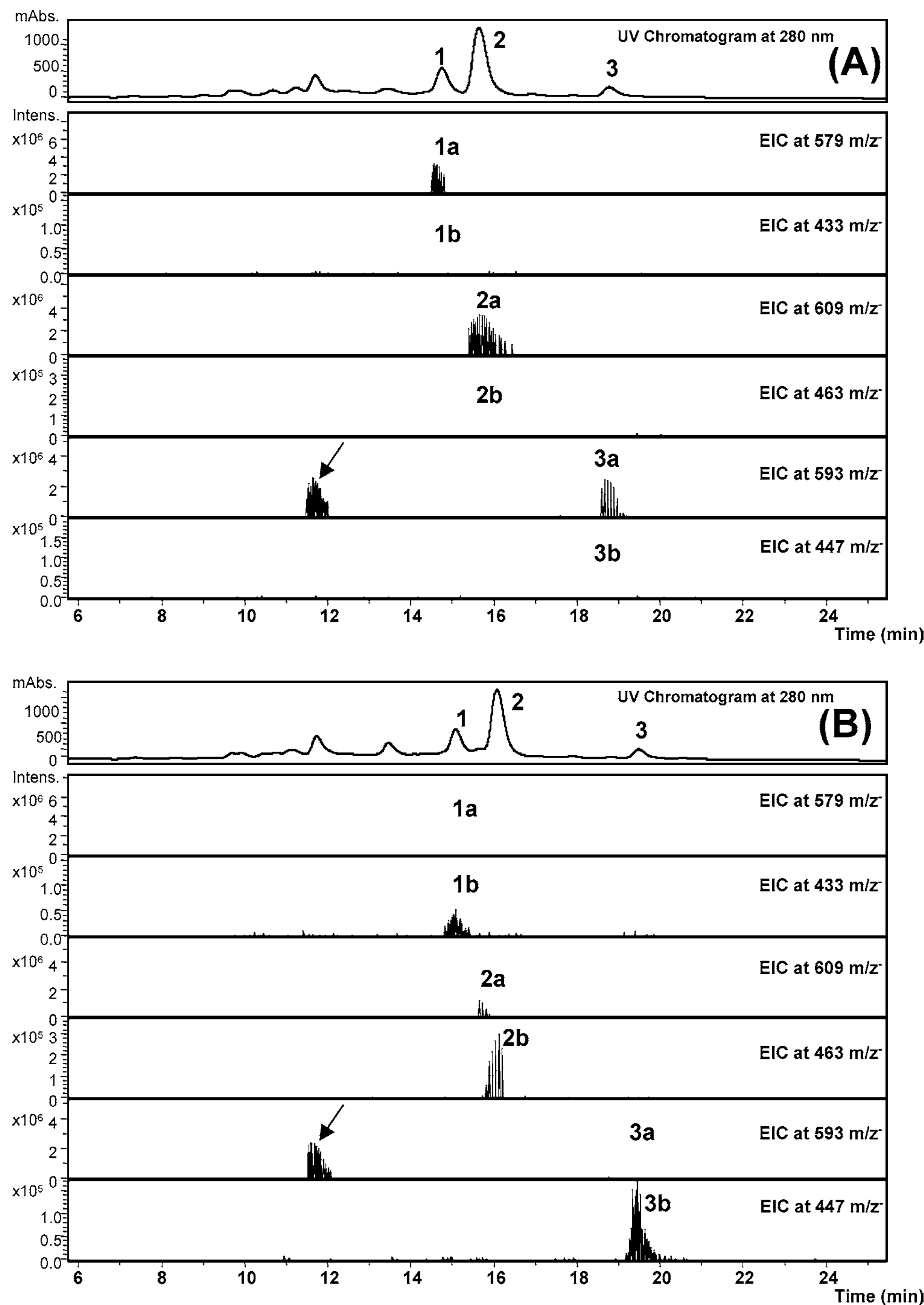

Figure 6. Flavanones from OJ in the absence (**A**) and presence (**B**) of rhamnosidase. (**A**) UV chromatogram showing the main flavanones in the absence of Rham and their corresponding extracted ion chromatograms (EIC). (**B**) UV chromatogram after incubation of OJ in the presence of 2 μg/mL RhaB at 30 °C for 3 h and their corresponding extracted ion chromatograms (EIC). (1) Narirutin + naringenin-7-*O*-glu (1a and 1b, respectively, in EIC); (2) HES + h-7-glu (2a and 2b, respectively, in EIC); (3) didymin + isosakuranetin-7-*O*-glu (3a and 3b, respectively, in EIC). The arrow designates the flavone vicenin-2 (apigenin 6,8-di-*C*-glucoside).

The disappearance of flavonoid rutinosides upon Rhams treatment was fitted by nonlinear regression via the same informatics program to the decreasing hyperbola

$$B = C - \frac{[B]t}{t_{1/2} + t} \quad (2)$$

where B is the flavonoid rutinoside concentration at any time, C is the initial flavonoid rutinoside concentration (in the absence of Rham treatment), $[B]$ is the final flavonoid rutinoside concentration reached, t is the incubation time of beverages with Rhams, and $t_{1/2}$ is the incubation time required to decrease to 50% flavonoid rutinoside concentration upon Rhams treatment.

Experimental data on anthocyanin stability at room temperature were fitted by nonlinear regression to a decreasing uniexponential equation:

$$D = E_0 e^{-\lambda t} \quad (3)$$

where D is the anthocyanin concentration at any time, E_0 is the initial anthocyanin concentration, λ is the apparent constant that describes the velocity of degradation, and t is the days of storage. The time required to decrease to half anthocyanin concentration ($t_{50\%}$) was also calculated as $t_{50\%} = (\ln 2)/\lambda$. The coefficient of variation in all the nonlinear regression fittings was always less than 10%.

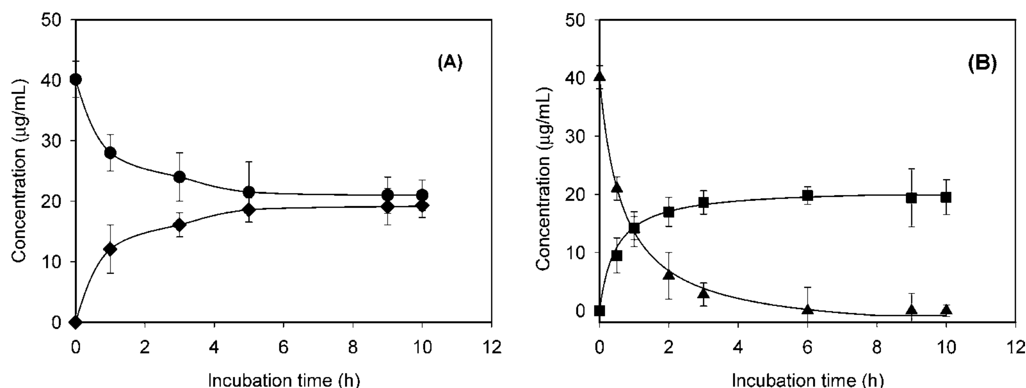

Figure 7. (**A**) Evolution of HES content in soluble (●) and cloud (◆) fractions of OJ in the absence of Rhams. (**B**) Time-course evolution of HES (▲) and h-7-glu (■) in total (soluble + cloud) OJ, in the presence of 2 μg/mL RhaA at 30 °C. Experimental data in panel B were fitted by nonlinear regression to eq 1 (h-7-glu) and eq 2 (HES).

RESULTS

Blackcurrant Juice. The main anthocyanins from BCJ (dp-3-rut, dp-3-glu, cy-3-rut, and cy-3-glu) (*22*) were separated under the chromatographic conditions described in the previous section. The incubation of BCJ with Rhams decreased the anthocyanin rutinoside content concomitant with the increase in anthocyanin glucosides (**Figure 1**). This evolution depended on the incubation time (**Figure 2**), and the corresponding $t_{1/2}$ of conversion from rutinoside to glucoside upon RhaA and RhaB treatment (**Table 1**) was calculated on the basis of these parameters. When a double amount of Rhams was used, $t_{1/2}$ values were proportionally reduced (results not shown).

Light, temperature, and pH are critical factors affecting anthocyanin stability. In addition, the stability has also been related to anthocyanin structure (*23*). To check a possible influence of Rhams treatment on anthocyanin stability, both the control and the Rham-treated BCJ were stored at room temperature (RT) and at 4 °C for 40 days (pH 2.75 and both protected from light). Anthocyanins from BCJ showed approximately the same degradation kinetics with 40% and 100% degradation after 40 days at 4 °C and RT, respectively (**Figure 3**). The kinetic parameters describing the stability of the different anthocyanins at room temperature—dp-3-rut ($\lambda = 0.087$ day^{-1} and $t_{50\%} = 8.7$ days), dp-3-glu ($\lambda = 0.1$ day^{-1} and $t_{50\%} = 7$ days), cy-3-rut ($\lambda = 0.083$ day^{-1} and $t_{50\%} = 8.2$ days), and cy-3-glu ($\lambda = 0.086$ day^{-1} and $t_{50\%} = 8.1$ days)—were determined on the basis of nonlinear regression fitting of the experimental data. Therefore, Rham treatment of BCJ did not affect the normal stability of anthocyanins, which should be stored at 4 °C. The fitting was not carried out at 4 °C because first-order conditions were not accomplished (results not shown).

Green Tea. Although up to 17 flavonols have been previously described in tea, not all of these compounds are present in each of the tea infusions (*24*). The main flavonoid rutinosides detected in the infusion of GT assayed were the flavonols quercetin 3-*O*-glucoside-rhamnoside-glucoside (q-glu-rham-glu; m/z^- at 771), kaempferol 3-*O*-glucoside-rhamnoside-glucoside (k-glu-rham-glu; m/z^- at 755), kaempferol 3-*O*-galactoside-rhamnoside-glucoside (k-gal-rham-glu; m/z^- at 755), rutin (m/z^- at 609), q-3-glu (m/z^- at 463), k-3-rut (m/z^- at 593), kaempferol 3-*O*-glucoside (k-3-glu; m/z^- at 447), myricetin 3-*O*-rut (my-3-rut; m/z^- at 625), and myricetin 3-*O*-glucoside (my-3-glu; m/z^- at 479) (**Figure 4**). Both RhaA and RhaB catalyzed the hyperbolic conversion of rutin and k-3-rut into their corresponding glucosides q-3-glu and k-3-glu, respectively (**Figure 5**; **Table 1**). RhaB proved to catalyze the removal of rutinosides in GT better than RhaA (**Table 1**). In the absence of Rhams, both rutin and k-3-rut were slightly unstable at 30 °C during the assay time (**Figure 5**), which could be due to the previous thermal treatment to get tea infusion. My-3-rut, a minor constituent in this GT, was also transformed into my-3-glu, although the conversion to glucoside was not kinetically characterized.

Orange Juice. The most representative polyphenols in OJ are the flavanone rutinosides narirutin (m/z^- at 579), hesperidin (HES, m/z^- at 609), and didymin (m/z^- at 593) (*19*). All these rutinosides were converted into their glucoside counterparts upon Rham treatment (**Figure 6**). Special attention was paid to HES, the most abundant polyphenol in OJ. RhaB was slightly better than RhaA to yield the corresponding h-7-glu from HES (**Table 1**). Due to the limited accessibility of the enzymes to HES, which was rather insoluble and readily precipitated, the total amount of rutinoside converted into glucoside was variable in the different assays. In fact, all the flavanone rutinosides analyzed showed the same behavior. The HES content in the soluble fraction progressively decreased concomitant with its increase in the cloud fraction in the absence of Rhams (**Figure 7A**). Therefore, Rhams treatment did not yield stoichiometrically h-7-glu from HES because of its poor solubility (**Figure 7B**).

DISCUSSION

Although α-L-rhamnosidases from *A. aculeautus* (RhaA and RhaB) have been effective in the removal of α-L-rhamnosides in model solutions (*18*), beverages containing flavonoid rutinosides, such as fruit juices and green tea, greatly differ from these model solutions. In this context, three representative beverages according to their flavonoid rutinoside content were selected: orange juice (OJ, pH 3.5); blackcurrant juice (BCJ, diluted 1:10, pH 2.75) and green tea (GT, pH 6.5). As RhaA and RhaB have different optimal pH values, 4.5 and 5, respectively, it is important to take in consideration the pH of the beverages. Besides the pH, there are several factors such as different substrates, osmolarity, possible endogenous inhibitors, etc., that could be useful to validate Rhams treatment as a biotechnological tool to improve flavonoid glucoside content in fruit juices and GT.

Although other phenolic rutinosides were also modified by Rhams treatment in the beverage assayed here (**Figures 4** and **6**), special attention was paid to the most abundant and representative ones: anthocyanin rutinosides in BCJ, quercetin and kaempferol rutinosides in GT, and hesperidin in OJ.

In all the different experiments on BCJ, the efficiency of RhaB to catalyze the conversion of rutinosides into glucosides was better than that of RhaA. This result could be explained in

part by the higher stability of RhaB at the pH 2.75 assayed here (*18*). In general, the efficiency of Rhams to convert the rutinoside into glucoside in BCJ was lower than that in GT and OJ (**Table 1**). These results do not necessarily imply that Rhams are less efficient in the conversion of anthocyanins than other substrates, because other factors including the low pH (pH 2.75; far from the optimum pH values for RhaA and RhaB, 4.5 and 5, respectively) and the higher osmolarity of BCJ compared to that in OJ and GT, could also be involved. Therefore, the specific comparison of catalytic efficiencies among different rutinosides should be carried out in model solutions (*18*). Although, in the present study, both RhaA and RhaB were very active in the conversion of HES into h-7-glu (**Figures 6** and **7**; **Table 1**), previous reports showed that Rhams are less efficient in the conversion of HES than rutin and naringin in model solutions (*18*). Both Rhams have been reported to be active on terminal nonreducing α-L-rhamnose residues in α-1,2 and α-1,6 linkage to the β-D-glucosides, as in the case of naringin (α-1,2), rutin and HES (α-1,6) (*18*). This was the reason both Rhams were not able to catalyze the hydrolysis of rhamnose residues from flavonol-hexosyl-rhamnosyl hexosides such as q-glu-rham-glu, k-glu-rham-glu, and k-gal-rham-glu, which were not affected by the incubation of GT with Rhams (**Figure 4**).

The results presented herein showed the great potential of fungal α-L-rhamnosidases to produce functional beverages based on their content in potentially bioavailable flavonoid glucosides.

LITERATURE CITED

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