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ENZYMATIC HYDROLYSIS AS AN ENVIRONMENTALLY FRIENDLY PROCESS COMPARED TO THERMAL HYDROLYSIS FOR INSTANT COFFEE PRODUCTION

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Abstract - Conventional production of instant coffee is based on solubilisation of polysaccharides present in roasted coffee. Higher process temperatures increase the solubilisation yield, but also lead to carbohydrate degradation and formation of undesirable volatile compounds. Enzymatic hydrolysis of roasted coffee is an alternative to minimize carbohydrate degradation. In this work, products obtained from thermal and enzymatic processes were compared in terms of carbohydrates and volatiles composition. Roasted coffee was extracted with water at 125 °C, and spent coffee was processed by thermal (180 °C) or enzymatic hydrolysis. Enzymatic hydrolysis experiments were carried out at 50 °C using the commercial enzyme preparations Powercell (Prozyn), Galactomannanase (HBI-Enzymes), and Ultraflo XL (Novozymes). These formulations were previously selected from eleven different commercial enzyme preparations, and their main enzymatic activities included cellulase, galactomannanase, galactanase, and β-glucanase. Enzymatic hydrolysis yield was 18% (dry basis), similar to the extraction yield at 125 °C (20%), but lower than the thermal hydrolysis yield at 180 °C (28%). Instant coffee produced by enzymatic hydrolysis had a low content of undesirable volatile compounds and 21% (w/w) of total carbohydrates. These results point to the enzymatic process as a feasible alternative for instant coffee production, with benefits including improved quality as well as reduced energy consumption.
Keywords: Instant coffee; Thermal extraction; Thermal hydrolysis; Enzymatic hydrolysis; Carbohydrate degradation.

INTRODUCTION

Coffee is a global commodity whose consumption and production are increasing every year. In the 2012/2013 crop year, worldwide production was 144.6 million sacks of 60 kg, while the international coffee trade had a turnover of about US$ 15.4 billion in 2009/10 (ICO, 2013). Coffee is consumed in many ways, including in the forms of roasted and ground coffee (RGC) and instant coffee (spray dried and freeze-dried). Although it is difficult to retain the same freshness as RGC in instant coffee beverages, their consumption has increased due to the ease of preparation (Ferdman, 2014). Instant coffee is also

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used as an ingredient for the preparation of cappuccinos, ready-to-drink beverages, cakes, and ice creams, amongst other possibilities. In addition to its flavour and taste, coffee also contains bioactive compounds such as caffeine and chlorogenic acids that may be beneficial to health (Torres-Mancera *et al*., 2011; Vignoli *et al*., 2011).

Among many coffee species, only two are grown on a commercial scale: *Coffea arabica* (arabica), with a mild taste and intense aromatic flavour, which corresponds to 70% of global production; and *Coffea canephora* (robusta), with a bitter taste and neutral flavour, corresponding to 30% of total production (Arya and Rao, 2007).

A typical composition of roasted arabica coffee is: carbohydrates (48.1%), fat (15.9%), protein (6.8%), melanoidins (25.0%) and other compounds (4.2%) (Oosterveld *et al*., 2003, Daglia *et al*., 2004). The main compounds derived from roasted coffee that are found in instant coffee are carbohydrates: mannose (51%), galactose (21%), glucose (16%), uronic acid (4%), and rhamnose (1%) (Oosterveld *et al*., 2003). These carbohydrates are present only as solubilized polysaccharides, because low weight coffee bean carbohydrates (oligosaccharides) are degraded at high roasting temperatures, leaving long chain polysaccharides in roasted coffee.

The process conditions employed for instant coffee production influence the yield as well as the quality of the resulting coffee beverage. Homemade extract of coffee (prepared with boiling water at 98 °C) has a yield below 20% (Martins *et al*., 2005). In industrial processes, the yield must be improved, both for economic reasons and to enrich the composition with high molecular weight solubilized polysaccharides that provide the final product with desirable physical-chemical properties, such as low hygroscopicity (Delgado *et al*., 2008).

Instant coffee is produced on an industrial scale using the GEA-NIRO process (Castle, 2002; GEA-NIRO, 2013), or similar, where thermal extraction at 125 °C is followed by a thermal hydrolysis at 180 °C to remove soluble solids from the RGC. The subsequent steps in instant coffee production involve concentration and freeze-drying or spray drying. A high quality coffee beverage with good flavour notes is produced by thermal extraction, although the yield is similar to that for homemade coffee. In order to achieve yields of 48% (arabica) and 54% (robusta), thermal extraction is followed by thermal hydrolysis. There are two main drawbacks of the GEA-NIRO process: high energy consumption and the production of undesirable compounds such as furfural, 5-hydroxymethylfurfural (5-HMF), and acetaldehyde from the thermal degradation of carbohydrates (De Maria *et al*., 1994). One possible solution to the problems of thermal hydrolysis is to use enzymatic technology to carry out hydrolysis at a low temperature, hence reducing energy consumption and avoiding carbohydrate degradation (Silver *et al*., 2007).

The carbohydrates present in roasted coffee are distributed between three main polysaccharides, namely galactomannan (GM), arabinogalactan (AG), and cellulose (Arya and Rao, 2007). An understanding of the chemical structures of these polysaccharides is crucial for selection of the most suitable hydrolytic enzymes. Mahammad and co-workers (Mahammad *et al*., 2007) studied the hydrolysis of GM and found that the enzymes *β*-mannosidase (EC 3.2.1.25), endo-1,4-*β*-mannanase (EC 3.2.1.78), and *α*-galactosidase (EC 3.2.1.22) operated in synergy, catalysing the hydrolysis at different points of the GM chain. As shown in Figure 1, the first enzyme hydrolyses terminal non-reducing bonds in the GM backbone, the second acts randomly on mannose linkages in the GM backbone, and the third enzyme acts between mannose and galactose linkages (Mahammad *et al*., 2007).

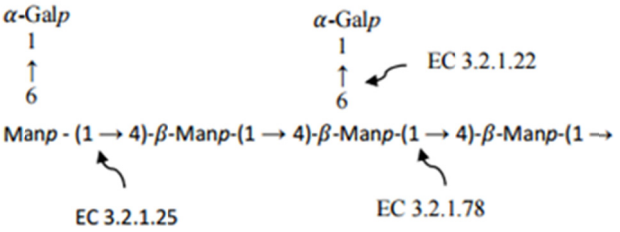

Figure 1: GM structure and cleavage sites (p: pyranose) (Mahammad *et al*., 2007).

Two enzymes have so far been identified for the hydrolysis of AG: endo-1,3-*β*-galactanase (EC 3.2.1.181), which acts on the AG backbone, and endo-1,6-*β*-galactanase (EC 3.2.1.164), which cleaves the AG side chains (Luonteri *et al*., 2003).

The cellulose present in the RGC structure appears to be intermingled with GM and AG in the cell walls (Kasai *et al*., 2006). Enzymes with cellulase and *β*-glucanase activities are therefore required in order to promote hydrolysis of the cell walls.

Although there has been some work concerning the use of enzymes to improve the quality of instant coffee (Delgado *et al*., 2008), the retrieval of soluble polysaccharides from RGC carbohydrates by the enzymatic route has not been fully explored. The main objectives of this study were to: i) identify commercial enzyme preparations with high potential for the hydrolysis of RGC biopolymers; and ii) com-

pare chemical composition (sugars and volatiles) of instant coffee produced from roasted arabica beans by the enzymatic process with similar products obtained by the conventional GEA-NIRO process.

MATERIALS AND METHODS

Roasted Coffee

Arabica coffee beans with a medium degree of roast (10% mass loss during roasting, on a dry basis) were used in all experiments. Arabica coffee was chosen because its aromatic compounds are more sensitive to the extraction temperature employed (180 ^{0}C) (Blank *et al*., 1991). The degree of roasting applied was specifically selected because it provides the release of an intense coffee aroma (Mayer *et al*., 2000).

Commercial Enzyme Preparations

Eleven commercial enzyme preparations with potential to hydrolyse coffee polysaccharides were evaluated: i) Cellulases: Celluclast 1.5 L (Novozymes Latin America, Brazil), Powercell, and Celumax C (Prozyn Biosolutions, São Paulo, Brazil); ii) Galactomannanases: Galactomannanase (HBI Enzymes, Japan) and Rhoapect B1-L (AB Enzymes, Germany); iii) *β*-glucanase: Ultraflo XL (Novozymes Latin America, Brazil); iv) Galactanases: Viscozyme L, Pectinex Ultra Clear, Pectinex Ultra SP-L, and Ultrazym AFP L (Novozymes Latin America, Brazil). Commercial pectinases were selected to search for galactanases because they usually have a side activity of these enzymes due to the presence of arabinogalactan, which is found in seeds, bulbs and leaves as a complex mixture with pectin (Luonteri *et al*., 2003). All the commercial preparations were characterized in terms of their main enzymatic activities according to the methods described below.

Analytical Methods

Enzymatic Activities

Cellulase activities were measured using the IUPAC method (Ghose, 1947), with filter paper (Whatman No. 1) and cellobiose (Sigma-Aldrich) as substrates. The initial rates method was employed to determine the galactomannanase, *β*-glucanase, and galactanase activities in the commercial preparations. All reactions were carried out in citrate buffer (50 mM), at pH 5.0 and 50 °C, using the following substrates: 0.5% locust bean gum (Sigma-Aldrich) for galactomannanases (Delgado *et al*., 2008); *β*-glucan from barley (Fluka) for *β*-glucanase (Yang *et al*., 2008); and 1.0% gum arabic for galactanase (Mahendran *et al*., 2008).

Moisture Determination

The moisture contents of all the solid raw materials and products were determined with a halogen moisture analyser (HB43-S Halogen, Mettler Toledo), which was calibrated to comply with the ISO 3726:1983 method (ISO 3726,1983).

Mass Fraction of Soluble Solids

After Brix determination using a refractometer with automatic temperature compensation to 25 °C (RX-5000, Atago), the mass fractions of soluble solids present in the coffee extracts produced using the enzymatic and thermal treatments were estimated using the correlation proposed by Sivetz and Desrosier (1979).

Carbohydrate Analysis

The solubilised carbohydrates were quantified in terms of free and total sugars using HPAE-PAD (high performance anion exchange chromatography-pulsed amperometric detection), according to the methodology described in ISO 11292:1995 (ISO 11292, 1995). For total sugars determination, the samples were first hydrolysed with 50 mL of HCl (1.0 M) at 98 °C for 2.5 hours. The concentration of soluble polysaccharides was estimated in terms of non-free sugars, calculated by subtracting the concentration of free sugars from the total sugars concentration.

Volatile Compound Analysis

Analysis of acetaldehyde, furfural, and 5-HMF present in a 3.0% (mass fraction) coffee extract solution was performed using HS-SPME-GC-MS (head space-solid phase microextraction-gas chromatography-mass spectrometry). The GC was fitted with an HP INNOVAX column (60 m x 320 μm x 0.25 μm). The temperature program was 40 °C for 5 min, followed by heating from 40 to 60 °C at 4 °C/min, and then from 60 to 250 °C at 8 °C/min. Helium was used as the carrier gas, at a flow rate of 1.2 mL/min. The MS detector operating conditions were an ionization energy of 70 eV, an interface temperature of 280 °C, a quadruple temperature of 150 °C, and an ion source temperature of 230 °C (Viegas and Bassoli, 2007).

Experimental Procedure

Roasted ground arabica coffee beans (RGC) were first extracted with hot water (125 °C) to produce an aroma extract (AE). The spent coffee (SC), which still contained a substantial quantity of roasted coffee polysaccharides, was used to produce soluble coffee by employing either enzymatic or thermal hydrolysis. The extracts obtained were denoted EHE and THE, respectively. A simplified flow diagram of the process is shown in Figure 2. The experimental conditions employed in each step are described below.

Thermal Extraction of Coffee Solids

Roasted ground coffee with particle sizes ranging from 0.5 to 2.0 mm was extracted in a pilot counterflow system consisting of three columns in series, with filters in the effluent streams. Hot water at 125 °C was used, with an extraction factor of 2.0 (2.0 kg of coffee extract from 1.0 kg of RGC) and an extraction cycle time of 30 min. The AE produced was immediately cooled to 25 °C and the concentrations of carbohydrates and volatiles were determined. The AE was freeze-dried, and the yield (Y) was calculated using Equations (1) and (2). The spent coffee (SC) residue remaining after thermal extraction (Figure 2) was then submitted to thermal hydrolysis or enzymatic hydrolysis, as detailed below.

$$Y = \frac{m_{ss}}{m_{RGC}} \tag{1}$$

$$m_{ss} = m_{AE} . x_{AE} \tag{2}$$

where

Y : yield (%)

m_{ss} : freeze-dried coffee solids on a dry basis (g), calculated using Equation (2)

m_{RGC} : RGC on a dry basis (g)

m_{AE} : amount of coffee aroma extract (g)

x_{AE} : mass fraction of soluble solids in aroma extract.

Thermal Hydrolysis

The spent coffee (SC) was hydrolysed and extracted in a pilot counterflow system consisting of four columns with filters in the exit streams. Water at 180 °C was used, with an extraction factor of 7.0 (7.0 kg of thermally hydrolysed coffee extract from 1.0 kg of RGC) and an extraction cycle time of 30 min. The extract containing thermally hydrolysed coffee (THE) was immediately cooled to 25 °C and analysed in terms of carbohydrates and volatiles. The extract was freeze-dried, and the yield (Y) was calculated using Equations (1) and (3).

$$m_{ss} = m_{THE} . x_{THE} \tag{3}$$

where

m_{THE} : amount of thermal hydrolysis extract (g)

x_{THE} : mass fraction of soluble solids in the thermal hydrolysis extract.

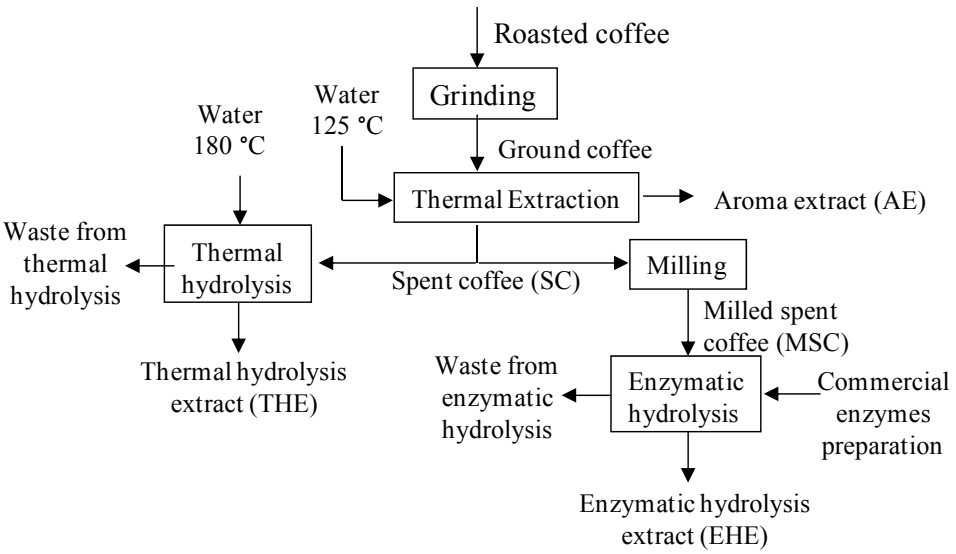

Figure 2: Flow diagram of soluble coffee production by thermal extraction, thermal hydrolysis and enzymatic hydrolysis.

Enzymatic Hydrolysis

The SC residue obtained after thermal extraction (Figure 2) was dried at 60 °C to a final moisture content of 3.0%. It was then cooled to room temperature, milled in a chilled (5 °C) laboratory mill (Model A10, IKA), and sieved to obtain milled spent coffee (MSC) particles smaller than 500 μm. The enzymatic hydrolysis was carried out in a 250 mL microreactor, employing 20 g of MSC and 200 mL of citrate buffer (50 mM, pH 5.0), under mechanical agitation at 350 rpm and 50 °C. The enzymatic hydrolysis was started by adding the previously selected commercial enzyme preparations containing the highest activities in terms of cellulase, galactomannanase and β-glucanase. The amounts of enzymes added were adjusted in order to provide final mass fractions of 0.06% galactomannanase, 0.06% cellulase, and 0.06% β-glucanase (weight of commercial enzyme preparation per weight of MSC) in the microreactor.

The experiments (in triplicate) were conducted for 71 h, and the progress of the hydrolysis was followed by measuring the reducing sugars released using the DNS method (Müller, 1959). The enzymatic hydrolysis was stopped by cooling the reactor to 5 °C and the reaction mixture was centrifuged at 3000 rpm for 20 min. The supernatant containing the soluble products of the enzymatic reaction was analysed in terms of carbohydrates and volatiles, and then freeze-dried. The enzymatic hydrolysis yield was calculated using Equations (1) and (4).

$$m_{ss} = m_{EHE}.x_{EHE} \quad (4)$$

where

m_{EHE} : amount of enzymatic hydrolysis extract (g)

x_{EHE} : mass fraction of soluble solids in the enzymatic hydrolysis extract.

Statistical Analysis

The products obtained from the RGC (AE, THE, and EHE) were compared in terms of the contents of carbohydrates (arabinose, glucose, galactose, and mannose) and undesirable volatile compounds (furfural, 5-HMF, and acetaldehyde), using the Tukey's test (Box *et al*., 1978) with $p<0.05$. All calculations were performed using STATISTICA 7.1 software (Statsoft, 2005), with a confidence interval (CI) of 95%.

RESULTS AND DISCUSSION

Selection of Commercial Enzyme Preparations

Eleven commercial enzyme preparations were evaluated to determine their cellulase, galactomannanase, β-glucanase, and galactanase activities. The results are shown in Table 1.

Powercell, Ultraflo XL, and Galactomannanase-HBI were selected to carry out the enzymatic hydrolysis step. Powercell showed the highest cellulase activities for both substrates (Table 1), Galactomannanase-HBI had the highest galactomannanase activity, and Ultraflo XL showed excellent β-glucanase activity (Table 1). Low (or zero) activities of galactanase were detected in all the enzymatic preparations.

Table 1: Activities of cellulase, galactomannanase, β-glucanase, and galactanase in eight enzymatic preparations.

Main activity	Preparation	Activity
Cellulase	Powercell	463 ± 15 FPU/g
		1297 ± 182 UI/g
	Celumax C	300 ± 7 FPU/g
		512 ± 25 UI/g
	Celluclast 1.5 L	108 ± 13 FPU/mL
		13 ± 1 UI/mL
Galactomannanase	Galactomannanase-HBI	18554 ± 564 UI/g
	Cellulax GM-L	51 ± 8 UI/g
	Rhoapect B1-L	8 ± 2 UI/mL
β-glucanase	Ultraflo XL	1028 ± 12 UI/g
Galactanase	Viscozyme L	4.0 ± 0.3 UI/g
	Pectinex Ultra Clear	ND
	Pectinex Ultra SP-L	ND
	Ultrazym AFP L	ND

Average ± CI (95%), n=3, ND: not detected, FPU: filter paper units.

Characterization of AE and THE

The products AE and THE (Figure 2) were analysed in terms of their contents of free (Table 2) and total sugars (Table 3). The non-free sugar contents were then obtained from these results (Table 4). The yields achieved in the thermal extraction and thermal hydrolysis steps were calculated using Equation (1) (Figure 3). The results for the product obtained by enzymatic hydrolysis (EHE) are also included in Tables 2-4 and Figure 3, but will be discussed separately. Both AE and THE showed low contents of free sugars, with overall concentrations of 1.0 and 3.7%, respectively (Table 2). In terms of total sugar composition (Table 3), THE contained about 36% of total sugars, with the highest concentration for galactose, followed by mannose and arabinose. These findings confirmed the efficiency of thermal hydrolysis for the solubilisation of polysaccharides.

Table 2: Free sugar concentrations in aroma (AE), thermal hydrolysis (THE) and enzymatic hydrolysis (EHE) extracts.

Free sugars	EA (% w/w)	THE (% w/w)	EHE* (% w/w)
Mannitol	0.09 ± 0.02	0.01 ± 0.00	ND
Fucose	0.02 ± 0.01	0.02 ± 0.01	ND
Arabinose	0.25 ± 0.07^{c}	2.3 ± 0.5^{a}	1.6 ± 0.1^{b}
Galactose	0.04 ± 0.01^{b}	0.6 ± 0.1^{a}	0.47 ± 0.02^{a}
Glucose	0.03 ± 0.02^{c}	0.09 ± 0.02^{b}	2.82 ± 0.03^{a}
Sucrose	0.45 ± 0.06	0.02 ± 0.01	ND
Mannose	0.04 ± 0.00^{c}	0.4 ± 0.1^{b}	7.2 ± 0.3^{a}
Fructose	0.02 ± 0.01	0.17 ± 0.05	0.05 ± 0.03
Ribose	ND	0.07 ± 0.03	0.04 ± 0.02
Total	1.0 ± 0.1^{c}	3.7 ± 0.8^{b}	12.2 ± 1.2^{a}

ND: not detected. Average ± CI (95%), n=3. Average values followed by different letters in the same line and in decreasing alphabetical order, are significantly different from each other, according to Tukey's test ($p<0.05$). *Refers to the hydrolysate obtained after 71 h of enzymatic reaction.

Table 3: Total sugar concentrations in aroma (AE), thermal hydrolysis (THE) and enzymatic hydrolysis (EHE) extracts.

Total sugars	AE (% w/w)	THE (% w/w)	EHE* (% w/w)
Mannitol	0.09 ± 0.02	0.11 ± 0.02	ND
Fucose	0.02 ± 0.01	0.19 ± 0.05	ND
Arabinose	3.1 ± 0.5^{b}	4.64 ± 0.04^{a}	2.7 ± 0.6^{b}
Galactose	2.6 ± 0.4^{c}	20.1 ± 2.4^{a}	7.2 ± 0.1^{b}
Glucose	1.53 ± 0.04^{b}	0.7 ± 0.1^{c}	2.6 ± 0.2^{a}
Mannose	0.9 ± 0.3^{c}	10.4 ± 0.8^{a}	8.9 ± 0.9^{b}
Total	8.2 ± 1.2^{c}	36.2 ± 3.2^{a}	21.4 ± 2.5^{b}

ND: not detected. Average ± CI (95%), n=3. Average values followed by different letters in the same line and in decreasing alphabetical order are significantly different from each other, according to Tukey's test ($p<0.05$). *Refers to the hydrolysate obtained after 71 h of enzymatic reaction.

Leloup and Liardon (1993) also characterized total sugars in roasted arabica coffee extracted at 95 °C and obtained a value of 7.0%, which is similar to the value of 8.2% obtained here (Table 3). In the same work, a total sugars content of 20% was reported for an extract produced directly from roasted coffee at 180 °C. In the present case, the thermal hydrolysis extract was produced at the same temperature, but from spent coffee (roasted coffee that had already been submitted to thermal extraction), which therefore had a higher content of total sugars (36.2%).

Knowledge of the total and free sugar contents of AE and THE enabled estimation of the non-free sugar contents (Table 4), so that conclusions could be drawn about the polysaccharide contents of the extracts. Arabinose, galactose, and glucose were the main non-free sugars in AE, while galactose and mannose were found at higher concentrations in THE. THE contained about 32.8% of non-free sugar, which revealed the efficiency of thermal hydrolysis in releasing high molecular weight solubilized polysaccharides (Table 4).

Application of Tukey´s test demonstrated that the compositions of AE and THE differed in terms of free sugars, total sugars, and non-free sugars. For all the samples evaluated, galactose, mannose, arabinose, and glucose were the main sugars present, reflecting the constitutions of the three polysaccharides present in the structure of coffee (Oosterveld *et al*., 2003). However, the composition was also directly influenced by the extraction/hydrolysis conditions. The milder conditions employed in the thermal extraction led to a recovery that was 77% inferior in terms of soluble polysaccharides, compared to thermal hydrolysis. It is also important to highlight that polysaccharides containing mannose and galactose (Table 4) were only efficiently produced under the high temperature conditions used for thermal hydrolysis. Leloup and Liardon (1993) also reported that GMs from coffee were preferentially solubilized at higher temperatures (180 °C). This requirement for high temperature reflects the difficulty in cleaving the GM and AG structures.

Table 4: Concentrations of volatiles and non-free sugars in aroma (AE), thermal hydrolysis (THE) and enzymatic hydrolysis (EHE) extracts.

Compounds	AE	THE	EHE*
Acetaldehyde (μ/kg)	5533 ± 221^{b}	14733 ± 453^{a}	1833 ± 52^{c}
Furfural (μg/kg)	24133 ± 482^{b}	48900 ± 733^{a}	167 ± 27^{c}
5-HMF (μg/kg)	667 ± 26^{b}	2667 ± 105^{a}	ND
Mannitol (%w/w)	ND	0.10 ± 0.02	ND
Fucose (% w/w)	ND	0.17 ± 0.05	ND
Arabinose (%w/w)	2.8 ± 0.5^{a}	2.3 ± 0.4^{a}	1.1 ± 0.6^{b}
Galactose (%w/w)	2.6 ± 0.4^{c}	19.5 ± 2.5^{a}	6.7 ± 0.1^{b}
Glucose (%w/w)	1.50 ± 0.04^{a}	0.6 ± 0.1^{b}	0.3 ± 0.2^{c}
Mannose (%w/w)	0.8 ± 0.3^{b}	10.1 ± 0.8^{a}	1.7 ± 0.9^{b}
non-free sugars (%w/w)	7.7 ± 1.2^{b}	32.8 ± 3.2^{a}	9.8 ± 2.8^{b}

ND: not detected. Average ± CI (95%), n=3. Average values followed by different letters in the same line and in decreasing alphabetical order are significantly different from each other, according to Tukey's test ($p<0.05$). *Refers to the hydrolysate obtained after 71 h of enzymatic reaction.

The yields achieved in each step (extraction, thermal hydrolysis, and enzymatic hydrolysis) were calculated using Equation (1) and are displayed in Figure 3. Thermal hydrolysis (180 °C) and extraction produced yields of 28 and 20%, respectively. These values were in agreement with the higher carbohydrate content of THE. It is important to note that, although AE contained only 7.7% of total carbohy-

drates, there were many other compounds present, such as caffeine, proteins, and organic acids, which were quantified as " " in the yield calculation of Equation (1) (Arya and Rao, 2007). The overall yield achieved (extraction plus thermal hydrolysis) was 48%, which is the standard value established by GEA-NIRO for arabica coffee beans (GEA-NIRO, 2013). In terms of the production of volatiles, THE presented a higher content of undesirable compounds (due to the higher temperatures employed), compared to AE (Table 4).

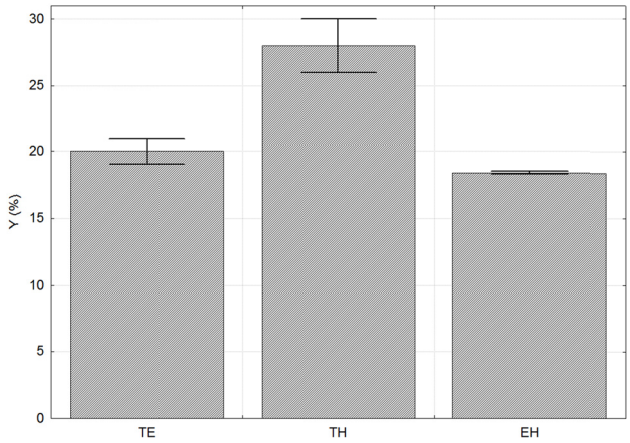

Figure 3: Yields (Y) obtained for thermal extraction (TE), thermal hydrolysis (TH) and enzymatic hydrolysis (EH), calculated from Eqs. (1) - (4). Average ± CI (95%), n=3, x_{AE} = 9.55 ± 0.02 (aromatic extract mass fraction of soluble solids in %) and x_{THE} = 3.84 ± 0.02 (thermal hydrolysis extract mass fraction of soluble solids in %)

Characterization of EHE

Enzymatic hydrolysis is an alternative to the thermal hydrolysis of polysaccharides from spent coffee (Figure 2). The time course of the reducing sugar concentration during enzymatic hydrolysis is shown in Figure 4. Tables 2-4 include the concentrations of free sugars, total sugars, non-free sugars and volatiles for the EHE product obtained after 71 h of enzymatic hydrolysis. Around 70% of the reducing sugar was produced within 25 h of reaction (Figure 4). This increase in the reducing sugar concentration was due to the action of the enzymes on solid spent coffee as well as on the polysaccharides that had already been solubilized.

The total sugar concentration obtained for THE (36.2%) was higher than for EHE (21.4%) (Table 3), reflecting the superior yield of the thermal hydrolysis step (28%, Figure 3). Furthermore, the total galactose content in EHE (7.2%, Table 3) was the main cause for the impaired yield of the enzymatic hydrolysis (18.4%, Figure 3). This indicates that the lack of galactanase activity in the commercial enzyme preparations hindered the release of polysaccharides containing galactose from the AG biopolymer.

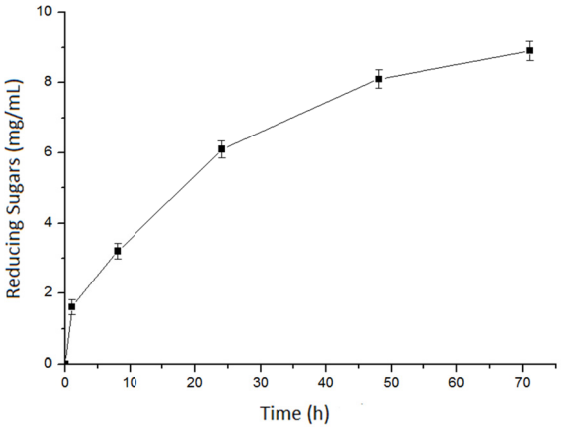

Figure 4: Time profile for the release of reducing sugars during enzymatic hydrolysis carried out at 50 °C and pH 5.0, using the commercial enzyme preparations Powercell, Galactomannanase-HBI, and Ultraflo XL. Vertical bars represent the standard deviation from triplicate experiments.

Comparison of EHE with AE showed that the former had a higher content of total sugars, with values of 21.4 and 8.2%, respectively (Table 3), as well as higher concentrations of total galactose, mannose, and glucose (Table 4), while the yields were similar (Figure 3).

The total non-free sugars content was similar for EHE and AE, at around 8% (Table 4), and was significantly higher for THE (~33%). The enzymes not only acted on the spent ground coffee, but also on the polysaccharides that were already soluble, as a result of which EHE had a higher content of free sugars (12.2%), compared to THE and AE (3.7 and 1.0%, respectively), as shown in Table 2.

A high content of free sugar in EHE could provide the final product (instant coffee) with desirable characteristics such as greater sweetness and faster solubilisation. On the other hand, a high content of free sugars can increase water absorption (due to greater hygroscopicity) and lead to product degradation, hence decreasing the shelf life of the product. A low content of non-free sugars reduces the viscosity of the coffee solution during concentration in an industrial evaporator, which improves the performance of the system. In addition, when the coffee extract is used to produce a ready-to-drink beverage, a high content of non-free sugars can lead to the formation of sediments at the bottom of the packaging,

decreasing the quality of the product (Delgado *et al*., 2008).

The most important feature of instant coffee produced by the enzymatic route is probably the increase in quality resulting from lower concentrations of undesirable compounds, as shown in Table 4. The concentrations of acetaldehyde, furfural, and 5-HMF in EHE were even significantly lower than the values obtained for the aroma extract (AE).

These preliminary results therefore suggest that enzymatic hydrolysis is a promising alternative procedure for the production of instant coffee. The overall yield (considering thermal extraction together with enzymatic hydrolysis) was up to 39.4% (Figure 4), which is ~80% of the value achieved with the standard GEA-NIRO process. However, the enzymatic hydrolysis yield could be further improved by the identification of galactanases and other enzymes capable of breaking down the structure of arabinogalactan. In addition, the enzymatic process offers versatility: the reaction time and the enzymatic formulation can be selected in order to obtain products containing different mixtures of free sugars and polysaccharides. Nevertheless, it is fundamental to carry out a sensory analysis of the enzymatic extract to fully establish the potential of the studied alternative technology for instant coffee production.

CONCLUSIONS

Updated industrial technologies have moved towards the incorporation of Green Chemistry principles, with reduced production of environmentally harmful compounds and lower energy consumption. The instant coffee currently marketed is obtained using the GEA-NIRO process, which is characterized by maximization of the yield at the expense of high consumption of energy and degradation of solubilized polysaccharides. The findings of the present work show that spent coffee can be used to obtain instant coffee by an environmentally friendly enzymatic route, using the commercially available enzymatic preparations Powercell (Prozyn Biosolutions), galactomannase (HBI Enzymes) and Ultraflo XL (Novozymes Latin America), which exhibited the highest enzymatic activities on celullases, galactomanannases and galactanases.

Comparison of the products manufactured by the conventional thermal process and enzymatic hydrolysis showed that the yield and the composition of the solubilized material, expressed as total or specific sugar compounds, were influenced by the process conditions. For the thermal processes, temperature was the key factor governing performance: the total sugar concentration increased from 8.2% (AE produced at 125 °C) to 36.2% (THE produced at 180 °C). On the other hand, the extract obtained from the enzyme-based process at 50 °C contained 21.4% of total sugars. Compositional analysis of the products (AE, THE, and EHE) also revealed that temperature was particularly crucial for the release of polysaccharides containing galactose, and that the enzyme pool must be improved in order to provide more efficient breakdown of spent coffee polysaccharides.

Enzymatic hydrolysis of spent coffee at 50 °C with the selected enzyme preparations resulted in a yield of 18.4%. This was similar to the yield achieved using extraction at 125 °C, but was associated with a reduced content of volatiles arising from the degradation of carbohydrates, due to the lower temperature employed. A high economic value is placed on low concentrations of undesirable process by-products (such as furfural, acetaldehyde, and 5-HMF) in instant coffee, due to their impact on product quality.

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