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Analytical Methods

Sediments in coffee extracts: Composition and control by enzymatic hydrolysis

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Abstract

The water-insolubility of some coffee extract components is one of the major limitations in the production of instant coffee. In this work, fractions from coffee extracts and sediments were prepared, and their chemical composition determined. Based on the carbohydrate analysis, galactomannan was found to be the main polysaccharide component of the insoluble fractions and probably responsible for sediment formation. The suitability of twelve commercial enzymes for the hydrolysis of the insoluble fractions was investigated. Pectinase 444L was the most effective enzyme in releasing sugars, mainly mannose and galactose, from these substrates. Biopectinase CCM, Rohapect B1L, Pectinase 444L and Galactomannanase ACH were found to be the most effective enzymes for reducing the sediment of coffee extracts. The highest sediment reduction was obtained using Rohapect B1L and Galactomannanase ACH, at enzyme concentrations of 0.3 and 0.1 mg protein/g substrate, respectively.

Keywords: Coffee polysaccharides; Galactomannans; Instant coffee; Enzymatic hydrolysis; Coffee composition; Sediment

1. Introduction

Coffee is one of the world's most widely consumed beverages. The chemical composition of the coffee cell wall has not been studied in detail, since it is difficult to dissolve, extract and digest (Kasai, Konishi, Iwai, & Maeda, 2006). Polysaccharides comprise nearly 50% of the green coffee bean weight (Fischer, Reimann, Trovato, & Redgwell, 2001; Nunes & Coimbra, 2001; Nunes, Reis, Domingues, & Coimbra, 2006), and those found in the coffee cell wall are mainly galactomannan, arabinogalactan and cellulose (Fischer et al., 2001; Oosterveld, Harmsen, Voragen, & Schols, 2003; Redgwell, Trovato, Curti, & Fischer, 2002). Arabinogalactans consist of a main chain of 1→3 linked galactose branched at C-6, with side chains containing arabinose and galactose. Galactomannans consist of a main chain of 1→4 linked mannan with galactose unit side chains linked at C-6, and different degrees of branching (Bradbury & Halliday, 1990; Navarini et al., 1999; Nunes et al., 2006). The structures of the polysaccharides of industrialized coffee products depend on the degree of roasting (Nunes & Coimbra, 2002; Oosterveld, Voragen, & Schols, 2003; Redgwell, Trovato, et al., 2002).

The main obstacle to characterizing the coffee cell wall is the high proportion of insoluble polymers (Bradbury & Atkins, 1997; Fischer et al., 2001; Redgwell, Curti, Fischer, Nicolas, & Fay, 2002). The solubility increases with increasing degree of branching and decreasing molecular weight (Nunes & Coimbra, 2001). Arabinogalactans dissolve better than do linear mannans, which can easily precipitate, and one of the reasons for this non-dissolution is an association of linear mannans to form crystalline regions (Bradbury & Atkins, 1997). This could be the reason for the formation of sediment during the manufacture of instant coffee. According to Fischer et al. (2001), the

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difficulty in dissolving the cell wall polysaccharides indicates an intimate association between some of the arabinogalactan, galactomannan and cellulose molecules.

Proteins are another important component of coffee extracts. The roasting process causes degradation of the proteins into smaller products (Nunes & Coimbra, 2001). In espresso coffee, the protein content was shown to be correlated with the foam volume (Nunes, Coimbra, Duarte, & Delgadillo, 1997), and the protein is usually covalently linked to arabinogalactans (Fischer et al., 2001; Redgwell, Curti, et al., 2002; Navarini et al., 1999; Redgwell, Schmitt, Beaulieu, & Curti, 2005). Information on coffee lipids is very limited, but it has been speculated that poor quality of coffee is also due to the hydrolysis of triacylglycerols (TAGs) with the release of free fatty acids, which, in turn, are oxidized (Jham, Velikova, Muller, Nikolova-Damyanova, & Cecon, 2001; Nikolova-Damyanova, Velikova, & Jham, 1998; Segall, Artz, Raslan, Jham, & Takahashi, 2005). The main classes of lipids present in green coffee are triacylglycerols (75%) and terpene esters (14%) (Jham et al., 2001; Nikolova-Damyanova et al., 1998). There are still no reports available on the lignin content of coffee, but the lignin is found closely associated with the cellulose and hemicellulosic polysaccharides (de Vries & Visser, 2001; Dóka, Bicanic, & Bunzel, 2004; Juhász, Szengyel, Réczey, Siika-aho, & Viikari, 2005).

In Brazil, coffee extracts are processed into instant coffee or concentrated extract for exportation. However, during storage and commercial circulation, sediment is sometimes observed in the extracts, which is considered to be a quality defect and limits the utilization of the product.

These days, enzymes are commonly used in many industrial applications, including the degradation of plant cell walls. Cellulases, hemicellulases and pectinases are industrially important enzymes that are sold in large amounts for many applications. Different authors have investigated the use of enzymes to hydrolyze coffee polysaccharides. Nunes et al. (2006) isolated the galactomannans from light and dark roasted coffee infusions, and hydrolyzed them with endo-mannanase, decreasing the molecular weight of these polysaccharides. Mannanase can also be used to reduce the viscosity of the extract in the production of instant coffee, improving the effectiveness of the concentration process and reducing drying costs (Sachslehner, Foidl, Foidl, Gübitz, & Haltrich, 2000). These authors hydrolyzed the coffee mannan with free and immobilized mannanase from *Sclerotium rofsii*.

The aim of this work was to determine the composition and study the enzymatic hydrolysis of coffee fractions using different commercial enzyme preparations, and then to apply the enzymatic treatment to the whole extract in order to reduce the sediment formed during coffee processing.

2. Materials and methods

2.1. Materials

Coffee extract containing sediment was supplied by Cia Iguaçu de Café Solúvel (Cornelio Procópio, Paraná, Brazil). Enzyme preparations were obtained from different sources, and are described in Table 1. Monosaccharide standards were purchased from Sigma and Fluka, and all other reagents and solvents were of the highest purity.

2.2. Preparation of the coffee and sediment fractions

In the process used by the Cia Iguaçu, green coffee beans were roasted and ground, and the ground coffee then percolated by hot water under high pressure to extract the solids. The extract obtained was stored in tanks at 4 °C, where the sediment formed. The whole extract (containing sediment) and the sediment alone were the samples used in the present work, being fractionated according to Fig. 1. All the fractions were freeze-dried.

2.3. Chemical analysis

Neutral and acidic sugars were analyzed according to the Saeman hydrolysis (Selvendran, March, & Ring, 1979). In this method, 10 mg of sample were first added to 0.5 ml of

Table 1
Source, major activity and protein content of the commercial enzyme preparations

Enzyme	Source	Major activity[a]	Protein content (mg/ml)
Econase CE	AB enzymes	Cellulase	110 ± 4.24
Protease GC 106	Genencor	Protease	86.0 ± 1.41
Novo Shape	Novozymes	Pectinase	28.2 ± 0.21
Pectinex 3XL	Novozymes	Pectinase	17.7 ± 0.42
Pectinex Ultra	Novozymes	Pectinase	50.6 ± 0.85
Biop. CCM	Biofincon	Pectinase	40.6 ± 1.70
Biop. Super 8x	Quest	Pectinase	57.9 ± 1.46
Pectinase 444L	Biocatalysts	Pectinase	16.9 ± 0.04
Rohapect B1L	AB enzymes	Pectinase	37.9 ± 0.14
Rohapect D5L	AB enzymes	Pectinase	12.9 ± 0.33
Rohapect 10L	AB enzymes	Pectinase	67.6 ± 2.62
Galactomannanase ACH	Sumizyme	Galactomannanase	0.3 ± 0.01[b]

[a] According to the manufacture.
[b] mg/mg (enzyme powder).

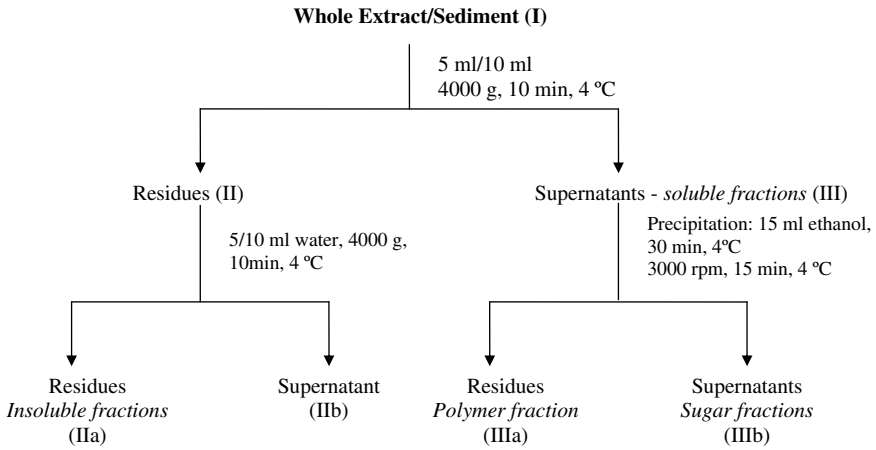

Fig. 1. Scheme for preparation of the coffee and sediment fractions.

72% H_2SO_4 and maintained at room temperature for 3 h. After this pre-treatment, distilled water was added to the mixture, to dilute the H_2SO_4 to 1 M, and incubated at 95 °C for 2 h. The monosaccharides were analyzed by high performance anion-exchange chromatography (HPAEC), using a Dionex DX 500 system (Dionex Corp. Sunnyvale CA), equipped with a GP40 gradient pump, ED40 electrochemical detector and AS 3500 autosampler. The analytical column was a CarboPac PA1 (250 × 4 mm) and the guard column was a CarboPac PA1 (25 × 3 mm). All determinations were carried out at 30 °C, using a flow rate of 1 ml/min and a gradient with pure water, NaOH and NaOAc. *Reducing sugars* were determined using the dinitrosalicylic acid (DNS) method (Bernfeld, 1955; Sumner, 1924). *Total protein content* was estimated by the Kjeldahl nitrogen method, and a factor of 6.25 was used to convert nitrogen into protein (Bernard, 1992; Buckee, 1994). *Ash* was analyzed by incineration in a programmable muffler oven from room temperature to 550 °C (4 h at peak temperature). *The insoluble lignin content* was estimated according to Browning (1967). *The lipid content* was analyzed according to Partanen, Hakala, Sjövall, Kallio, and Forssell (2005), using a Maran 23 MHz proton NMR spectrometer. The *soluble protein* concentration of the enzymes was determined according to the Lowry assay against a standard curve of bovine serum albumin (Lowry, Rosebrough, Farr, & Randall, 1951), after precipitation of the protein from the samples using trichloroacetic acid. All the analyses were carried out in duplicate. The data were analyzed by ANOVA followed by Tukey's test, at a significance level of 0.05. Statistical analyses were performed using the software STATISTICA 7.0.

2.4. Enzymatic hydrolysis of insoluble fractions of the extract and sediment

Three-millilitre aliquots of insoluble fraction preparations, IIa (1% w/v in 50 mM citrate buffer, pH 5.0) were treated with 100 µl of different enzyme solutions (10 mg protein/g substrate) at 45 °C for 20 h. The reaction was stopped by heating the mixture in a boiling water bath for 10 min. The sugars released were determined by HPAEC. Control treatments (with no enzyme) were done in parallel to the enzymatic ones.

2.5. Enzymatic hydrolysis of the whole extract to reduce the sediment

To investigate the effect of the enzymatic treatment on the sediment contents, 4 ml of the whole extract were incubated with 100 µl of enzyme solution (initially 5 mg protein/g substrate). The solutions were then cooled to 0 °C, maintained at this temperature for 5 min and then centrifuged at 3000 rpm, 4 °C for 10 min. The supernatant was discarded and the sediment dried overnight at 105 °C. Before centrifugation, a small aliquot (25 µl) was collected for the reducing sugar analysis by the DNS method. The temperatures studied ranged from 35 to 55 °C. Control treatments were made under similar conditions except that no enzyme was added. The sediment content was calculated according to the following equation:

$$\text{SEDIMENT}(\%) = \frac{\text{dried weight of residue}}{\text{dried weight of initial substrate}} \times 100.$$

3. Results and discussion

3.1. Chemical composition of the extract and sediment fractions

The chemical compositions of the whole extract and of the insoluble fractions isolated from the coffee extract and sediment are shown in Table 2. The whole extract (I) contained 43.9% of carbohydrate, the insoluble fraction of the extract 54.7% and the insoluble fraction of the sediment 12.9%. Thus the carbohydrate concentration was approximately 10% higher in the insoluble fraction of the extract than in the whole extract, and approximately 30% smaller in the insoluble fraction of the sediment. Concerning the whole extract, similar results have been described in

Table 2
Chemical composition of the whole extract and insoluble fractions % (w/w)

Fraction	Total sugars	Free sugars	Protein	Lipids	Ash	Insoluble lignin
Whole extract	43.4 ± 1.34^{a}	4.7 ± 0.14^{a}	19.2 ± 0.28^{a}	~0.0	10.5 ± 0.69^{a}	3.8 ± 0.32^{a}
Insoluble fraction of extract	55.9 ± 1.93^{b}	3.4 ± 0.14^{b}	15.2 ± 0.57^{b}	~0.0	6.2 ± 0.31^{b}	5.2 ± 0.35^{a}
Insoluble fraction of sediment	12.8 ± 0.08^{c}	0.6 ± 0.03^{c}	30.5 ± 1.41^{c}	12.2 ± 0.5	3.4 ± 0.14^{c}	27.6 ± 2.26^{b}

Different letters in the same column are significantly different ($P < 0.05$) according to Tukey's test.

the literature on roasted coffee (Oosterveld, Harmsen, et al., 2003; Redgwell, Trovato, et al., 2002).

The protein content of the whole extract was found to be higher, 19%, than the value reported by Oosterveld, Harmsen, et al. (2003), which was only 6.8%, but was closer to the value reported by Franca, Mendonça, and Oliveira (2005), which was 14.9%. As the protein content was calculated from the total nitrogen content of the samples, it may have been overestimated due to the presence of other nitrogen-containing substances (caffeine, trigonelline, free amines and amino acids). In addition to polysaccharides and proteins, the whole extract also contained ash (10.5%) and insoluble lignin (3.8%). Lipids were not found.

The insoluble fraction of the extract showed higher contents of carbohydrate (54.7%) and insoluble lignin (5.2%), and lower contents of protein (15.2%) and ash (6.2%), when compared to the whole extract, whereas the insoluble fraction of the sediment had relatively lower contents of sugar (12.9%) and ash (3.4%) and higher contents of protein (30.5%), lipid (12.2%) and insoluble lignin (27.6%). Since the insoluble lignin content was higher in the insoluble fraction of the sediment than in the other fractions, this leads us to believe that the lignin may be bound to the cellulose, hemicellulose and protein, forming a large amount of insoluble particles. Besides polysaccharides, proteins, lipids, ash and insoluble lignin, the material could also contain soluble lignin, chlorogenic acids, aliphatic acids, humic acids and melanoidins (Charles-Bernard, Kraehenbuehl, Rytz, & Roberts, 2005; Smith, 1985).

The monosaccharide profiles of the coffee fractions are shown in Table 3, and were composed mainly of galactose, arabinose and mannose, building blocks of the galactomannans and arabinogalactans.

The predominant sugars in the whole extract (I) were galactose and mannose (44.1% and 33.1%, respectively), followed by arabinose (10.6%). The ratios of galactose:mannose and arabinose:galactose were, respectively, 1.3 and 0.24. Oosterveld, Harmsen, et al. (2003) examined the chemical structure of the polysaccharide fractions obtained after the hot water extraction of roasted coffee. After an extraction with water at 90 °C, the authors found 49% of mannose, 33% of galactose and 9% of arabinose. After extraction at 170 °C, the sugar profile was 32% of mannose, 53% of galactose, and 8% of arabinose; the ratios galactose:mannose and arabinose:galactose were 1.7 and 0.15, respectively, values close to the present results. Acidic sugars were also found in the whole extract (methylglucuronic acid, galacturonic acid and glucuronic acid), representing 7% of the total carbohydrate content. According to Redgwell, Trovato, et al. (2002) and Redgwell, Curti, et al. (2002), rhamnose and galacturonic acid are components of pectin or rhamnogalacturonan, that can be present in the coffee bean cell wall, while glucuronic acid can exist as terminal residues on the side chains of arabinogalactans.

Concerning the insoluble fractions (IIa), it was found that the main polysaccharide was a galactomannan. The ratios of galactose:mannose were 0.41 and 0.37 for the extract and sediment insoluble fractions, respectively. Therefore, the galactomannan present in these fractions is less branched than that found in the whole extract. The low solubility of these fractions may result from the low degree of branching (Buckeridge, Tiné, Santos, & Lima, 2000; Oosterveld, Harmsen, et al., 2003). The polymer fractions (IIIa) were both primarily composed of galactose units, indicating the presence of arabinogalactans and galactans. In the sugar fractions (IIIb), the main sugar was mannose, followed by galactose and arabinose. The ratio of mannose:galactose was approximately 1, suggesting the high solubility of these fractions.

3.2. Enzymatic hydrolysis of the insoluble fractions

Insoluble fractions (IIa), previously isolated from the coffee extract and sediment, were treated with twelve commercial enzyme preparations containing cellulases, hemicellulases, pectinases and protease. The protein content of the enzymes was also investigated in order to standardize suitable concentrations for them in the coffee treatments (Table 1). These enzyme preparations are complex mixtures of various different hydrolytic enzymes, but the major activity according to the manufacture is also given.

Tables 4 and 5 show the monosaccharide compositions of the products resulting from enzymatic hydrolysis of the insoluble fractions of the extract and sediment, respectively. The content of each sugar released after acid hydrolysis of these fractions is also included, for comparison. The free sugar contents of the non-hydrolyzed (control treatments) insoluble fractions were also determined, in order to compare with the values obtained after the enzymatic treatments.

As shown in Table 4, the control of the insoluble fraction of the extract (without the addition of enzyme) was found to have 3.35% of free sugars, composed mainly of arabinose, followed by galactose and mannose. After treatment with Econase, the free sugar content increased to 5.33%, glucose being the main sugar released by this

Table 3
Monosaccharide composition of the coffee fractions after acid hydrolysis (% w/w)

Sample	Rha	Ara	Gal	Glu	Xyl	Man	Fru	MeGlcA	GalA	GlcA	Total carbohydrate (mg/100 mg solids)
Extract											
Whole	1.0 ± 0.07^{a}	10.5 ± 0.18^{a}	44.1 ± 0.33^{a}	3.2 ± 0.01^{a}	0.4 ± 0.03^{a}	33.2 ± 0.10^{a}	1.2 ± 0.07^{a}	0.6 ± 0.02^{a}	5.2 ± 0.02^{a}	0.6 ± 0.02^{a}	43.4 ± 1.34^{a}
Insoluble fraction	0.6 ± 0.04^{b}	6.1 ± 0.05^{b}	25.3 ± 0.24^{b}	2.1 ± 0.01^{b}	0.2 ± 0.02^{b}	61.3 ± 0.10^{b}	0.7 ± 0.00^{b}	0.5 ± 0.02^{a}	3.0 ± 0.18^{b}	0.5 ± 0.02^{b}	55.9 ± 1.93^{b}
Polymer fraction	0.8 ± 0.02^{c}	5.4 ± 0.10^{c}	60.4 ± 0.35^{c}	1.8 ± 0.01^{c}	0.3 ± 0.01^{a}	22.0 ± 0.08^{c}	0.3 ± 0.00^{c}	0.7 ± 0.42^{a}	7.9 ± 0.30^{c}	0.4 ± 0.00^{c}	64.6 ± 0.37^{c}
Sugar fraction	1.6 ± 0.05^{d}	20.0 ± 0.08^{d}	33.5 ± 0.19^{d}	6.2 ± 0.02^{d}	0.6 ± 0.02^{c}	36.1 ± 0.05^{d}	2.0 ± 0.14^{d}	Na	Na	Na	24.9 ± 0.70^{d}
Sediment											
Insoluble fraction	1.3 ± 0.04^{e}	8.2 ± 0.00^{e}	21.5 ± 0.14^{e}	4.3 ± 0.07^{e}	0.3 ± 0.01^{a}	57.4 ± 0.08^{e}	0.7 ± 0.03^{b}	2.0 ± 0.01^{b}	2.5 ± 0.02^{b}	2.0 ± 0.01^{d}	12.8 ± 0.08^{e}
Polymer fraction	1.1 ± 0.01^{a}	6.5 ± 0.06^{f}	65.2 ± 0.03^{f}	$2.0 \pm 0.01^{b,c}$	0.3 ± 0.03^{a}	24.6 ± 0.01^{f}	0.3 ± 0.00^{c}	Na	Na	Na	57.1 ± 1.55^{b}
Sugar fraction	1.7 ± 0.01^{d}	20.9 ± 0.01^{g}	33.0 ± 0.07^{d}	6.2 ± 0.11^{d}	0.7 ± 0.01^{d}	35.3 ± 0.09^{g}	2.2 ± 0.03^{e}	Na	Na	Na	23.4 ± 0.43^{d}

Values expressed on dry bases. Na, not analyzed.
Rha, rhamnose; Ara, arabinose; Gal, galactose; Glu, glucose; Xyl, xylose; Man, mannose; Fru, fructose; MeGlcA, methylglucuronic acid; GalA, galacturonic acid; GlcA, glucuronic acid.
Different letters in the same column are significantly different ($P < 0.05$) according to Tukey's test.

enzyme. Econase is a preparation of cellulases obtained from *Trichoderma reesei*, containing several endoglucanases and exoglucanases and various hemicellulases (Suutarinen et al., 2003). Hydrolysis with Protease CG106 produced mainly mannose, followed by galactose and glucose, which were certainly released due to the presence of other hydrolytic activities apart from the protease activity. Galactose and mannose were found to be the main sugars released by hydrolysis with Pectinex 3XL, Pectinex Ultra, Biopectinase CCM, Biopectinase Super 8x, Pectinase 444 and Rohapect B1L. It was evident that these pectinase preparations were mixtures of various enzymes, which hydrolyzed mannans, galactans and other carbohydrates. Of all the pectinases tested, Rohapect D5L, which is used in fruit juice processing, achieved the lowest yield of released sugars. After hydrolysis with Pectinase 444L, the mannose content increased significantly (42 times) and 80% of the total carbohydrates was present as free sugars, indicating the hydrolysis of mannans.

When the insoluble fraction of the coffee sediment (Table 5) was treated with the commercial enzymes, similar results to those obtained with the insoluble fraction of the extract were found, although lower amounts of reducing sugars were observed. As shown in Table 3, both the extract and sediment insoluble fractions were composed of the same carbohydrates, but the extract fraction had a higher carbohydrate concentration than had the sediment fraction.

Two other enzyme complexes (Novo Shape and Rohapect 10L) were also tested, but apparently had no effect on the carbohydrate composition of the substrates (data not shown).

The glucose and fructose contents were higher after enzymatic hydrolysis than after acid hydrolysis. Acid hydrolysis probably degraded part of these sugars, but if the acid hydrolysis conditions had been less drastic, complete hydrolysis of the polysaccharides might not have been possible. According to Puls (1993), after TFA and HCl hydrolysis, part of the sample may still not have been hydrolyzed, whilst with H_2SO_4, the hydrolysis of the neutral sugars is complete but some of the monomer units may degrade.

Therefore it was concluded, from the results, that Pectinase 444L was the most efficient enzyme in releasing sugars from insoluble coffee materials.

3.3. Enzymatic hydrolysis of the whole extract to reduce the sediment

As it is important to reduce the amount of sediment during the production of instant coffee, the efficiencies of the different enzymes on the coffee extract were studied. The contents of the main components found in the whole extract can be seen in Table 2. The performance of the enzymes in the hydrolysis of the coffee extract can depend on factors, such as substrate concentration, enzyme type and concentration, and process conditions, such as pH,

Table 4
Monosaccharide composition of the insoluble fraction of the extract after enzymatic hydrolysis (mg in 100 mg of dried substrate)

Enzyme	Rha	Ara	Gal	Glu	Xyl	Man	Fru	Total
Control treatment[a]	0.04	1.24	0.79	0.17	0.04	0.77	0.29	3.35
Total sugars (acid hydrolysis)[b]	0.31	3.30	13.67	1.16	0.09	33.12	0.35	52.00
Econase CE	0.04	1.65	1.19	0.58	0.17	1.45	0.31	5.38
Protaese GC 106	0.04	1.55	2.69	0.57	0.06	6.72	0.39	12.0
Pectinex 3XL	0.04	2.17	9.30	0.12	0.04	26.4	0.72	38.8
Pectinex Ultra SP-L	0.05	1.96	8.68	0.71	0.04	9.30	0.53	21.3
Biopectinase CCM	0.04	2.07	8.06	0.90	0.04	25.2	0.74	37.1
Biopectinase Super 8x	0.04	2.07	6.92	0.82	0.04	28.9	0.73	39.6
Pectinase 444L	0.04	2.17	9.63	1.32	0.04	30.4	0.85	44.4
Rohapect B1L	0.04	1.76	2.89	1.24	0.04	25.9	0.71	32.6
Rohapect D5L	0.04	1.71	1.50	0.64	0.08	1.27	0.32	5.56

Rha, rhamnose; Ara, arabinose; Gal, galactose; Glu, glucose; Xyl, xylose; Man, mannose; Fru, fructose.
[a] Free sugars of the control treatment (no enzyme).
[b] Total sugars obtained after acid hydrolysis of the insoluble fraction of the extract.

Table 5
Monosaccharide composition of the insoluble fraction of the sediment after enzymatic hydrolysis (mg in 100 mg of dried substrate)

Enzyme	Rha	Ara	Gal	Glu	Xyl	Man	Fru	Total
Control treatment[a]	0.04	0.24	0.15	0.04	0.04	0.13	0.05	0.69
Total sugars (acid hydrolysis)[b]	0.17	1.05	2.77	0.54	0.03	7.35	0.09	12.01
Econase CE	0.04	0.42	0.30	0.30	0.04	0.42	0.06	1.59
Protaese GC 106	0.04	0.33	0.69	0.24	0.04	2.69	0.07	4.10
Pectinex 3XL	0.04	0.59	1.76	0.04	0.04	6.30	0.13	8.89
Pectinex Ultra SP-L	0.04	0.49	1.65	0.31	0.04	2.48	0.11	5.13
Biopectinase CCM	0.04	0.51	1.55	0.37	0.04	6.30	0.15	8.96
Biopectinase Super 8x	0.04	0.51	1.40	0.29	0.04	6.20	0.13	8.61
Pectinase 444L	0.07	0.53	1.64	1.31	0.04	6.47	0.23	10.3
Rohapect B1L	0.04	0.44	0.62	0.73	0.04	6.72	0.15	8.74
Rohapect D5L	0.04	0.42	0.39	0.34.	0.04	0.40	0.07	1.72

Rha, rhamnose; Ara, arabinose; Gal, galactose; Glu, glucose; Xyl, xylose; Man, mannose; Fru, fructose.
[a] Free sugars of the control treatment (no enzyme).
[b] Total sugars obtained after acid hydrolysis of the insoluble fraction of the sediment.

temperature, mixing rate and reaction time. The pH of the industrialized coffee extract is around 5.0, which was maintained, since this is the optimum pH of the enzymes studied, and due to the difficulty of adjusting the pH during the industrial manufacture of instant coffee.

The first set of experiments was conducted in order to determine which enzymes most efficiently reduced the amount of sediment in the whole extract, employing an enzyme concentration of 5 mg protein/g substrate at 45 °C for 2 h. The effects of the enzymes on the sediment and on the reducing sugars are shown in Fig. 2.

The amount of sediment obtained in the non-enzymatically treated coffee was 14% at 45 °C. Treatments with Econase, Protease, Novo Shape, Pectinex 3XL, Rohapect 5DL and Rohapect 10L had no effect on the amount of sediment. Hydrolysis with Pectinex Ultra, Biopectinase CCM, Pectinase 444L, Rohapect B1L and Galactomannanase ACH resulted in approximately the same efficiency (≈4% of the amount of sediment). Fig. 2a and b shows that the profiles of the amount of sediment and of the sugars released were not related, i.e., Pectinase 444L was shown to release the highest concentration of sugars.

Equivalent conversions can be achieved with lower concentrations of enzymes during longer treatments; therefore the influence of enzyme concentration on the hydrolysis was evaluated. Hydrolysis experiments with the four best enzyme preparations (Biopectinase CCM, Pectinase 444L, Rohapect B1L and Galactomannanase ACH) were performed at 50 °C and the results can be seen in Fig. 3a–d, respectively. Hydrolysis with Biopectinase CCM was carried out in a concentration range from 1–4 mg protein/g substrate, as shown in Fig. 3a. Increasing enzyme concentrations resulted in decreasing amounts of sediment. However, when 4 mg protein/g substrate was used, the final sediment content was 5% (w/w), and so this enzyme was not considered to be economically viable for an industrial process, since quite a large amount of enzyme would be required for a long period of time. Similar hydrolysis performances were obtained by Pectinase 444L, as shown in Fig. 3b, the maximum rate being achieved with an enzyme concentration of 4 mg protein/g substrate, which represents 16% (w/w) of enzyme per quantity of dried substrate, a value considered too high for a commercial application. However, when Rohapect B1L was employed (Fig. 3c), it

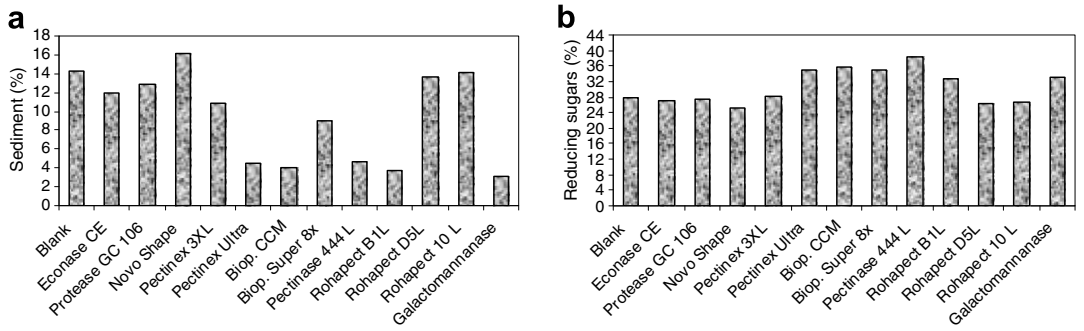

Fig. 2. Screening of commercial enzymes for sediment reduction. The effect of enzymatic hydrolysis on: (a) the amount of sediment and (b) the release of reducing sugars.

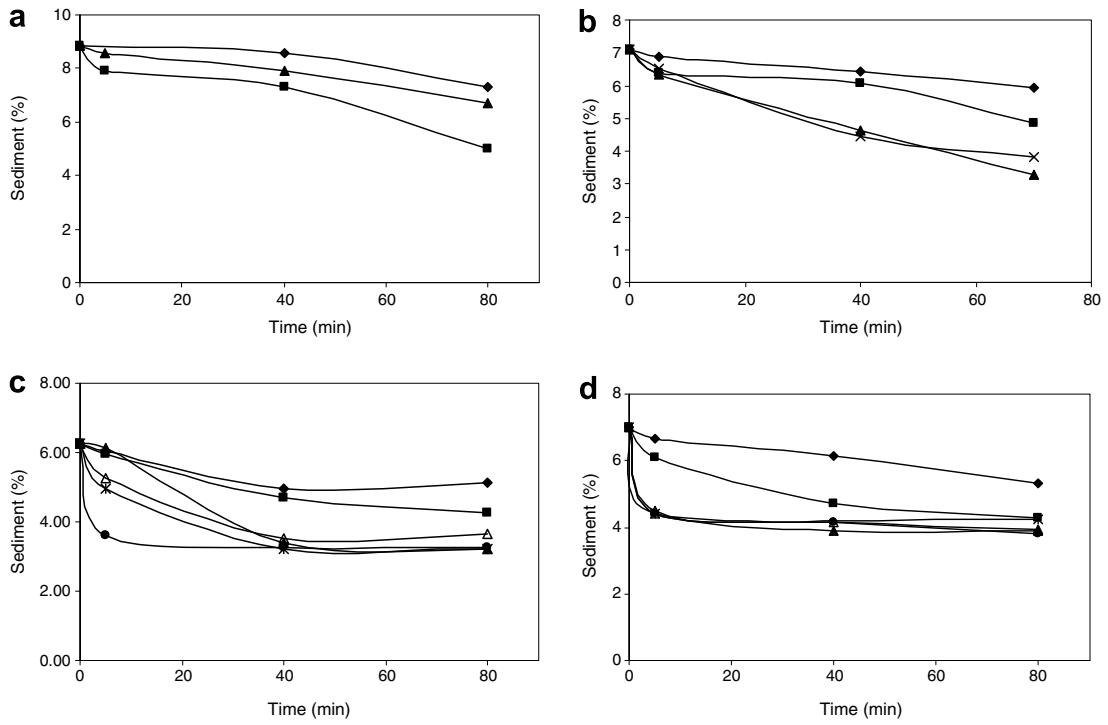

Fig. 3. Time course curves of the enzymatic hydrolysis of coffee extracts at 50 °C. Effect of the enzyme concentrations on the amounts of sediment. (a) Biopectinase CCM (-♦-) 1 mg prot/g substrate, (-▲-) 2 mg prot/g substrate, (-■-) 4 mg prot/g substrate; (b) Pectinase 444L (-♦-) 0.5 mg prot/g substrate, (-■-)1.0 mg prot/g substrate, (-▲-) 2.0 mg prot/g substate, (-×-) 4.0 mg prot/g substrate; (c) Rohapect B1L (-♦-) 0.05 mg prot/g substrate, (-■-) 0.1 mg prot/g substrate, (-▲-) 0.3 mg prot/g substrate, (-Δ-) 0.5 mg prot/g substrate, (-×-) 1.0 mg prot/g substrate, (-●-) 2.0 mg prot/g substrate; (d) Galactomannanase ACH – (-♦-) 0.05 mg prot/g substrate, (-■-) 0.1 mg prot/g substrate, (-▲-) 0.5 mg prot/g substrate, (-Δ-) 1.0 mg prot/g substrate, (-×-) 2.0 mg prot/g substrate, (-●-) 4.0 mg prot/g substrate.

was observed that, after 40 min of incubation, a significantly lower amount of enzyme (0.3 mg protein/g substrate), which represents 0.9% (w/w) of enzyme per amount of substrate, resulted in a decreased amount of sediment (approximately 3.5%). Further increases in the enzyme concentration did not result in a better effect. Galactomannanase ACH treatment (Fig. 3d) resulted in a high and fast sediment reduction. The lowest enzyme concentration to obtain the minimum sediment, 3.5% after 1 h, was 0.1 mg protein/g substrate, which represents 0.03% (w/w) of enzyme per amount of substrate. Above this, the enzyme concentration did not appear to enhance the hydrolysis yield; however, when using higher concentrations of enzyme, the same yield was achieved in a shorter period of time. Therefore, the present results indicated that, potentially, Rohapect B1L and Galactomannanase ACH could be employed for sediment reduction in coffee processing.

The effect of temperature on the dissolution of the sediment was also studied, since during the manufacture of coffee the sediment is mostly observed at low temperatures. The kinetic curves for the release of reducing sugars and the amount of sediment are shown in Figs. 4 and 5 for the enzymes Rohapect B1L and Galactomannanase ACH, respectively, at five different temperatures (35, 40, 45, 50 and 55 °C).

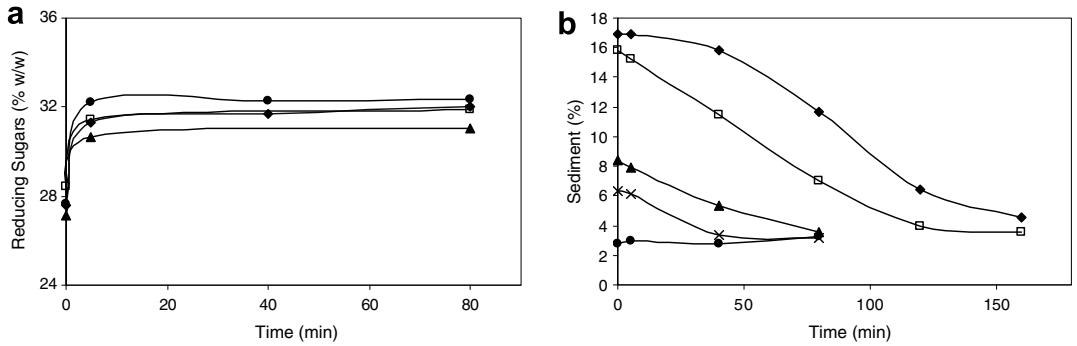

Fig. 4. Time course curves of the enzymatic hydrolysis of coffee extracts with Rohapect B1L. The effect of temperature on: (a) the release of reducing sugars and (b) the amount of sediment. (-♦-) 35 °C, (-□-) 40 °C, (-▲-) 45 °C, (-×-) 50 °C, (-●-) 55 °C.

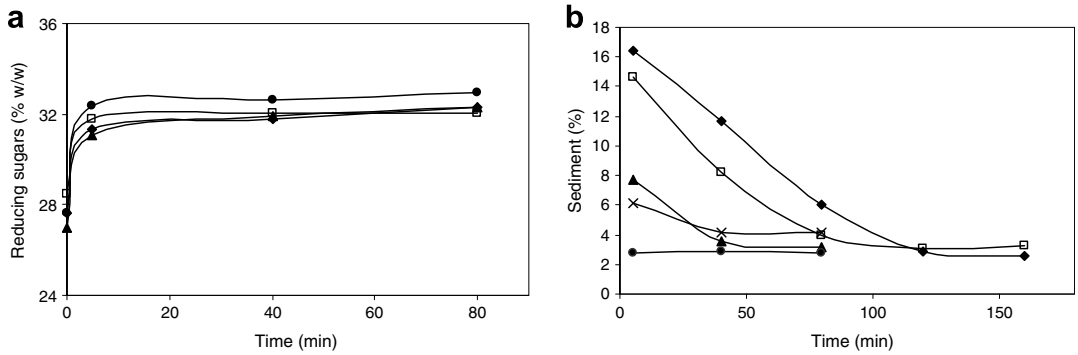

Fig. 5. Time course curves of the enzymatic hydrolysis of coffee extracts with Galactomannanase ACH. The effect of temperature on: (a) the release of reducing sugars and (b) the amount of sediment. (-♦-) 35 °C, (-□-) 40 °C, (-▲-) 45 °C, (-×-) 50 °C, (-●-) 55 °C.

Hydrolysis started quickly, as can be deduced from the reducing sugars profiles. The concentration of reducing sugars increased during the first 5 min and remained constant until the end of the reaction, independent of temperature. However, while the formation of total reducing sugars ceased after 5 min, the decrease in sediment showed different kinetics, depending on which temperature was used for hydrolysis. The fact that the reduction in sediment persisted while the release of reducing sugars had finished, indicates that the commercial preparations used in this work contained a mixture of enzymes with a wide spectrum of different activities, such as high activities of endo-enzymes, for example, endo-mannanase, and other activities.

Sensory evaluation of enzymatically treated coffee extracts has been studied and is the objective of another publication. Our studies showed that practically none or few differences were observed by the trained panellists and consumers (Delgado, 2008).

The present experiments showed that the initial amount of sediment decreased from 16% to 3.5% when the temperature increased from 35 to 55 °C. With an increase in extract temperature, the amount of sediment decreases, but according to the manufacturer becomes insoluble again on cooling. In the present work, the coffee extract was treated at five different temperatures, but the best temperature should be determined by the manufacturer.

4. Conclusions

This study showed that both the whole extract and its insoluble faction were composed mainly of carbohydrates and proteins, while the insoluble fraction of the sediment, besides these compounds, contained considerable amounts of insoluble lignin and lipids.

Analyzing the sugar compositions of the coffee fractions, it was observed that all of them consisted mainly of galactose, mannose and arabinose, which are the building blocks of galactomannans and arabinogalactans. The galactomannan present in the insoluble fractions had a lower galactose:mannose ratio than that found in the whole extract, which may explain the lower solubility of the insoluble fractions.

The insoluble fractions of the extract and sediment were treated with commercial enzymes. After analysis of the sugar composition, it was shown that the main sugars released were arabinose, galactose and mannose.

Four enzymes, Biopectinase CCM, Pectinase 444L, Rohapect B1L and Galactomannanase ACH, were considered efficient in treating the sediment but, of these, only Rohapect B1L and Galactomannanase ACH were economically viable for an industrial application. The lowest enzyme concentration required to reduce the sediment to approximately 3.5% was 0.3 and 0.1 mg protein/g substrate, for Rohapect B1L and Galactomanannase ACH, respectively.

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