

Analytical Methods

Extraction and characterization of pectins from cocoa husks: A preliminary study

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Received 9 October 2006; received in revised form 8 June 2007; accepted 5 September 2007

Abstract

Cocoa husks, a by-product of cocoa processing, were investigated as a source of pectins. Preliminary results of pectin recovery and characterization are shown; they constitute the first part of a study for the optimization of pectin extraction from this by-product. Husks of two different origins (Ghana and Venezuela) were used whole or minced and pectins were extracted under various conditions (pH 7.0, 4.0, 2.5, 1.5 and 1.0; extraction periods 1–3 h): the highest yield is obtained with minced husks after 1 h of extraction at pH 2.5. A preliminary characterization of pectins, in terms of methyl and acetyl ester contents, was also carried out in order to investigate the influence of different extraction conditions on the chemical composition of the extracts.

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Keywords: Cocoa husks; Pectins; Extraction; Characterization

1. Introduction

Pectins are complex carbohydrate molecules; they are used mainly as gelling agents in the food industry, however pharmaceutical and cosmetic uses are also known. Usually, industrial pectins are extracted from citrus peels and apple pomace with a multiple-stage physical-chemical process characterized by an extraction step with hot dilute mineral acid and recovery through alcohol precipitation (Daniel, Voragen, & Pilnik, 1994; May, 1990). Recently, non-traditional pectin sources have been investigated. Pectins have been extracted from various food industry by-products and, considering that food processing is characterised by large amounts of waste material, by this way the extraction process could represent an efficient and environmental friendly matter recovery for the production of functional compounds (Kroyer, 1995; Schieber, Stintzing, & Carle, 2001).

In the present study, cocoa husks, a by-product of the chocolate industry, have been investigated as a potential source of pectins. Cocoa husks represent a disposal problem, since increased processing of cocoa beans brings increasing wastes and this is a good reason to come up with a useful outlet for this by-product (Redgwell et al., 2003; Serra Bonvehì & Escolà Jordà, 1998). Cocoa husks contain a lower amount of pectins (about 9% dry weight) than citrus peels and apple pomace (about 30% and 15%, respectively), hence it is necessary to optimise the extraction process for improved yield, purity and functional properties, but at the same time, due to their low humidity, they present many advantages (e.g. they can be stored and transported without further processing). Cocoa husks pectins can be recovered by adapting the hot acid extraction method used for production from conventional raw materials; however, considering that little is known about this recovery, it is necessary to investigate the influence of extraction parameters on pectin yield and quality and their possible improvement through additional processing (e.g. pre-treatment of the raw material with microwave heating or enzymes). Finally, impurities like pigments or residual

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acids contained in the precipitated pectins, must be determined and reduced (Kratchanova, Pavlova, & Panchev, 2004; Sahari, Akbarian, & Hamedi, 2003).

The main objectives of this research were to define optimum conditions for cocoa husks pectins extraction and to give a preliminary chemical characterisation of the obtained product in terms of quality and purity.

2. Materials and methods

2.1. Raw materials and chemicals

Husks of cocoa imported from Ghana and Venezuela were utilized; both whole or minced husks were applied in the extraction procedure (husks were ground in a grinding mill).

All the chemicals and reagents were of analytical grade (Sigma-Aldrich S.r.l. Milano, Italy). The kit for the enzymatic analysis of acetic acid (Cat. No. 10 148 261 035) was purchased by Boehringer Mannheim/R-BIOPHARM AG, Darmstadt, Germany.

2.2. Pectins extraction and recovery

Pectins extraction was based on the method proposed by De Giorgi, Tomasicchio, and Andreotti (1985). Husks were extracted with water (1:25, w:v), acidified or not with HCl, at 95 °C, for 1 h or more. The complete set of experiments and extraction conditions is summarized in Table 1.

After extraction, the slurries were filtered through four layers of gauze and then the filtrate was passed through a Büchner funnel under vacuum with a Whatman No.1 qualitative filter paper. Pectins were precipitated by adding seven volumes of boiling ethanol 99.8%. The precipitate was filtered under vacuum through a mixed cellulose ester filter (0.45 µm) (Advantec MFS, Inc., USA) and washed with 10 volumes of an ethanol solution (63%). Coagulated pectins were finally solubilized with 0.05 N NaOH.

2.3. Microwave heating of cocoa husks

One set of extraction experiments was carried out by pre-treating Ghana cocoa husks by microwave heating (Kratchanova et al., 2004). Minced husks (20 g) were hydrated with 50 ml of distilled water; after 30 min the excess water was discharged and husks were heated in a microwave oven for 15 min at maximum power (750 W).

Table 1
Extractions conditions

Extraction time (hours)	Cocoa husks						
	Ghana			Venezuela			
	Whole pH	Minced pH	2.5	Minced pH	4.0	2.5	1.5
1	7.0	7.0	2.5	–	4.0	2.5	1.5
2	7.0	7.0	–	–	–	–	–
3	7.0	7.0	–	7.0	–	–	–

The entire quantity of irradiated ground husks was extracted as described above (extraction at pH 2.5 for 1 h).

2.4. Pectins characterization

Anhydrogalacturonic acid (%AGA) content of pectins was determined by the colorimetric phenyl–phenol method (Blumenkrantz & Asboe-Hansen, 1973) with an UV/VIS spectrophotometer, at 524 nm (ABS₅₂₄).

The determination of the ester methoxyl content (%DM) was carried out by two different analytical methods. It was firstly determined by saponification with 0.25 N NaOH and titration with 0.1 N NaOH in the presence of Hinton's indicator according to the method proposed by McCready, 1970. This content was also determined from the methanol liberated upon its alkaline hydrolysis through an enzymatic method with alcohol oxidase, peroxidase and the chromogen 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (Mangos & Haas, 1996).

Acetyl ester content was determined by an enzymatic UV-method based on the formation of an amount of NADH, measured by the increase in light absorbance at 340 nm, which is proportional to the acetic acid concentration.

The presence of polyphenols in solubilized pectins was determined with an UV/VIS spectrophotometer, at 325 nm, as reported by Petrus and Dougherty (1973).

All analyses of pectins were performed in triplicate.

3. Results and discussion

3.1. Influence of the extraction pH and time on pectin yield

The extraction process for cocoa husks pectins was optimised on the basis of two different conditions: pH and extraction time.

A first set of extractions was performed with Ghana cocoa husks. Firstly, whole or minced husks were extracted at a neutral pH and at three different extraction times (1–3 h); in this way the applicability of the process was demonstrated. Secondly, taking into account that hot acid extraction is usually the most efficacious, the procedure was repeated with minced husks at pH 2.5 for 1 h. Fig. 1 shows the obtained results expressed as %AGA by husks dry weight. Considering the extraction at neutral pH with whole husks, the pectins quantity is influenced by the extraction time; in fact, when the time passes from 1 to 2 h also the extracted quantity is doubled (2.0% and 4.0%, respectively), and when the extraction lasts 3 h a little improvement is obtained (4.7%). When the same runs are repeated with minced husks, as expected, the amount of recovered pectins rises appreciably because of the higher contact surface during the extraction; as a consequence, all the subsequent experiments were conducted with minced husks. On the other hand, the influence of time was lower with respect to results obtained with whole husks; in this case, pectins quantity passes from 5.0% to 6.0% for 1 and 2 h of extraction, respectively, while with 3 h 6.5% is

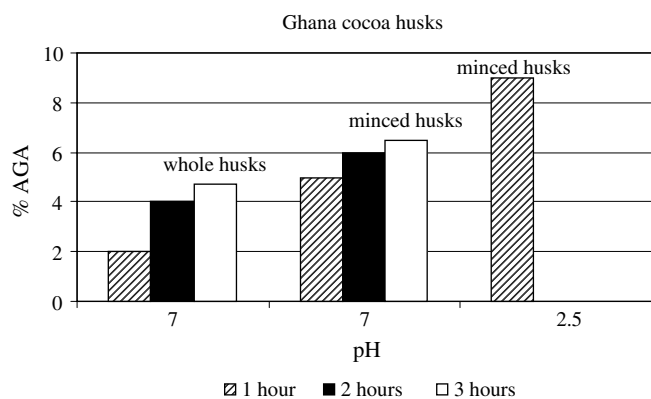


Fig. 1. Pectins extracted (expressed as %AGA) from Ghana cocoa husks.

obtained. Cocoa husks pectins content, is about 8–9%; therefore, 6.5%, the maximum quantity obtained at neutral pH, is lower, but they will represent only the water extractable pectins, which represent the major fraction of the total content. When the extraction is carried out at pH 2.5, the yield is improved, in fact for the same extraction time (1 h) the result is about doubled (9.0%) with respect to that obtained at neutral pH (5.0%). Considering that at pH 2.5 about all the extractable pectins are recovered, it is possible to affirm that the hot acid extraction is the most efficacious also for a matrix different from the conventional ones.

Subsequent experiments were carried out with Venezuela cocoa husks, due to supplying reasons. Results obtained up to now are shown in Fig. 2. At first minced husks were subjected to extractions at pH 7.0 for 3 h and at pH 2.5 for 1 h because those parameters enable to obtain best results with Ghana husks. The quantities recovered in this case are little lower: for the extraction at neutral pH, 6.0% is obtained (6.5% for Ghana cocoa husks), while at 2.5 pH, the result is 8.0% (9.0% for Ghana cocoa husks). This fact is not interpreted as a limitation because differences are not statistically remarkable, moreover the different origin of the raw material has to be taken into account (Sahari et al., 2003).

Considering literature information about extractions carried out at low pH values, the possibility to improve the quantity of recovered pectins was evaluated repeating the extraction at pH 1.5 and 1.0 for 1 h (Berardini,

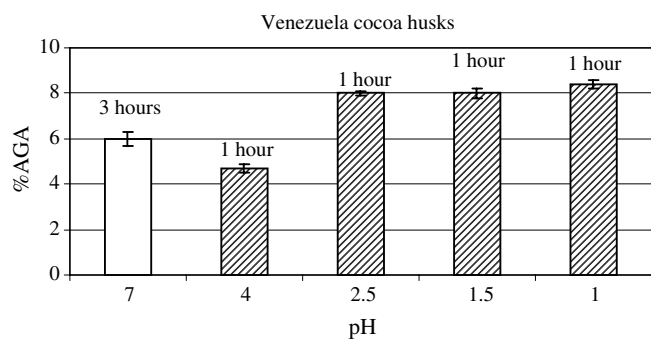


Fig. 2. Pectins extracted (expressed as %AGA) from Venezuela cocoa husks.

Knödler, Schieber, & Carle, 2005). Results obtained at pH 1.5 and 2.5 are identical (8.0%), while the run at pH 1.0 gave a little improvement (8.4%) but not significantly so. In addition, during the execution of extractions at pH 1.5 and 1.0, a problem arose: the solution containing pectins and separated after the extraction had a red color. Probably, other substances (e.g. tannins, one of the constituents of cocoa husks) are extracted together with pectins. This fact could also interfere with the procedure used for the determination of %AGA (the pectins quantity, obtained with an extraction conducted at pH 1.0 for 3 h, was lower than that obtained with 1 h extraction, data not shown); for all those reasons results obtained at low pH values (1.5 and 1.0) need further investigations.

In order to examine the probable trend of pectins extraction versus pH, some extractions were conducted at pH values between 7.0 and 2.5 for 1 h: Fig. 2 shows, as an example, the value obtained at pH 4.0 (the other data are not shown). From the obtained results it appears that lowering the pH from neutrality the improvement is limited and it becomes significant when the pH 2.5 is reached; this corresponds to the maximum quantity of pectins that is extractable. Even if the reliability of those results should be further corroborated, the boundary characteristic of pH 2.5 is evident. For this reason, at present, the extraction at pH 2.5 for 1 h is considered the reference one for the recovery of pectins from Venezuela cocoa husks.

3.2. Effect of microwave heating

The pre-treatment of Ghana cocoa husks through microwave heating had little influence on the quantity of pectins recovered; in fact, the amount corresponds to 9.3% and it is not so significantly different from 9.0% obtained without pre-treatments under the same experimental conditions. For this reason it was decided not to process husks before the extraction.

3.3. Pectins characterization: methyl ester and acetyl ester contents

Pectins samples extracted at pH 2.5 for 1 h and at pH 7.0 for 3 h were characterized in terms of degree of methylation and acetylation. As concerned pectins extracted at pH 2.5, the %DM was firstly determined by the titrimetric method which provided a low value ($25.0\% \pm 3.0$) if compared with conventional commercial pectins. Then the %DM was determined by the enzymatic method, which gave a value that was a little higher ($31.1\% \pm 0.5$). It is clear that cocoa husks pectins extracted at acid conditions are low methoxylated (LM; DM < 50%), probably due to the hot acid extraction itself that can cause demethylation and fragmentation of the polygalacturonic chain (Cheftel & Cheftel, 1988). Taking into account that extraction conditions could have important effects on the chemical structure of pectins, it was decided to evaluate the %DM also for pectins recovered at neutral

pH. In this case samples are at the boundaries between LM and high methoxylated (HM) pectins ($54.2\% \pm 5.2$ for the titrimetric method; $46.7\% \pm 0.6$ for the enzymatic method). The enzymatic method was tested on HM commercial apple pomace pectins and gave results that agreed with the manufacturer's specifications within experimental errors; for the titrimetric method the errors were higher. This fact was observed also in relation to cocoa husks pectins. With the first method an error of about 10% was calculated, while with the second the error was only about 1.5%. For this reason the enzymatic determination was chosen as the reference value.

The other evaluated parameter was the acetylation degree. Also in this case, extraction conditions slightly influence results obtained for different pH and extraction times. Both samples have an acetyl content, similar to that found in sunflower pectins, lower than 4% ($1.7\% \pm 0.4$ for the extraction at pH 2.5 for 1 h; $3.2\% \pm 0.5$ for the extraction at pH 7.0 for 3 h), which is considered a critical limit for gel formation (Iglesias & Lozano, 2004).

Possible impurities in pectins extracts were also investigated. In particular it was thought that polyphenols could be present because they are one of the constituents of husks (Serra Bonvehì & Escolà Jordà, 1998). Polyphenols are absent in both analysed samples extracted at pH 2.5 for 1 h and pH 7.0 for 3 h, therefore it is possible to deem that they could be efficiently removed by the ethanol washing step during extraction procedure. It has also to be remembered that the hot extraction could interfere with the presence of polyphenols, since the temperature affects the compounds presence both in terms of decomposition and stability (Moure et al., 2001).

4. Conclusions

The hot acid extraction, usually utilized for commercial pectins production, is highly suitable for the recovery of these compounds from cocoa husks. The best result, in terms of extraction yield, is obtained with pH 2.5 and extraction time of 1 h. When the extraction conditions are pH = 7 and time = 3 h, the yield is lower, but pectins are characterised by slightly higher values of methyl and acetyl ester contents. Because some problems arose during extractions at low pH values (1.5, 1.0), for example the probable presence of tannins, more investigations into the optimization of the extraction parameters are highly desirable. Further investigations need also to be directed at the characterization of the extracted samples in order to reveal possible undesirable aspects and to correct them through chemical modifications during extraction.

Acknowledgement

Part of cocoa husks was supplied by "Cioccolato Peyrano S.P.A.", Torino: the authors wish to thank the company and in particular Mr. Peyrano.

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