



Enzymatic conversion of date fruit fiber concentrates into a new product enriched in antioxidant soluble fiber



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ABSTRACT

Soluble dietary fiber (SDF) has got increasing interest because of its prebiotic effects and technological applications. The enzymatic hydrolysis is an effective treatment to convert insoluble dietary fiber (IDF) into SDF. Date fiber concentrate (DFC) is a very good source of dietary fiber, but its IDF/SDF ratio is very high. In this study an enzymatic treatment has been optimized to enrich DFC in SDF: Viscozyme[®] L from Novozymes A/S at 2.38 mL enzyme/100 mL reaction volume, in a solid/liquid ratio 1 g/35 mL, at 55 °C during 30–60 min. In these conditions, the amount of SDF increased from 1.8–6.3 to 5.4 g/100 g, and the ratio IDF/SDF changed from 19 to 2–3. In the SDF, besides an increase in the antiradical activity, gluco-, manno-, and xylo-oligosaccharides have been identified. Their molecular weights varied in a wide range, depending of hydrolysis duration, which suggests that this enzymatic treatment could be a promising process for obtaining tailor-made prebiotic oligosaccharides.

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1. Introduction

Plant tissues discarded at harvesting or after industrial processing are a good source of dietary fiber and phytochemicals. Their upgrading into products with added value allows them to contribute to environmental protection and to recover valuable nutrients. Dietary fiber plays an important role in human health and has shown beneficial effects in the prevention of several diseases, such as cardiovascular diseases, diverticulosis, and others (Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006).

Although traditional dietary fiber sources have consisted on

cereals, fruits and vegetables, new ones are being developed, such as artichoke by-products (Fissore et al., 2014) or spent coffee grounds (Campos-Vega, Loarca-Piña, Vergara-Castañeda, & Oomah, 2015). In this context, date palm (*Phoenix dactylifera* L.) fruit could be also considered as an important fiber source. The annual production was about 7.5 million tons in 2012 (FAO, 2016), but there is approximately two million tons per year, as wastes (fruits with imperfect appearance, secondary varieties not suitable for human consumption, and by-products from date processing). From Tunisian secondary date fruit varieties, attempts have been made to value them as dietary fiber source. Their dietary fiber content ranged between 4.7 and 7 g/100 g, with water- and oil-holding capacities higher than 17 and 4 mL/g fibre, respectively, which make them suitable for being used as additives in fibre-enriched food (Mrabet et al., 2012). From these unused varieties, a fibrous solid has been obtained after applying some hydrothermal pre-treatments (Mrabet et al., 2015). These date fiber concentrates (DFC) have been successfully added to bakery products (Mrabet et al., 2016), but they had a insoluble to soluble dietary fiber (IDF/SDF) ratio very far from the optimum range (1–2.3) to have beneficial physiological effects (Spiller, 1986). Conversion of IDF into SDF

Abbreviations: DFC, date fiber concentrate; DP, degree of polymerization; EC₅₀, efficient concentration; HDFC, hydrolyzed date fiber concentrate; IDF, insoluble dietary fiber; RI, refractive index; S/L, solid:liquid; SDF, soluble fiber; TEAC, Trolox equivalent antioxidant capacity; TDF, total dietary fiber; TFA, trifluoroacetic acid.

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can be achieved by chemical and enzymatic treatments. Indeed, enzymatic hydrolysis is recognized to be the most promising and preferred technology due to higher product yields and low energy requirements. It could be considered as an environmentally friendly process because it does not involve solvents or chemical reagents (Meyabadi & Dadashian, 2012). Napolitano et al. (2006) showed that the conversion of IDF into SDF from cereal fiber and coffee silver skin by enzymatic treatment was accompanied by an increase in the free phenolic concentration, water-soluble antioxidant activity, and phenol compound bioavailability.

The present study was carried out to modify DFC by enzymatic hydrolysis. The treatment conditions have to be optimized in order to obtain a ratio IDF/SDF as beneficial as possible. Factors as the ratio solid/liquid, enzyme amount and time of hydrolysis were studied. The hydrolyzed DFCs (HDFC) were chemically characterized to determine the potential of the modified DFC as functional food ingredients.

2. Materials and methods

2.1. Chemicals

Viscozyme[®] L was kindly given by Novozymes A/S (Bagsvaerd, Denmark) representative in Spain. 4-Morpholinoethanesulfonic acid (MES), protease from *Bacillus licheniformis*, amyloglucosidase solution from *Aspergillus niger*, tris(hydroxymethyl) aminomethane (TRIS), 2,2-diphenyl-1-picrylhydrazyl (DPPH• free radical), trifluoroacetic acid, 3-phenylphenol, anthrone, Folin-Ciocalteu phenol reagent, were purchased from Sigma-Aldrich Química (Madrid, Spain). Amylase thermostable Thermamyl 120 L was from Novo Nordisk Pharma (Madrid, Spain). Sodium hydroxide, sodium carbonate, acetic acid, and hydrochloric acid were from Panreac Química S.A. (Barcelona, Spain). Sulfuric acid was from Sharlau (Barcelona, Spain). Ethanol was purchased from Alcoholes del Sur (Córdoba, Spain). Standards of gallic acid, and inositol were purchased from Sigma-Aldrich Química (Madrid, Spain). Dextran for calibration, maltose, and blue dextran were from Fluka (Buchs, Switzerland).

2.2. Preparation of DFC

Dates fruits of secondary varieties were hydrothermally pretreated as described previously (Mrabet et al., 2015). Briefly, the steam treatment was carried out using a 100-L capacity reactor which operates at temperatures between 50 and 190 °C by direct heating, and at a maximum pressure of 882 kPa. Samples consisting in 4 kg of a mixture of several secondary date varieties were treated in duplicate at 140 °C during 30 min. The wet treated material was centrifuged at 4700g and freeze-dried. Seed pieces higher than 4 mm were removed from the dried solid fractions by sieving. The material under 4 mm was considered the DFC to be used as enzymatic digestion substrate.

2.3. Enzymatic hydrolysis of DFC

The hydrolysis of DFC was carried out using commercial Viscozyme[®] L. The key enzyme activity is endo- β -glucanase (100 fungal β -glucanase units) that hydrolyzes (1,3)- or (1,4)-linkages in β -D-glucans. Xylanase, cellulase, and hemicellulase are also declared as side activities (Viscozyme L, 2014). Solid/liquid (S/L) ratio (g/mL), enzyme concentration (mL/100 mL reaction volume), and treatment duration were the studied factors to optimize the hydrolysis conditions. 400 mg of DFC were weighted in duplicate in 50 mL Falcon tubes. Enzyme solution and water were added to reach the hydrolysis conditions specified in Table 1, and then incubated in a

Table 1

Solid/liquid ratio and percentage of enzyme in the different hydrolysis condition assayed of date fiber concentrate.

Assay	S/L ratio (g/mL)	Viscozyme [®] L (mL/100 mL)
1	1/12	6.67
2	1/12	0.67
3	1/20	2.08
4	1/35	2.38
5	1/35	0.24

S/L: solid/liquid.

stirring water bath at 55 °C, for 30, 60, 180, and 360 min at 70 oscillations/min. Tubes without enzyme preparation were used as a blank for each treatment time. After digestion, the tubes were immediately heated at 100 °C for 10 min to inactivate the enzymes, even blanks. The samples were centrifuged at 14,000g during 20 min, and the residue washed with distilled water and centrifuged in the same conditions, and then frozen and freeze-dried. Each residue and the original DFC underwent a Saeman hydrolysis with sulfuric acid (Jiménez et al., 2001a) in order to evaluate the enzymatic digestion progress. Samples of 10 mg of the freeze-dried residue were hydrolyzed with 0.5 mL of 72 g/100 g sulfuric acid 2 h at 40° C. Afterwards, 5.5 mL of distilled water were added and the hydrolysis continued during 3 h at 100 °C. 100 μ L (in duplicate) of the hydrolysates were neutralized with 2 mol/L NH₄OH, inositol (25 μ g) was added as internal standard, and the mixture was reduced and acetylated for neutral sugars quantification by gas chromatography (Dos-Santos, Jiménez-Araujo, Rodríguez-Arcos, & Fernández-Trujillo, 2011). The results were expressed as mg of sugar remaining in the solid residue, after subtracting the values obtained for the blank without enzyme addition to each corresponding enzymatic digestion sample.

After choosing the optimized conditions, enzymatic hydrolysis of 4 g DFC were developed. Two replicates were directly frozen and freeze-dried and the residue was named as HDFC (hydrolyzed date dietary fiber). Four more replicates were centrifuged as described above to obtain the soluble fraction.

2.4. Proximate composition and antioxidant activity of selected fiber concentrates

DFC and selected HDFCs were analyzed for their content in moisture, protein, ethanol-soluble sugars, cellulose, non-cellulosic sugars, uronic acids, ethanol-soluble phenols, and dietary fiber. The antiradical activity was determined from the ethanol-soluble fraction and the residue.

The moisture was determined in a moisture analyzer MB45 (Ohaus, Switzerland). Protein content was determined by the Kjeldahl method and applying a factor of 6.25 to convert the total nitrogen into protein content. Non-cellulosic sugars composition was determined by hydrolysis with 2 mol/L trifluoroacetic acid (TFA) at 121 °C for 1 h. The released sugars were quantified by gas chromatography (Dos-Santos et al., 2011). Cellulose was quantified from the TFA-insoluble residue after sulfuric acid hydrolysis by the anthrone method (Dische, 1962). Uronic acids were quantified using the phenyl-phenol method after sulfuric acid hydrolysis (Blumenkrantz & Asboe-Hansen, 1973).

For ethanol-soluble sugars, total phenols and ethanol-soluble antiradical activity quantifications, an extraction with 80 mL/100 mL ethanol had to be done, in a solid liquid ratio of 1 g/40 mL. Ethanol-soluble sugars were determined using the anthrone method (Dische, 1962). The total phenol content was quantified for each ethanol extract according to the Folin-Ciocalteu method, using gallic acid as a reference (Singleton & Rossi, 1965). The total

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