Title: 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 to 6.3-5.4 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.

Keywords: date fruit, date fiber concentrate, soluble dietary fiber, enzymatic hydrolysis, prebiotic oligosaccharides

Abbreviations used: 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.

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, Santo Domingo, Pujol, Damonte, Rojas & Gerschenson, 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 4.7-7 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, Rodríguez-Arcos, Guillén-Bejarano, Chaira, Ferchichi & Jiménez-Araujo, 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 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). Dextrans 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-liter 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. Product data sheet, 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 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 14000g 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, Rodríguez, Fernández-Caro, Guillén, Fernández-Bolaños & Heredia, 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 µL (in duplicate) of the hydrolysates were neutralized with 2 mol/L NH₄OH, inositol (25 µg) 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 phenolic content of the samples was expressed as gallic acid equivalents (g/100 g). Soluble antiradical activity was determined from the ethanol extracts by the DPPH· method (Rodríguez et al, 2005). The efficient concentration EC₅₀ was calculated from a calibration curve by linear regression for each sample.

The antiradical activity of the insoluble residue after ethanol extraction was evaluated by the QUENCHER-DPPH· method as described by Serpen, Capuano, Fogliano & Gökmen (2007) with some modifications (Fuentes-Alventosa et al, 2009). As for soluble antiradical activity, EC₅₀ was also calculated. Both activities were expressed as millimoles of Trolox equivalent antioxidant capacity (TEAC) per kilogram of sample by means of a dose–response curve for Trolox.

The amount of dietary fiber was determined using the protocol described by Lee, Prosky & De Vries (1992), with slight modifications. Samples (1 g x 4) were suspended in 40 mL MES-TRIS buffer and treated with 50 µL Thermamyl (heat stable alpha-amylase) at 100° C for 15 min and then digested with 100 µL of a 50 mg/mL protease solution (60° C, 30 min), followed by incubation with 100 µL amyloglucosidase (60° C,

1 h). Two replicates were filtered on a sintered glass crucible (no. 2) using the Fibertec E system from Tecator (Hillerød, Denmark) consisting of the 1023 Filtration Module to determinate IDF. The residue was dried overnight at 105° C. Four volumes of 96 mL/100 mL hot ethanol were added to the other two replicates to precipitate SDF. Total dietary fiber (TDF) was recovered by filtration in the same conditions. The residue was then washed with 80 and 96 mL/100 mL ethanol and dried overnight at 105° C and weighed. The values of IDF and TDF were corrected with their ash and protein content (ash was determined according to the AOAC method 923.03 by incinerating samples in a muffle furnace at 550° C to white ash, and protein as described above). SDF was calculated as the difference between TDF and IDF.

2.5. Ultra-filtration through a membrane of 1000 Da.

The soluble fractions obtained after centrifugation of the selected enzymatic digestion were subjected to ultra-filtration using an Amicon 8400 stirred cell (Millipore Corporation, Bedford, MA, USA) through 1000 Da molecular weight cut off membranes. The retained solutions were washed with water until 500 mL of permeate was collected. Two fractions were obtained: the eluted fraction, with molecular weight <1000 Da, and the retained one (molecular weight >1000 Da). Both fractions were analyzed for their content in neutral sugars, uronic acids, total phenol and antiradical activity as above. For the determination of monomeric sugars present in the eluted fraction, an aliquot of this fraction was directly reduced and acetylated for gas chromatography quantification without TFA hydrolysis.

2.6. High-Performance Size Exclusion Chromatography (HPSEC) and evaluation of the molecular weight distribution of SDF from HDFC.

An aliquot of molecular weight >1000 Da fractions was freeze-dried and redissolved in 0.05 mol/L TRIS–hydrochloric acid. The method used was described by Jiménez,

Rodríguez, Fernández-Caro, Guillén, Fernández-Bolaños & Heredia (2001b) with slight modifications. The molecular weight was measured in a Jasco equipment (LC-Net II ADC, Kyoto, Japan) with a refractive index (RI) detector (Jasco RI-1530) and injection valve (Rheodyne, loop 20 μ L, Cotati, CA). Two different columns in sequence were used: TSKgel GMPWxl and TSKgel G3000PWxl (300 x 7.8 mm i.d., Tosoh Bioscience LLC, King of Prussia, PA) after calibration with dextrans of 500, 110, 40, 6 kDa and maltose. Blue dextran was used to test the void volume of the column. The elution buffer was 0.05 mol/L TRIS–hydrochloric acid at a flow rate 0.4 mL/min.

2.7. Statistical analysis.

The results are expressed as the average value of at least three repetitions. To assess the differences among samples, a multiple-sample comparison was performed using the Statgraphics Plus program Version 2.1. Multivariate analysis of variance (ANOVA), followed by Duncan's multiple comparison test, was performed to differentiate among the groups ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Study of hydrolysis conditions.

The assayed hydrolysis conditions are summarized in Table 1. The ratios S/L are among most used in bibliography, from 1/4 (Landbo & Meyer, 2001) to 1 g/100 mL (Fissore et al, 2014). In the present experiences the volume has been kept as low as possible in order to shorten the subsequent lyophilization step. Ratios lower than 1 g/12 mL were assayed but due to the high water holding capacity of the starting material (8.50 mL water/g dry matter (Mrabet et al, 2015)) the sample shaking during hydrolysis was not effective. The enzyme percentage was similar to other published works on okara (Kasai, Murata, Inui, Sakamoto & Kahn, 2004) and oil palm empty fruit bunches (Nomanbhay,

Hussain & Palanisamy, 2013), but higher than used to release ferulic acid from sweet potato stems (Min et al, 2006).

The hydrolysis was developed from 30 to 360 min, and the solid residues were studied for their glycosidic composition (Figure 1). All the quantified sugars decreased with the exception of mannose which did not show the same behavior. The treatment 2 was the less effective, being treatments 1, 3, and 5 similar for most sugars. The highest degree of hydrolysis was obtained with treatment 4: the minimum amount of sugars was reached after 1 h, although at 30 min there were also very good results. At 1 h, all the sugars decreased to 4-26% of the initial amount, and at 30 min the 21-27%.

Viscozyme® L has got β -endoglucanase as main declared activity (Viscozyme L. Product data sheet. 2014), but also xylanase, cellulase and hemicellulase activity were declared as side-activities. In fact, xylose and arabinose were the sugars which showed the highest decreases, reaching 4-8% respectively of their initial amounts after 60 min hydrolysis.

But mannose did not have the same progress. Glucomannans have been described in date pits (Ishrud, Zahid, Ahmad & Pan, 2001), and during date fruit hydrothermal pretreatment, these polysaccharides are quantified as non-cellulosic sugars of the obtained DFC (Mrabet et al, 2015). Viscozyme® L has not mannanase activity, so they must remain in the insoluble residue after enzymatic digestion. In some conditions, the amount of quantified mannose was even higher than that found in the original DFC. It is widely known that cellulose and mannans have interactions which form stronger composites than those of cellulose-xyloglucans (Whitney, Brigham, Darke, Reid & Gidley, 1998), so a little percentage of mannan may remain not hydrolyzed in the original material. After each Viscozyme® L treatment, cellulose was partially digested,

and therefore mannans could be released from their composites, hydrolyzed by sulfuric acid, and quantified as mannose.

The treatment 4 at 30 and 60 min (30-HDFC and 60-HDFC, respectively) were chosen to continue the study.

3.2. Chemical composition of DFC and HDFCs.

The chemical composition of both HDFCs and that of the original DFC are presented in Table 2. Moisture and protein increased with enzymatic treatments, but there were no significant differences between both HDFCs. The total sugar content was studied in three different groups: ethanol-soluble sugars, non-cellulosic sugars, and cellulose. Only cellulose decreased with the enzymatic digestion. Although the highest differences were quantified between DFC and both HDFCs, between 30- and 60-HDFC there were also significant differences in ethanol-soluble sugars and cellulose. In the three samples, the total sugar amount was around 40 g/100 g, but the relative percentages of each group of sugars varied among the samples. In the original DFC they were 3.7-61.9-34.1 g/100 g of total sugars for ethanol-soluble sugars, non-cellulosic sugars and cellulose respectively. Ethanol-soluble sugars increased to 9.6 and 10.7 g/100 g with 30 min and 60 min treatments, non-cellulosic sugars increased to 77.7 and 81 g/100 g, and cellulose decreased to 12.7 and 8.2 g/100 g. It is clear that an important quantity of cellulose was hydrolyzed to oligomers, which were recovered in the group of ethanol-soluble sugars or non-cellulosic sugars depending on their degree of polymerization (DP).

The composition of non-cellulosic sugars was determined by gas chromatography (Table 3). Glucose was the main sugar, in a percentage of 75 g/100 g of quantified sugars in untreated sample and around 80 g/100 g of quantified sugars in HDFCs. There were not significant differences in any sugar between both HDFC. In general, all sugars

decreased with digestion but only mannose increased probably due to a more complete hydrolysis of mannans, as it was explained above.

Uronic acids (Table 2) were also quantified. Their increase could be also related to a better yield of the acid hydrolysis after Viscozyme® L treatment and to their subsequent quantification in higher amounts from HDFC than from DFC.

Phenols and antiradical activity were determined from the ethanol-soluble fraction. The phenols which were entrapped or structurally linked to fiber could be partially released after digestion and were quantified in the ethanol-soluble fraction. In fact, enzyme-assisted extraction are usually applied for enhancing the yield of phenolic production from agricultural byproducts such as black currant pomace (Landbo & Meyer, 2001). These results were confirmed when the antiradical activity was discussed: the ethanol-soluble activity increased with treatment as a consequence of the decrease of the activity linked to residue. The total antioxidant activity did not change due to the enzymatic digestion (124, 120.6, and 122 mmol TEAC/kg fiber for DFC, 30-HDFC, and 60-HDFC respectively). This level of antiradical activity was similar to that of several citrus fiber concentrates (Marín, Soler-Rivas, Benavente-García, Castillo & Pérez-Álvarez, 2007) and much higher than that of spent coffee fiber (Campos-Vega et al, 2015) which are considered useful as potential antioxidant dietary supplements.

3.3. Dietary fiber content of DFC and HDFCs and process mass balance.

The dietary fiber content decreased with the treatment, from 36 to near 22-23 g/100 g (Table 2). A part of the original IDF would be solubilized due to enzymatic digestion, increasing in this way the SDF, but also the SDF could be partially hydrolyzed to smaller fragments which did not precipitate with hot ethanol during TDF isolation.

Working with carrot pomace, Yoon, Cha, Shin, & Kim (2005) considered this lost fraction as alcohol-soluble dietary fiber which increased from 14 to 36 g/100 g after

enzymatic treatment. In fact, some institutions from European Union include carbohydrate polymers of 3-9 DP as a part of the dietary fiber definition (Menezes, Giuntini, Tanasov Dan, Hoffman Sardá & Lajolo, 2013). Therefore, this fraction soluble in ethanol should be considered mostly as dietary fiber and, in the case of HDFC, the amount of TDF would not decrease in fact so much.

The ratio IDF/SDF decreased from around 20 to 2.5-3, depending on the duration of hydrolysis. Taking into account both results, it could be concluded that the total amount of SDF increased from 1.8 g/100 g in DFC to 6.3 and 5.4 g/100 g in 30-HDFC, and 60-HDFC respectively. Similar results were found working with IDF coming from artichoke byproducts (Fissore et al, 2014), where SDF increased up to 3.5 times after a treatment with hemicellulases. Lower results were described by Napolitano et al. (2006), reaching an increase of 2.4 times in barley spent grain SDF. With the Viscozyme® L treatment on DFC very good results have been obtained: the ratio IDF/SDF was near the optimum range (1-2.3) (Spiller, 1986) and the amount of SDF was three-fold higher in HDFCs than in DFC, pointing to an important improvement in the physiological quality of these modified date fibers.

The optimized conditions and the mass balance of the enzymatic digestion are presented in Figure 2. The yield of the insoluble fraction was the same after 30 or 60 min hydrolysis. The reaction time had high influence on the molecular weight of the soluble fraction, the retained fraction after ultrafiltration (DP>6) decreasing from 40 to 20 g/100 g DFC, and consequently increasing the eluted one (DP<6). The digestion time is a key parameter for the control of the molecular weight distribution of the soluble fraction especially that of the oligosaccharides present in the SDF, as discussed below.

3.4. Composition of the HDFC soluble fraction.

The solubilized fraction after Viscozyme® L digestion was subjected to ultra-filtration through a 1000 Da (DP>6) molecular weight cut-off. Eluted and retained fractions were analyzed for neutral sugars, uronic acids, total phenols and antiradical activity and the results are summarized in Table 4. Neutral sugars present as monomers have been also quantified from fractions with DP<6.

The total amount of neutral sugars rose up as the digestion duration increased (7.59 and 9.16 g/100 g for 30-HDFC and 60-HDFC, respectively). The molecular weight distribution also changed depending on treatment length: DP>6 and DP 2-6 fractions decreased and consequently monomers increased with longer treatments. The relative percentage of each fraction went from 11.98, 50.7, and 37.4% (DP>6, 2-6, and monomers respectively) in 30-HDFC to 6.1, 47.2, and 46.6% in 60-HDFC. The same results were described when carrot pomace was digested (Yoon et al, 2005), where high molecular weight polysaccharides were converted into low molecular weight as reaction time was prolonged. The other analyzed components (uronic acids and phenols) had the same behavior than neutral sugars: the longer treatments, the lower amount in DP>6 fractions. The antiradical activity had the same behavior than total phenol content.

When the glycosidic composition was studied (Table 5) important differences were found. DP>6 fraction from 30-HDFC was the only one where glucose was not the main sugar. As Viscozyme® L has got β -endoglucanase as main declared activity; cellulose fragments of different molecular weight could be released from DFC and recovered in the soluble fraction. Mannose was the second sugar in quantity (and the major in DP>6 fraction from 30-HDFC) but no mannanase activity is declared in Viscozyme® L. This release of mannan fragments could be a consequence of cellulose network loosening, where mannan are very strongly linked (Witney et al, 1998). In Japan, some studies have shown that manno-oligosaccharides could have beneficial effects on human health

(Campos-Vega et al, 2015). These results have led to their approbation as FOSHU (Food for Specified Health Uses) in Japan (Fukami, 2010). Besides them, xylo-oligosaccharides seemed to be present, especially in DP>6 fraction. Taking into account that arabinoxylans are the main hemicelluloses quantified in date fruit dietary fiber (Mrabet et al, 2012; Mrabet et al, 2015), and that xylanase is another side activity declared in Viscozyme® L, it was expected the presence of xylo-oligosaccharides in the soluble fraction. Xylo- and gluco-oligosaccharides are also considered as emerging prebiotics, xylo-oligosaccharides having also the category of FOSHU in Japan (Rastall, 2010). Therefore, it seems to be of great interest the presence of gluco-, manno- and xylo-oligosaccharides in HDFC from both technological and physiological points of view, which molecular weight could be tailored by controlling digestion conditions.

3.5. Molecular weight distribution of DP>6 fraction.

The fraction with DP>6 was analyzed by HPSEC due to its interesting glycosidic composition. The obtained RI profiles are presented in Figure 3. Three different fractions were isolated: F1, with the highest molecular weight, centered in 110 kDa; F2, with molecular weight between 6-40 kDa, and F3, lower than 6 kDa. As digestion time increased, F1 and F2 decreased and F3 went up. Besides, the maximum lecture in RI of F2 shifted down to lower molecular weight, so the average molecular weight of this fraction also decreased with longer treatments. In the inner table of Figure 3, the relative percentages of the three isolated fractions are presented, showing the diminution of F1 and F2 and the increase in F3 as digestion was prolonged. The chemical composition of these three fractions has been also studied (Table 6). The relative percentages of neutral sugar quantified changed from 10.3-66.4-23.24% for F1, F2 and F3 respectively at 30 min hydrolysis to 7.8-61.5-30.6% at 60 min. The same happened for phenols, which changed from 22.8-44.9-32% to 11.5-45.0-43.4%. Uronic acids were only present in F2

fraction and decreasing from 120 to 34 mg/100 g HDFC in 30-HDFC and 60-HDFC, respectively.

The glycosidic composition of the three isolated fractions (Table 7) revealed that manno-oligosaccharides were the main components of F2 (higher than 40% of total sugar quantified), but gluco-oligosaccharides were predominant in F3. In F1 there were important percentages of galactose and rhamnose, which could indicate the presence of pectic polymers in this minor fraction. Xylo-oligosaccharides were especially present in F2 also (xylose and arabinose accounted for near 30 g/100 g of total sugars quantified). Therefore, manno- and xylo-oligosaccharides had higher molecular weight than those of glucose, probably due to the higher activity of β -endoglucanase present in Viscozyme® L, existing slight differences between 30 min and 60 min digestions.

4. CONCLUSIONS

Viscozyme® L has been successfully applied on DFC in order to increase the proportion of SDF. The optimized conditions consist in the enzymatic complex at 2.38 mL enzyme/100 mL reaction volume, a solid/liquid ratio 1 g/35 mL, at 55° C during 30-60 min. Although the amount of TDF decreased, the ratio IDF/SDF went from near 20 to 2-3 after 30 min hydrolysis, and as a consequence the amount of SDF increased from 1.8 g/100 g in DFC to 6.3 g/100 g in 30-HDFC. This characteristic could enhance the physiological effects of the new fiber concentrate. Besides, gluco-oligosaccharides were the main SDF component obtained with this treatment due to the high β -endoglucanase activity, but also manno- and xylo-oligosaccharides were quantified in significant amounts. These three groups are considered as emerging prebiotics, so *in vitro* studies and human trials are needed in order to assess their health positive effects. The enzymatic digestion of DFC in the proposed conditions is a promising beginning for the amelioration of the beneficial health effects of date fiber consumption. In addition, this

process could promote the use of this new fiber concentrate, enriched in SDF, as a prebiotic and antioxidant functional food ingredient.

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FIGURE CAPTIONS

Figure 1.- Release of the different sugars from date fiber concentrate by Viscozyme® L enzymatic hydrolysis, expressed as mg of sugars remaining in the residue after digestion.

◆ Treatment 1; ■ Treatment 2; ▲ Treatment 3; □ Treatment 4; △ Treatment 5.

511 Figure 2.- Scheme of the enzymatic process and mass balance of the different fractions
512 isolated from date fiber concentrate after 30 and 60 minutes of treatment. The yield of
513 each fraction is expressed in the rectangles as g/100 g DFC.

514 DFC: date fiber concentrate; S/L: solid/liquid; HDFC: hydrolyzed date fiber
515 concentrate; DP: degree of polymerization.

516

517 Figure 3.- HPSEC elution profile (refractive index) of the soluble fraction with degree
518 of polymerization higher than 6 units from the selected hydrolyzed date fiber
519 concentrates (30 and 60 min hydrolysis). — 30-HDFC; — 60-HDFC.

520 Standards 1: 500kDa; 2: 110 kDa; 3: 40 kDa; 4: 6 kDa; 5: Maltose; HDFC: hydrolyzed
521 date fiber concentrate; RI: refractive index.

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

Table 2.- Chemical composition (g/100g) and antiradical activity (mmol TEAC/kg product) of the original date fiber concentrate before enzymatic hydrolysis (DFC) and of the products after 30 and 60 min of hydrolysis (30-HDFC and 60-HDFC respectively).

	DFC	30-HDFC	60-HDFC
Moisture	7.1±0.2 a	8.3±0.1 b	8±0.2 b
Protein	7±0.3 a	9.5±0.4 b	9.9±0.2 b
Ethanol-soluble sugars	1.40±0.04 a	4±0.1 b	4.3±0.1 c
Cellulose	13±1 c	5.3±0.4 b	3.3±0.2 a
Non-cellulosic sugars	23.3±0.9 a	32.3±0.6 b	33±1 b
Uronic acids	1.9±0.2 a	4.5±0.3 b	4.2±0.6 b
Ethanol-soluble phenols	1.3±0.1 a	1.7±0.02 b	1.99±0.05 c
Antiradical activity			
Ethanol-soluble	86±7 a	101±3 b	106±1 b
Residue	38±2 c	19.6±0.4 b	16±1 a
Total dietary fiber	36±2 b	23±2 a	21.7±0.3 a
IDF/SDF	19.65	2.46	3.06

Values are the means of at least triplicate assays. Means bearing the same symbol are not significantly different at the 5% level as determined by the Duncan multiple-range test ($P<0.05$). TEAC: Trolox equivalent antioxidant capacity; IDF: insoluble dietary fiber; SDF: soluble dietary fiber.

Table 3.- Percentages in weight of each neutral sugar on total sugar quantified by gas chromatography from the original date fiber concentrate before enzymatic hydrolysis (DFC) and of the products after 30 and 60 min of hydrolysis (30-HDFC and 60-HDFC respectively).

	DFC	30-HDFC	60-HDFC
Rhamnose	0.9±0.1 b	0.65±0.03 a	0.72±0.04 a
Fucose	0.6±0.07 b	0.28±0.02 a	0.35±0.05 a
Arabinose	3.5±0.4 b	2±0.04 a	1.8±0.4 a
Xylose	13±1 b	8.4±0.7 a	8.7±0.5 a
Mannose	3.87±0.09 a	6.2±0.2 b	6±0.2 b
Galactose	3.3±0.3 b	2.27±0.08 a	2.25±0.05 a
Glucose	75±2 a	80.3±0.9 b	80.2±0.8 b

Values are the means of at least triplicate assays. Means bearing the same symbol are not significantly different at the 5% level as determined by the Duncan multiple-range test ($P<0.05$). DFC: date fiber concentrate; HDFC: hydrolyzed date fiber concentrate.

Table 4.- Composition (g/100g HDFC) and antiradical activity (mmol TEAC/kg HDFC) of recovered fractions after 1000Da ultrafiltration process (degree of polymerization higher and lower than 6 units). The ultrafiltered samples were the soluble fractions obtained after centrifugation of 30 and 60 min hydrolysates (30-HDFC and 60-HDFC respectively). Monomers were determined from the fractions with DP<6 by direct reduction and acetylation without trifluoroacetic acid hydrolysis.

			Neutral sugars	Uronic acids	Total phenols	Antiradical activity
30-HDFC	DP>6		0.9±0.02 b	0.14±0.02	0.45±0.01 b	20.5±0.8 b
	DP<6	DP 2-6	3.85±0.04 a	2.6±0.2 a	1.09±0.03 a	38.5±0.4 a
		Monomers	2.84±0.04 a			
60-HDFC	DP>6		0.56±0.02 a	t	0.15±0.01 a	6.8±0.2 a
	DP<6	DP 2-6	4.30±0.05 b	2.7±0.2 a	1.96±0.05 b	59.6±0.5 b
		Monomers	4.3±0.3 b			

Values are the means of at least triplicate assays. Means bearing the same symbol are not significantly different as determined by the Duncan multiple-range test (P<0.05).

HDFC: hydrolyzed date fiber concentrate; DP: degree of polymerization; t: traces.

Table 5.- Percentages in weight of each neutral sugar on total sugar quantified from recovered fractions after 1000Da ultrafiltration process (degree of polymerization higher and lower than 6 units). The ultrafiltered samples were the soluble fractions obtained after centrifugation of 30 and 60 min hydrolysates (30-HDFC and 60-HDFC respectively).

	DP<6					
	DP>6		DP 2-6		Monomers	
	30-HDFC	60-HDFC	30-HDFC	60-HDFC	30-HDFC	60-HDFC
Rhamnose	3.3±0.3 a	2.6±0.1 a	0.89±0.06 a	0.59±0.03 a	0.7±0.05 a	0.71±0.05 a
Fucose	0.91±0.03 b	0.73±0.04 a	0.2±0.02 a	0.14±0.02 a	0.3±0.1 a	0.33±0.03 a
Arabinose	12.5±0.4 b	8.1±0.03 a	0.5±0.06 a	0.6±0.1 a	1.89±0.06 a	2.1±0.01 a
Xylose	17.31±0.03 b	11.67±0.01 a	0.23±0.06 a	0.4±0.04 a	2.37±0.09 a	2.95±0.09 a
Mannose	34.3±0.1 b	25.2±0.5 a	19.9±0.5 a	21±3 a	5.9±0.4 a	6.08±0.08 a
Galactose	3.94±0.04 b	3.27±0.08 a	1.49±0.04 a	1.5±0.2 a	2.6±0.1 a	2.7±0.06 a
Glucose	27.7±0.4 a	48.4±0.6 b	76.8±0.6 a	76±4 a	86±2 a	85±1 a

Values are the means of at least triplicate assays. Means bearing the same symbol are not significantly different as determined by the Duncan multiple-range test (P<0.05).
HDFC: hydrolyzed date fiber concentrate; DP: degree of polymerization.

Table 6.- Composition (mg/100g HDFC) of isolated fractions after HPSEC analysis of DP>6 soluble fractions from 30 and 60 minutes hydrolyzed date fiber concentrates.

		Neutral sugars	Uronic acids	Total phenols
30-HDFC	F1	63±5 b	n.d.	62±0.5 b
	F2	406±4 b	121±5 b	122±1 b
	F3	142±1 b	n.d.	88±0.9 b
60-HDFC	F1	24±1 a	n.d.	12.04±0.08 a
	F2	185±12 a	34±7 a	47.1±0.4 a
	F3	92±5 a	n.d.	45.4±0.9 a

Values are the means of at least triplicate assays. Means bearing the same symbol are not significantly different as determined by the Duncan multiple-range test (P<0.05).

HDFC: hydrolyzed date fiber concentrate; HPSEC: high-performance size exclusion chromatography; n.d.: not detected.

Table 7.- Percentages in weight of each neutral sugar on total sugar quantified from the three fractions isolated after HPSEC analysis of DP>6 soluble fractions from 30 and 60 minutes hydrolyzed date fiber concentrates.

	DP>6					
	F1		F2		F3	
	30-HDFC	60-HDFC	30-HDFC	60-HDFC	30-HDFC	60-HDFC
Rhamnose	16.6±0.7 b	14.2±0.1 a	5±0.2 a	6.5±0.2 b	3±1 a	5.8±0.5 a
Fucose	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Arabinose	12.8±0.5 b	9.6±0.3 a	16±0.3 a	14±1 a	8.1±0.2 b	4.1±0.1 a
Xylose	12±3 a	10±1 a	17±0.6 a	16.52±0.08 a	8±4 a	5.2±0.6 a
Mannose	18.3±0.6 a	13±5 a	43.2±0.6 a	41±1 a	1.26±0.09 a	21.1±0.5 b
Galactose	24±3 a	40±5 b	13±1 a	18.4±0.1 b	17±2 a	10±1 a
Glucose	16±5 a	14±1 a	6±1 a	3.8±0.2 a	64±2 b	53.7±0.9 a

Values are the means of at least triplicate assays. Means bearing the same symbol are not significantly different as determined by the Duncan multiple-range test ($P<0.05$).

HPSEC: high-performance size exclusion chromatography; HDFC: hydrolyzed date fiber concentrate; DP: degree of polymerization; n.d.: not detected.

Figure 1

Figure 1

Figure 2

Figure 2

Figure 3

Figure 3