Title: Enzymatic conversion of date fruit fiber concentrate into a new product enriched in antioxidant soluble fiber.

ABSTRACT

Soluble fiber (SF) has got increasing interest because of its prebiotic effects. The enzymatic hydrolysis is an effective treatment to convert insoluble fiber (IF) into SF. Date fiber concentrate (DFC) obtained by hydrothermal treatments isa very good source of dietary fiber, but it IF/SF ratio is very high. In this study an enzymatic treatment has been optimized to enrich DFC in SF: Viscozyme® L from Novozymes A/S at 2.38%, in a solid/liquid ratio 1:35, at 55º C during 30-60 min. In these conditions, the amount of SF increased from 1.8% to 6.3-5.4%, and the ratio IF/SF changed from 19 to 2-3. In the SF, 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 processfor obtaining tailor-made prebiotic oligosaccharides.

Keywords: secondary date varieties, date fiber concentrate, soluble fiber, enzymatic hydrolysis, prebiotic oligosaccharides

Running title:

1.Introduction

Vegetable tissues discarded at harvesting or after industrial processing could be reused, since they are a good source of dietary fiber and phytochemicals. The upgrading of these wastes into products with added value allows them to contributing to environmental protection (reduce pollution) and to recover valuable nutrients.Nowadays, the demand of consumers for healthier foods leads to explore new sources of dietary fiber. This food component plays an important role in human health and has shown beneficial effects in the prevention of several diseases, such as cardiovascular diseases, diverticulosis, coronary heart disease, constipation, irritable colon, colon cancer, atherosclerosis, obesity and diabetes (Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006).

In addition to traditional dietary fiber sources (cereals(Baixauli, Salvador, Hough, & Fiszman, 2008; Gómez, Moraleja, Oliete, Ruiz, & Caballero, 2010) or fruits and vegetables(Massodi, Sharma, & Chauhan, 2002; Vergara-Valencia, Granados-Pérez, Agama-Acevedo, Tovar, Ruales, & Bello-Pérez, 2007)), new ones are being developed, such ascactus cladodes (Ayadi, Abdelmaksoud, Ennouri, & Attia, 2009; Kim, Lee, Lee, Lim, Imm, & Suh, 2012),green tea leaves(Lu, Lee, Mau, & Lin, 2010), 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 considered as an important fiber source. The annual production of dates was about 7.5 million tons in 2012. Among this quantity, there is approximately two million tons per year, as wastes (FAO, 2016) (fruits with imperfect appearance, secondary varieties not able for human consumption, and by-products from syrup, jam or paste factories). From Tunisian

secondary date fruit varieties, attempts have been made in order to value them as dietary fiber source. It has been reported that their content in dietary fiber ranged between 4.7-7%, 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).Applying some hydrothermal pre-treatments to these unused varieties, two different extract were obtained: a liquid one, rich in sugars and phenols (Mrabet, Jiménez-Araujo, Fernández-Bolaños, Rubio-Senent, Lama-Muñoz, Sindic, et al., 2016), and the solid residue, considered as an antioxidant fiber (Mrabet, Rodríguez-Gutiérrez, Guillén-Bejarano, Rodríguez-Arcos, Ferchichi, Sindic, et al., 2015). These date fiber concentrates (DFC) have been successfully added to bakery products:the fiber-enriched muffins obtained good scores in the sensory evaluation and also had high antioxidant capacity, which could help in increasing their self-life (Mrabet, Rodríguez-Gutiérrez, Rodríguez-Arcos, Guillén-Bejarano, Ferchichi, Sindic, et al., 2016). However, these DFCs had a insoluble to soluble fiber ratio (IF/SF) very far (higher than 20) from the optimum range (1-2.3) to have beneficial physiological effects with the fiber consumption (Spiller, 1986). Conversion of IF into SF can be achieved by chemical and enzymatic treatments. Indeed, enzymatic hydrolysis is recognized to be the most promising hydrolysis technology and the most preferred 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; Siqueira, Bras, & Dufresne, 2010; Wingren, Galbe, Roslander, Rudolf, & Zacchi, 2005). Napolitano et al. (Napolitano, Lanzuise, Ruocco, Arlotti, Ranieri, Knutsen, et al., 2006)showed that the conversion of IF into SFfrom cereal fiber and coffee silver skinby enzymatic treatment was accompanied by an increase in the

74 free phenolic concentration, water-soluble antioxidant activity, and phenol compound
75 bioavailability. In addition, the SF released from durum wheat IF had potential prebiotic
76 activity (Napolitano, Costabile, Martin-Pelaez, Vitaglione, Klinder, Gibson, et al.,
77 2009). A very exhaustive review recently published (Tejada-Ortigoza, Garcia-
78 Amezquita, Serna-Saldívar, & Welti-Chanes, 2015)about the advances in extraction of
79 dietary fiber pointed that enzymatic extractions use minimal treatment conditions to
80 obtain high yields in short periods of time, unlike the chemical methods.
81 The present study was carried out to modify DFC by enzymatic hydrolysis. The
82 treatment conditions have to be optimized in order to obtain a ratio IF/SF as beneficial
83 as possible. Factors as the ratio solid/liquid, enzyme amount and time of hydrolysiswere
84 studied. The hydrolyzed DFCs (HDFC) were chemically characterized to determine the
85 potential of the modified DFC as functional food ingredients.
86

87 **2. Materials and methods**

88 **2.1. Preparation of DFC.**

89 Dates fruits of secondary varieties not used for human consumption were
90 hydrothermally pretreated as described previously (Mrabet, et al., 2015). Briefly, the
91 steam treatment was carried out using a 100-liter capacity reactor which can operate at
92 temperatures between 50 and 190 °C by direct heating, and at a maximum pressure of 9
93 Kg/cm^2. 4 Kg of a mixture of several secondary date varieties were treated in duplicate
94 at 140º C during 30 min. The wet treated material was filtered by centrifugation at 4700
95 g (Comteifa, S.L., Barcelona, Spain) and freeze-dried. Seed pieces higher than 4 mm
96 were removed from the dried solid fractions by sieving. The material under 4 mm was
97 considered the DFC to be used as enzymatic digestion substrate.
98

99 **2.2. Enzymatic hydrolysis of DFC.**

100 The hydrolysis of DFC was carried out using commercialViscozyme® L, kindly given
101 by Novozymes A/S (Bagsvaerd, Denmark) representative in Spain. In this product the
102 key enzyme activity is provided by endo-β-glucanase (100 fungal beta-glucanase units -
103 FBG) that hydrolyzes (1,3)- or (1,4)-linkages in β-D-glucans. Xylanase, cellulase, and
104 hemicellulase are also declared as side activities ("Viscozyme L. Product data sheet,"
105 2014). Solid/liquid (S/L) ratio, % of enzyme, and treatment duration were the studied
106 factors to optimize the hydrolysis conditions. 400 mg of DFC were weighted in
107 duplicate in 50 mL Falcon tubes. Enzyme solution and water were added to reach the
108 hydrolysis conditions specified in Figure 1, and thenincubated in a stirring water bath at
109 55 ºC, for 30, 60, 180, and 360 min at 70 oscillations/min. Tubes without enzyme
110 preparationwere used as a control for each treatment time. After digestion, the tubes
111 were immediately heated at 100ºC for 10 min to inactivate the enzymes. The samples
112 were centrifuged at 14000g during 20 min, and the residue washed with distilled water
113 and centrifuged in the same conditions, and then frozen and freeze-dried. Each residue
114 and the original DFC underwent a Saeman hydrolysis with sulfuric acid(Jiménez,
115 Rodríguez, Fernández-Caro, Guillén, Fernández-Bolaños, & Heredia, 2001a) in order to
116 evaluate the enzymatic digestion progress. Samples of 10 mg of solid residue were
117 hydrolyzed with 0.5 mL of 72% sulfuric acid during 2 h at 40º C. Afterwards, 5.5 mL of
118 distilled water were added and the hydrolysis continued during 3 h at 100º C. 100 μL (in
119 duplicate) of the hydrolysates were neutralized with 2 N NH_4OH, inositol (25 mg) was
120 added as internal standard, and the mixture was reduced and acetylated for neutral
121 sugars quantification as their alditol acetates derivatives by gas chromatography. The
122 results were expressed as mg of each neutral sugar remaining in the solid residue, after

subtracting the values obtained for the control to each corresponding enzymatic digestion sample.

After choosing the optimized conditions, enzymatic hydrolysis of 4 g DFC were developed. Two replicates were directly freeze-dried and the residue was named as HDFC, the modified date dietary fiber. Four more replicates were centrifuged as describedabove to obtain the soluble fraction to be studied in detail.

2.3. Proximate composition and antioxidant activity of selected fiber concentrates.

DFC and selected HDFCs were analyzed for their content in moisture, protein, ethanol-soluble sugars (ESS), cellulose, non-cellulosic sugars (NCS), uronic acids, lignin, 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. NCS composition was determined by hydrolysis with 2N trifluoroacetic acid (TFA) at 121 °C for 1h. The released sugars were quantified as alditol acetates by gas chromatography.Cellulose was quantified from the TFA-insoluble residue after sulfuric acid hydrolysis by the anthrone method. Uronic acids were quantified using the phenyl−phenol method after sulfuric acid hydrolysis.Klason lignin levels were determined gravimetrically as the acid-insoluble material remaining after a two-stage sulfuric acid hydrolysis.

For ESS, total phenols and ethanol-soluble antiradical activity determinations, an extraction with 80% ethanol had to be done. ESS were determined using the anthrone method. The total phenol content was quantified for each ethanol extract according to the Folin− Ciocalteu spectrophotometric method, using gallic acid as a reference

standard compound. 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, Jaramillo, Rodríguez, Espejo, Guillén, Fernández-Bolaños, et al., 2005). The efficient concentration EC_{50}, which represents the amount of antioxidant necessary to decrease the initial absorbance by 50%, was calculated from a calibration curve by linear regression for each sample.

The antiradical activity of the insoluble residue after ethanol extraction was evaluated as described by Fuentes-Alventosa et al. (Fuentes-Alventosa, Jaramillo-Carmona, Rodríguez-Gutiérrez, Rodríguez-Arcos, Fernández-Bolaños, Guillén-Bejarano, et al., 2009).As for soluble antiradical activity, EC_{50} wasalso calculated. Both activities were expressed as millimoles of Trolox equivalent (TE) antioxidant capacity per kilogram of sample by means of a dose−response curve for Trolox.

The amount of DF was determined using the protocol described by Lee, Prosky, and De Vries (Lee, Prosky, & De Vries, 1992), with slight modifications.Samples (1 g each x 4) were suspended in 40 mL MES-TRIS buffer and treated with 50 µL Thermamyl (heat stable alpha-amylase) at 100 ºCfor 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) to remove protein and starch. Two replicates were filtered on a sintered glass crucible (no. 2)using the Fibertec E system consisting of the 1023 Filtration Module to determinate the insoluble fiber portion (IF). The residue was dried overnight at 105º C. Four volumes of 96% hot ethanol were then added to the other two replicates to precipitate the soluble fiber (SF). Total fiber (TF) was recovered by filtration in the same conditions. The residue was then washed with 80% ethanol and 96% ethanol and dried overnight at 105 ºC in an air oven and then weighed. The values

of IF and TF were corrected with their ash and protein content. SF was calculated as the difference between TF and IF.

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

The soluble fractions obtained after centrifugation of the selected enzymatic digestion mixtures were subjected to ultra-filtration at room temperature 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: Fraction A (<1000 Da) and Fraction B (>1000 Da).Both fractions were analyzed for their content in neutral sugars, uronic acids, total phenol and antiradical activity as it was describe above. For the determination of monomeric sugars present in Fraction A, an aliquot of this fraction was directly reduced without TFA hydrolysis.

2.5. High-Performance Size Exclusion Chromatography (HPSEC) and evaluation of the molecular weight (MW) distribution of SF from HDFC.

Analliquot of Fraction B was freeze-dried and redissolvedin 0.05 Mtris–hydrochloric acid. The method used for HPSEC analysis was that described by Jiménez et al.(Jiménez, Rodríguez, Fernández-Caro, Guillén, Fernández-Bolaños, & Heredia, 2001b) with slight modifications. The MW was measured in Jasco equipment (LC-Net II ADC, Kyoto, Japan) with a refractive index detector (Jasco RI-1530) and injection valve (Rheodyne, loop 20 µL, Cotati, CA). Two different columns in sequence were used: TSKgelGMPWxl and TSKgel G3000PWxl (300 x 7.8 mmi.d., Tosoh Bioscience LLC, King of Prussia, PA) after calibration with 500, 110, 40, 6 kDa and maltose (Fluka, Buchs, Switzerland). Blue dextran was used to test the void volume (V0) of the

column. The elution buffer was 0.05 Mtris–hydrochloric acid at a flow rate 0.4 mL/min.Fractions of 250 μL were collected using a Redifrac® fraction collector (Pharmacia Biotech, Uppsala, Sweden). Fractions were assayed for neutral sugars by the anthronemethod.

2.6. 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. The level of significance was $P<0.05$.

3. Results and discussion

3.1. Study of hydrolysis conditions.

In Figure 1 the assayed hydrolysis conditions are summarized. The ratios S/L are among most used in bibliography, from 1:4 (Landbo & Meyer, 2001) to 1:100 (Fissore, Santo Domingo, Pujol, Damonte, Rojas, & Gerschenson, 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 1:12 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 in the water bath 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 improve the extraction of sugars from date

fruits (Bahramian, Azin, Chamani, & Gerami, 2011), or to release ferulic acid from sweet potato stems (Min, Kang, Park, Kim, Jung, Yang, et al., 2006).

The hydrolysis was developed from 30 to 360 min., and the solid residues after enzymatic hydrolysis, washing and lyophilization 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 in most of sugars. The highest degree of hydrolysis was obtained with treatment 4: after 1 h, the minimum amount of sugars was reached, although at 30 min there were also very good results. At 1 h, all the sugars were between 4-26% of the initial amount, and at 30 min 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. This could be the reason why glucose is not the only released sugar. In fact, xylose and arabinose were the sugars which showed the highest decreases, reaching 4-8% of their initial amounts, respectively.

As it has been said above, mannose did not have the same progress. Glucomannans have been described in date pits (Ishrud, Zahid, Ahmad, & Pan, 2001), andduring date fruit hydrothermal pretreatment these polysaccharides are released from pits and accounted for the non-cellulosic sugars of the obtained fiber concentrate(Mrabet, et al., 2015). The used enzymatic complex (Viscozyme® L) has not mannanase activity, so they could not be hydrolyzed and must remain in the insoluble residue after enzymatic digestion. In some conditions, the amount of quantified mannose was even higher than initially found in the original date fiber concentrate. This fact could be due to the underestimation of mannose in the initial sample: it is widely known that cellulose and mannans have interactions which form stronger composites than cellulose-xyloglucans

246 do(Whitney, Brigham, Darke, Reid, & Gidley, 1998; Winkworth-Smith & Foster,
247 2013), so it could be possible that a little percentage of mannan-cellulose remained not
248 hydrolyzed in the original material. Aftereach Viscozyme® L treatment, cellulose was
249 partially digested by enzymes, and therefore mannans could be released from their
250 composites, hydrolyzed by sulfuric acid, and quantified as mannose.
251 The treatment 4 at 30 and 60 min long (30-HDFC and 60-HDFC, respectively),were
252 chosen to continue the study. Treatments longer than 60 min did not increase the sugar
253 solubilization, and 30 min one would generate soluble polysaccharides with higher
254 molecular weight and a lower amount of oligosaccharides with a degree of
255 polymerization (DP) lower than 4. In this way the ratio IF/SF would decrease the most,
256 which was the main objective of the present study.
257

258 **3.2. Chemical composition of DFC and HDFCs.**

259 The chemical composition of both HDFCs and that of the original DFC are presented in
260 Table 1. Moisture and protein increased with enzymatic treatments, but there were no
261 significant differences between both HDFCs. The total sugar content was studied in
262 three different groups: ESS, NCS, and cellulose. Only cellulose decreased with the
263 enzymatic digestion, ESS and NCS increasing. Although the highest differences were
264 quantified between DFC and both HDFCs, between 30- and 60-HDFC there were also
265 significant differences in ESS and cellulose. In the three samples, the total sugar amount
266 was around 40% w/w, but the relative percentages of each group of sugars varied
267 among the samples. In the original DFC they were 3.7-61.9-34.1% for ESS, NCS and
268 cellulose respectively. ESS increased to 9.6 and 10.7% with 30min and 60min
269 treatments, NCS increased to 77.7 and 81%, and cellulose decreased to 12.7 and 8.2%.

It is clear that an important quantity of cellulose was hydrolyzed to oligomers, which were recovered in the group of ESS or NCS depending on their DP.

The composition of NCS was determined by GC (Figure 2). Glucose was the main sugar, in a percentage near 75% in untreated sample and around 80% in the digested ones. There were not significant differences between both of them in any sugar. In general, all sugars decreased due to enzymatic digestion. Only mannose increased probably due to the disorganization of DFC which led to a more complete hydrolysis of mannans, as it was explained above.

Uronic acids (Table 1) were also quantified after a hydrolysis step with sulfuric acid. 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 the original DFC. In addition to the increase in mannose and uronic acids, the already commented better accessibility of different polymers to acid hydrolysis after enzymatic treatment couldlead to another unexpected result:lignin content decreased significantly after digestion. It is widely referred in bibliographythat the Klason lignin overestimates the values of "true lignin" (Fukushima & Hatfield, 2004; Hatfield, Jung, Ralph, Buxton, & Weimer, 1994) quantifying as "lignin" very different compound groups (cutin, suberin, waxes, proteins, Maillard reaction products, etc)(Bunzel, Schüßler, & Saha, 2011). The disorganizing effect of enzymatic treatment on DFC structure could lead to a release of interfering substances, conducting in this way to a decrease in Klason lignin.

Phenols and antiradical activity were determined from the ethanol soluble fraction. Phenols increased significantly due to Viscozyme® L treatment, although no differences were found between both HDFC. The phenols which were entrapped or structurally linked to fiber could be partially released after digestion and were quantified

295 in the ethanol soluble fraction. In fact, enzyme-assisted extraction are usually applied
296 for enhancing the yield of phenolic production from agricultural byproducts such as
297 black currant pomace (Landbo & Meyer, 2001) and sweet potato stems(Min, et al.,
298 2006). Theseresultswere confirmed when the antiradical activity was discussed: the
299 ethanol soluble activity increased with treatment as a consequence of the decrease of the
300 activity linked to residue. The total antioxidant activity did not change after enzymatic
301 digestion (123.48, 120.90, and 122.44 mmols TE/Kg fiber for DFC, 30-HDFC, and 60-
302 HDFC respectively). This level of antiradical activity was similar to that of several
303 citrus fiber concentrates (Marín, Soler-Rivas, Benavente-García, Castillo, & Pérez-
304 Álvarez, 2007) and much higher than cocoa bean husks (Lecumberri, Mateos,
305 Izquierdo-Pulido, Rupérez, Goya, & Bravo, 2007) and spent coffee fiber (Campos-
306 Vega, Loarca-Piña, Vergara-Castañeda, & Oomah, 2015) which are considered useful
307 as potential antioxidant dietary supplements.
308 The dietary fiber content decreased with the treatment, from 36.19 to near 22%. A part
309 of the original IF would be solubilized due to enzymatic digestion, increasing in this
310 way the SF, but also the SF could be partially hydrolyzed to smaller fragments which
311 did not precipitate with hot ethanol during TF isolation. Working with carrot pomace,
312 Yoon et al. (Yoon, Cha, Shin, & Kim, 2005) considered this lost fraction as alcohol-
313 soluble dietary fiber (ASDF). In carrot, ASDF increased from 14 to 36% after
314 enzymatic treatment, which could lead to a reduction in TF.The ratio IF/SF decreased
315 from around 20 to 2.5-3, depending on the duration of hydrolysis. Taking into account
316 both results, it could be concluded that the total amount of SF increased from 1.8% in
317 DFC to 6.3 and 5.4% in 30-HDFC, and 60-HDFC respectively, despite the net loss of
318 TF. SF increased more than three times because of treatment. Similar results were found
319 working with IF coming from artichoke byproducts (Fissore, Santo Domingo, Pujol,

320 Damonte, Rojas, & Gerschenson, 2014), where SF increased up to 3.5 times after a
321 treatment with hemicellulases. Lower results were described by Napolitano et al.
322 (Napolitano, et al., 2006), reaching an increase of 2.4 times in barley spent grain SF.
323 With the Viscozyme® L treatment on DFCvery good results have been obtained: the
324 ratio IF/SF was near the optimum range to have beneficial physiological effects (1-2.3)
325 (Spiller, 1986) and the amount of SF was three-fold higher in the hydrolyzed fiber than
326 in the initial one, implying in this way an important improvement in the physiological
327 quality of date fiber.
328

329 **3.3. Composition of the HDFC soluble fraction.**

330 The solubilized fractionafter Viscozyme® L digestion was subjected to ultra-filtration
331 through a 1000 Da (DP>6) molecular weight cut-off membrane. Eluted (Fraction A) and
332 retained (Fraction B) fractions were analyzed for neutral sugars, uronic acids, total
333 phenols and antiradical activity and the results are summarized in Table 2 (upper table).
334 Neutral sugars present as monomers have been also quantified from fractions with
335 DP<6 (Fraction A).
336 The total amount of neutral sugars rose up as the digestion duration increased (7.59 and
337 9.11% for 30-HDFC and 60-HDFC, respectively). The distribution of molecular weight
338 also changed depending on treatment length: DP>6 and DP 2-6 fractions decreased and
339 consequently monomers increased. The relative percentage of each fraction in the total
340 neutral sugar quantified went from 11.98, 50.7, and 37.4% (DP>6, 2-6, and monomers
341 respectively) in 30-HDFC to 6.1, 47.2, and 46.6% in 60-HDFC. The same results were
342 described when carrot pomace was treated with edible-snails crude enzymes (Yoon,
343 Cha, Shin, & Kim, 2005), where high molecular weight polysaccharides were converted
344 to 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 of DP>6 fractions. The antiradical activity had the same behavior than total phenol content.

When the glycosidic composition was studied (Figure 3, subfigures A) important differences were found among fractions and digestion length. 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("Viscozyme L. Product data sheet," 2014), cellulose fragments of different molecular weight could be released from DFC structure during digestion 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(Whitney, Brigham, Darke, Reid, & Gidley, 1998; Winkworth-Smith & Foster, 2013). In Japan, some studies have shown that manno-oligosaccharides (MOS) could have beneficial effects on human health: promotion of intestinal bifidobacteria growth, reduction of abdominal and subcutaneous fat, inhibition of intestinal fat absorption by increasing its excretion, etc.(Campos-Vega, Loarca-Piña, Vergara-Castañeda, & Oomah, 2015). These results have led to the approbation of coffee MOS as Food for Specified Health Uses (FOSHU) oligosaccharide functional food ingredient in Japan (Fukami, 2010). Even β-mannanases are being isolated from microorganisms in order to produce MOS with DP 2-6 from locust bean gum (Zang, Xie, Wu, Wang, Shao, Wu, et al., 2015).

Besides MOS, xylo-oligosaccharides (XOS) seemed to be present, especially in DP>6 fraction but also in DP 2-6 one. Taking into account that arabinoxylans are the main hemicelluloses quantified in date fruit dietary fiber (Mrabet, Rodríguez-Arcos, Guillén-

370 Bejarano, Chaira, Ferchichi, & Jiménez-Araujo, 2012; Mrabet, et al., 2015), and that
371 xylanase is another side activity declared in Viscozyme® L, it was expected the
372 presence of XOS in the soluble fraction. XOS and gluco-oligosaccharides (GOS) are
373 also considered as emerging prebiotics, XOS having also the category of FOSHU in
374 Japan (Rastall, 2010). Therefore, it seems to be of great interest the presence of GOS,
375 MOS and XOSin HDFC from both technological and physiological points of view,
376 which molecular weight could be tailored by controlling enzymatic digestion
377 conditions.
378

379 **3.4. Molecular weight distribution of DP>6 fraction.**

380 The fraction with DP>6 was analyzed by HPSEC due to its interesting glycosidic
381 composition. The obtained IR profiles are presented in Figure 4. Three different
382 fractions were isolated: F1, with the highest MW, centered in 110 KDa; F2, with MW
383 between 6-40 KDa, and F3, lower than 6 KDa. As digestion time increased, F2
384 decreased and F3 went up. Besides, the maximum lecture in RI of F2 shifted down to
385 lower MW, so the average MW of this fraction also decreased with longer treatments. In
386 the inner table of Figure 4, the relative percentages of the three isolated fractions are
387 presented, showing in this way the diminution of F1 and F2 and the increase in F3 as
388 digestion time was prolonged. The chemical composition of these three fractions has
389 been also studied (Table 2, lower table). The proportion of neutral sugars changed from
390 10.3-66.4-23.24 for F1, F2 and F3 respectively at 30min hydrolysis to 7.8-61.5-30.6 at
391 60min. The same happened for phenols, which changed from 22.8-44.9-32 to 11.5-45.0-
392 43.4. Uronic acids were only present in F2 fraction and decreasing from 1.2 to 0.34
393 g/Kg HDFC.

394 The glycosidic composition of the three isolated fractions (Figure 3, subfigures B)
395 revealed that MOS were the main components of F2 (higher than 40%), but GOS were
396 predominant in F3. In F1 there were important percentages of galactose and rhamnose,
397 which could indicate the presence of pectic polymers in this minor fraction. XOS were
398 especially present in F2 also: in this fraction xylose and arabinose accounted for near
399 30% of total sugars quantified. Therefore, MOS and XOS had higher MW than GOS,
400 probably due to the higher activity of β-endoglucanase present in Viscozyme® L,
401 existing slight differences between 30min and 60min digestions. The thorough control
402 of time hydrolysis could lead to a tailor-made oligosaccharide composition to obtain the
403 best health beneficial effects.
404

405 **4. Conclusion**

406 Viscozyme® L has been successfully applied on DFC in order to increase the
407 proportion of SF in this fiber concentrate. The optimized conditions consist in the
408 enzymatic complex at 2.38%, a solid/liquid ratio 1:35, at 55º C during 30-60
409 min.Although the amount of total dietary fiber decreased, the ratio IF/SF went from
410 near 20 to 2-3 after 30min hydrolysis in the optimized conditions, and as a consequence
411 the amount of SF increased from 1.8% in untreated fiber to 6.3% in 30-HDFC. This
412 characteristic could enhance the physiological effects of the new fiber concentrate.
413 Besides, GOS were the main oligosaccharides obtained with this treatment due to the
414 high β-endoglucanase activity present in the reaction mixture, but also MOS and XOS
415 were quantified in significant amounts. These three groups of oligosaccharides are
416 considered as emerging prebiotics, so *in vitro* studies and human trials are needed in
417 order to assess their health positive effects. Anyway, the enzymatic digestion of DFC in
418 the proposed conditions is a promising beginning for the amelioration of date fiber

419 beneficial health effects and the use of this new soluble fiber concentrate as a prebiotic
420 and antioxidant functional food ingredient.
421
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425
426 **References**
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584 **FIGURE CAPTIONS**

585

586 **Figure 1**.-Hydrolysis conditions and release of the different sugars throughout
587 Viscozyme® L enzymatic hydrolysis.

588

589 **Figure 2**.- Glycosidic composition (%) of non-cellulosic sugars from the original date
590 fiber concentrate and from the selected hydrolyzed ones (30 and 60 min hydrolysis).
591 Values are the means of at least triplicate assays. Means bearing the same symbol are
592 not significantly different at the 5% level as determined by the Duncan multiple-range
593 test.DFC: date fiber concentrate; HDFC: hydrolyzed date fiber concentrate; Rha:
594 rhamnose; Fuc: fucose; Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose;
595 Glu: glucose.

596

597 **Figure 3**.-Glycosidic composition (%) of the fractions obtained from soluble fractions.
598 Subfigures A: after 1000 Da ultrafiltration from the soluble fraction of the selected
599 hydrolyzed date fiber concentrates (30 and 60 min hydrolysis). Subfigures B: after
600 HPSEC analysis from DP>6 soluble fractions.
601 Values are the means of at least triplicate assays. * indicates significant differences at
602 the 5% level as determined by the Duncan multiple-range test.HDFC: hydrolyzed date
603 fiber concentrate; DP: degree of polymerization;Rha: rhamnose; Fuc: fucose; Ara:
604 arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glu: glucose.

605

606 **Figure 4**.- HPSEC elution profile (refraction index) of the soluble fraction with degree
607 of polymerization higher than 6 unitsfrom the selected hydrolyzed date fiber
608 concentrates (30 and 60 min hydrolysis).

609 1: 500KDa; 2: 110 KDa; 3: 40 KDa; 4: 6 KDa; 5: Maltose; HDFC: hydrolyzed date

610 fiber concentrate.

611

612

Table 1.-Chemical composition (g/100g) and antiradical activity (mmolsTrolox equivalent/Kg product) of the original date fiber concentrate and of the selected hydrolyzed ones (30 and 60 min hydrolysis).

	DFC	30-HDFC	60-HDFC
Moisture	7.08±0.19 a	8.34±0.10 b	8.02±0.23 b
Protein	7.03±0.26 a	9.53±0.40 b	9.94±0.25 b
Ethanol-soluble sugars	1.40±0.04 a	4.01±0.12 b	4.34±0.11 c
Cellulose	12.83±0.99 c	5.26±0.36 b	3.32±0.15 a
Non-cellulosic sugars	23.26±0.90 a	32.25±0.64 b	32.68±0.96 b
Uronic acids	1.94±0.21 a	4.46±0.30 b	4.15±0.55 b
Lignin	27.34±1.87 b	18.45±0.40 a	20.82±0.45 a
Ethanol-soluble phenols	1.34±0.12 a	1.70±0.02 b	1.99±0.05 c
Antiradical activity			
Ethanol-soluble	85.54±7.02 a	101.35±3.00 b	106.07±1.05 b
Residue	37.94±1.72 c	19.55±0.37 b	16.37±1.05 a
Dietary fiber	36.19±2.23 b	22.46±1.67 a	21.73±0.31 a
IF/SF	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.DFC: original date fiber concentrate; HDFC: hydrolyzed date fiber concentrate; IF: insoluble fiber; SF: soluble fiber

Table 2.- Composition (g/KgHDFC) and antiradical activity (mmols Trolox equivalents/Kg HDFC) of recovered fractions after 1000Da ultrafiltration process from soluble fractions after enzymatic hydrolysis (upper table), and after HPSEC fractionation from DP>6 fractions (lower table).

			Neutral sugars	Uronic acids	Total phenols	Antiradical activity
30-HDFC	DP>6		9.05±0.22 b	1.43±0.18	4.52±0.12b	20.50±0.81 b
	DP<6	DP 2-6	38.54±0.47 a	26.07±1.81 a	10.92±0.34 a	38.52±0.39 a
		Monomers	28.41±0.42 a			
60-HDFC	DP>6		5.65±0.25 a	t	1.52±0.10 a	6.82±0.23a
	DP<6	DP 2-6	43.05±0.48 b	27.22±2.00 a	19.64±0.49b	59.57±0.52 b
		Monomers	42.47±3.30 b			

30-HDFC	DP>6	F1	0.63±0.05 b	n.d.	0.62±0.01 b	n.d.
		F2	4.06±0.04 b	1.21±0.05 b	1.22±0.01 b	n.d.
		F3	1.42±0.01 b	n.d.	0.88±0.01 b	n.d.
60-HDFC	DP>6	F1	0.34±0.01 a	n.d.	0.12±0.00 a	n.d.
		F2	1.85±0.12 a	0.34±0.06 a	0.47±0.00 a	n.d.
		F3	0.92±0.04 a	n.d.	0.45±0.00 a	n.d.

Values are the means of at least duplicate assays. Means bearing the same symbol are not significantly different at the 5% level as determined by the Duncan multiple-range

test.HDFC: hydrolyzed date fiber concentrate; DP: degree of polymerization; t: traces;n.d.: not detected.

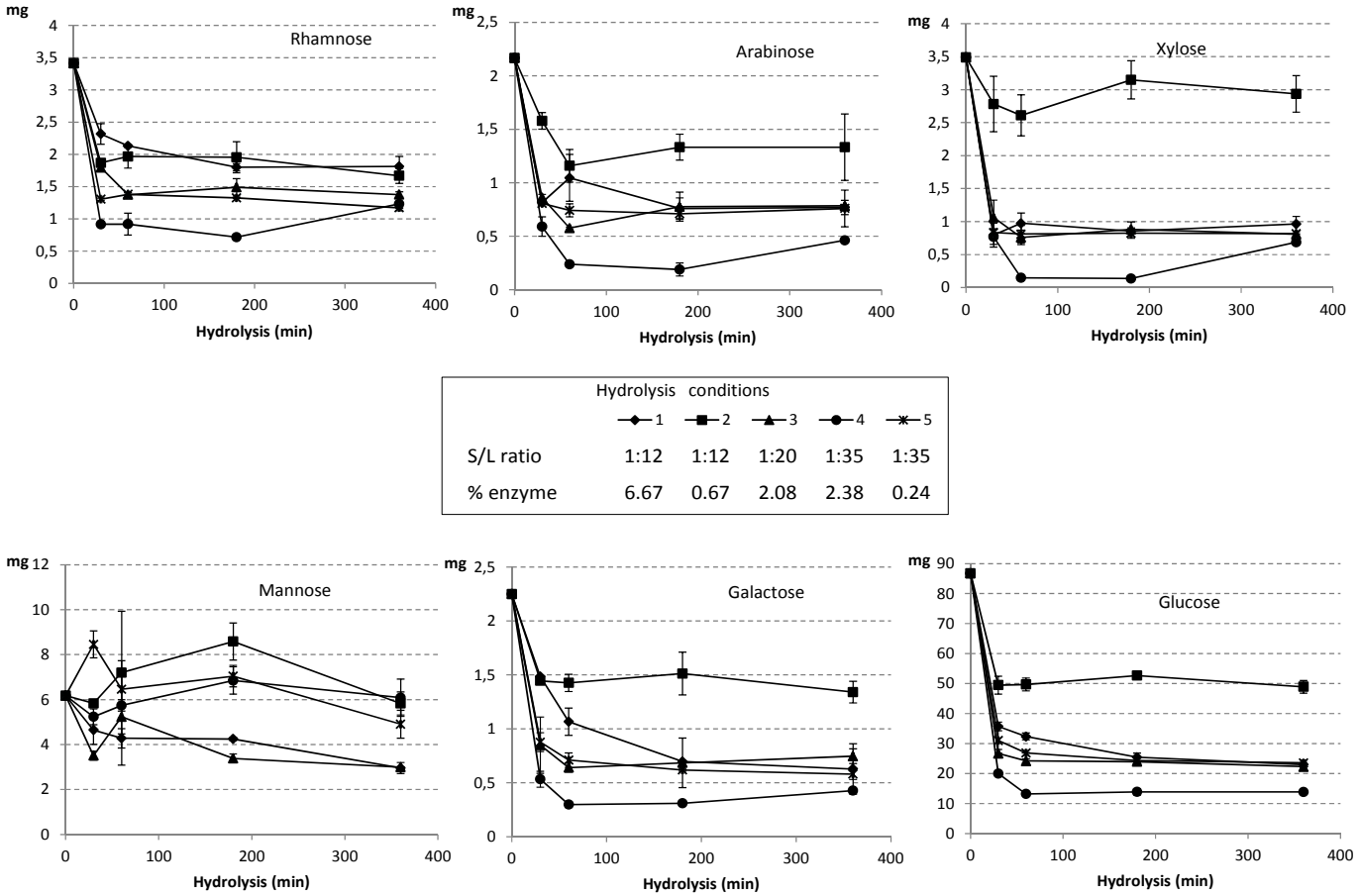

mg
Rhamnose
Hydrolysis (min)
mg
Arabinose
Hydrolysis (min)
mg
Xylose
Hydrolysis (min)
Hydrolysis conditions
1 2 3 4 5
S/L ratio 1:12 1:12 1:20 1:35 1:35
% enzyme 6.67 0.67 2.08 2.38 0.24
mg
Mannose
Hydrolysis (min)
mg
Galactose
Hydrolysis (min)
mg
Glucose
Hydrolysis (min)

Figure 1

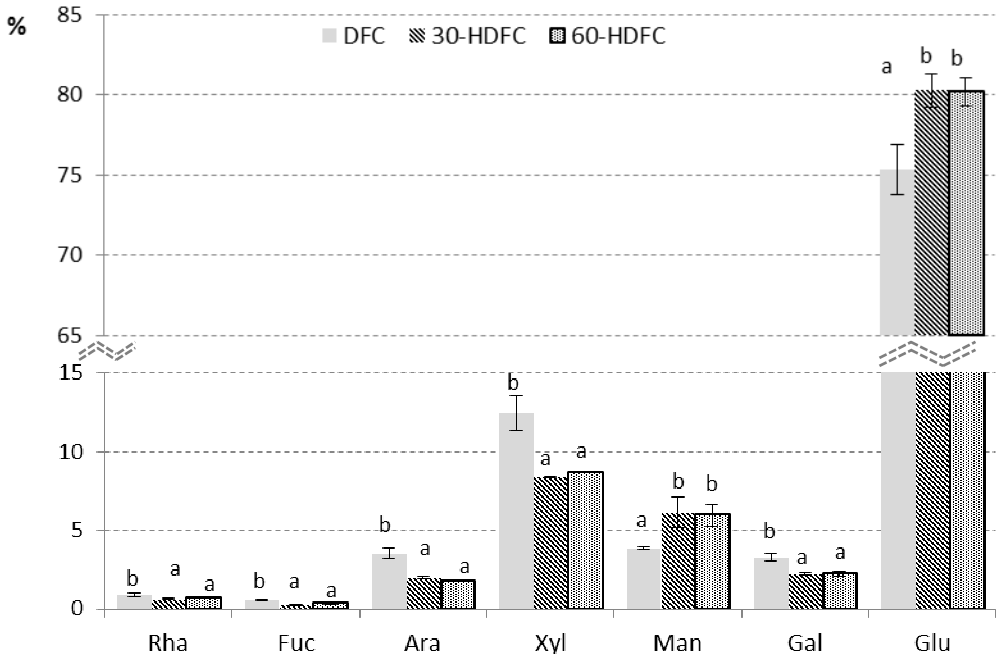

%
DFC
30-HDFC
60-HDFC
85
80
75
70
65
15
10
5
0
Rha
Fuc
Ara
Xyl
Man
Gal
Glu

Figure 2

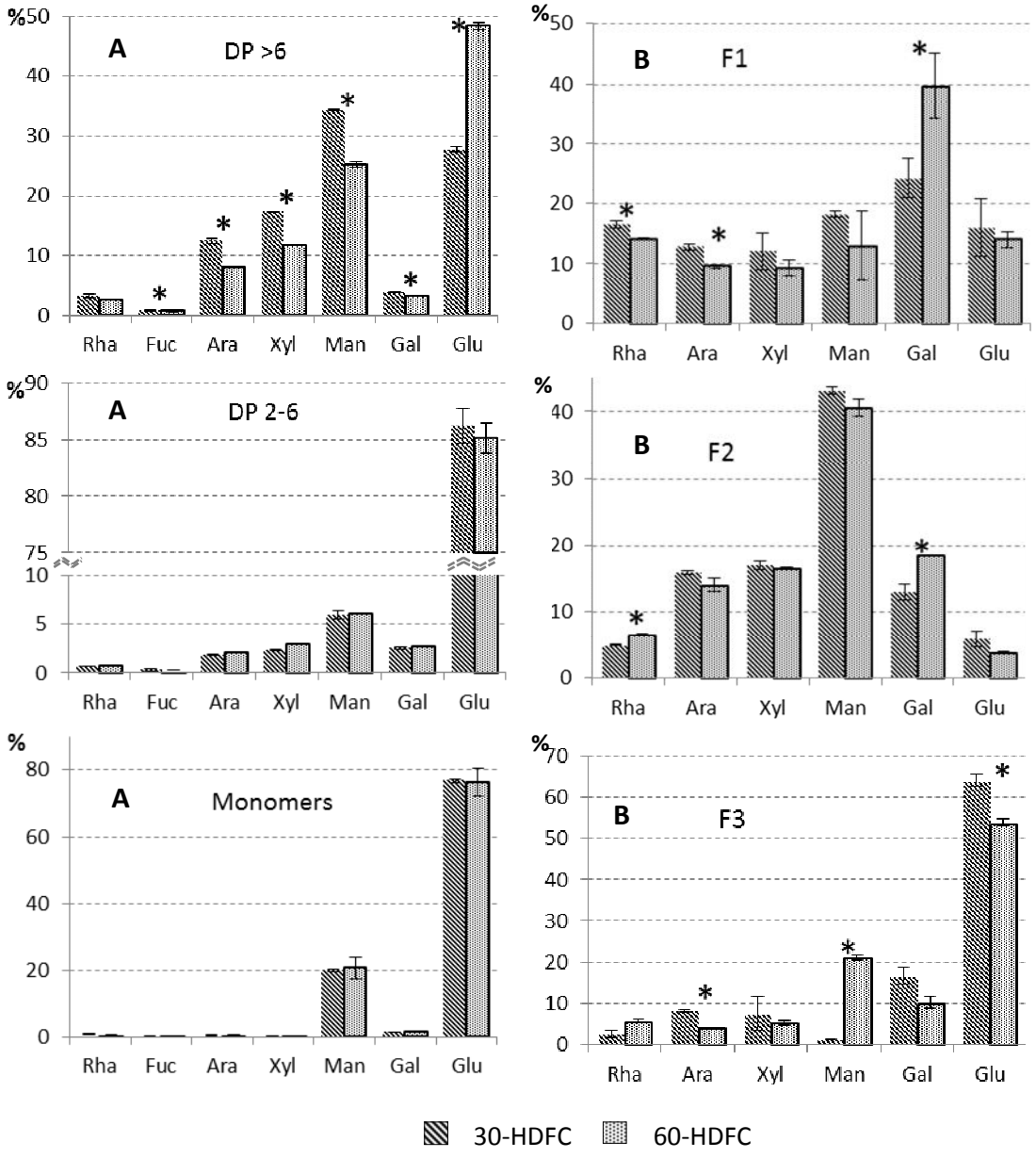
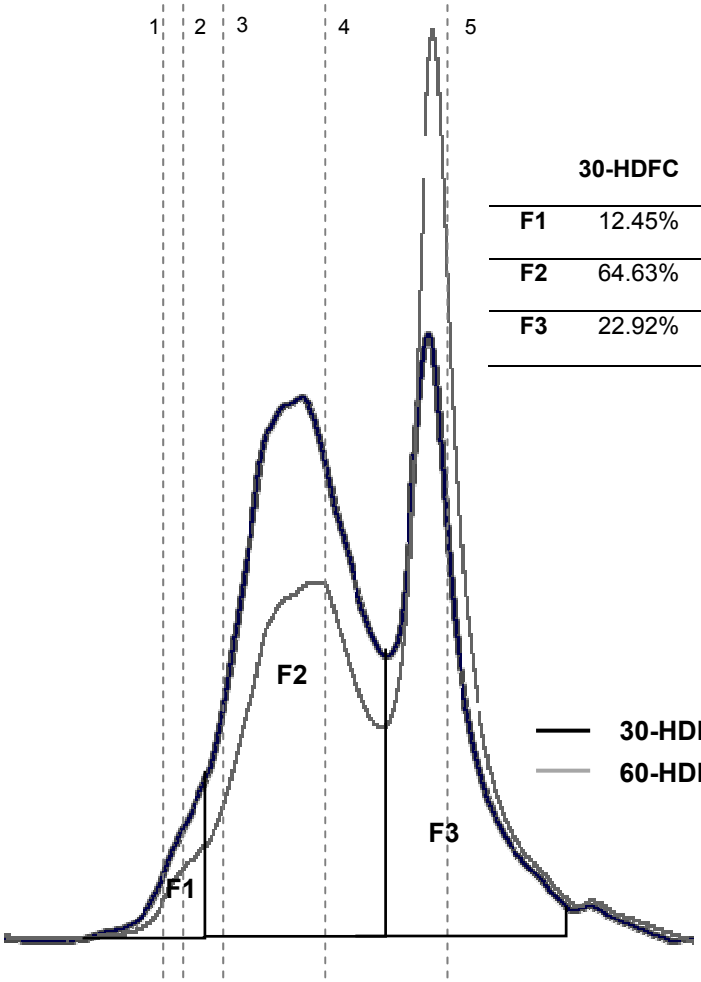

A
DP >6
%
Rha
Fuc
Ara
Xyl
Man
Gal
Glu
B
F1
A
DP 2-6
B
F2
A
Monomers
B
F3
30-HDFC
60-HDFC

Figure 3



	30-HDFC	60-HDFC
F1	12.45%	8.12%
F2	64.63%	60.60%
F3	22.92%	31.28%

Figure 4