Microwave-assisted synthesis of prebiotic di-α-fructose dianhydride-enriched caramels

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**A R T I C L E   I N F O**

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**A B S T R A C T**

The synthesis of prebiotic caramels involving the use of microwaves as the activating/heating source has been achieved. The yields in di-fructose dianhydrides (DFAs) in caramels were measured. The aim of this study was twofold: first to check the feasibility of the process, and second to determine the conditions to obtain an optimum response with microwave heating. The study showed that it was possible to obtain a yield of almost 50% of DFAs in a reaction time that was 10 times shorter than a previous study; i.e. 5–10 min for microwave activation compared to 60–120 min for conventional heating. It was shown that the radiation time and the radiation power were linked. The simultaneous determination of the values of these two factors was therefore necessary to obtain significant yields. This technique demonstrates the advantage of activation for mixtures such as caramels.

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**1. Introduction**

A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health (Figueroa-Gonzalez, Quijano, Ramyrez, & Cruz-Guerrero, 2011; García-Moreno, Benito, Ortiz Mellet, & García Fernández, 2008). In addition to fructans such as inulin and fructo-oligosaccharides (FOS) which are the main carbohydrate prebiotics, difructose dianhydrides (DFAs) (Fig. 1) and their glycosylated derivatives (glycosyl-DFA) can also be included (García-Moreno et al., 2008; Ortiz Mellet & García Fernández, 2010). Some individual DFA representatives have been shown to behave as prebiotics, promoting bifidobacteria growth and mineral absorption in the small and large intestine of rats (Mineo et al., 2004; Mitamura & Hara, 2005; Strojny et al., 2011) and humans (Tomita et al., 2007). Likewise, products containing DFAs and glycosylated (glucosyl or fructosylated) DFAs (up to 40% together), obtained by the thermolysis of sucrose, inulin, or isomaltulose (6-O-α-α-glucopyranosyl-α-fructofuranose) in the presence of citric acid, have been shown to claim the beneficial microbiota (Arribas et al., 2010). DFAs have been isolated from plants and microorganisms and are also produced enzymatically or chemically from fructose or fructose-containing raw materials (Arribas et al., 2010; Defaye & García Fernández, 1994). Recently, DFAs and glycosyl-DFAs as well as a mixed α-fructose-α-glucose dianhydride were identified in a commercial caramel of sucrose (Fig. 1) in which they constituted the major components of the saccharide fraction (Manley-Harris & Richards, 1996). Caramel is the product of the heat treatment of a food-grade sugar under particular conditions. The final product is complex and consists of 5–10% of volatile fraction and 90–95% of non-volatile fraction, consisting mainly of unreacted sugar and DFAs (Arribas et al., 2010; Defaye & García Fernández, 1994). DFA-enriched caramels have prebiotic properties and are intended to be used as food additives. DFAs do not need to be isolated from caramels. Moreover, glycosyl-DFAs, present in caramel, are currently being studied and are also thought to possess prebiotic properties. Health-promoting caramels are directly incorporated in food matrices such as yoghurt for instance. Recently (Idri, Havet, Garcia Fernandez, & Porte, 2011), an efficient synthesis of DFA-enriched caramels, based on a patent using fructose as raw material and a Lewatit resin as catalyst, was described. Scale-up and optimisation led to interesting results. The caramels produced in a 100 ml flask contained about 30% by mass of DFAs, while in a 1 L reactor the yield reached 50% with better rheological conditions (lower proportion of resin and higher proportion of water). The heating required the circulation of a thermal fluid in a double-jacketed vessel. Because of the high viscosity, homogenisation of the mixture was particularly difficult, as were heat transfers; the efficiency of the process was therefore limited. To circumvent these difficulties, the use of a microwave source was considered in order to heat or activate the pasty mixture to the core.

Since the initial publications by Gedye (Gedye et al., 1986) and Giguere (Giguere, Bray, Duncan & Majetich, 1986) in 1986, the use of microwave has progressively emerged as a popular non-conven-
tional heating source in the field of organic synthesis (Gedye et al., 1986; Kappe & Stadler, 2005; Loupy, 2002). Recently microwave irradiation was used for the synthesis of several organic compounds in the carbohydrate series (Hansen, Woodley, & Riisager, 2009; Li, Le, Cheng, Wang, & Shi, 2006). Using a microwave process also leads to a lower formation of by-products, easier work-up matching with the goal of green chemistry, solvent-free organic transformations, atom economy and selectivity of reactions (Ferroud, Godart, Ung, Borderies, & Guy, 2008; Maiti, Kaith, Jindal, & Jana, 2010). Beside hydroxyl protection/deprotection sequences, microwaves can be successfully employed in alkylation/glycosylation reactions. Microwave-accelerated Fischer glycosylation, under acidic conditions, affords good yields in a single step (Richel, Laurent, Wathelet, Wathelet, & Paquot, 2011). However, the application of microwaves in the area of caramel chemistry is less well documented. To our knowledge, the synthesis of DFA-enriched caramels involving the use of microwaves as the activating/heating source has never been reported in the literature.

This method can be advantageously compared to conventional heating for the implementation of the synthesis of caramels. Heat transfer by radiation typically follows a rate law proportional to the fourth power of the source temperature. A microwave is particularly suitable to overcome the high constraints of the medium rheology that decrease heat transfer. In addition, the effect of the chemical activation of organic functions by the microwave enables this technology to be used for caramel synthesis. The objective of this study is therefore twofold: first to check the feasibility of the process, and second to determine the requisite conditions to obtain an optimum response with microwave heating.

2. Materials and methods

2.1. Products

Anhydrous α-fructose (99% purity) was purchased from Danisco Sweeteners. Phenyl-β-d-glucopyranoside (Internal standard, I.S.), pyridine, hydroxylamine, hexamethyldisilazane and trimethylchlorosilane were purchased from Sigma–Aldrich.

Di-α-β-fructofuranosane 1,2;2,3-dianhydride (DFA 1), α-β-fructopyranosane-β-α-fructopyranosane 1,2;2,1′-dianhydride (DFA 5), α-β-fructofuranosane-β-α-fructopyranosane 1,2;2,1′-dianhydride (DFA 9) and α-β-fructofuranosane-β-β-fructofuranosane 1,2;2,1′-dianhydride (DFA 10) were synthesized and purified at the Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla. The ion-exchange resin Lewatit® 2328 was purchased from Lanxess. The use of this resin makes it possible to work at temperatures below those currently used for the caramelization process. Before use, an activation step by treatment with hydrochloric acid solution is necessary.

2.2. Conditioning of the acid resin

500 g of Lewatit® 52328 ion-exchange resin was introduced into a glass column and then eluted (3 ml min⁻¹) with deionized water (2 L), 1 N aqueous HCl (2 L) and methanol (1 L). Drying was first effected under air current at room temperature for 8 h, then in an oven at 90 °C for 16 h (Suarez-Pereira, Rubio, Pilard, Ortiz Mellet, & Garcia Fernandez, 2010).

2.3. Experimental setup and operating conditions

In the method reported here, fructose conversion was accomplished using a microwave oven (CEM Discover™) under pressure and was monitored by ChemDriver®. This enables several parameters such as temperature, pressure, microwave period, and power to be monitored.

The DFA-enriched caramels were synthesized: α-fructose was first dissolved in water to the desired concentration by mild heating (60 °C). The appropriate amount of catalyst was then added. Compounds were then mixed in a sealed reaction tube which was immediately introduced into the microwave oven and irradiated at various powers for different times. Conversion of fructose to the corresponding product was monitored by GC. Two experimental conditions were tested for microwave treatment: Condition 1 corresponds to the optimal conditions in a 1 L reactor with conventional heating and Condition 2 corresponds to the optimal...
Idri et al. (2011). The reaction time is considered during the period of microwave activation. During this period, the temperature reaches its setpoint then oscillates around it. All the experiments were repeated at least twice. The mean error was 4% on the yield and the conversion ratio.

For conventional heating, the DFA-enriched caramels were produced in a round-bottom flask (250 ml) by heating in a silicon oil bath in the presence of the acid resin under the conditions described in a previous patent (Castillo et al., 2010). α-fructose (100 g corresponding to 3% wt) was first dissolved in water (96% with x + y = 100%) to the desired concentration by mild heating (60 °C). The appropriate amount of catalyst (2%) was then added; z was calculated from the final amount of fructose that represented 100%. For example, if z = 10%, then the amount of resin was 10 g. The experimental time was 2 h.

2.4. Sample preparation

For GC analysis, caramel samples, as well as the reference samples used for identification, were converted into their corresponding per-O-trimethylsilyl (TMS; non-reducing sugars) or per-O-trimethylsilylated oxime (TMS–oximes; reducing sugars) derivatives (Mohar Perl, Katona, & Sass, 1999; Silva & Ferraz, 2004). The method described by Sweeley et al. (Sweeley, Bentley, Makita, & Wells, 1963) was followed with minor modifications. The samples were diluted with deionized water (1 ml), the resin (in caramel cases) was separated by centrifugation (13,000 rpm, 30 min) and the aqueous solutions were freeze-dried. The dried sugar or caramel was diluted in deionized water to a concentration of 16 mg ml⁻¹. To 100 µl of the resulting solution was then added 100 µl of internal standard (L.S.; 4 mg ml⁻¹ phenyl-β-D-glucopyranoside in acetone-water; 1:9; v/v) and the final solution was freeze-dried at –60 °C. The residue was then oximated by treatment with 1 ml of a solution of hydroxylamine in pyridine (20 mg ml⁻¹) at 60 °C over 50 min. The cooled sample was then trimethylsilylated by reaction with a mixture of hexamethyldisilazane (200 µl) and trimethylchlorosilane (100 µl) at 60 °C over a further 40 min period. The white precipitate obtained during this operation was then separated by centrifugation (13,000 rpm, 5 min) (Ratsimba, 2000; Ratsimba, García Fernández, Defaye, Nigay, & Voilley, 1999). It is worth noting that following oximation–trimethylsilylation derivatization, reducing compounds (e.g. residual α-fructose and 5-hydroxymethyl-2-furfural) provide two peaks in the GC chromatograms, corresponding to the syn- and anti-TMS-oximes, while nonreducing derivatives (e.g. DFAs and the L.S.) provide a single peak (Suarez-Pereira et al., 2010).

2.5. GC-FID analysis

2.5.1. Qualitative analysis

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2.5. GC-FID analysis

2.5.1. Qualitative analysis

GC-FID was carried out using an Agilent 7820A series chromatograph equipped with an FID detector (Agilent Technologies). A cross-linked 5% phenyl-dimethylsiloxane column (HP-5; 30 m x 320 μm x 0.25 μm) was used. Operating conditions were: injection port temperature 310 °C; splitting ratio 25:1; injection volume 1 µl of derivatized samples; column oven temperature from 180 to 310 °C (5 °C min⁻¹) and held at 310 °C for 5 min; carrier gas helium (constant flow at 1.2 ml min⁻¹); detector port temperature 310 °C. Total acquisition time was 31 min.

2.5.2. Quantitative analysis

Calibration lines were established from pure fructose and DFAs (1, 5, 9 and 10). The injections were repeated three times. Quantification was done by the method of internal standard. The response factors (RF) for fructose and four individual DFAs were determined (Table 2) and used for quantification of their relative proportion in caramels.

2.5.3. Definition of responses

The yield of total DFAs, with regard to the reproducibility of the results between the two laboratories LGPPEES (Paris) and CSIC (Seville), was determined using the average RF value calculated by the Sevillian team from calibration curves of the 13 isomers of DFA (Table 2).

3. Results and discussion

For the first experiments performed under Condition 1, the irradiation power ranged from 100 to 300 W (Table 3). The fructose was almost totally consumed (conversion ratios about 90–98.5%) but not transformed into DFA (yields between 2% and 5%), whereas with conventional heating, after 3–5 min of reaction, the conversion of fructose was very low (below 10%) for the same yield of DFA (less than 5%). In these conditions (entries 1–5 in Table 3) the microwave treatment seemed to be too intensive. Two parameters could be involved: the radiation power and radiation time, for the caramelization reaction. First, to improve DFA production, the reactions were performed at 70 °C at lower powers (5–25 W), and the wattage was automatically adjusted so as to maintain the desired temperature. In these experimental conditions, the yields in DFA increased for a moderate conversion of fructose. The decrease in power was therefore beneficial for the synthesis and the results were improved; 17% and 29% DFA yields were obtained, respectively, for 25 W (2 min) and 10 W (4 min). A last test

Table 1

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Condition 1</th>
<th>Condition 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose (g)</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>Water (g)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Water (g)</td>
<td>0.25</td>
<td>0.11</td>
</tr>
<tr>
<td>Resin (g)</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Reaction</th>
<th>RF LGPPEES (Paris)</th>
<th>RF CSIC (Seville)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>1.26</td>
<td>1.42</td>
</tr>
<tr>
<td>DFA 1</td>
<td>0.71</td>
<td>0.89</td>
</tr>
<tr>
<td>DFA 5</td>
<td>0.65</td>
<td>0.77</td>
</tr>
<tr>
<td>DFA 9</td>
<td>0.70</td>
<td>0.69</td>
</tr>
<tr>
<td>DFA 10</td>
<td>0.79</td>
<td>0.76</td>
</tr>
<tr>
<td>Average RF value for DFAs</td>
<td>0.71ᵃ</td>
<td>0.78ᵇ</td>
</tr>
</tbody>
</table>

ᵃ Calculated from 4 RF. ᵇ Calculated from 13 RF.
was conducted at 5 W for 15 min; the DFA yield (13%) decreased. Second, these powers (25 and 10 W) were monitored to check the kinetic evolution of the products over time. A set of tests was therefore performed for 1–6 min (Fig. 2). The conversion of fructose was faster at 25 than at 10 W; 60%, the maximum value, was reached after 4 min at 25 W and after 6 min at 10 W. For the two powers, the yield curves followed the same profile and passed by maxima of 17% for 2 min at 25 W and 29% for 4 min at 10 W. Moreover, by reducing the power from 25 to 10 W, the efficiency was further increased for an experimental time that was slightly longer. Beyond these times, the yields decreased. This result could be explained by the fact that a prolonged treatment led to a consumption of the desired products. One hypothesis was that the DFAs were converted into their glycosyl-DFA derivatives (Suarez-Pereira et al., 2010). The results presented in Table 3 shows the need to determine the best power/time couple to obtain the appropriate conversion.

When the reaction was carried out with conventional heating for a reaction time of 15 min, the DFA yields never exceeded 12% under Condition 1.

Other caramels were produced under Condition 2 (Table 1) which involved working in a medium that was less hydrated, and enriched in catalyst. Three kinetic studies were performed with 3, 5 and 10 W from 0 to 20 min (Fig. 3). The curves shown in Fig. 3 exhibit the same profile and show a maximum around 40–46% of DFA yield. Fig. 3 also shows that the best results are obtained between 5 and 10 min. Since the parameters of time and power were linked, the determination of the values of these two factors was necessary. Under microwave activation, the conversion was high (around 80%) and very similar for the three powers: the DFA yields varied between 40% and 47%. The ratio of yield to conversion showed that the microwave, for these experimental conditions, led to the selective synthesis of DFAs. The selectivity was expressed by the specific conversion of fructose to DFAs, and was calculated by the quotient of the amount of DFAs to the amount of products formed. The selectivity increased in relation to time up to an optimum. At 10 W, the maximum selectivity was 53%, at 5 W, 58% and at 3 W, 63% (Table 5). The decrease in power therefore improved the conversion to DFAs. The reaction time to reach the optimum is a key factor because beyond this time, the DFAs are consumed.

For conventional heating, the conversion remained low and reached a maximum of 45% at 20 min (Table 4). The yields obtained, also shown in Fig. 3, were always lower than those obtained with microwave activation. For this type of heating, under the same conditions, it was previously shown that 120 min of reaction time was necessary to obtain similar yields (Idri et al., 2011; Suarez-Pereira et al., 2010).
The comparison of the results obtained at 10 W under Condition 1 and 2 (Figs. 2 and 3) showed that Condition 2 had more potential, as the conversion of fructose was higher, 75% at 5 min compared to 35% at 4 min, and the DFA yields were also higher, 40% at 5 min compared to 29.5% at 4 min. The beneficial effect of increasing the concentration of α-fructose is consistent with the bimolecular character of the reaction leading to DFA formation. Moreover, the use of higher proportions of the acid resin catalyst favours the homogeneous caramelization in the solution. High local concentrations would then translate not only into higher caramelization rates but would also favour the dimerization route leading to DFAs, limiting the nonspecific dehydration processes leading to volatiles and melanoidines. These conditions are therefore more efficient for the production of prebiotic caramels despite the rheological constraints. For these experiments, the viscosity was no longer a problem; this is the advantage of using microwave activation.

The most abundant DFAs present in caramel are the compounds 1, 5, 6, 9 and 10. Firstly, two caramels produced under Condition 2, conventional heating at 75 min (Caramel 1) and microwave activation at 7 min (Caramel 2) were compared. The results are reported in Fig. 4. The proportions of the majority DFAs were slightly different but the ratio of the majority DFAs to total DFAs was 75% for the first case and 67% for the second. The quality of caramels was therefore similar. Then the two most caramel-enriched DFAs produced with 10 W microwave activation, under Condition 1 after 4 min (Caramel 3) and under Condition 2 after 5 min (Caramel 4) were compared. The results are reported in Fig. 5. The proportions of DFAs were notably different. Under Condition 1, 83% of DFAs were the majority DFAs, whereas under Condition 2 only 67% of DFAs were majority DFAs. The minority DFAs were present in a greater amount in the second caramel.

**4. Conclusion**

The caramelization process was previously described in a batch reactor and proved to be difficult to implement because the mixture is a viscous paste. The synthesis of DFA-enriched caramels by microwave is a very promising alternative to the batch production method using conventional heating. It leads to significant yields of DFA (nearly 50%) in a reaction time that is 10 times shorter (5–10 min for microwave activation compared to 60–120 min for conventional heating (Idri et al., 2011)). This technique exhibits other advantages such as heating the mixture to the core, which can be an important asset given the nature of the medium. This technology makes it possible to work under difficult operating conditions (more solid catalyst, less water), conditions which raise problems under classical heating.

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