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Qualitative and quantitative evaluation of mono- and disaccharides in D-fructose, D-glucose and sucrose caramels by gas–liquid chromatography–mass spectrometry
Di-D-fructose dianhydrides as tracers of caramel authenticity

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Abstract

The monosaccharide (D-fructose, D-glucose, anhydrosugars), disaccharide (glucobioses) and pseudodisaccharide (di-D-fructose dianhydrides) content of D-fructose, D-glucose and sucrose caramels has been determined by gas–liquid chromatography–mass spectrometry (GLC–MS) of their trimethylsilyl (TMS) or TMS–oxime derivatives. The chromatographic profiles revealed significant differences in the disaccharide/pseudodisaccharide distribution depending on the caramel source: a D-fructose caramel contains prominent proportions of di-D-fructose dianhydrides, a D-glucose caramel mainly D-glucobioses, and a sucrose caramel similar proportions of both disaccharide/pseudodisaccharide series. It is noteworthy that di-D-fructose dianhydrides are found in all three types of caramels and might then be used as specific tracers of the authenticity of caramel, i.e., a product resulting from the controlled heat treatment of food-grade carbohydrates for use as food additives.

Keywords: Caramel; Food analysis; Carbohydrate; Difructose dianhydrides

1. Introduction

Caramelization commonly occurs when sugars are heated, either dry or in concentrated solution, either alone or with certain additives. Caramel has been used for millennia to impart color and flavor to food and beverages. Nowadays, caramel is designed as a food additive or ingredient and its manufacture for commercial use is an important technological process that takes place under rather strictly controlled conditions [1–3]. The caramel's origin, preparation parameters, permissible additives and final properties are laid down by the food laws of various countries or economic unions [4–8]. Among others, the French

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norm AFNOR distinguishes between two types of caramel: (i) aromatic caramel and (ii) caramel color, based essentially on their use, either as a flavor ingredient or a coloring additive, respectively [8]. Attempts to further standardize caramel based on its molecular composition have met with little success however [3], due to the limited knowledge in this field; although the volatile low-molecular-mass fraction is pretty well known [3,9–12], the non-volatile components, representing more that 95% (w/w), remain poorly characterized. Some recent findings, however, suggest that a particular family of non-reducing pseudodisaccharides, namely di-D-fructose dianhydrides[1] (DFAs), could be used as tracers of caramelization [13].

Difructose dianhydrides are the cyclic products of condensation of two D-fructose molecules with the loss of two molecules of water, which results in a 1,4-dioxane intersaccharide ring as the main structural feature. Formation of these intermolecular anhydrides upon acidic treatment of D-fructose and inulin has been known for some time [14–16] and more systematic studies have reported their formation in high yield when sucrose and positional isomers are treated with various acids [17–19]. The presence of DFAs in caramel was first suggested by Tschiersky and Baltes in 1989 [20], based on mass spectrometry evidence. Five years later, Defaye and García Fernández [21] isolated five isomeric DFAs from a commercial sucrose caramel and established their structure by comparison with authentic samples. Moreover, fast atom bombardment (FAB) mass spectrometry and ^{13}C NMR spectroscopy of a crude acetylated sample indicated that the non-volatile fraction of sucrose caramel consisted, mainly, of DFAs and poly(glycosyl)DFAs, together with a series of reducing oligosaccharides [22]. This general scheme was further confirmed by Manley-Harris and Richards [23], who identified up to thirteen DFAs in the product of thermal treatment of acidified sucrose and inulin by a combination of analytical and spectroscopic techniques. DFAs have been found in commercial chicory [22]. Interestingly, DFAs have recently been shown to exhibit favourable nutritional properties, promoting the growth of bifidobacteria [13,24–26] and a DFA-enriched sucrose caramel has found application as a feed supplement to enhance health and performance in fowl [27,28].

We now propose an analytical methodology for the separation of the monosaccharide, disaccharide and pseudodisaccharide (DFAs) components of caramel using gas–liquid chromatogrpahy–mass spectrometry (GLC–MS), based on derivatization into the corresponding per-*O*-trimethylsilyl (TMS) or per-*O*-trimethylsilyl oxime (TMS–oxime) derivatives. The reported procedure does not need preliminary fractionation of the caramel sample, is suitable for routine analysis and allows quantification and identification of the major caramelization products. A main target of these results is to use the disaccharide and pseudodisaccharide distribution, especially DFAs, as a tool to prove caramel identity and authenticity. Fructose, glucose and sucrose caramels have been included in our study since they exhibit the best organoleptic properties [3] and are the most frequently used as food ingredients and additives.

2. Experimental

2.1. Chemicals and reagents

1,6-Anhydro-β-D-glucopyranose (levoglucosan), D-fructose, D-glucose, D-mannose, sucrose, 4-*O*-α-D-glucopyranosyl–D-glucose (maltose), 4-*O*-β-D-glucopyranosyl–D-glucose (cellobiose), 6-*O*-β-D-glucopyranosyl–D-glucose (gentiobiose), 6-*O*-α-D-glucopyranosyl–D-glucose (isomaltose), 6-*O*-α-D-glucopyranosyl–D-fructofuranose (palatinose, isomaltulose), α-D-glucopyranosyl α-D-glucopyranoside (α,α-trehalose) and 3-*O*-α-D-glucopyranosyl–D-fructose (turanose) were purchased from Sigma–Aldrich (Steinheim, Germany). 1,6-Anhydro-β-D-glucofuranose was a gift from Prof. P. Köll (University of Oldenburg, Germany). α-D-Fructopyranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**5**), α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**9**), α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride (**10**) and di-β-D-fructopyranose 1,2′:2,1′-dianhydride (**14**) were synthesized by

[1]The nomenclature of this family of compounds has been the subject of frequent confusion over the years. For the present IUPAC–IUBMB recommendations, see A.D. MacNaught, Pure Appl. Chem. 52 (1997) 1919.

protonic activation of fructose or sucrose with hydrogen fluoride (HF) or the pyridinium poly(hydrogen fluoride) complex, following previously reported methods [16,29,30]. β-D-Fructofuranose α-D-glucopyranose 1,1′:2,2′-dianhydride and β-D-fructopyranose α-D-glucopyranose 1,1′:2,2′-dianhydride were prepared by protonic activation of 1-*O*-α-D-glucopyranosyl–D-fructose (trehalulose) [31,32]. The identity of the compounds was checked by melting and mixed melting point determination, FAB mass spectrometry and ^{1}H and ^{13}C NMR spectroscopy.

Phenyl β-D-glucopyranoside (internal GLC standard, I.S.), hydroxylamine hydrochloride, hexamethyldisilazane and trimethylchlorosilane were purchased from Sigma–Aldrich and were stored at room temperature.

2.2. Caramels

The fructose and glucose caramels were prepared in a pilot plant replica of the industrial induction electric oven used by Nigay for their batch process. For fructose caramel, a mixture of D-fructose (levulose; Roquette, F-62136 Lestrem, 5 kg), water (500 ml) and citric acid (50 g) was heated up to 150°C in 120 min. Additional water (1300 ml) was added at the end of the cooking process. For glucose caramel, D-glucose (dextrose monohydrate; Cerestar, F-59482 Haubourdin, 2.5 kg) in water (250 ml) was heated up to 170°C in 170 min. Additional water (900 ml) was finally added to cool down the caramel. The sucrose caramel was a commercial aromatic caramel produced by Nigay (Feurs, ref. Nigay 1395 SMA6) conforming with the AFNOR NF V 00-100 norm [8]. The physicochemical characteristics of these products are summarized in Table 1. For comparative purposes and in order to assess the utility of DFAs as specific tracers of caramel authenticity, a commercial topping product denominated 'sauce' caramel, obtained by the addition of a pigment to a mixture of sugars that had not been submitted to heat-induced caramelisation, was comparatively managed.

2.3. Derivatization of sugars in caramels as their TMS or TMS–oxime derivatives

For GLC–MS analysis, caramels samples, as well as the reference samples used for identification purposes, were converted into their corresponding per-*O*-trimethylsilyl (TMS; nonreducing sugars) or per-*O*-trimethylsilylated oxime (TMS–oximes; reducing sugars) derivatives. The method described by Sweeley et al. [33] was followed with minor modifications. A sugar or caramel was diluted in distilled water to a concentration of 16 mg ml^{-1}. To an aliquot (100 μl) of this solution in a small vial, a solution of phenyl β-D-glucopyranoside in acetone–water (1:9, v/v) (4 mg ml^{-1}, 100 μl) was added. The final solution was evaporated to dryness at 60°C (drying oven) for 1 h. The residue was then oximated by treatment with a solution of hydroxylamine in pyridine (20 mg ml^{-1}; 1 ml) at 60°C over 45 min, with mixing at intervals. The cooled sample was then trimethylsilylated by reaction with a mixture of hexamethyldisilazane (200 μl) and trimethylchlorosilane (100 μl) at 60°C for 30 min and transferred to chromatographic flasks.

2.4. Instrumentation

The GLC system consisted of an HP 6890 chromatograph equipped with an HP 5672 mass detector (Hewlett-Packard, Walbronn, Germany). Ionization was carried out by electron impact. For identification purposes, the mass spectrum of each

Table 1
Physico-chemical characteristics of caramels

	(Nigay ref. 1395 SMA6)			
	D-Fructose caramel	D-Glucose caramel	Sucrose caramel	'Sauce' caramel
Dry matter (° Brix)	79.2	81.7	79.2	81.4
pH (50% in demineralized water)	3.03	3.08	2.8	3.66
Color (absorbance at 520 nm)	2.32	8.18	6.04	7.65

peak was recorded in the total ion current mode (TIC) of the mass spectrometer, within a mass range of 50 to 700. For quantification of saccharides, the mass spectrometer was scanned in the selected ion monitoring (SIM) mode with monitoring of ions at m/z 147, 204 and 217, common to the mass spectra of TMS–oxime derivatives of reducing carbohydrates and of per-*O*-TMS derivatives of DFAs.

The capillary column used was an HP5MS (5% phenyl-methylsiloxane; 30 m×0.25 mm I.D.) with a 0.25-μm film thickness (Hewlett-Packard). Operating conditions were: injection port temperature, 310°C; interface temperature, 280°C; column oven temperature, programmed from 180 to 310°C at 5°C min^{-1} with a 25-min hold at 310°C; carrier gas helium (constant flow at 1.2 ml min^{-1}); splitting ratio, 1:50 and injection volume, 1 μl setting on an autosampler.

3. Results and discussion

3.1. Qualitative and quantitative analysis of sugars in caramels

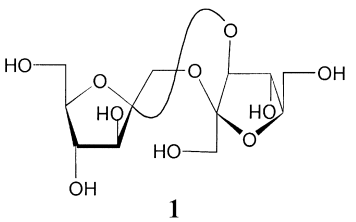
For identification and quantification of both reducing and non-reducing sugars in caramels, an analytical GLC procedure was employed that involves successive derivatizations into the corresponding oximes and per-*O*-TMS oxime derivatives. Reducing saccharides, such as D-fructose or D-glucose, provide up to six peaks corresponding to per-*O*-TMS derivatives upon direct silylation, i.e. the cyclic α,β pyranose/furanose forms and the acyclic hydrate/carbonyl forms [34]. After sequential oximation and silylation reactions, each reducing sugar gives rise only to two peaks, corresponding to the per-*O*-TMS syn- and anti-oximes, respectively [35]. In contrast, nonreducing sugars such as sucrose or DFAs (structures are shown in Fig. 1) result only in single peaks in both cases, corresponding to the per-*O*-TMS derivatives. Comparison of the GLC chromatographic profiles, using the above two derivatization protocols, allows rapid discrimination between peaks arising from reducing or nonreducing carbohydrates. Identification of individual carbohydrates was carried out by comparison of the retention times and the mass spectra of the chromatographic peaks with those of authentic samples.

The respective monosaccharide (D-fructose, D-glucose, 1,6-anhydro-β-D-glucopyranose and 1,6-anhydro-β-D-glucofuranose), DFA (based on the two major components, **9** and **10**) and glucobiose (based on gentiobiose and isomaltose) content of caramels was quantified from the corresponding peak area relative to that obtained for the internal standard (phenyl β-D-glucopyranoside), using linear equations obtained after a calibration process. For this purpose, standard solutions of pure samples of each of the above sugars, in variable concentrations, were run three times. The average peak areas were calculated and plotted, and linear regression analysis was performed (correlation coefficients>0.990). The quantitative results obtained for the fructose, glucose and sucrose caramels by replicate injection of the derivatized (oximation–silylation) sample are given in Table 2.

3.2. Fructose caramel

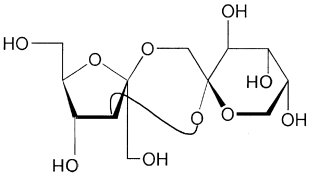
The GLC profile of the TMS–oxime derivatives of the sugars formed by caramelisation of D-fructose is shown in Fig. 2. It illustrates the presence of residual D-fructose and 13 peaks corresponding to dimeric products. The mass spectra of each of these peaks on the TIC chromatogram display, on the one hand, ions at m/z 73, 147, 204 and 217, which are found in the spectra of most trimethylsilylated glycosides [36] and, on the other hand, ions at m/z 362 and 509, previously reported as being characteristic of per-*O*-TMS DFAs [37]. Actually, the chromatographic profile was similar to that recently reported by Manley-Harris and Richards [23] for the product of the thermal treatment of acidified inulin. Direct comparison of both chromatograms and inspection of the corresponding mass spectra allowed the formal identification of peaks 1–4, 6, 7 and 11–13. Peaks 5 and 6 were not separated in the chromatogram shown in the above reference, and DAF **14** had not been identified previously in caramel. Structures **5**, **9**, **10** and **14** have been ascribed to the corresponding peaks by comparison of retention times and mass spectra with those of authentic samples obtained by chemical synthesis.

In order to further corroborate the DFA nature of



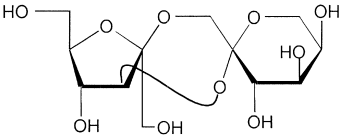
1

α-D-Fru*f*-1,2':2,3'-β-D-Fru*f*



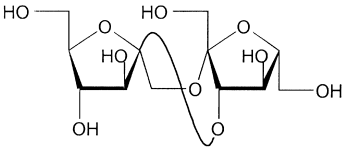
2

β-D-Fru*f*-2,1':3,2'-α-D-Fru*p*



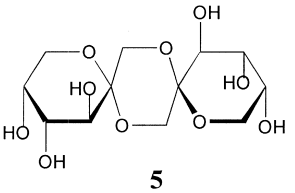
3

β-D-Fru*f*-2,1':3,2'-β-D-Fru*p*



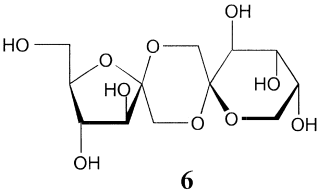
4

β-D-Fru*f*-1,2':2,3'-β-D-Fru*f*



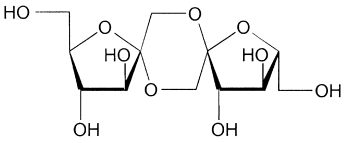
5

α-D-Fru*p*-1,2':2,1'-β-D-Fru*p*



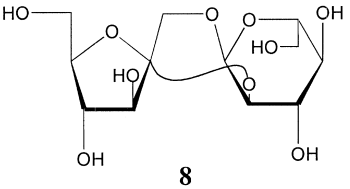
6

β-D-Fru*f*-1,2':2,1'-α-D-Fru*p*



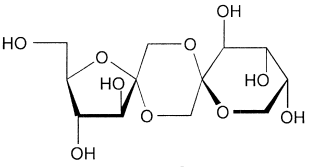
7

α-D-Fru*f*- 1,2':2,1'-α-D-Fru*f*



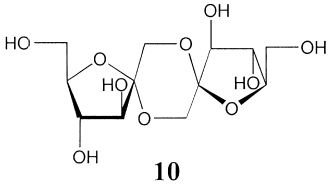
8

α-D-Fru*f*-1,1':2,2'-α-D-Glc*p*



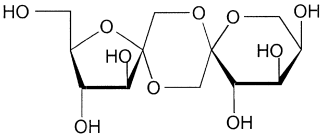
9

α-D-Fru*f*-1,2':2,1'-β-D-Fru*p*



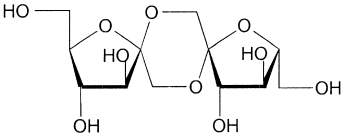
10

α-D-Fru*f*-1,2':2,1'-β-D-Fru*f*



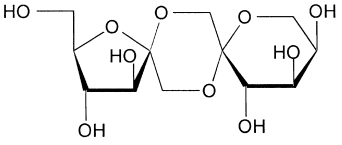
11

α-D-Fru-1,2':2,1'-α-D-Fru*p*



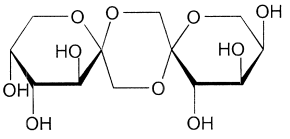
12

β-D-Fru*f*-1,2':2,1'-β-D-Fru*f*



13

β-D-Fru*f*-1,2':2,1'-β-D-Fru*p*



14

β-D-Fru*p*-1,2':2,1'-β-D-Fru*p*

Fig. 1. Structural drawings for difructose dianhydrides found in fructose, glucose and sucrose caramels. Formula no. corresponds to peak no. in GLC profiles and is assigned according to its elution sequence.

Table 2
Sugar concentration (% of dry matter) in D-fructose, D-glucose and sucrose caramels calculated by internal standardization of TMS–oxime derivatives after GLC–MS separation on an HP5MS capillary column[a]

Sugar	D-Fructose caramel			D-Glucose caramel			Sucrose caramel		
	m[b] (%)	SD	RSD (%)	m[b] (%)	SD	RSD (%)	m[a] (%)	SD	RSD[c](%)
1,6-Anydro-β-D-glucopyranose				1.55	0.05	3.13	0.3	0.01	3.29
1,6-Anhydro-β-D-glucofuranose				1.62	0.02	1.51	0.31	0.007	2.4
D-Fructose	21.98	0.37	1.66	0.55	0.02	3.72	14.66	0.24	1.65
D-Glucose				17	0.21	1.23	27.82	0.35	1.27
DFA 9	17.42	1.13	6.48	0.14	0.02	14.05	4.14	0.41	9.97
DFA 10	21.72	0.92	4.22	0.22	0.03	15.1	5.22	0.4	7.6
Gentiobiose				5.32	0.43	8.01	1.15	0.17	14.99
Isomaltose				7.04	0.43	6.13	1.21	0.15	12.24

[a] Internal standard (I.S.), phenyl β-D-glucopyranoside.
[b] Concentration (% on dry matter), mean of five replicate injections.

the di- and pseudodisaccharides peaks, a sample of this fructose caramel was acetylated by treatment with 1:1 acetic anhydride–pyridine and the peracetylated product was subjected to column chromatography on silica gel using 1:1→3:1 ethyl acetate–light petroleum ether, following the separation protocol recently applied by Defaye and García Fernández [21,22] in the analysis of a sucrose caramel. The fraction containing the per-*O*-acetylated DFAs was deacetylated using the Zemplén

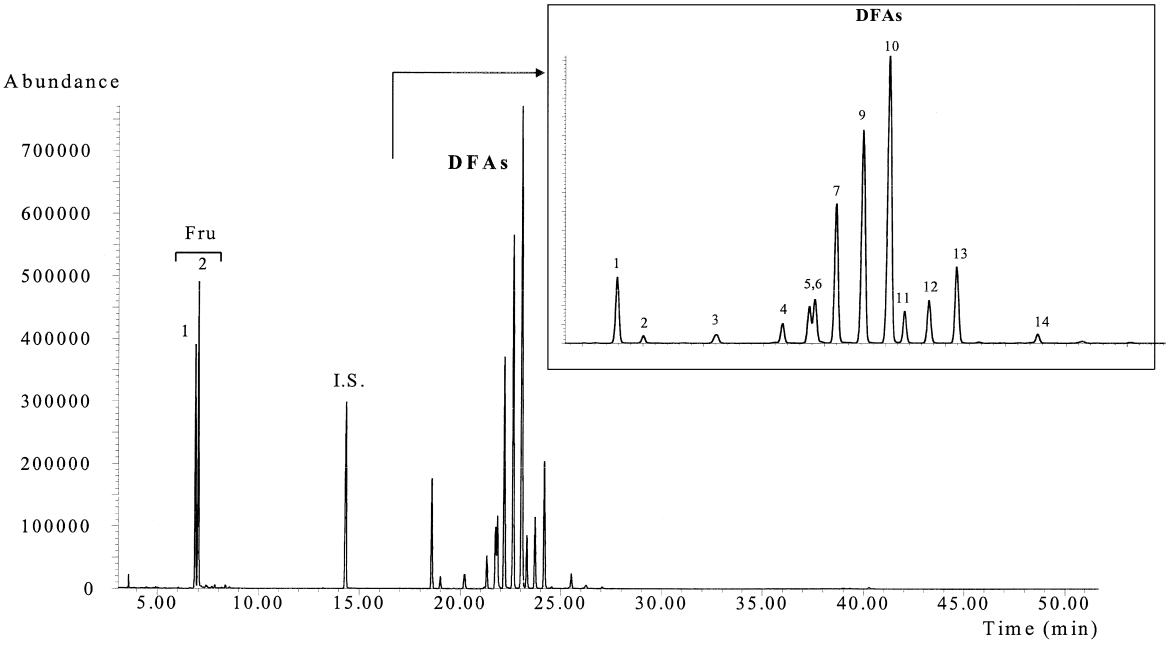


Fig. 2. GLC profile for a D-fructose caramel upon successive oximation and trimethylsilylation with, inset, the expanded region for di-D-fructose dianhydrides; I.S., internal standard; Fru 1,2: D-fructose; DFA numbering refers to Fig. 1. Chromatographic conditions are the same as given in Experimental.

technique (methanolic sodium methoxide, 0.1 equiv., 2 h) and the composition of the mixture was unequivocally established by FAB–MS and ^{13}C NMR spectroscopy (data not shown). Comparison of the relative proportions of compounds **1**–**7** and **9**–**14**, as obtained by integration of the ^{13}C anomeric signals (C-2 and C-2′) using an antigate pulse sequence, and from the GLC chromatogram of the same fraction after silylation, agreed with the above assignment.

The dimeric (pseudodisaccharide) fraction of fructose caramel consists, therefore, of a mixture of DFAs **1**–**7** and **9**–**14**, which are fully separated by GLC from their per-*O*-TMS derivatives under the conditions stated under Experimental. Compounds **9** and **10**, known to be the major kinetic products of D-fructose dimerization under protonic activation conditions [16,29], accounted for about 40% of this caramel on a dry basis. In addition, the FAB mass spectrum exhibited pseudomolecular peaks for pseudotrisaccharides and pseudotetrasaccharides, which might result from the incorporation of one or two fructosyl residues, respectively, to a DFA core. Identification and setting up of an appropriate analytical method for these higher-molecular-mass components of fructose caramel is currently under development in our laboratories.

3.3. Glucose caramel

The analysis of the glucose caramel after the oximation–trimethylsilylation reaction sequence by GLC–MS showed the presence of 1,6-anhydro-β-D-glucopyranose (levoglucosan) and 1,6-anhydro-β-D-glucofuranose (Fig. 3). These non-reducing monosaccharides result evidently from intramolecular dehydration reactions of D-glucose. In addition to the expected peaks for residual D-glucose, the chromatogram displayed peaks for the TMS–oxime derivatives of D-fructose and D-mannose. Formation of these reducing monosaccharides can be explained by

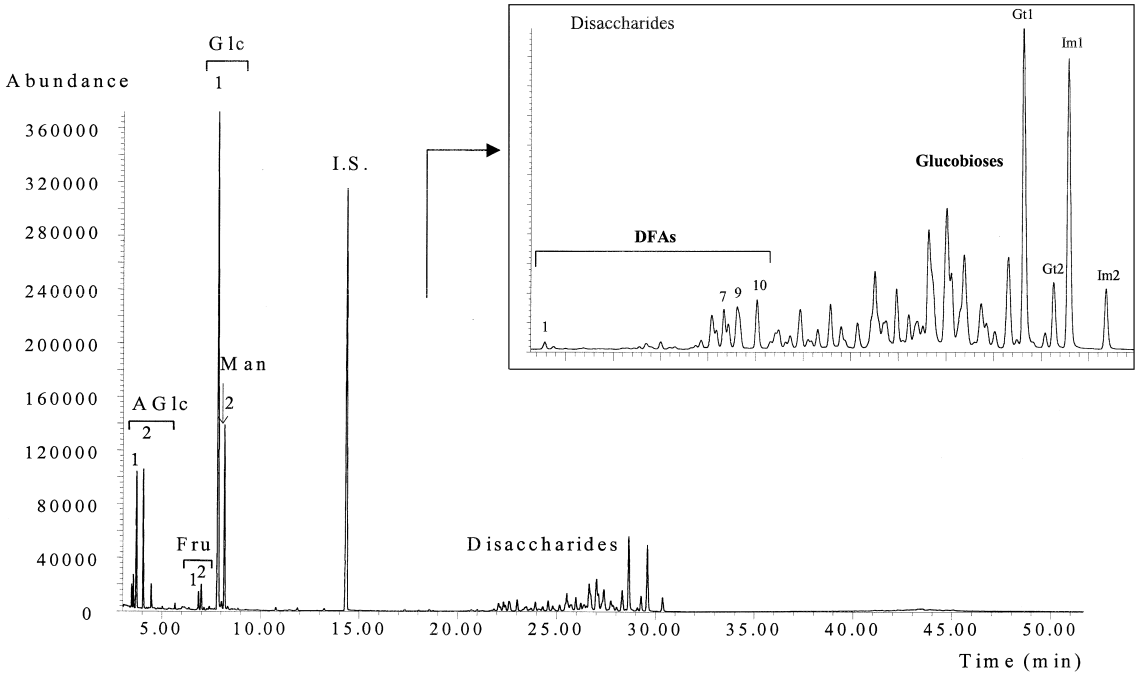


Fig. 3. GLC profile for a D-glucose caramel upon successive oximation and trimethylsilylation with, inset, the expanded region for pseudodisaccharides and disaccharides. I.S.: internal standard; AGlc 1: 1,6-anhydro-β-D-glucopyranose; AGlc 2: 1,6-anhydro-β-D-glucofuranose; Fru 1,2: D-fructose; Glc 1,2: D-glucose; Man: D-mannose; Gt 1,2: gentiobiose; Im 1,2: isomaltose; DFAs numbering refers to Fig. 1. Non-labelled peaks correspond to unidentified components. Chromatographic conditions are given in Experimental.

assuming a Lobry de Bruyn–Alberda van Ekenstein enolization process for D-glucose [38], resulting either in isomerization to the corresponding 2-ketose or in C-2 epimerization, respectively. Although this enolization reaction is expected to be favored in the presence of alkali, it is also known to occur upon acid catalysis and is presumably promoted by the inherent acidity of caramel.

The dimeric region of the glucose caramel chromatogram showed a much more complex pattern compared to that of the fructose caramel. The majority of the peaks corresponded to reducing disaccharides that arose from acid-catalysed autoglycosylation (reversion) reactions of D-glucose molecules. Although the whole set of glucodisaccharides listed under Chemicals and reagents was tried, the heavy overlapping of peaks precluded a conclusive identification except for the (1→6)-linked glucobioses, i.e. gentiobiose and isomaltose. These two disaccharides represented more than 12% of the glucose caramel and were the major components of the dimer fraction. Their formation must be favoured by the higher accessibility of the primary hydroxyl group at C-6 in glycosylation reactions of D-glucose compared to the secondary positions C-1–C-4.

It is noteworthy that significant proportions of DFAs **1**, **7**, **9** and **10** were also found in glucose caramel, probably originating from the acid-catalysed dimerization of D-fructose molecules that formed in the early stages of the caramelization reaction. The present results suggest that this might be a major carbohydrate transformation route under caramelization conditions, even in the case of aldoses.

3.4. Sucrose caramel

Nigay Caramel ref. 1395 SMA6 was chosen as a standard sucrose aromatic caramel. Defaye and García Fernández [21] have previously reported that this commercial caramel contains about 18% of DFAs, from which, compounds **4**, **5**, **7**, **9** and **10** were isolated after acetylation and fractionation on a silica gel column. The GLC–MS analysis of this caramel after derivatization as above showed the presence of 1,6-anhydro-β-D-glucofuranose and 1,6-anhydro-β-D-glucopyranose, D-fructose and D-glucose in the monosaccharide region (Fig. 4). The disaccharide and pseudodisaccharide domain of the chromatogram displayed two well-defined regions corresponding to the per-*O*-TMS derivatives of DFAs and to the per-*O*-TMS oxime derivatives of reducing disaccharides, respectively. Peaks for DFAs **1**–**7** and **9**–**13** as well as for gentiobiose and isomaltose were identified, the structural assignment being confirmed as described for the fructose and glucose caramels. The presence of DFA **14** could not be conclusively established nor discarded since the corresponding peak overlapped with the lower retention time peaks of reducing disaccharides.

Comparison of the chromatographic profiles for fructose, glucose and sucrose caramel strongly supports the view that the composition of the disaccharide fraction of the latter can be seen, essentially, as the addition of the caramelization products of their monosaccharide constituents, that is, cleavage of the anomeric linkage probably precedes sucrose caramelization. The D-fructose subunit is then highly prone to form intermolecular cyclic acetals whereas the D-glucose moiety preferentially undergoes classical glycosylation (reversion) reactions. Furthermore, the simultaneous presence of both monosaccharides in the caramelization batch is expected to result in crossed-hetero-oligomers. Thus, the reported FAB mass spectrum of the acetylated sucrose caramel [22] exhibited prominent pseudomolecular peaks for glycosyl-DFAs up to DP (degree of polymerization) 8, together with peaks for reducing oligosaccharides. In the dimer region, this crossed-reactivity is reflected by the presence of a new peak, noted 8, which was absent in the chromatograms of both fructose and glucose caramels. The non-reducing α-D-fructofuranose α-D-glucopyranose 1,1′:2,2′-dianhydride (structure **8**) for this peak was assigned by comparison of the retention time and mass spectrum with data for the product recently isolated by Manley-Harris and Richards [23]. It must, however, be pointed out that, under these chromatographic conditions, the per-*O*-TMS derivative of sucrose results in a signal that is superimposed with that for peak 8. In the particular case of the Nigay caramel used in our study, the content of sucrose has been determined separately after acetylation and separation by silica gel column chromatography and found to be not higher than 0.6%. A GLC analysis of the sucrose-free DFA fraction confirmed the pres-

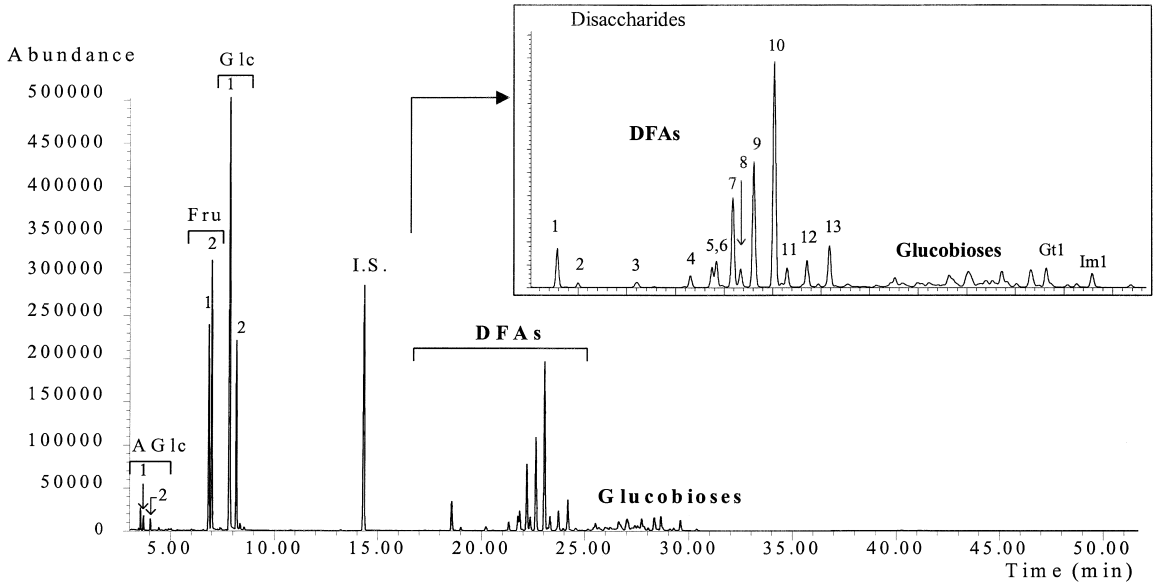


Fig. 4. GLC profile for Nigay 1395 SMA6 sucrose caramel upon successive oximation and trimethylsilylation with, inset, the expanded region for pseudodisaccharides and disaccharides. I.S.: internal standard; AGlc 1: 1,6-anhydro-β-D-glucopyranose; AGlc 2: 1,6-anhydro-β-D-glucofuranose; Fru 1,2: D-fructose; Glc 1,2: D-glucose; Gt 1: gentiobiose; Im 1: isomaltose; DFAs numbering refers to Fig. 1. Non-labelled peaks correspond to unidentified components. For chromatographic conditions, see Experimental.

ence of **8** in the sucrose caramel. Its formation seems to be highly specific, since the isomeric mixed glucose–fructose dianhydrides β-D-fructofuranose α-D-glucopyranose 1,1′:2,2′-dianhydride and β-D-fructopyranose α-D-glucopyranose 1,1′:2,2′-dianhydride, available by synthesis [32], were not formed under these conditions. Reducing glucosylfructoses, such as palatinose and turanose, were also absent from the caramelization product.

3.5. Determination of caramel origin and authenticity by GLC–MS

The comparative GLC–MS analysis of fructose, glucose and sucrose caramels provides evidence that the disaccharide region of the chromatographic profiles provides valuable information about the source used for the manufacture of the respective caramel. Interestingly, the formation of DFAs and reversion compounds under weakly acidic conditions, due either to the presence of added acid promoters or to the inherent acidity developed upon caramelization, requires heat treatment of the carbohydrate material. Therefore, identification of these components in a given caramel might be used simultaneously as a mean to demonstrate its authenticity and as a proof of identity. Di-D-fructose dianhydrides are particularly promising tracers towards this aim, since these pseudodisaccharides are present in all three types of caramels studied and, furthermore, are, at least presently, not commercially available.

The French norm AFNOR NF V00-100 [8] defines aromatic caramel as ‘a pale to brown syrup or solid exclusively obtained upon controlled heat treatment of food-grade carbohydrates and intended for use in food flavoring. Small amounts of food carboxylic acid may be added in order to promote hydrolysis of the saccharide. The inherent acidity of caramel may be neutralised after the caramelization process’. Despite this restrictive definition, however, some commercial products are marketed with the label ‘caramel’ even though they are only syrups containing various sugar components to which color and flavor have been added, without involvement of further heat treatment. We have performed a qualitative chromatographic analysis of such a product (Fig. 5, lower profile) and compared the results with those obtained for original sucrose caramel Nigay

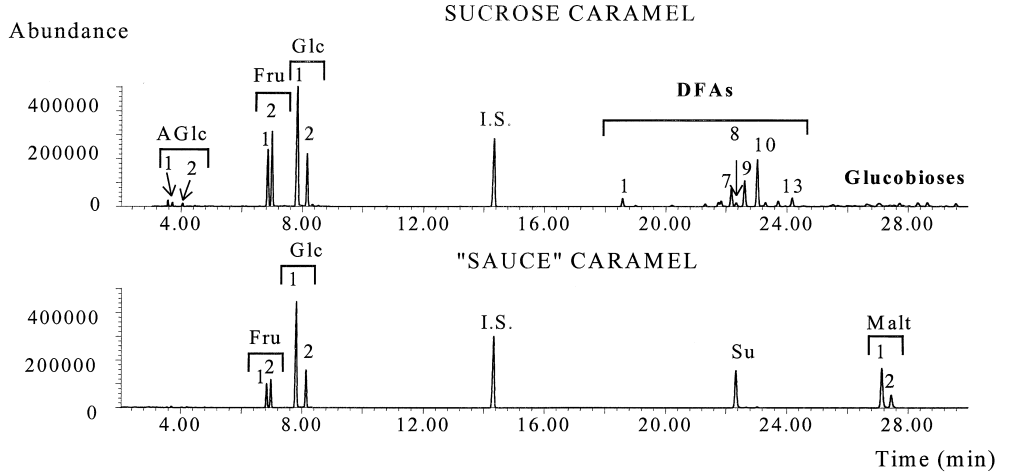


Fig. 5. Comparative GLC profile for commercial Nigay 1395 SMA6 sucrose caramel and a commercial fake 'sauce' caramel. I.S.: internal standard; AGlc 1: 1,6-anhydro-β-D-glucopyranose; AGlc 2: 1,6-anhydro-β-D-glucofuranose; Fru 1,2: D-fructose; Glc 1,2: D-glucose; Su: sucrose; Malt 1,2: maltose; DFA numbering refers to Fig. 1. Non-labelled peaks correspond to unidentified components. For chromatographic conditions, see Experimental.

ref. 1395 SMA6 (Fig. 5, upper profile). Comparison of the chromatographic profiles clearly confirms that the fake 'sauce' caramel contained D-fructose, D-glucose, sucrose and maltose. The numerous peaks corresponding to disaccharides and pseudodisaccharides (glucobioses and DFAs, respectively) seen in the SMA6 sucrose caramel were absent from the 'sauce' caramel. This confirms that the product was not subjected to heat treatment and, therefore, does not conform to the present regulations. At this time, where authenticity of food products is becoming increasingly important, DFAs appear to be suitable tracers of caramelization. Taking into consideration the promising nutritional properties of DFAs, the GLC–MS analytical method now described might be, additionally, a useful tool for quality control in the industrial manufacture of caramels, particularly for those to be enriched in DFAs.

4. Conclusions

Sequential oximation–trimethylsilylation followed by GLC–MS analysis of the resulting oximes and per-*O*-TMS oxime derivatives allows the separation and identification of the monosaccharide, di- and pseudodisaccharide components in various caramels. The distribution of the dimers formed upon heat-induced caramelization has been shown to depend on the carbohydrate source: a fructose caramel contains high proportions of difructose dianhydrides, a glucose caramel mainly glucobioses and a sucrose caramel both types of compounds in similar proportions. In all caramels studied, DFAs were found as constituents, whereas they were absent in the case of a 'fake' caramel. Our results support the proposal for the use of this analytical protocol to prove the identity and authenticity of caramels.

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References

[1] R. Truhaut, G. Vitte, V. Lassalle-Saint Jean, Bull. Soc. Pharm. Bordeaux 101 (1962) 97.
[2] V. Lassalle-Saint Jean, Rev. Port. Quim. 99 (1968) 77.
[3] P. Tomasik, M. Palasinski, S. Wiejak, Adv. Carbohydr. Chem. Biochem. 47 (1989) 203.
[4] Nutr. Meet. Rep. Ser., No. 57, FAO, Geneva, 1977
[5] WHO Tech. Rep. Ser., No. 617, World Health Organization, Geneva, 1977.
[6] International Technical Caramel Association (ITCA)–European Technical Caramel Association (EUTCA) Specifications for Caramel Color, Meet. Sub. Comm. Specifications 3rd., 4th May 1979, Paris.
[7] Directive no. 95/45/CE of the European Parliament and Council: July 26, 1995; J. Off. Commun. Eur., no. L226, 1 July 1995.
[8] Standard AFNOR NF V00-100, Association Française de Normalisation, Paris, 1988.
[9] L. Cottier, G. Descotes, C. Neyret, H. Nigay, Ind. Aliment. Agric. 106 (1989) 567.
[10] A.L. Patey, G. Shearer, M.E. Knowles, H.B. Denne, Food Addit. Contam. 2 (1985) 237.
[11] H. Binder, C. Wentzel, H. Junk, M. Mittelbach, Z. Lebensm. Unters. Forsch. 184 (1987) 187.
[12] F. Collino, S. Volpe, Biol. Chim. Farm. 121 (1982) 375.
[13] M. Manley-Harris, G.N. Richards, Adv. Carbohydr. Chem. Biochem. 52 (1997) 207.
[14] E.J. McDonald, Adv. Carbohydr. Chem. 2 (1946) 253.
[15] H.W. Hilton, Methods Carbohydr. Chem. 2 (1963) 199.
[16] J. Defaye, A. Gadelle, C. Pedersen, Carbohydr. Res. 136 (1985) 53.
[17] J. Defaye, J.M. García Fernández, Carbohydr. Res. 237 (1992) 223.
[18] J. Defaye, J.M. García Fernández, Carbohydr. Res. 251 (1994) 1.
[19] J. Defaye, J.M. García Fernández, Carbohydr. Res. 251 (1994) 17.
[20] H. Tschiersky, W. Baltes, Z. Lebensm. Unters. Forsch. 189 (1989) 132.
[21] J. Defaye, J.M. García Fernández, Carbohydr. Res. 256 (1994) C1.
[22] J. Defaye, J.M. García Fernández, Zuckerindustrie (Berlin) 120 (1995) 700.
[23] M. Manley-Harris, G.N. Richards, Carbohydr. Res. 287 (1996) 183.
[24] S. Kobayashi, K. Seki, K. Haraguchi, M. Kashimoto, K. Nagata, K. Honbo, K. Kainuma, M. Kadoma, JP 63,269,962 (1988); Chem. Abstr. 111 (1989) 56162g.
[25] J. Kashimura, T. Fujisawa, Y. Nakajima, K. Nishio, T. Mitsuoka, Nippon Eiyo Shokuryo Gakkaishi 44 (1991) 54; Chem. Abstr. 115 (1991) 25751b.
[26] M. Tanaka, Y. Nakajima, K. Nishio, H. Hashimoto, J. Carbohydr. Chem. 12 (1993) 49.
[27] M. Manley-Harris, G.N. Richards, Zuckerindustrie (Berlin) 129 (1994) 924.
[28] J.A. Patterson, J.I. Orban, A.L. Sutton, G.N. Richards, Poultry Sci. 76 (1997) 497.
[29] J.M. García Fernández, J. Defaye, A. Gadelle, Carbohydr. Res. 265 (1994) 249.
[30] J.M. García Fernández, R.-R. Schnelle, J. Defaye, Tetrahedron: Asymmetry 6 (1995) 307.
[31] J.M. García Fernández, J. Defaye, Tetrahedron Lett. 33 (1992) 7861.
[32] J.M. García Fernández, C. Ortiz Mellet, J. Defaye, J. Org. Chem. 63 (1998) 3572.
[33] C.C. Sweeley, R. Bentley, M. Makita, W.W. Wells, J. Am. Chem. Soc. 85 (1963) 2497.
[34] H.C. Curtius, M. Müller, J.A. Völlmin, J. Chromatogr. 37 (1968) 216.
[35] G. Petersson, Carbohydr. Res. 33 (1974) 47.
[36] D.C. De Jongh, T. Radford, J.D. Hribar, S. Hanessian, M. Bieber, G. Dawson, C.C. Sweeley, J. Am. Chem. Soc. 91 (1969) 1728.
[37] M. Manley-Harris, G.N. Richards, Carbohydr. Res. 226 (1992) 327.
[38] J.C. Speck Jr., Adv. Carbohydr. Chem. 13 (1958) 63.