**DIFRUCTOSE ANHYDRIDES AS QUALITY MARKERS OF HONEY AND
COFFEE.**

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ABSTRACT

Difructose anhydrides (DFAs) are pseudodisaccharides produced by condensation of two fructose molecules by means of caramelization reaction which takes place during heating of sugars or sugar-rich foodstuffs. The aim of this research was to evaluate the feasibility of DFAs as chemical markers of honey authenticity and sugar-roasted torrefacto coffee. DFAs were analysed by gas chromatography coupled to mass spectrometry after conversion to their trimethylsilyl (TMS) derivatives. α -D-fructofuranoside-1,2':2,1'- α -D-fructofuranoside (DFA7) and α -D-fructofuranoside-1,2':2,1'- β -D-fructopyranoside (DFA9) can be used as quality markers of honey and coffee. DFA7 and DFA9 were detected in honey added with 5% fructose and sucrose caramels and 15% of glucose caramels. Torrefacto coffees showed DFAs values ranged from 0.195 to 0.570 g/100g whereas only traces were found in natural roasted coffees. Quantities from 0.073 to 0.189 g/100g were measured in blends of natural and torrefacto roasted coffees. A relationship between DFAs content in torrefacto coffees and roasting conditions was observed. In conclusion, this study indicated that DFAs are useful chemical indicators to control honey authenticity and torrefacto coffee roasting.

KEYWORDS: Difructose anhydrides, caramelization, honey, coffee, authenticity, torrefacto.

INTRODUCTION

In recent times, food quality assurance is getting a great importance for consumers, producers and regulatory authorities. Food quality requires the control of nutritional value, sensorial properties, authenticity and safety. Various chemical compounds can be used as quality markers. By definition, good chemical indicators of quality may be considered those natural food components which concentrations are modified during processing such as proteins, fats, or aminoacids; those formed during processing and storage, or those incorporated to foods as contaminants and adulterants (Lee and King, 1996). Carbohydrate breakdown products formed by isomerization, Maillard and caramelization reactions have been widely employed as very good markers of quality in a great number of processed foods (Corzo, Delgado, Troyano, Olano, 1994; Sanz, del Castillo, Corzo, Olano, 2000).

Difructose anhydrides (DFAs) are pseudodisaccharides formed during caramelization reaction consisting of two fructose residues and possessing physiological functions (Manley-Harris & Richards, 1997; Saito, Tomita, 2000). This reaction takes place during heating of sugars or food products rich in sugars, and results in a minor volatile fraction and a major proportion of non-volatile components (90-95 %) including DFAs. The relative proportion of DFAs depends on food composition and processing (Defaye, Fernandez, Ratsimba, 2000). DFAs analysis in caramels was first described by Tschiersky and Baltes (1989) and more studies have been conducted to give insight to their chemical structure (Defaye & García-Fernández, 1994; Defaye & García-Fernández, 1995). Methodologies have been settled for analysis of DFAs as suitable tracers of caramelization in food and food additives as caramels, chicory and dehydrated fruits (Defaye et al., 2000). DFA content in caramels prepared from fructose, glucose and sucrose may be determined by gas chromatography-mass spectrometry (GC-MS) of

75 their trimethylsilyl (TMS) derivatives (Ratsimba, Fernandez, Defaye, Nigay, Voilley,
76 1999).

77 Honey is a natural food elaborated by honeybees either from nectar of blossoms
78 (nectar honey) or from secretions of living parts of plants or excretions of plant sucking
79 insects on the living part of plants (honeydew honey). Honeydew honeys usually have
80 darker colours than nectar honeys and in many areas are more valued than these ones
81 (Prodoliet & Hischenhuber, 1998). There are evidences of honey frauds by caramels
82 addition to nectar honeys to enhance their colour. Detection of honey adulteration is not
83 always easy and represents a problem to solve. DFA analysis by GC may provide an
84 inexpensive easy adequate approach to be employed for the routine quality control of
85 this food.

86 For coffee companies is very important to check the roasting degree of coffee
87 beans because it strongly affects taste, acidity, body, aroma and flavour. Coffee beans
88 may be roasted following two main ways known as "conventional or natural" without
89 sugar addition and "torrefacto" when sugar is added. The roasting degree in each
90 country is determined by preferences of consumers. In general, North European
91 countries mainly consume light-roasted, American medium-roasted and South European
92 countries dark-roasted coffees; some South American countries, Portugal and Spain
93 mainly consume blends of natural and torrefacto coffees (Maetzu, Andueza, Ibáñez, de
94 Peña, Bello, Cid, 2001). Natural and torrefacto roasted coffee present differences in the
95 volatile fraction perceived by sensorial analysis in the intensities of the nutty, roasty,
96 earthy, burnt and caramel notes (Sanz, Meatzu, Zapelena, Bello, Cid, 2002). Torrefacto
97 roasting also contributes to the brownish colour of the coffee brew (Maetzu et al.,
98 2001).

Very few references related to quality control of torrefacto coffees are available. As it is known, Arabica coffees are more valued by consumers than Robusta coffees because their sensorial properties. Previous studies have observed that torrefacto roasting mask the poor sensorial properties of Robusta coffee (Maetzu et al., 2001) and could be used as a fraud. The influence of torrefacto roasting on the volatile fraction of coffee and its sensorial properties has been described (Sanz et al., 2002); however, studies related to the non-volatile compounds formed by caramelization in this type of coffee have not been conducted yet. Therefore, since caramelization takes place during torrefacto roasting, DFAs may be formed and provide useful information related to the degree of torrefacto roasting and the composition of blends.

In our knowledge, DFAs have not been previously determined either in honey or in torrefacto coffee. Consequently, the aim of this research was to find out the feasibility of DFAs as chemical indicators of honey authenticity and control torrefacto roasting.

MATERIALS AND METHODS

Reagents

Glucose, fructose, sucrose and phenyl- β -glucoside were acquired from Sigma (St. Louis, USA). High-purity water was produced in-house using a Milli-Q Synthesis A10 system (Millipore, Bellerica, Mass., USA) and was used throughout.

Samples

Honey samples (n=20): 16 nectar honeys from different botanical origins (citrus, rosemary, heather, acacia, eucalyptus and multifloral) and 4 honeydew honeys were purchased to beekeepers and local markets.

Coffee samples (n=17): 6 natural (N), 4 torrefacto (T) and 7 coffee blends including 20N/80T (n=1), 30N/70T (n=1) and 50N/50T (n=5), from industrial brands were purchased at local markets.

In order to evaluate the influence of roasting on DFAs formation, Arabica coffee beans were roasted under controlled conditions by a coffee company (Café La Mexicana, Rodríguez y Mateus, S.A Madrid) to obtain 3 natural and 2 torrefacto coffees. Natural roasting was carried out at temperatures ranged from 150°C to 198°C for 15 min. Torrefacto coffees were obtained adding sugar to Arabica beans during roasting at 203-214°C for 18 min. In addition, coffee blends (20N/80T (n=1), 30N/70T (n=1) and 50N/50T (n=1)) were produced in the laboratory from commercial coffees having known DFA contents. A comparative analysis of DFAs values obtained for commercial and produced in-house blends was carried out.

Sample preparation

Acid caramels:

10g of glucose, fructose and sucrose were separately mixed with 0.1g of citric acid and 1mL of water and heated at 160°C for 150 minutes, 140°C for 35 min and 140°C for 25 min, respectively. 3mL of additional water were added to each sample at the end of the cooking process and heated for two minutes at the same temperatures.

Honey

Acacia honey was added with 5, 10 and 15% of caramel. Caramels and honey samples (0.5 g) were diluted to 25mL with 70% methanol, prior to DFAs analysis.

Coffee extracts

Hot water (75°C, 60 mL) was added to ground coffee (10g), stirred for 5 min and filtered through Whatman no.4 filter paper. 3mL of filtrates were deproteinated and defatted by stirring with 25mL of methanol followed by standing for at least 1h. The clear supernatants obtained were employed for DFAs analysis.

Analytical methods

All analyses were carried out in duplicate.

Gas chromatography analysis

Methanol solutions of caramels (0.5mL), honey (0.5mL) and coffees (2mL) were derivatised, prior to GC analysis. 0.5 mL of 1 mg/mL phenyl- β -D-glucoside, internal standard, was added to the samples. Afterwards, methanol was evaporated under vacuum at 38-40 °C. 2.5% Hydroxylamine chloride in pyridine (350 μ L) was added to the dry samples and kept at 75°C for 30 min. The oximes obtained in this step were silylated with hexamethyldisilazane (350 μ L) and trifluoroacetic acid (35 μ L) and kept at 45°C for 30 min (Brobs and Lott, 1966). The mixtures of reaction were centrifuged at 7000g at 5°C for 5 min (Li and Schumman, 1981; Baños, Olano, Corzo, 2000). The supernatants containing TMS-DFAs were either directly injected or stored at 4°C until analysis.

GC was performed with a Hewlett-Packard gas chromatograph (HP6890) equipped with a flame ionisation detector (FID). A 25m x 0.25 mm x 0.25 μ m fused silica column coated with CP-SIL 5CB (methyl silicone from Chrompack, Middelburg, The Netherlands) was used. The carrier gas (nitrogen) flow rate was 1.2 mL/min. Injector and detector temperatures were 300°C. Oven temperature was programmed as

follows: ramp from 180°C to 200°C at 5°C/min, hold for 10 min, ramp to 270°C at 5°C/min, ramp to 282°C at 1°C/min, ramp to 290 at 15°C/min and hold for 25 min. Samples were injected in split mode (split flow 48mL/min). An HPChemStation acquisition system (Hewlett-Packard, Palo Alto, CA, USA) was employed.

DFAs concentrations were directly estimated from the areas of peaks and internal standard. Results were expressed as g of DFAs/100 g of product.

Mass spectrometry analysis

A HP-5890 chromatograph coupled to a MD 5971 quadrupole mass detector (Hewlett-Packard, Palo Alto, CA, USA) was employed. DFAs separation was performed under the same chromatographic conditions described above. Helium was used as carrier gas. Samples were injected in split mode (40 mL/min). Mass spectrometer was operated in EI mode at 70 eV. Mass spectra were acquired using a HP-G1034C MS ChemStation software (Hewlett-Packard, Palo Alto, CA, USA).

Browning development

Brown pigments were measured in caramel (100mg/mL), honey (100 mg/mL) and coffee (10mg/mL) samples according to Meydav, Saguy & Kopelman, (1977) by measuring of the absorbance values at 420 nm. This measurement is employed as convenient index of the development of caramelization and Maillard reactions.

RESULTS AND DISCUSSION

Caramels

Glucose, fructose and sucrose caramels were analysed by GC-MS. Chromatographic profiles were similar to those reported by Manley-Harris and Richards

(1996) and Ratsimba et al. (1999). Peaks were numbered according to the last authors. Mass spectrometric analysis confirmed those peaks eluting from 26 to 31min corresponded to DFAs, excepting that with retention time of 30.1 min which was assigned as a mixture of DFA10 and sucrose. Thirteen DFA peaks were detected in fructose and sucrose caramels and only five peaks in glucose caramel. DFAs with retention times of 26 min (DFA1), 29.5min (DFA 7), 29.9 min (DFA 9) and 30.1 min (DFA 10) were detected in all caramels. Therefore, only DFA 7 and DFA 9, eluting without chromatographic interferences and in relatively high amount, were employed for quantitative purposes. DFAs 7 and 9 were previously assigned as α -D-fructofuranoside-1,2':2,1'- α -D-fructofuranoside and α -D-fructofuranoside-1,2':2,1'- β -D-fructopyranoside by Ratsimba et al. (1999). Figure 1 shows their structures and table 1 summarizes the main ions obtained by MS for both compounds in caramels. As occur in disaccharides, the same fragments are found in all the series, and compounds have to be distinguished on the basis of retention time and abundance ratios. Fragments at low m/z values (less than 190) have in general few diagnostic values. A big fragment at 217 (TMSO-CH=CH-CH=OTMS including carbons 1-2-3) indicates two furanose rings, whereas similar abundance of 217 and 204 ions (TMSO-CH=CH-OTMS including carbons 2 and 3) reveals the presence of both furanose and pyranose rings. DFAs spectra are clearly different from those of disaccharides which present a higher 361 ion ($C_{13}H_{33}O_4Si_3$). Results agreed with those published by Manley Harris and Richards (1992, 1997).

Table 2 shows DFAs content of caramels. Glucose caramel showed the lowest amounts of these compounds (0.15g DFA7/100g caramel and 0.09g DFA9/100g caramel) and fructose caramel the greatest values (3.93g DFA7/100g caramel and 9.49g DFA9/100g caramel) as previously reported by Ratsimba et al. (1999) for DFA 9.

Honey adulteration

Figure 2 presents the chromatographic profiles of an acacia genuine honey and its adulteration with glucose, fructose and sucrose caramels. DFA7 and DFA9 contents of the samples under study are shown in table 2. DFAs were not detected in the genuine honey sample and in the sample adulterated with 5% glucose caramel. However, DFAs could be measured in 10% glucose caramel additions. Adulterations of 5% fructose and sucrose caramels were easily detected as DFAs. Contents of these compounds progressively increased with the higher addition of acid caramels.

Moreover, absorbance values at 420 nm of all studied samples were also evaluated. A slight increase of absorbance as a function of caramel addition was observed in honey sample ($A_{420\text{nm}}$ 0.10). Values of absorbance of 0.19, 0.25 and 0.37 were obtained adding 15% of glucose, sucrose and fructose caramels, respectively.

Nineteen quality guaranteed honeys of different botanical origins were also analysed to determine if presence of DFAs could be observed in these samples. Absence of these compounds was found in all the analyzed honeys. Therefore, these compounds could be suggested as chemical markers of adulteration of honeys with acid caramels.

Coffee quality

Figure 3 shows the GC chromatograms acquired for natural, torrefacto and blended (20N/80T) coffees. DFA values obtained for the studied coffee samples are given in Table 3. Very small quantities of DFAs were found in conventionally roasted coffees (0.010 - 0.016g DFA7/100g and 0.000 – 0.006g DFA9/100g), intermediate in coffee blends (0.047 – 0.137g DFA7 /100g and 0.024 – 0.053g DFA9/100g) and the greatest values in torrefacto coffees (0.114 - 0.403g DFA7/100g and 0.081 – 0.167g

DFA9/100g). No linear relationship between torrefacto content and DFAs values were observed for commercial coffee blends, probably due to different manufacture practices.

In general, data obtained for the samples produced under controlled conditions agreed with that obtained for commercial samples. All natural conventionally roasted coffees under study (6 purchased at the local markets and 3 produced by the coffee company under controlled conditions) showed similar DFAs contents. Different DFAs values were detected in torrefacto coffees purchased at local market and those roasted at 204-213°C for 18 min. Differences in quantity of sugar added to coffee beans during roasting and the employed roasting conditions in each case may explain these results.

As can be observed by analysis of absorbance (Table 3), the greatest production of brown pigments took place under torrefacto conditions; intermediate values were obtained for coffee blends and lower for natural roast coffees. Blends data indicated a good relationship between percentage of torrefacto and colour. In general, absorbance data agreed with DFAs values; however, absorbance values obtained for natural and blended coffees (20T/80N) are quite similar. Caramelization of sugar contributes to the brownish colour of the torrefacto coffee brews (Maeztu et al., 2001); however sugar caramelization is not the unique responsible for that. The influence of the conventional roasting on the melanoidin formation (brown pigments) has been previously studied and demonstrated (Ottinger and Hoffman, 2001). Therefore, coffee colour is not a good parameter to discriminate coffee blends (20T/80N) from natural roasted coffees. DFAs may be employed as an adequate indicator of coffee quality in order to avoid frauds or torrefacto addition to mask poor beans quality and to determine the degree of torrefacto roasting.

REFERENCES

1. Baños, J.L.G., Olano, A., & Corzo, N. (2000) Determination of mono and disaccharide content of enteral formulations by gas chromatography. *Chromatographia* 52 (3-4), 221-224.
2. Brobst, K.M., & Lott, C.E. (1966) Determination of some components in corn syrup by gas-liquid chromatography of trimethylsilyl derivatives. *Cereal Chemistry* 43, 35-43.
3. Corzo, N., Delgado, T., Troyano E. & Olano, A. (1994) Ratio of lactulose to furosine as indicator of quality of commercial milks *Journal of Food Protection* 57 (8), 737-739.
4. Defaye, J., & García Fernández, J.M. (1994) Protonic and thermal-activation of sucrose and the oligosaccharide composition of caramel *Carbohydrate Research*, 256 (2) C1-C4.
5. Defaye, J., & García Fernández, J.M. (1995) The oligosaccharide components of caramel. *Zuckerindustrie* 120 (8), 700-704.
6. Defaye, J., García Fernández, J.M., & Ratsimba, V. (2000) Les molecules de la caramelization: structure et méthodologies de détection et d'évaluation L'actualité chimique. Novembre 24-27.
7. Lee, T.C. & King, H.J. (1996) Preface. In: Chemical markers for processed and stored foods. Lee T.C., Kim, H.J. (eds) pp. ix-xi.
8. Li, B.W., & P.J. Schumann. (1981) Gas chromatographic analysis of sugars in granola cereals *Journal of Food Science* 46: 425-427.
9. Maetzu, L., Andueza, S., Ibáñez, C., de Peña, M. P., Bello, J. & Cid, C. (2001) Multivariate methods for characterization and classification of espresso coffees

- from different botanical varieties and types of roars by foam, taste, and mouthfeel
Journal of Agricultural and Food Chemistry 49: 4743-4747.
10. Manley-Harris, M., & Richards, G.N. (1992) Mass spectra of some di-D-fructose
dianhydride derivatives *Carbohydrate Research* 226: 327-330.
11. Manley-Harris, M., & Richards, G.N. (1996) Di-D-fructose dianhydride and
related oligosmers from thermal treatments of inulin and sucrose. *Carbohydrate
Research* 287: 183-202.
12. Manley-Harris, M. & Richards, G.N (1997) Dihexulose dianhydrides. *Advances in
Carbohydrate Chemistry and Biochemistry* 52, 207-266
13. Meydav, S., Saguy, I. and Kopelman, I.J. (1977) Browning determination in citrus
products. *Journal of Agricultural and Food Chemistry* 25: 602-604.
14. Prodolliet, J., & Hischenhuber, C. (1998) Food authentication by carbohydrate
chromatography *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung A*.
207, 1-12.
15. Ottinger, H. & Hoffman, T. (2001). Influence of roasting on the melanoidin
spectrum in coffee beans and instant coffee. *Melanoidins in Food and Health*.
Volume 2. Office for Publications of the European Communities, Luxembourg,
pp119-125.
16. Ratsimba, V., García Fernández, J.M., Defaye, J., Nigey, H., & Voilley A. (1999)
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 anhydrides as tracers of caramel authenticity. *Journal
of Chromatography A* 844, 283-293.

17. Saito, K., & Tomita, F. (2000) Difructose anhydrides: their mass-production and physiological functions. *Bioscience Biotechnology and Biochemistry* 64 (7), 1321-1327.
18. Sanz, M.L., del Castillo, M.D., Corzo, N. & Olano, A. (2000) Presence of 2-Furoylmethyl Derivatives in Hydrolysates of Processed Tomato Products *Journal of the Agriculture and Food Chemistry* 48, 468-471.
19. Sanz, C., Maeztu, L., Zapelena, M.J., Bello, J., & Cid, C. (2002) Profiles of volatile compounds and sensory analysis of three blends of coffee: influence of different proportions of Arabica and Robusta and influence of roasting coffee with sugar *Journal of the Science of Food and Agriculture* 8, 840-847.
20. Tschiersky, H., & Baltes, W. (1989) Investigations of caramel - curiepoint pyrolysis of caramel syrups and other investigations of structure. *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung* 189, 132 .

332 Table 1. Characteristic ion pattern of trimethylsilylated DFA7 and DFA9.

m/z	Abundance (%)	
	DFA 7	DFA9
73	91.0	100.0
75	73.7	27.9
103	19.4	15.9
129	14.5	16.5
147	21.8	33.0
169	8.1	5.9
191	2.6	2.9
204	3.0	58.2
217	100.0	66.7
218	17.7	13.8
230	5.7	3.9
361	0.1	0.2
362	0.3	0.6
509	0.5	0.6
510	0.3	0.3

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Table 2. DFA7 and DFA9 content (n=2) in caramels and honeys (g/100 g product).

Sample	DFA7	DFA9
Glucose caramel (GAC)	0.15 (0.03)*	0.09 (0.01)
Fructose caramel (FAC)	3.93 (0.23)	9.49 (0.74)
Sucrose caramel (SAC)	1.68 (0.11)	2.77 (0.19)
Genuine honey	0.00	0.00
Honey +5% GAC	0.00	0.00
Honey + 10% GAC	0.01 (0.00)	0.01 (0.00)
Honey + 15% GAC	0.02 (0.00)	0.02 (0.00)
Honey +5% FAC	0.19 (0.08)	0.48 (0.10)
Honey + 10% FAC	0.36 (0.01)	1.00 (0.00)
Honey + 15% FAC	0.60 (0.03)	1.55 (0.04)
Honey +5% SAC	0.08 (0.01)	0.14 (0.01)
Honey + 10% SAC	0.15 (0.02)	0.28 (0.02)
Honey + 15% SAC	0.24 (0.03)	0.41 (0.02)

* Standard deviation in brackets

Table 3. DFAs content expressed as g/100g product and absorbance measure at 420nm of roasted coffees.

Samples	DFA7	DFA9	Absorbance 420nm
Commercial samples			
Natural roast (n=6)	0.013 (0.002)*	0.000	1.17 (0.22)
Torrefacto roast (n=4)	0.236 (0.010)	0.108 (0.026)	2.57 (0.42)
Blends			
20/80 (n=1)	0.062 (0.010)	0.024 (0.001)	1.49 (0.01)
30/70 (n=1)	0.047 (0.004)	0.026 (0.007)	1.63 (0.07)
50/50 (n=5)	0.088 (0.032)	0.037 (0.010)	1.83 (0.04)
Samples produced under known conditions			
Natural roast (n=3)	0.014 (0.010)	0.05 (0.000)	0.83 (0.03)
Torrefacto roast (n=2)	0.376 (0.038)	0.154 (0.018)	2.54 (0.10)
Blends			
20/80 (n=1)	0.071 (0.020)	0.029 (0.004)	1.27 (0.02)
30/70 (n=1)	0.113 (0.010)	0.048 (0.010)	1.35 (0.01)
50/50 (n=1)	0.168 (0.010)	0.074 (0.000)	1.43 (0.02)

* Standard deviation in brackets

345 Figure 1.- Structural drawings of DFA 7 and DFA 9.

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DFA 7 (α -D-fructofuranoside-1,2':2,1'- α -D-fructofuranoside)

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DFA 9 (α -D-fructofuranoside-1,2':2,1'- β -D-fructopyranoside)

349 Figure 2.- Gas chromatographic profiles of genuine honey (A) and honey adulterated
 350 with 5% fructose (B), 10% glucose (C) and 5% sucrose (D) caramels. (1) phenyl β - D
 351 glucoside (internal standard), (2) DFA7 (3) DFA9, (4) sucrose, (5) DFA10.

353 Figure 3.- Gas chromatographic profiles of natural roasted (A) and torrefacto roasted
 354 coffees (B) and 20 natural : 80 torrefacto coffee blend (C). (1) phenyl β - D glucoside
 355 (internal standard), (2) DFA7 (3) DFA9, (4) sucrose, (5) DFA10.

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