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Food Chemistry 78 (2002) 157–161

Food Chemistry

www.elsevier.com/locate/foodchem

Evolution of fructose and glucose in honey over one year: influence of induced granulation

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Received 7 August 2001; accepted 26 November 2001

Abstract

Evolution of fructose and glucose over 1 year has been evaluated in 30 honey samples from Burgos (N. Spain). The influence of the induced granulation process in this evolution was also determined. Each sample was divided into two aliquots of 500 g and aseptically bottled. One aliquot was directly stored and the second induced to crystallise by seeding with 10% of finely crystallised honey. Analyses of moisture content, pH, fructose and glucose were carried out over 1 year, once each 4 months. Both, fructose and glucose increased in most samples. Induced-crystallised samples did not show any significant differences in the evolution of the two sugars in comparsion with directly stored samples. Linear correlations were found, for both fructose and glucose, between samples directly stored and honeys in which granulation was induced. These results are clearly different from those reported in previous papers where decrease of monosaccharides below their original values was described. pH of honey might promote reversion of monosaccharides and the formation of disaccharides and trisaccharides. This investigation has demonstrated the possibility of formation of monosaccharides, by the hydrolysis of higher sugars, as a process predominant over the reversion. No statistical relationship was found between pH of honey samples and their fructose and glucose content evolution.

Keywords: Honey; Fructose; Glucose; Evolution; Induced granulation

1. Introduction

More than 95% of the solids of floral and honeydew honeys are carbohydrate in nature, largely simple sugars or monosaccharides, fructose and glucose being the major constituents (White, 1992; chap 21). In nearly all honey types, fructose predominates and only a few honeys, such as rape (*Brassica napus*), dandelion (*Taraxacum officinale*) and blue curls (*Trichostema lanceolatum*) appear to contain more glucose than fructose. These two sugars together account for 85–95% of honey carbohydrates (White, 1979).

The sugars of honey are responsible for many of the physicochemical properties of honey, such as viscosity, hygroscopicity and granulation.

As most honeys are supersaturated solutions of glucose, this sugar trends to crystallise spontaneously at room temperature in the form of glucose monohydrate. Crystallisation of honey, commonly called granulation, is an undesirable process in liquid honey because it affects the textural properties, making it less appealing to the consumer, and, in many cases, it results in increased moisture of the liquid phase which can allow naturally occurring yeast cells to multiply, which causes fermentation of the honey (Donner, 1977). The rate at which this process occurs depends on several composition parameters (glucose, fructose, moisture and water activity) as well as the processing and handling methods. To solve those problems, fine seed crystals can be introduced into liquid honey, acting as nuclei for growth under a process of controlled crystallisation. This process, known as induced granulation, is normally carried out by seeding the liquid honey with 10% of

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0308-8146/02/$ - see front matter
PII: S0308-8146(01)00393-4

finely crystallised honey, which shows crystallisation compatibility when moisture is less than 18.5% and ratio glucose/water higher than 1.80 (Gonnet, 1992).

The purpose of this study was to determine evolution of fructose and glucose in honey over one year, its normal commercialisation period, and the influence of the induced granulation on this evolution. pH, moisture and botanical origin influences are also studied.

2. Material and methods

2.1. Samples

Thirty unheated samples of honey were collected from Burgos, a Spanish Province with a typical continental climate. The botanical origin of the samples was determined according to the Louveaux et al. (1978) procedure, after treating and dying the honey sediment by the Terradillos, Muniategui, Sancho, Huidobro, and Simal-Lozano (1994) method. Seventy-one taxa were found. Thirteen samples were unifloral. Eight samples were heather honeys (Ericaceae). Four samples were thyme honeys (*Thymus* L. sp.). One sample was sunflower honey (*Helianthus annus* L.). Seventeen samples were polyfloral honeys, being the secondary pollen types (16–45%), Ericaceae, Leguminosae Type *Trifolium* L. sp,. Rosaceae Type *Rosa* L. sp., *Rubus* L. sp., Compositae Type *Helianthus annus* L., *Morus* L. sp., Cistaceae Type *Cistus ladanifer* L., Cruciferae Type *Sinapis arvensis* L., Leguminosae Type *Genista* sp. and Leguminosae Type *Onobrychis* sp., according to Louveaux et al. (1978) and Sáinz-Laín and Gómez-Ferreras (2000).

Each sample (1 kg) was divided into two aliquots of 500 g and aseptically bottled. One of the aliquots was labelled as "A" and was directly stored. In the second one, labelled as "B", crystallisation was induced by seeding with 10% of finely crystallised honey, which showed crystallisation compatibility on the basis of moisture less than 18.5% and ratio glucose/water higher than 1.80 (Gonnet, 1992). Both honeys were mixed avoiding air bubbles, which could spoil the *newly crystallised* honey. Complete and homogeneous granulation was reached between 3 and 20 days from the seeding. The texture observed was very fine-grained in all samples. Samples were kept in darkness and stored at room temperature.

The analyses were carried out four times over 1 year, i.e. at 0, 4, 8 and 12 months. The first 4 months after harvesting were necessary for collecting all the samples and selecting the samples for inducing the crystallisation. Moisture and sugars in honeys used for inducing crystallisation were previously analysed.

2.2. Methods

Moisture was determined by measuring refractive index at 20 °C with an ATAGO 3T refractometer coupled to an ultra thermostatic bath (Grant W28) according to the AOAC (2000) method. (Method 925.45).

pH was determined by using a pH meter Crison micropH 2001 with an electrode Crison ref. 104053931, according to the AOAC (2000) method for acidity of honey. (Method 962.19).

Fructose and glucose were determined by the Boehringer-Mannheim (1995) enzymatic test Cat No. 139106 (Huidobro & Simal, 1984) with a Kontron 930 uvikon double beam spectrophotometer.

3. Results and discussion

Most of the "A" samples (25 of 30) crystallised before the end of the study. The result of this process was a coarse, granulated product with a lower commercial value than the samples where crystallisation was induced.

Table 1 shows the moisture of the samples and its evolution over the year. The initial moisture mean value for "A" samples was 16.9%, ranging from 15.0 to 18.7% in all samples but two. These two samples showed values of 21%, which indicated their immaturity at the harvesting time. Mean moisture was 0.9% lower in "B" samples than in "A" samples. This decrease is higher than that expected according to the moisture contents in the samples and in the honey used for inducing granulation in "B" samples (as the latter had lower moisture contents, the mixture explains a decrease of 0–2%). The difference implies that induced granulation caused important changes of the water retention in honeys. In both samples "A" and "B", moisture was constant over the year.

Table 2 shows pH of the samples and its evolution over the year. Its mean value was 4.0 for all analyses but

Table 1
Moisture (%)of samples and its evolution over 1 year

No. Honey	M_{A1}	M_{A2}	M_{A3}	M_{B1}	M_{B2}	M_{B3}
X	16.9	16.9	16.9	16.0	16.0	16.0
Sn-1	1.5418	1.5401	1.5993	1.4618	1.4972	1.4867
Min.	15.0	15.1	15.2	14.2	14.1	14.1
Max.	21.6	21.6	21.9	20.6	20.7	20.6

Table 2
pH of samples and its evolution over 1 year

No. Honey	pH_{A1}	pH_{A2}	pH_{A3}	pH_{B1}	pH_{B2}	pH_{B3}
X	3.9	4.0	4.0	4.0	4.0	4.0
Sn-1	0.2587	0.2694	0.2735	0.2714	0.2547	0.2175
Min.	3.6	3.7	3.5	3.6	3.6	3.7
Max.	4.6	4.7	4.7	4.7	4.6	4.6

in "A" samples, where the mean value was 3.9, it ranged from 3.5 to 4.7 and in "B" samples from 3.6 to 4.7.

Table 3 shows the fructose content of the samples and its evolution over the year. Fructose contents increased twice in 8 "A" samples. In seven samples, fructose contents decreased. The rest of the samples showed constant values or slight differences in fructose contents, below the CV% of the method (1%). A similar evolution was found in "B" samples, with a clear increase in the fructose content in 15 samples and a decrease in only one sample.

In all the analyses, a linear correlation between the fructose content of honeys was found both in directly stored honeys ("A") and honeys in which granulation was induced ("B"). For the third analysis the equation obtained was:

$$y = 0.573x + 16.60$$

where: y = fructose contents of "A" samples; x = fructose contents of "B" samples; $r = 0.7569$ and $P < 0.0001$.

"A" samples can be classified into seven groups according to their fructose content evolution. In 14 samples, fructose content remained constant in both analyses. Eight samples increased their fructose content between the first and second analyses and remained constant between the second and third analyses. Three samples increased their fructose content between the first and the second analysis and decreased it between the second and third analysis. In two samples, fructose content was constant between the first and the second analysis and decreased between the second and third analysis. The other three samples all showed, an initial decrease between the first and second analysis and then the first one showed a decrease, the second one an increase and the last one a constant value between the second and third analysis.

Trends of evolution in the fructose content of "B" samples were similar to those observed for "A" samples. "B" samples can be classified into six groups according to their fructose content evolution. In 10 samples, fructose content remained constant in both analyses. Seven samples increased their fructose contents between the first and the second analysis and remained constant between the second and third analysis. Six samples increased their fructose contents between the first and the second analysis and decreased it between the second and third analysis. In three samples, fructose content was constant between the first and the second analysis and increased between the second and third analysis. Three samples showed an initial decrease between the first and second analysis and then an increase between the second and third analysis. Finally one sample showed a continuous increase.

During evolution of fructose in the studied honey samples, the slope of the evolution curves, obtained adjusting the data from the three analyses by linear regression, is noteworthy.

"A" samples (36.7%) (numbers 1, 3, 4, 5, 7, 8, 18, 19, 25, 28 and 29) and 27.7% of "B" samples (1, 5, 7, 8, 18, 19, 20 and 21) show a negative slope for fructose evolution. Sample 1A is represented in Fig. 1 as a representative example for this group of honeys. The rest of the samples show a positive slope for fructose

Table 3
Fructose (%) content of samples and its evolution over 1 year

No. Honey	F_{A1}	F_{A2}	F_{A3}	F_{B1}	F_{B2}	F_{B3}
X	37.7	38.2	37.7	37.7	38.1	38.2
Sn-1	1.5117	1.1407	1.4433	1.3169	1.4276	0.9747
Min.	34.7	35.8	34.2	35.1	34.7	35.3
Max.	42.6	41.0	39.9	42.0	40.6	40.1

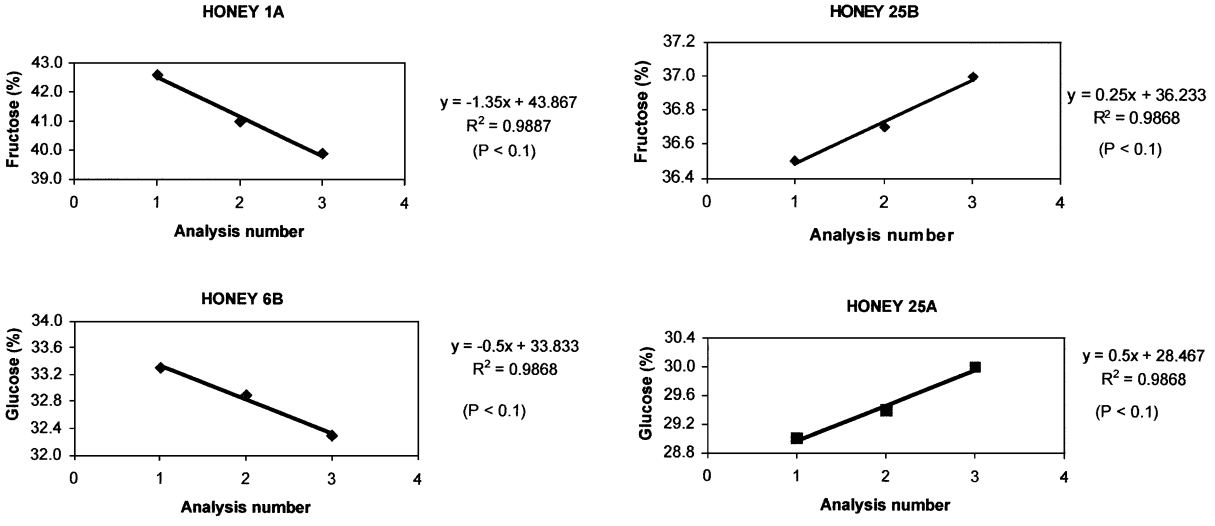

Fig. 1. Representative samples for fructose and glucose evolution.

Table 4
Glucose (%) content of samples and its evolution over 1 year

No. Honey	G_{A1}	G_{A2}	G_{A3}	G_{B1}	G_{B2}	G_{B3}
X	31.0	31.6	31.8	31.1	31.8	31.8
Sn-1.	2.2199	2.0403	2.0911	1.9944	1.9328	1.8227
Min.	26.9	26.6	26.3	27.7	28.7	28.2
Max.	34.8	34.9	34.7	34.8	35.9	35.5

evolution. As representative of this group, sample 25B has been selected (Fig. 1).

Table 4 shows the glucose content of the samples and its evolution over the year. Glucose contents increased twice in 21 "A" samples and decreased only in 6 samples. The rest of the samples showed constant values or slight differences in glucose contents, below the CV% of the method (0.72%). "B" samples showed a similar evolution. A clear increase was observed in 22 samples and only two samples showed a decrease in the glucose content.

Glucose content evolution followed a linear correlation between the directly stored honeys ("A") and honeys in which induced granulation was applied ("B") in all three analyses. For the third analysis the equation obtained was:

$$y = 0.850x + 4.97$$

where: y = glucose contents of "A" samples; x = glucose contents of "B" samples; $r = 0.9050$ and $P < 0.0001$.

According to their glucose content evolution, "A" samples can be classified into eight groups. Eleven samples increased glucose contents between the first and the second analysis and remained constant between the second and third analysis. In five samples glucose content remained constant in both analyses. Four samples showed a continuous increase in this parameter. Three samples decreased their glucose contents between the first and the second analysis and it remained constant between the second and third analysis. In three samples, glucose content was constant between the first and second analysis and increased between the second and third analysis. Two samples showed an initial increase between the first and second analysis and then a decrease between the second and third analysis. One sample showed a continuous decrease and another showed a constant glucose content between the first and second analysis and then a decrease between the second and third analysis.

"B" samples can be classified into six groups according to their glucose content evolution. Ten samples showed an initial increase between the first and second analysis and then a decrease between the second and third analysis. In six samples, this parameter increased between the first and the second analysis and remained constant between the second and third analysis. In four samples, glucose content remained constant in all analyses. Four samples showed an initial decrease followed by an increase. In three samples, glucose content increased continuously. Finally, glucose content was constant between the first and second analysis and decreased between the second and third analysis, in three samples.

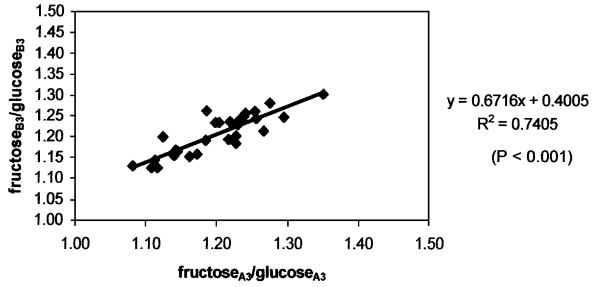

Fig. 2. Correlation in the third analysis between the ratio fructose/glucose for "A" samples and the relation fructose/glucose for "B" samples.

In glucose evolution curves, obtained adjusting the data of the three analyses by linear regression in each sample, only 26.7% of "A" samples (numbers 1, 2, 4, 5, 6, 13, 19 and 21) and 13.3% of "B" samples (numbers 2, 4, 5, and 6) showed a negative slope. As representative of this group, sample 6B is shown in Fig. 1. The rest of the samples showed a positive slope for glucose evolution. As representative of this group sample 25A is shown in Fig. 1.

Finally, the ratio fructose/glucose showed a linear correlation for both "A" and "B" samples in all analyses. Fig. 2 shows the correlation, in the third analysis, between the ratio fructose/glucose for "A" samples that are represented on the x axis and the ratio of fructose/glucose for "B" samples that are represented on the y axis.

4. Conclusions

Induced granulation caused important changes of water retention in honeys, as mean moisture was significantly lower in "B" samples than in "A" samples.

pH was constant over the year in both "A" and "B" samples. No differences in this parameter, were observed between "A" and "B" samples.

Induced-crystallised samples showed no significant differences, in the evolution of both sugars, from directly stored samples.

No statistical relationship was observed between moisture and sugar evolution in samples.

No statistical relationship was found between the pH values and the fructose and glucose evolution.

No statistical correlations between botanical origin of samples and fructose and glucose evolution were found.

Increases in fructose and glucose contents were observed in most samples. These results are clearly different from those reported in previous papers (Donner,

1977; Jimenez, Mateo, Huerta, & Mateo, 1994), where decreases of monosaccharides below their original values have been described. These authors suggested that the acidic pH of honey could promote reversion of monosaccharides and the formation of disaccharides and trisaccharides. Our investigation has demonstrated that formation of monosaccharides by the hydrolysis of higher sugars predominantes over reversion.

Acknowledgements

Thanks are due to the "Comisión Interministerial de Ciencia y Tecnología" for the Project ALI96-1108. To all the beekeepers that have provided a sample for this study.

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