Original article

Evolution of monosaccharides of honey over 3 years: influence of induced granulation

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Summary Evolution of fructose and glucose over 30 months was researched in sixty fresh and unheated samples of honey collected in areas with typical Continental and Oceanic climates. Samples were stored at room temperature and analysed every 5 months. Influence of induced granulation process was also studied. Evolution patterns of both sugars were similar for all samples. We observed that both monosaccharides decreased with time being the evolution highly dependent on the initial pH of the samples. The lower the initial pH, the greater the decrease of monosaccharides. Induced granulation showed no influence on the evolution of fructose and glucose.

Keywords Evolution, fructose, glucose, honey, induced granulation.

Introduction

More than 95% of the solids of floral and honeydew honeys are carbohydrate in nature, largely simple sugars or monosaccharides, being fructose and glucose the major constituents. The sugars of honey are responsible for many of the physical and chemical properties of honey such as viscosity, hygroscopicity and granulation. 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 have been widely analysed (White *et al*., 1961; Donner, 1977; Huidobro & Simal, 1984; Pérez-Arquillué *et al*., 1990; Martínez-Gómez *et al*., 1993; Hisil & Bagdatlioglu, 1994; Jiménez *et al*., 1994; Bogdanov *et al*., 1997; Cavia *et al*., 2002; Serrano *et al*., 2004; Kenjeric *et al*., 2006; Ruoff *et al*., 2007; among others), most of the times in respect of the characterisation of this food commodity, using several chemometric methods (Tzouros & Arvanitoyannis, 2001; Serrano *et al*., 2004; Arvanitoyannis *et al*., 2005).

Because of the fact that most honeys are supersaturated solutions in glucose, this sugar usually crystallises 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 negatively affects the textural properties and allows the fermentation (Donner, 1977). The rate at which granulation occurs mainly depends on such parameters as glucose, fructose, moisture and water activity, as well as the processing and handling method. To solve those problems fine seed crystals can be introduced in liquid honey acting as nuclei for growth under a process of controlled crystallisation (Gonnet, 1992).

The purpose of this study has been to research the evolution patterns of honey's main monosaccharides over 3 years, studying the influence on the evolution of other quality control parameters of this foodstuff, as well as the potential influence of induced granulation. This work is part of a project on 'best-before dates' for honeys (Cavia *et al*., 2002, 2004, 2007, 2008a,b; Alonso-Torre *et al*., 2006).

Materials and methods

Samples

Sixty fresh and unheated honeys were collected from individual apiaries. Thirty-five samples were originating in Burgos, a Spanish province with typical continental climate (CC samples), and twenty-five in Galicia, a

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Spanish area with typical oceanic climate (OC samples). All samples originated from the same year of harvest. The botanical origin of the samples was determined according to Von der Ohe *et al*. (2004) procedure, after treating and dying the honey sediment by Terradillos *et al*. (1994) method. Six CC samples were unifloral (two Ericaceae, one Compositae Type *Helianthus annus* L., one *Lavandula* sp., one *Thymus* sp. and Leguminosae Type *Trifolium* L. sp. Four OC samples were unifloral (one *Castanea sativa* sp., two *Eucalyptus* sp. and other one *Rubus* sp.). Induced granulation (Cavia *et al*., 2008a,b) was carried out taking into account the crystallisation compatibility (Gonnet, 1992). The aliquots labelled 'A' were directly stored after harvesting. The aliquots labelled 'B' were the samples in which granulation was induced. In these 'B' samples 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 (between 15 and 25 °C).

Analyses were carried out over 3 years each 5 months, at 5, 15, 20, 25 and 30 months after honey bottling. The first 5 months after harvesting were necessary to collect all the samples and selecting the samples for induced granulation. Moisture and sugars in the honeys used to induce the crystallisation were previously analysed.

Methods

Fructose and glucose were determined by employing Boehringer-Mannheim enzymatic test Cat No. 139106 (Huidobro & Simal, 1984) with a Kontron 922 Uvikon double beam spectrophotometer (Kontron Instruments, Milan, Italy). All samples were analysed in duplicate. Determination of monosaccharides was repeated for every sample until the difference between the results of duplicate analysis of each sample (obtained by the same procedure under the same conditions in rapid succession) did not exceed the maximum value given in the precision tables of Harmonised Methods of the European Honey Commission (Bogdanov *et al*., 1997).

Statistical approaches were developed with STATGRAPHIC plus software 5.1 (Manugistics Inc., 2005). Multifactor analysis of variance (ANOVA) (95% confidence level, $P < 0.05$, $n = 2$) followed by LSD Fischer's *post-hoc* test, was applied to research the effects of storage, other parameters, as well as induced granulation on fructose and glucose evolution. Possible relationships between different parameters were studied (99% confidence level, P-value < 0.01). Taking into account the coefficient of variation of the method, a confidence interval has been calculated for each fructose and glucose value for a confidence level of 95%. The confidence level for each value at a level of 95% ($\alpha = 0.05$) will be $X \pm 0.2$ for fructose content and $X \pm 0.1$ for glucose content.

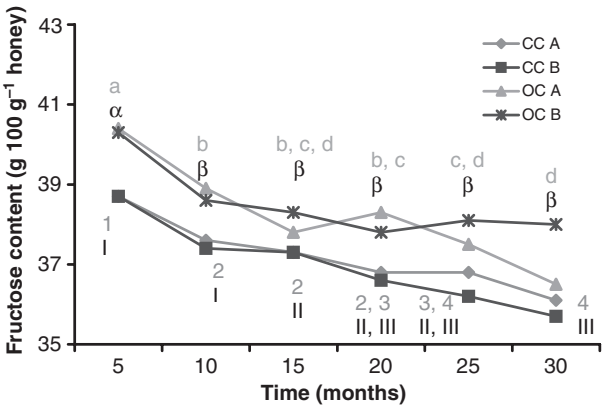

Figure 1 Evolution of fructose content averages for 'A' and 'B' samples originated from continental climate area (CC) and oceanic climate area (OC). a, b, c, d evidence significant differences ($P < 0.05$) in samples OC A. α, β, evidence significant differences ($P < 0.05$) in samples OC B. 1, 2, 3, 4 evidence significant differences ($P < 0.05$) in samples CC A. I, II, III evidence significant differences ($P < 0.05$) in samples CC B. Points with different letters or numbers differ significantly ($P < 0.05$).

Results and discussion

Figure 1 shows the evolution of fructose content averages of the samples over 30 months and the significant differences that were found between the values at different months. Standard deviations of all points of analyses varied from 1.3 to 3.1. Fructose contents were higher in honeys OC (average 40.4 ± 1.6 g/100 g) than in honeys CC (average 38.7 ± 1.4 g/100 g). These results agree with the values found in the literature regarding melissopalinology and fructose content (Serra, 1989; Pérez-Arquillué *et al*., 1990; Martínez-Gómez *et al*., 1993), because OC samples contained more pollens of *Castanea sativa* and *Eucalyptus* sp. than CC honeys. CC samples contained more pollens of Ericaceae and Leguminosae type *Trifolium* sp. than OC honeys.

Fructose contents of CC honeys gradually decreased in all samples during the studied period, showing a similar evolution in both 'A' and 'B' samples. Along the 30 months period fructose content decreased in 94.3% of 'A' samples, from an average of 38.7 ± 1.44 g/100 g to an average of 36.1 ± 2.11 g/100 g. During the same period, all samples 'B' also decreased, from an average of 38.7 ± 1.28 g/100 g to an average of 35.7 ± 1.90 g/100 g.

Also OC honeys showed a decrease in fructose content, with the exception of a slight increase of fructose content of 'A' samples at 20 months. Fructose content of OC honeys decreased in 100% of 'A' samples and in 96.0% for 'B' samples throughout 30 months, from an average of 40.4 ± 1.55 g/100 g to an average of 36.5 ± 3.21 g/100 g and from an average of

4.0 ± 1.40 g/100 g to an average of 38.0 ± 1.81 g/100 g, respectively.

The slope of the evolution curves of fructose content in individual honeys was studied. Most samples (71.4% of 'A' CC samples and 80.0% of 'B' CC samples; 68.0% of 'A' OC honeys and 52.0% of 'B' OC honeys) showed a linear regression, with a negative slope, being $P < 0.01$.

Statistical analysis made it clear that storage had an effect on fructose content. On the contrary, induced granulation did not show any influence on this parameter (Table 1).

These results agree with those reported by others authors (White *et al*., 1961; Hisil & Bagdatlioglu, 1994; Jiménez *et al*., 1994; Cherchi *et al*., 1997). Fructose content is supposed to decrease because of the dehydration of fructose and formation of hydroxymethylfurfural, as well as by formation of oligosaccharides by action of the enzyme transglucosilase.

According to the literature (White *et al*., 1961) honeys' pH has an influence on fructose dehydration to form HMF, so this possible influence was also researched, noticing that the lower the pH, the greater the variation rates (Table 2).

Jiménez *et al*. (1994) observed that fructose decrease was related to initial moisture percentage of honey, but we did not find this relationship in this study.

The evolution of glucose averages over 30 months, as well as the significant differences found between values at different months is showed in Fig. 2. Standard deviations of all points of analyses varied from 1.4 to

Table 1 ANOVA of fructose and glucose contents in samples A and B of CC and OC areas

			Induced granulation	Time storage	Interaction
Source		**DF**	**1**	**5**	**5**
Fructose content	CC	F	1.62	17.77*	0.29
	OC	F	1.45	12.25*	1.58
Glucose content	CC	F	0.73	5.62*	0.22
	OC	F	17.85*	13.20*	0.83

*Significant differences at *P*-value < 0.05. DF, degrees of freedom; *F*, *F*-ratio of ANOVA.

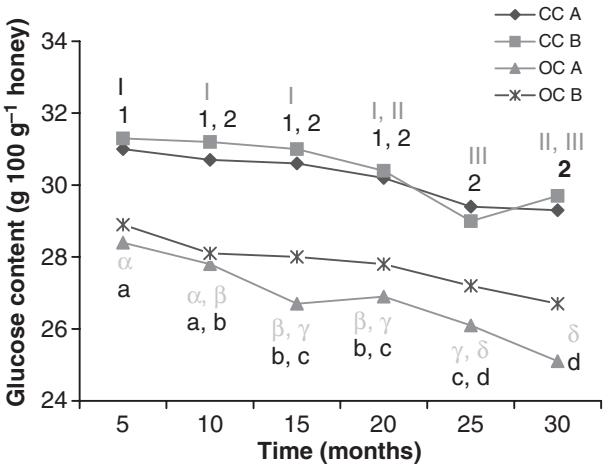

Figure 2 Evolution of glucose content averages for 'A' and 'B' samples originated from continental climate area (CC) and oceanic climate area (OC). a, b, c, d evidence significant differences ($P < 0.05$) in samples OC A. α, β, γ, δ evidence significant differences ($P < 0.05$) in samples OC B. 1, 2 evidence significant differences ($P < 0.05$) in samples CC A. I, II, III evidence significant differences ($P < 0.05$) in samples CC B. Points with different letters or numbers differ significantly ($P < 0.05$).

Table 2 Coefficients of correlation and equations between initial pH and percentages of variation of fructose content observed at the different analysis that been carried out

Time (months)	CC honeys		OC honeys	
	A	**B**	**A**	**B**
10	−0.4176*	−0.3702*	−0.5848**	−0.4617*
	$Y = 9.727 - 3.061X$	$Y = 8.420 - 2.855X$	$Y = 12.387 - 3.858X$	$Y = 12.326 - 3.964X$
15	−0.4026*	−0.4280*	−0.4631*	−0.4552*
	$Y = 14.251 - 4.328X$	$Y = 11.227 - 3.579X$	$Y = 24.566 - 7.432X$	$Y = 19.411 - 5.850X$
20	−0.5259**	−0.4141*	−0.7486**	−0.4628*
	$Y = 13.060 - 4.349X$	$Y = 11.652 - 4.121X$	$Y = 18.071 - 5.544X$	$Y = 24.958 - 7.491X$
25	−0.5379**	−0.1455	−0.2673	−0.5227**
	$Y = 34.304 - 9.530X$	$Y = 1.556 - 1.957X$	$Y = 12.098 - 4.633X$	$Y = 19.624 - 6.010X$
30	−0.4460**	−0.3881*	−0.7116**	−0.3106
	$Y = 13.798 - 5.019X$	$Y = 9.621 - 4.195X$	$Y = 44.743 - 13.008X$	$Y = 7.687 - 3.189X$

Y: percentage of variation of fructose content at each time, starting at 5 months. *X*: pH at 5 months.
*Significant correlation at 95%.
**Significant correlation at 99%.

3.2. Glucose content has been higher in CC honeys (average 31.0 ± 2.6 g/100 g) than in OC samples (average 28.4 ± 1.4 g/100 g). The different botanical origin of CC and OC honeys could be responsible for these differences (Serra, 1989; Pérez-Arquillué *et al.*, 1990; Martínez-Gómez *et al.*, 1993).

Glucose contents of CC honeys decreased for 30 months in almost all samples (80.0% of 'A' samples and 88.6% of 'B' samples), from an average of 31.0 ± 2.60 g/100 g to 29.3 ± 3.18 g/100 g in 'A' samples, and an average of 31.3 ± 2.32 g/100 g to 29.7 ± 2.63 g/100 g in 'B' samples. Glucose in 'A' samples did not decrease steadily, because between 10 and 15 months, and 25 and 30 months glucose content remained constant. For 'B' samples a slight increase was observed from 25 to 30 months.

In respect of OC samples, both groups 'A' and 'B' showed similar evolution patterns. Glucose content decreased at the end of study in 100% of 'A' samples and in 96.0% of 'B' samples, from an average of 28.4 ± 1.42 g/100 g to 25.1 ± 2.55 g/100 g in 'A' samples, and from an average of 28.9 ± 1.26 g/100 g to 26.7 ± 2.05 g/100 g in 'B' samples.

When studying the slope of the evolution curves of glucose content in individual honeys, most samples (51.4% of 'A' samples and 62.9% of 'B' samples of CC honeys; 80.0% of 'A' samples and 68.0% of 'B' samples of OC honeys) showed a linear regression, with a negative slope, being $P < 0.01$.

Statistical analysis of Table 1 clearly shows that storage had an effect on glucose evolution. Conversely, induced granulation showed no influence at all over glucose evolution on CC honeys, and only little influence on OC honeys.

Overall, the results found for glucose evolution agree with those reported in the literature (White *et al.*, 1961;

Table 3 Coefficients of correlation and equations between initial pH and percentages of variation of glucose content observed at the different analysis that been carried out

Time (months)	CC honeys		OC honeys	
	A	B	A	B
10	−0.2471	−0.1945	−0.6429***	−0.5416***
	$Y = 11.219 - 3.030X$	$Y = 6.535 - 1.700X$	$Y = 18.924 - 4.987X$	$Y = 15.614 - 4.478X$
15	−0.3134 *	−0.1744	−0.5764***	−0.6407***
	$Y = 16.802 - 4.456X$	$Y = 7.358 - 2.003X$	$Y = 38.445 - 10.582X$	$Y = 31.781 - 8.460X$
20	−0.5707***	−0.3573**	−0.5402***	−0.7872***
	$Y = 21.211 - 5.863X$	$Y = 14.592 - 4.241X$	$Y = 28.305 - 7.991X$	$Y = 25.685 - 7.141X$
25	−0.2072	−0.1312	−0.5652***	−0.3149
	$Y = 15.320 - 5.029X$	$Y = 11.444 - 4.585X$	$Y = 43.339 - 12.287X$	$Y = 9.794 - 3.843X$
30	−0.4018**	−0.3791**	−0.6606***	−0.7447***
	$Y = 23.093 - 6.957X$	$Y = 20.072 - 6.192X$	$Y = 49.399 - 14.546X$	$Y = 50.021 - 13.918X$

Y: Percentage of variation of glucose content at each time, starting at 5 months. *X*: pH at 5 months.
*Significant correlation at 90%.
**Significant correlation at 95%.
***Significant correlation at 99%.

Table 4 Relationships between initial value of fructose content of CC and OC honeys and showed values at different analysis

Time (months)	CC honeys		OC honeys	
	A	B	A	B
10	$F2 = 1.003 \times F1 - 1.228$	$F2 = 0.954 \times F1 + 0.499$	$F2 = 0.975 \times F1 - 0.535$	$F2 = 1.054 \times F1 - 3.834$
	$r = 0.8397$*	$r = 0.8060$*	$r = 0.8672$*	$r = 0.8287$*
15	$F3 = 1.162 \times F1 - 7.629$	$F3 = 1.035 \times F1 - 2.691$	$F3 = 1.017 \times F1 - 3.342$	$F3 = 1.121 \times F1 - 6.810$
	$r = 0.7814$*	$r = 0.8070$*	$r = 0.5988$*	$r = 0.7278$*
20	$F4 = 1.023 \times F1 - 2.746$	$F4 = 1.028 \times F1 - 3.106$	$F4 = 1.1299 \times F1 - 7.3385$	$F4 = 1.077 \times F1 - 5.573$
	$r = 0.8138$*	$r = 0.7504$*	$r = 0.8794$*	$r = 0.6221$*
25	$F5 = 1.517 \times F1 - 21.87$	$F5 = 0.744 \times F1 + 7.397$	$F5 = 0.942 \times F1 - 0.615$	$F5 = 1.149 \times F1 - 8.154$
	$r = 0.7080$*	$r = 0.5144$*	$r = 0.5428$*	$r = 0.7673$*
30	$F6 = 1.082 \times F1 - 5.806$	$F6 = 1.144 \times F1 - 8.468$	$F6 = 1.475 \times F1 - 23.45$	$F6 = 0.959 \times F1 - 0.575$
	$r = 0.7391$*	$r = 0.7712$*	$r = 0.7139$*	$r = 0.7432$*

F is fructose content. Number mark analysis carried out (i.e. two mark analysis at 10 months, three analysis at 15 months).
*Statistically significant relationship at *P*-value < 0.01.

Table 5 Relationships between initial value of glucose content of CC and OC honeys and showed values at different analysis

Time (months)	CC honeys		OC honeys	
	A	B	A	B
10	G2 = 0.983 × G1 + 0.146 r = 0.8874*	G2 = 0.908 × G1 + 2.733 r = 0.9357*	G2 = 1.086 × G1 − 2.994 r = 0.9133*	G2 = 1.029 × G1 − 1.699 r = 0.8870*
15	G3 = 0.951 × G1 + 1.054 r = 0.8643*	G3 = 0.882 × G1 + 3.406 r = 0,8960*	G3 = 1.092 × G1 − 4.277 r = 0.6869*	G3 = 1.049 × G1 − 2.387 r = 0.7730*
20	G4 = 1.014 × G1 − 1.316 r = 0.9302*	G4 = 0.918 × G1 + 1.701 r = 0.8873*	G4 = 1.159 × G1 − 5.934 r = 0.7853*	G4 = 0.967 × G1 − 0.181 r = 0.8546*
25	G5 = 0.966 × G1 − 0.603 r = 0.7078*	G5 = 0.832 × G1 + 2.956 r = 0.5010*	G5 = 1.143 × G1 − 6.378 r = 0.6458*	G5 = 1.035 × G1 − 2.787 r = 0.7946*
30	G6 = 1.007 × G1 − 1.924 r = 0.8236*	G6 = 0.929 × G1 + 0.570 r = 0.8206*	G6 = 1.164 × G1 − 7.947 r = 0.6500*	G6 = 1.07 × G1 − 4.259 r = 0.6596*

G is glucose content. Number mark analysis carried out (i.e. two mark analysis at 10 months, three analysis at 15 months).
*Statistically significant relationship at P-value < 0.01.

Hisil & Bagdatlioglu, 1994; Jiménez *et al*., 1994; Cherchi *et al*., 1997; Cavia *et al*., 2002). The decrease of glucose at the end of the study in almost all samples may be caused by the formation of oligosaccharides from glucose due to the enzyme transglucosidase and yeasts' activity. The increase found in a small number of samples could be explained by the invertase activity on honey sucrose, whose effect could be stronger than the one due to the processes that lead to degradation of glucose.

A possible influence of other honey parameters on glucose evolution was also researched; finding a relationship between the initial pH values and the variation of glucose content in OC honeys. In these samples, the lower the initial pH, the more marked the decrease of glucose (Table 3). Moisture percentages had not influence on the glucose evolution of the sixty samples.

Significant relationships between initial values of fructose and glucose contents and the values of these parameters at each time of analysis were found (Tables 4 and 5). These relationships were significant for both 'A' and 'B', CC and OC samples. This means that it would be possible to know, at a given time, the value of both fructose and glucose contents on the basis of the initial value of these parameters. Those possible relationships are very interesting fields to research. If same relations were found in different honeys from different botanical sources, geographical origins and harvesting years, aging of honey due to fructose and glucose contents could be predictable.

Conclusions

Fructose and glucose of honey decreased with time. The lower the initial pH is, the greater the decrease of fructose is. Glucose decreased more steadily than fructose in honeys from Continental Climate. In samples from Oceanic Climate, the evolution of glucose was also pH dependent. Induced granulation showed no effect on the evolution of honey monosaccharides.

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