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Posprint del documento publicado en Food Control Nº 17, 2006

Posprint. Publicación definitive en Food Control, 17 : 59-64, (2006)

"Determination of water activity change due to crystallization in honeys from Argentina"

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Abstract

As most honeys are supersaturated solutions of glucose, this sugar may crystallize spontaneously at room temperature in the form of glucose monohydrate. Crystallization of honey lowers the glucose concentration in the liquid phase and thus increases the water activity (a_w) which sometimes can allow naturally occurring yeasts cells to multiply, causing fermentation of the honey. It is the purpose of present work to measure the water activity of 49 samples of crystallized honeys from Argentina, as well as the shift in water activity (Δa_w) when the samples were re-dissolved upon heating. It was found that Δa_w for most samples studied was in the range 0.03-0.04 a_w. Studies with sugar model systems resembling honey confirmed that the observed change in water activity quantitatively corresponded to that caused by glucose crystallization.

Keywords: honey / water activity/ crystallization/ glucose/ fructose

1. Introduction

It is well known that sugars represent the largest portion of honey composition (i.e. more than 95 % of the honey solids); the monosaccharides fructose and glucose are the most abundant (typically about 80- 85 % of the solids in the honey) while small amounts of disaccharides (maltose and sucrose) are also present. Other disaccharides (i.e. trehalose, isomaltose, etc) and higher sugars (trisaccharides and oligosaccharides) are also present, although in quite small quantities The sugar composition depends highly on the types of flowers used by the bees, as well as regional and climatic conditions (White, Riethof, Subers, & Kushnier, 1962; Mendes, Brojo Proenca, Ferreira, & Ferreira, 1998; Mateo, & Bosch-Reig, 1998). Honey may be essentially described as a highly concentrated water solution of two sugars, glucose and fructose, with small amounts of various more complex sugars. Many other substances such as acids, some protein, minerals, etc. and a number of other minor components including pigments, flavor and aroma substances, sugar alcohols, colloids and vitamins also occur in honey.

Fermentation of honey is caused by the action of "sugar-tolerant" yeasts upon the sugars fructose and glucose resulting in formation of ethyl alcohol and carbon dioxide. The alcohol in the presence of oxygen then may be broken down into acetic acid and water; as a result, honey that has fermented may taste sour. The yeasts responsible for fermentation occur naturally in honey and *Saccharomyces spp*. represents the dominant yeast found but other genera have been also reported (Snowdon & Cliver, 1996). In the honey industry it is recognized that water content of a honey is a key factor concerned in spoilage by fermentation. However, Scott’s (1953) classical demonstration that it is not the water content but the water activity (a_w) of a food system which governs microbial growth, showed that microorganisms have a limiting water activity below which they will not grow or produce toxins (Troller & Christian, 1978; Beuchat, 1983). Due to its high content of monosacharides (fructose + glucose) and relatively low moisture content, the water activity of honey is usually below 0.60 which is enough to inhibit the growth of osmophilic yeasts (Ruegg & Blanc, 1981).

As most honeys are supersaturated solutions of glucose, this sugar may crystallize spontaneously at room temperature in the form of glucose monohydrate. Crystallization of honey, commonly called granulation, lowers the solute (glucose) concentration in the liquid phase and thus increases the water activity which can allow naturally occurring yeasts cells to multiply, causing fermentation of the honey. Nearly all kinds of honey will crystallize in time; some honeys granulate uniformly throughout, while others will leave a liquid portion at the top.

It is the purpose of present work to measure the water activity of several crystallized honeys from Argentina, and the a_w shift due to crystallization. The implications on the likehood of honey spoilage (fermentation) due to this shift are discussed.

2. Materials and Methods

2.1. Honey samples

Crystallized honeys used in the present study were of the following origin: honey for retail sales (packaged in glass or plastic jars) which were obtained from supermarkets in Buenos Aires area; natural honey samples obtained directly from honey exporters located in Buenos Aires. The crystallized honey samples corresponded to one or the other of the following types: a) solidly granulated or crystallized, b) partially crystallized honey; i.e. a mixture of liquid honey and crystallized honey, c) honey processed by controlled crystallization to a smooth, spreadable consistency, also called "creamed". Export samples were all partially crystallized honey, while samples for retail sales included all types of crystallized forms. Crystallized honey samples were also measured after liquefaction; the honeys were liquefied at about 42-45 °C in hermetically sealed glass containers.

2.2. Sugars

The following sugars (analytical reagent grade) were used for the model systems: D (+) glucose (anhydrous) and sucrose were obtained from Cicarelli, Sta. Fe, Argentina; levulose (fructose) was from Anedra, Buenos Aires, Argentina and maltose was from Carlo Erba, Italy.

2.3. Determination of water activity

Water activity of honeys (crystallized or liquefied) was measured at 25 ° C (± 0.2 °C) using an electronic dew-point water activity meter, Aqualab Series 3 model TE (Decagon Devices, Pullman, Washington, USA), equipped with a temperature-controlled system which allow to have a temperature stable sampling environment. The equipment was calibrated with saturated salt solutions in the a_w range of interest (Favetto, Resnik, Chirife & Ferro Fontán, 1983). For each determination four/five replicates were obtained and the average reported; under these conditions accuracy of this meter is about ± 0.003 a_w (Fontana, 2001). In order to speed up measurement time, honey samples in plastic sample holders were first equilibrated at 25 °C by putting on an electronic chilling/heating plate (Decagon Devices, Model 40510, Pullman, Washington, USA).

3. Results and Discussion

Many sugars crystallize out from a food system (i.e. honey) if their concentration is above the saturation level and there are no other hindrances that might affect crystallization (Bhandari & Bareyre, 2003). Since most honeys are supersaturated with respect to glucose, spontaneous crystallization may occur in the form of glucose monohydrate. The rate at which glucose crystallization occurs in honey depends on many factors (to be discussed later), among them the most important is the ratio glucose/water (Manikis & Thrasivoulou, 2001). Fig.1 shows the glucose concentration in the water of honey (g glucose/100 g water in honey); it was calculated from compositional data reported in the literature for honeys from different countries (White et al., 1962; Joshi, Pechhacker, Willam & von der Ohe, 2000; Mossel, Bhandari, D'Arcy & Caffin, 2003; Mendes et al., 1998; Spettoli, Bottacin, Pescioa & Girolami, 1982; Mateo & Bosch-Reig, 1998; Oddo & Piro, 2004). It can be seen that the concentration of glucose is in all cases examined well above the equilibrium saturation value at 25 °C which is 103.3 g glucose/100 g water (Pancoast & Junk, 1980). If we define the glucose supersaturation value**,** $\mathbf{S_G}$ = actual glucose concentration in honey (g glucose in 100 g honey water) /saturation value (g glucose in 100 g water), most S_v values in honey are in the approximate range of 1.5 to 2.5, as observed in Fig. 1.

On the contrary, fructose in honey is always below its saturation value which at 25º C is 405.1 g fructose /100g water (Bubnik, Kadlec, Urban & Bruhns, 1995). For example, highest values reported for fructose concentration in the water of honeys from different botanical/geographical sources, were in the range 222–295g fructose in 100g water (White et al., 1962; Mesallam & El-Shaarawy, 1987; Spettoli, Bottacin, Pescioa & Girolami, 1982; Mendes et al., 1998; Mateo & Bosch-Reig, 1998).

Each individual compound possesses its own and constant equilibrium water activity value at saturation, and for the case of glucose it is 0.891 at 25 °C (Ruegg & Blanc, 1981); however if a saturated glucose solution gets supersaturated (i.e. upon heating) the water activity decreases. Since supersaturation is a thermodynamically nonequilibrium (metastable) state, the excess solute above saturation will tend to crystallize out and the water activity of the solution will increase up to the level of the equilibrium solubility concentration. Consequently, if a mixture of crystal-liquid slurry (i.e. crystallized honey) is warmed up to re-dissolve the glucose crystals, the water activity of this supersaturated solution will decrease, since water activity is a colligative property (i.e. its depends on the number of moles in solution). Table 1 shows the water activity (at 25 °C) of 49 samples of crystallized and re-dissolved honeys from Argentina; as expected, the water activity of the re-dissolved samples is always lower than that of the crystallized ones. It is noteworthy that several honey samples which in the liquid (re-dissolved) condition may be considered safe as regard to fermentation (i.e. a_w about ≤ 0.61), increased to an "unsafe" value upon crystallization.

Fig. 2 shows an histogram of the water activity shift (a_w crystallized honey – a_w re-dissolved) in Argentine honeys; it can be seen that in the major number of samples the shift is in the approximate range of 0.03-0.04 $a_{w.}$ Ruegg and Blanc (1981) studied the change in water activity of honeys from various (non-identified) countries and found that the average water activity of the liquid (re-dissolved) samples was 0.562 (standard deviation 0.041) as compared to a mean value of 0.589 (standard deviation 0.038) for the crystallized ones.

Verification of the water activity change due to crystallization: sugar model systems

The observed shift in water activity of Argentine honeys due to crystallization may be examined in the following way. The water activity of honey is largely determined by the molal concentration of main sugars, basically the monosaccharides fructose and glucose, and to a much lesser extent by the disaccharides maltose and/or sucrose; the contribution to a_w-lowering of higher saccharides as well as other compounds of relatively high molecular weight is not significant (Chirife, 1978; Chirife, Ferro Fontan & Benmergui, 1980; Ruegg & Blanc, 1981; Vega-Mercado, Romanach & Barbosa-Canovas, 1992). Thus, the a_w increase upon glucose crystallization may be quantitatively analyzed from the sugar composition of honey. Mesallam and El-Shaarawy (1987), White et al. (1962), and Mateo and Bosch-Reig (1998) reported the sugar composition of various types of honeys from Saudi Arabia, USA and Spain, respectively. Their data were recalculated on a water basis, (g sugar/100 g honey water) both for the original composition and after allowance was made for the amount of glucose that theoretically will crystallize (all these honeys were supersaturated in glucose).

On the basis of following assumption, a) all glucose above the solubility at 25 °C (103.3 glucose/100 g water) crystallizes out, b) glucose crystallizes as monohydrate, c) after crystallization glucose concentration in water is equal to the equilibrium solubility, a mass balance performed for components allows us to calculate the concentration of the different sugars after excess glucose crystallized. Table 2 compares the calculated (after crystallization) and original concentration of each sugar in water. It can be seen that the concentration of non-glucose sugars in water somewhat increased after glucose crystallization due to water taken up from the solution to form the glucose monohydrate. However, total solute (all sugars) concentration in water, decreased after crystallization, or in other words, the moisture content of the liquid phase increased. The water activity change associated with those solute concentrations was experimentally determined using model systems of water, fructose, glucose, maltose and sucrose having the composition of honeys shown previously (Table 2). Results are shown on Table 3; as observed, changes in a_w after allowance is made for glucose crystallization, are well within the range of values observed for Argentine honeys (Table 1).

The crystallization behavior of glucose in honey (and corresponding changes in a_w) is very difficult to predict. As mentioned before, honey is essentially a highly concentrated (sugars) non-equilibrium system because it is supersaturated in glucose. It is known that a supersaturated sugar solutions may or may not crystallize. Before crystallization proceeds it is necessary to attain a given supersaturation, but not every supersaturated solution will crystallize during the time available for observation; i.e. the time scale required for crystal formation to occur may be too long to be observed on a practical scale (Hartel, 2001). The driving force for glucose crystallization in honey is the degree of supersaturation ($\mathbf{S_G}$) which as shown before (Fig.1) is in the range 1.5 - 2.5 for different honeys. However, the mobility of glucose in honey also plays an important role in determining how high a degree of metastability may be maintained. At increasing concentrations all sugar solutions become increasingly viscous and the mobility of sugar molecules decreases as the viscosity increases; due to lack of mobility, sugar (i.e. glucose) solutions may not crystallize despite a high degree of supersaturation. Also, different honeys are known to have widely different viscosities due to differences in moisture content and/or presence of colloids (Munro, 1943) which in turn influences the metastable state. In addition to this, there are many other factors which may also affect the metastability: agitation, storage temperature, and presence of foreign matter enhance crystallization, while some soluble impurities, complex carbohydrates and proteins inhibit crystallization. The presence of fructose in honey is particularly important factor because it inhibits glucose crystallization.

The experimentally determined changes in water activity after crystallization for several Argentine honeys may be utilized by producers to keep safety margins as regarding the critical water activity to inhibit fermentation.

Acknowledgments

The authors acknowledge to Dr. Diego Sabatini for providing several samples of crystallized honey.

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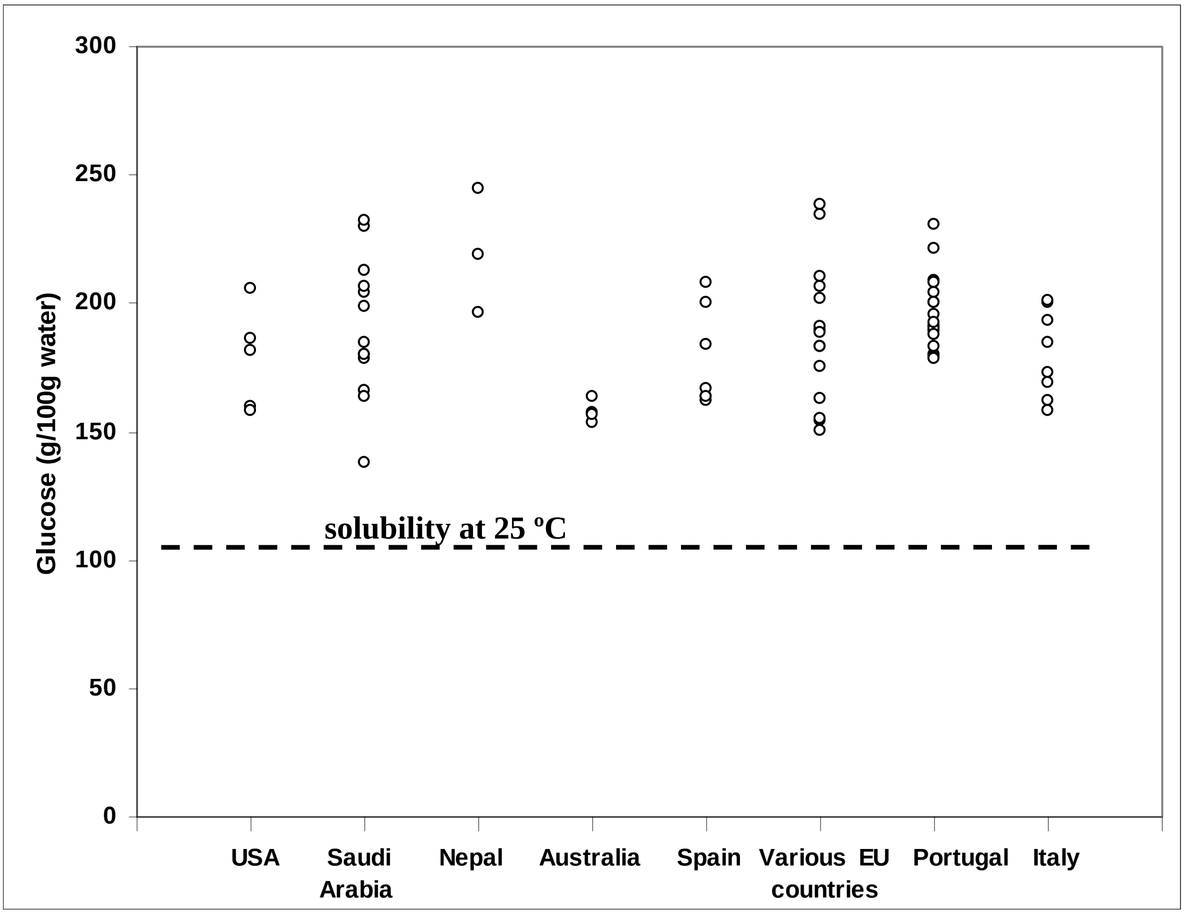

Fig.1.

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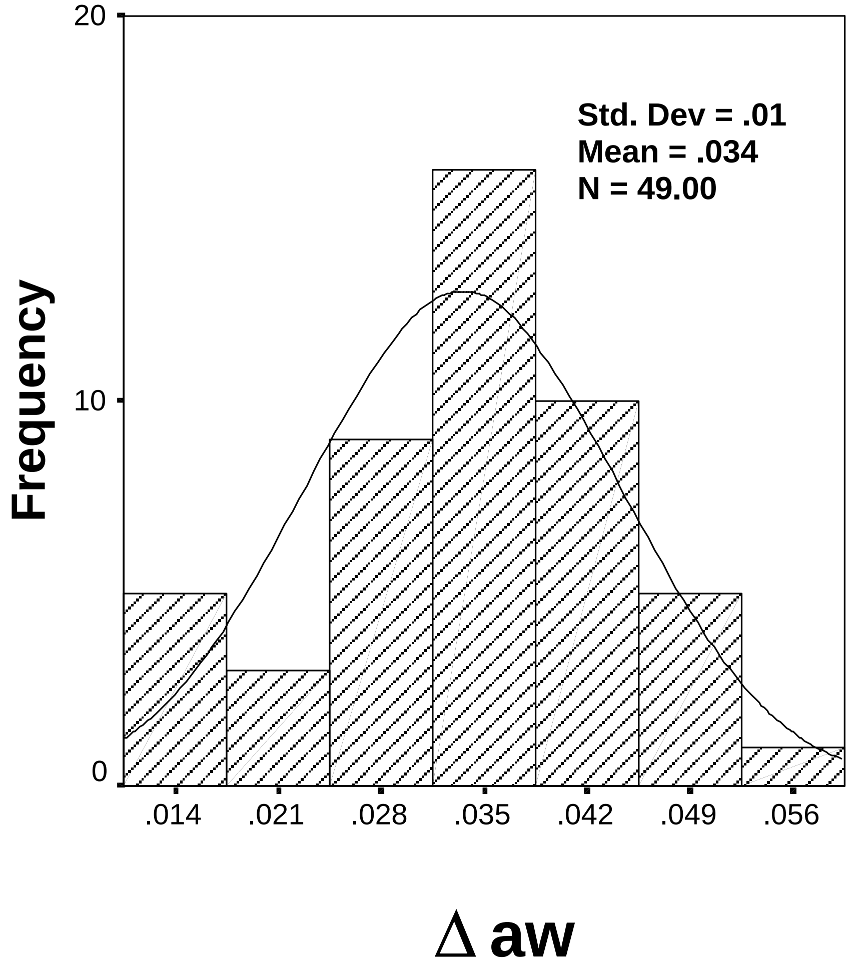

Fig. 2

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Figures captions

Fig.1. Glucose concentration in the water of honey as compared with the solubility of glucose at 25 ºC; sources of compositional data for the various honeys were from references White et al., 1962; Joshi, Pechhacker, Willam & von der Ohe, 2000; Mossel, Bhandari, D'Arcy & Caffin, 2003; Mendes et al., 1998; Spettoli, Bottacin, Pescioa & Girolami, 1982; Mateo & Bosch-Reig, 1998; Oddo & Piro, 2004.

Fig. 2. Histogram of the water activity shift in water activity for crystallized Argentine honeys.

Table 1 – Water activity [*] (at 25 ºC) of crystallized and re-dissolved honeys from Argentina.

Honey characteristics	*Expiration date*	a_w crystallized	a_w re-dissolved
Internal market *(packaged in glass or plastic jars)*			
(1) Multifloral, creamy, province Buenos Aires	26/08/2005	**0.599**	**0.562**
(2) Multifloral, creamy, province of Buenos Aires	29/05/2005	**0.592**	**0.536**
(3) Multifloral, creamy, province Buenos Aires	29/05/2005	**0.589**	**0.549**
(4) Multifloral, creamy, province of Buenos Aires	26/08/2005	**0.598**	**0.564**
(5) Creamy, province of Buenos Aires	19/09/2005	**0.579**	**0.546**
(7) Creamy, pasteurized, [1]	-----------	**0.575**	**0.537**
(8) Creamy, pasteurized, [1]	03/2005	**0.573**	**0.536**
(9) Creamy, pasteurized, [1]	03/2005	**0.606**	**0.559**
(10) Creamy, pasteurized [1]	03/2005	**0.573**	**0.543**
(12) Creamy, pasteurized,[1]	03/2005	**0.573**	**0.541**
(13) Artisan honey, [1]	-----------	**0.626**	**0.619**
(14) Artisan honey, [1]	-----------	**0.633**	**0.620**
(15) Artisan honey, [1]	-----------	**0.601**	**0.586**
(16) Creamy, [1]	25/12/2005	**0.610**	**0.576**
(17) Artisan honey, [1]	-----------	**0.607**	**0.571**
(19) Artisan honey, [1]	------------	**0.620**	**0.581**
(20) Artisan honey, [1]	------------	**0.592**	**0.553**
(21) [1]	09/06/2005	**0.573**	**0.549**
(22) [1]	09/06/2005	**0.577**	**0.551**
(23) [1]	09/06/2005	**0.569**	**0.549**
(24) [1]	09/06/2005	**0.571**	**0.546**
(25) [1]	09/06/2005	**0.589**	**0.543**
(26) Monofloral (caldén), province of La Pampa	23/02/2005	**0.555**	**0.520**

(27) Monofloral (cardo), province of Buenos Aires	23/12/2005	**0.588**	**0.551**
(52) Province of Córdoba	2006	**0.614**	**0.566**
(53) Province of Córdoba	2006	**0.621**	**0.575**
***Export samples**, province of Buenos Aires; crop 2003*			
(32)		**0.632**	**0.607**
(35)		**0.602**	**0.574**
(38)		**0.565**	**0.537**
(39)		**0.548**	**0.527**
(40)		**0.617**	**0.579**
(41)		**0.569**	**0.536**
(42)		**0.605**	**0.570**
(37)		**0.623**	**0.582**
(44)		**0.595**	**0.547**
(45)		**0.588**	**0.561**
(46)		**0.605**	**0.563**
(47)		**0.591**	**0.553**
(50)		**0.595**	**0.561**
(54)		**0.603**	**0.593**
(55)		**0.622**	**0.607**
(56)		**0.658**	**0.622**
(57)		**0.629**	**0.589**
(58)		**0.635**	**0.601**
(59)		**0.653**	**0.614**
(60)		**0.638**	**0.594**
(61)		**0.667**	**0.625**
(62)		**0.671**	**0.642**

[*] average of five replicates

[1] geographical origin not stated in the label

Table 2 – Reported sugar composition of some honeys

Saudi Arabia [(1)]	% of honey	Original composition of honey g/100 g water	Assuming excess glucose crystallized g/100 g water
Moisture	15.5		
Fructose	42.1	271.61	309.46
Glucose	33.0	212.90	103.3
Sucrose	2.8	18.07	20.58
Maltose	3.2	20.64	23.51
Total sugars		523.22	456.84
USA [(2)]			
Moisture	16.1		
Fructose	38.62	239.88	274.49
Glucose	34.85	216.46	103.3
Sucrose	2.41	14.97	17.13
Maltose	5.43	33.73	38.59
Total sugars		505.03	433.52
Spain [(3)]			
Moisture	19.1		
Fructose	36.2	189.53	203.14
Glucose	31.2	163.35	103.3
Sucrose	1.6	8.38	8.99
Maltose	3.90	20.42	21.89
Total sugars		381.67	337.30

[(1)] Mesallam and El-Saarawy (1987); obtained from producer in the province of KSA, S. Arabia.

[(2)] White et al. (1962); “average” USA honey

[(3)] Mateo and Bosch-Reig (1998); rosemary honey

Table 3 – Observed change in water activity in sugar model systems after allowance is made for glucose crystallization

Honey model system	Water activity of the initial formulation	Water activity after allowance is made for glucose crystallization	**Δ a_w**
Saudi Arabia [1]	0.553	0.594	**0.041**
USA [2]	0.566	0.635	**0.069**
Spain [3]	0.640	0.675	**0.035**

[1], [2] [3]: see Table 2.