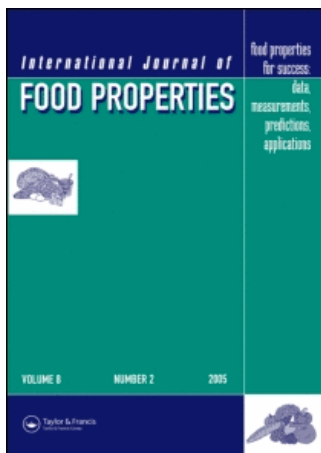


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### Crystallization of Honey at -20°C

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## CRYSTALLIZATION OF HONEY AT $-20^{\circ}\text{C}$

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*Honeys from different regions of the province of Buenos Aires were stored at  $-20^{\circ}\text{C}$ , and factors that affect crystallization were analyzed. Crystals were observed by light microscopy. Firmness, adhesivity and viscosity of the samples were measured. Honey was characterized by determining the water activity, turbidity, moisture, fructose, and glucose contents. Results show that the viscous characteristics of the samples depend on the number, size, and disposition of crystals. Various honey samples exhibited Newtonian, pseudoplastic, and thixotropic behaviors. Crystallization was favored at higher moisture contents, suggesting that the parameters that affect honey crystallization at room temperature have a different effect at freezing temperatures. Honey that presented higher values of firmness had a moisture content lower than 17%, and a linear inverse relationship was observed between the adhesivity and firmness of honey samples.*

**Keywords:** *Honey, Crystallization.*

## INTRODUCTION

Nearly all honeys are in liquid form as stored by the bees in the comb, but after beekeepers extract it from the comb, many honeys crystallize within a few days or weeks. Crystallization of honey is a complex phenomenon, being a matter of interest for beekeepers, honey handlers, and processors. When crystallization occurs during storage in an undesired and uncontrolled fashion, it causes the product to be cloudy and, therefore, less appealing to the consumer.<sup>[1,2,3]</sup> While crystallization is usually undesirable in liquid honey, controlled crystallization can be used to make a desirable product, as creamed honey, in which there are a large number of crystals of very small size, so that they will not be perceived by the palate.<sup>[4]</sup>

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Although it is necessary to attain supersaturation before crystallization can occur, not every supersaturated solution will crystallize during a reasonable period of time.<sup>[5]</sup> At some point with increased concentration or reduced temperature, the supersaturated solution goes through a transition to a glassy state. Crystallization of the solute occurs mainly in the region between the solute solubility and the glass transition curves.<sup>[4,6]</sup> At conditions of temperature and concentration close to the solubility curve, crystallization rates increase with increasing supersaturation. However, as a solution moves closer to the glass transition curve, either by concentration or cooling, the viscosity increases dramatically and the crystallization rates decrease due to diffusion limitations. Thus, in the granulation of honey, two opposite trends have been observed: when temperature decreases, the solubility of sugars decreases,<sup>[7]</sup> favoring granulation, but at the same time viscosity increases,<sup>[8]</sup> retarding the mobility of molecules, thereby resulting in lower granulation rates.<sup>[9]</sup> As the glass transition curve is reached, crystallization does not occur over any reasonable period of time due to the limited mobility of molecules. This is a dynamic (kinetically constrained) state but not an equilibrium state.<sup>[4]</sup>

There are many factors that affect crystallization, such as composition and temperature. Honey is composed mainly of fructose and glucose. Most honeys are supersaturated with respect to glucose, which is less soluble than fructose; thus, there exists the possibility to crystallize spontaneously at room temperature in the form of glucose monohydrate.<sup>[2]</sup> Several attempts were made to predict the tendency of a honey to crystallize, taking into account their moisture, glucose, and fructose contents. Most of the published reports, however, indicate that all of the predictive methods for crystallization are not sufficiently accurate; thus, further investigation is needed.<sup>[3]</sup> Moreover, these parameters are proposed to predict the tendency of a honey to crystallize at room temperature, whereas there is very little information concerning the crystallization of honey stored at freezing temperatures. The objective of this article was to characterize honey from different regions of the province of Buenos Aires, Argentina, stored at  $-20^{\circ}\text{C}$  and to correlate different parameters with honey crystallization. According to the state diagram for honey,<sup>[10]</sup> samples analyzed in the present study, with moisture contents lower than 25%, stored at  $-20^{\circ}\text{C}$ , were in the region between the solubility curve and the glass transition curve.

## MATERIALS AND METHODS

### Materials

One hundred forty eight samples of honey were collected from different zones of the province of Buenos Aires, Argentina. Honey samples were obtained by cold extraction at the laboratory in order to ensure that samples were not modified during the extraction procedures; they were stored at  $-20^{\circ}\text{C}$  for about four years.

### Analytical

Moisture was determined with an Abbe refractometer reading at  $20^{\circ}\text{C}$ , obtaining the corresponding value of moisture from the Chatway Table.<sup>[11]</sup> Determinations were performed in duplicate. Glucose and fructose were determined by HPLC (HP1100, Agilent, Palo Alto, CA, USA), consisting of a vacuum degasser, a binary pump, a Rheodyne 7125 manual injector (San Francisco, CA, USA), and a variable wavelength UV detector equipped with a microcell (1  $\mu\text{L}$ ). An amino propylsilica column Supelco ( $250 \times 4.6$  mm

i.d., particle diameter 5  $\mu\text{m}$ , Bellefonte, US) at  $30^{\circ}\text{C}$  was used. The eluting solvent was acetonitrile:water, 83:17. Detection was set at 195 nm. Honey was diluted 1:50 with distilled water, and then samples were filtered through 0.22  $\mu\text{m}$ -Nylon membrane filters (Micron Separations, MSI, Inc., Westborough, MA, USA) before injecting.

### Light Microscopy

Stored honey samples were directly observed at room temperature with a Leica DMLB microscope (Wetzlar, Germany) at a magnification of 400 $\times$ . Four to eight photographs were taken of each honey sample.

### Turbidity of Honey

Honey was put into a cuvette with a path-length of 1 cm. The absorbance was measured at 660 nm at room temperature with a Cintra 5 UV-visible double beam spectrometer (GBC Scientific Equipment, Victoria, Australia).<sup>[9]</sup>

### Water Activity

Water activity was measured in duplicate at  $25^{\circ}\text{C}$  with an Aqualab Model Series 3 TE (Decagon Devices Inc., Pullman, Washington, USA).

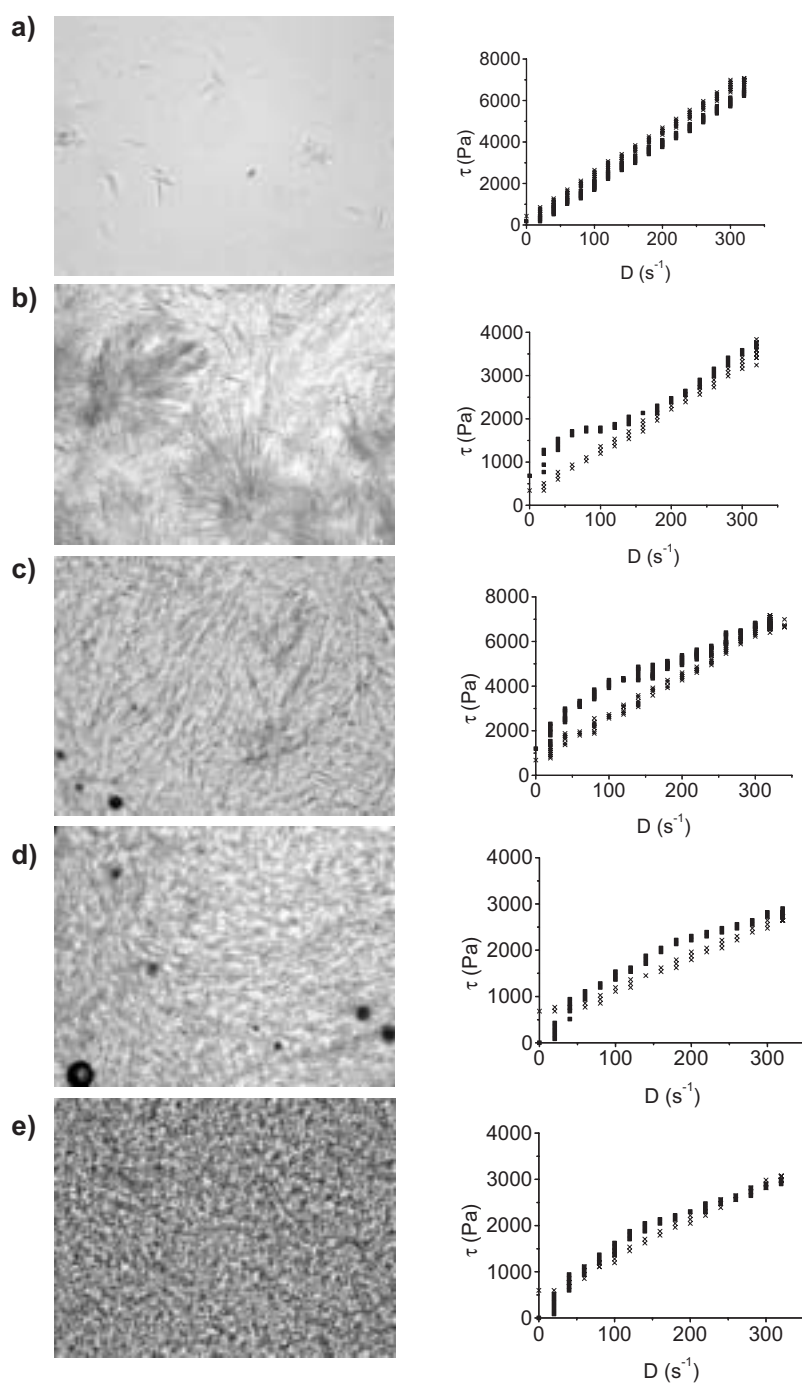
### Rheology

Firmness and adhesivity of honey were determined at room temperature by using a TA.XT2 Texture analyzer (Stable Micro Systems Ltd., Surrey, UK) in the compression mode. Compression was exerted by a cylindrical probe with a flat section (12 mm diameter) at a displacement speed of 0.5 mm/s. Firmness was defined as the force  $F_0$  (Newtons) measured at 7 mm compression, according to Shinn and Wang,<sup>[12]</sup> and adhesivity was calculated as the negative force area obtained after the compression cycle, representing the work necessary to pull the compressing plunger away from the sample.<sup>[13]</sup> For each sample, four to seven replicates were measured. Viscosity measurement was done in non-equilibrium conditions according to Anupama et al.<sup>[14]</sup> Measures were performed in honey samples at  $25^{\circ}\text{C}$  by using a Haake Rotavisco RV2 viscosimeter (Karlsruhe, Germany), with a cone plate sensor system Pk I,  $03^{\circ}$ . The rotation rate was increased from 0 to 320  $\text{s}^{-1}$  in 1.5 min, maintained for 2 minutes at maximum speed, and then returned to 0 in 1.5 minutes. The shear stress  $\tau$  (Pa) was plotted against the shear rate  $D$  ( $\text{s}^{-1}$ ). Two or three measures were performed of each honey sample.

## RESULTS AND DISCUSSION

### Crystallization and Viscosity

Fig. 1 shows different honey samples of the province of Buenos Aires stored at  $-20^{\circ}\text{C}$ , observed with light microscopy, and the corresponding viscosity diagrams of shear stress vs. shear rate. Most of samples presented needle crystals, arranged as bouquets or randomly disposed. With respect to viscous characteristics, samples showed a Newtonian behavior when decreasing shear the rate from 320 to 0  $\text{s}^{-1}$ . Fig. 1a shows a honey with very few crystals. This sample presented a Newtonian behavior and a high viscosity, due



**Figure 1** Light microscopy and viscosity of honey from different regions of the province of Buenos Aires, Argentina, stored at  $-20^\circ\text{C}$  for about 4 years.  $\tau$ : shear stress;  $D$ : shear rate. ( ) Upward shear rate sweep; (×) Downward shear rate sweep.

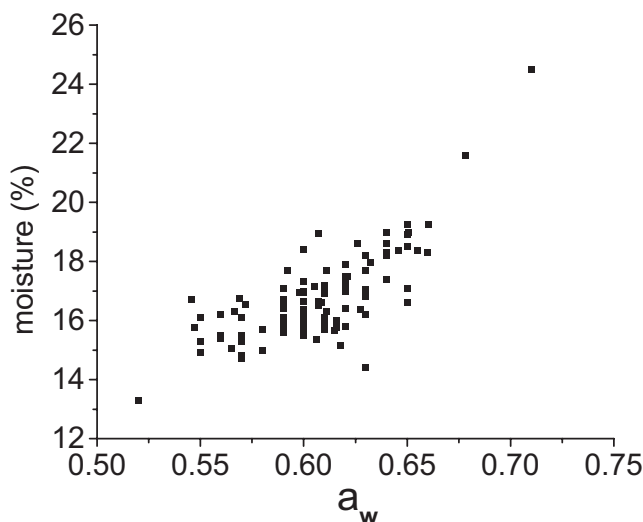
probably to the fact that, as there are very few crystals, it is expected to have a high sugar concentration in solution. This is similar to the results previously reported for liquid honey.<sup>[15,16]</sup> Fig. 1b shows a sample with big crystals disposed as bouquets, which exhibited a pseudoplastic behavior in the first portion of the upper curve, obtained by increasing the shear rate. On the other hand, at higher shear rates the behavior became Newtonian. The lower curve, generated by decreasing the shear rate, also showed a Newtonian behavior. A hysteresis was thus observed in the first portion of the curve, which is characteristic of a thixotropic material. These results suggest that the initial structure was broken during the viscosity assay. Big crystals randomly disposed, as can be seen in Fig. 1c, were associated with pseudoplastic and thixotropic characteristics in the first portion of the curve of shear stress vs shear rate. This behavior was maintained in a higher shear rate range than the sample with big crystals disposed as bouquets (Fig. 1b). As crystals became smaller in the honey sample showed in Fig. 1d, hysteresis decreased, until practically disappeared in the sample showed in Fig. 1e, in which crystals were very small.

### Water Activity ( $a_w$ ) and Moisture Content of Honey Samples

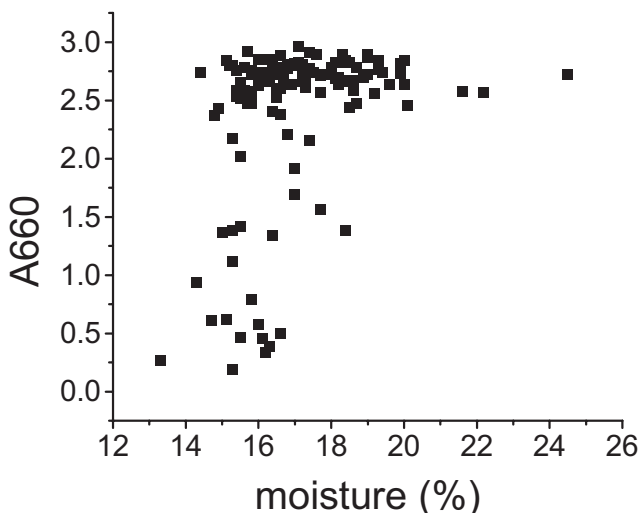
The moisture content and water activity ( $a_w$ ) of 102 samples of honey from different regions of the province of Buenos Aires were analyzed. Moisture contents varied from 13.3 to 24.5%, and  $a_w$  from 0.52 to 0.71. A linear relationship was observed between  $a_w$  and moisture content in this range (Fig. 2), with  $R^2 = 0.57$ . Most of the samples had  $a_w$  values between 0.55 and 0.65, a little higher than the average values of 0.5–0.6, mentioned by Gonnet.<sup>[17]</sup>

### Relationship Between Honey Granulation and Moisture, Glucose, and Fructose Contents

Fig. 3 depicts the absorbance at 660 nm as a function of the moisture content of 140 samples of honey from different regions of the province of Buenos Aires, stored at  $-20^{\circ}\text{C}$ .



**Figure 2** Moisture content of 102 honey samples stored at  $-20^{\circ}\text{C}$ , as a function of their water activity ( $a_w$ ).

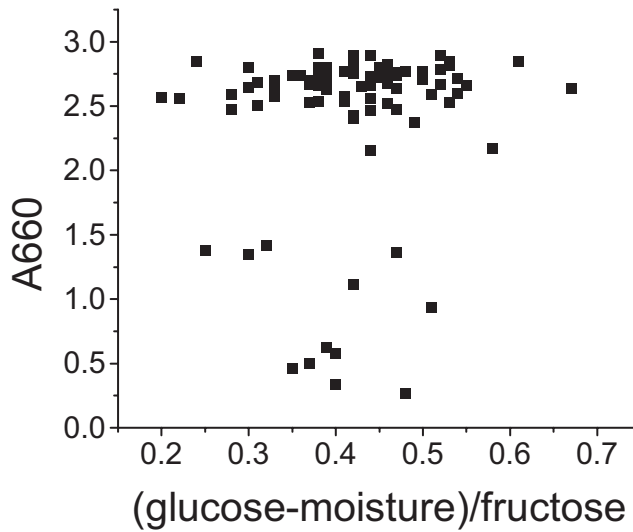


**Figure 3** Absorbance at 660 nm (A660) as a function of moisture content of 140 samples of honey from different regions of the province of Buenos Aires, stored at  $-20^{\circ}\text{C}$ .

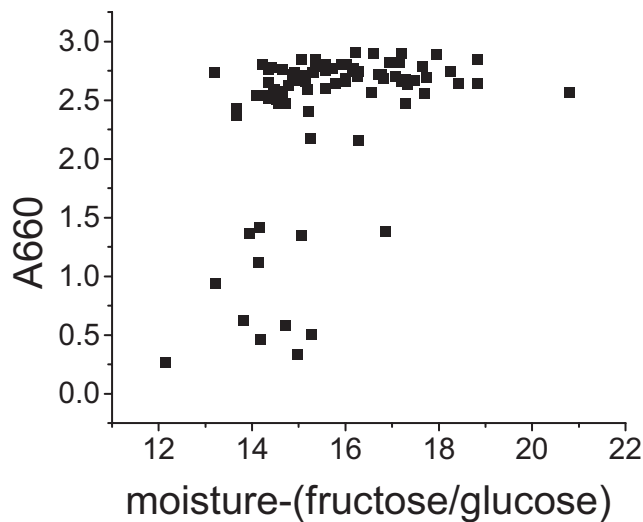
The turbidity, measured as the absorbance at 660 nm, can be taken as an indicator of honey granulation.<sup>[9]</sup> It can be observed that crystallization appears to be favored at higher moisture contents. This fact can be explained taking into account that when temperature decreases, viscosity increases, retarding crystallization. Water can act as a plasticizer, decreasing the viscosity of honey and favoring crystallization. At room temperature, on the contrary, crystallization compatibility was taken on the basis of moisture contents less than 18.5%.<sup>[17,18]</sup> No relationship was observed between the absorbance at 660 nm and the relation  $[(\text{glucose} - \text{moisture}) / \text{fructose}]$  (Fig. 4), which is one of the parameters usually used to predict crystallization at room temperature.<sup>[19]</sup> On the other hand, samples with lower values of the relation  $[\text{moisture} - (\text{fructose}/\text{glucose})]$ , in which moisture content plays an important role, showed a lower tendency to crystallize (Fig. 5). Even taking into account that the absorbance method is approximate, these results suggest that the parameters that affect honey crystallization at room temperature have a different effect than at a freezing temperature.

### Firmness and Adhesivity of Honey Samples

Fig. 6 depicts the firmness of 111 honey samples as a function of their moisture content. Honey that presented higher values of firmness had moisture contents lower than 17%, and were both crystallized and non-crystallized. The high firmness would be the result of a high sugar concentration in solution in non-crystallized honey, or a structure formed by crystals in some crystallized honey samples. A linear inverse relationship was observed between the adhesivity and firmness of honey samples, with  $R^2 = 0.94$  ( $N = 119$ ) (Fig. 7). Adhesivity represents the work necessary to overcome the attractive forces between the surface of the food, and the surface of other material with which the food comes in contact.<sup>[20]</sup> A theory of adhesion proposes that materials adhere because of interatomic and intermolecular forces, such as covalent or ionic bonds or van der Waals



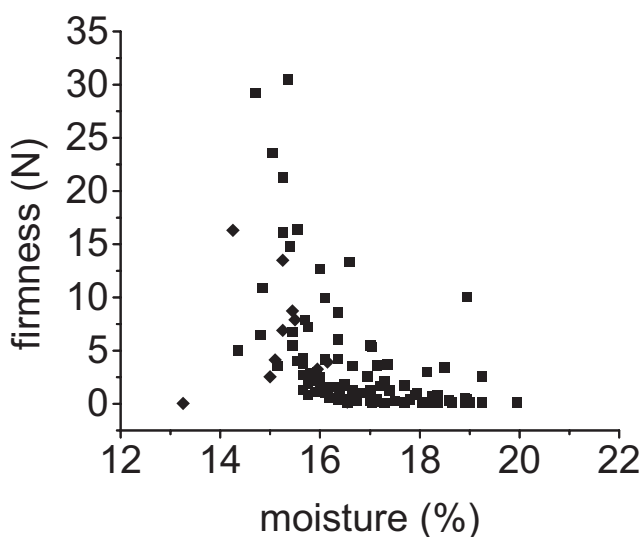
**Figure 4** Absorbance at 660 nm (A660) as a function of the relation [(glucose - moisture)/ fructose] of 88 honey samples stored at  $-20^{\circ}\text{C}$ .



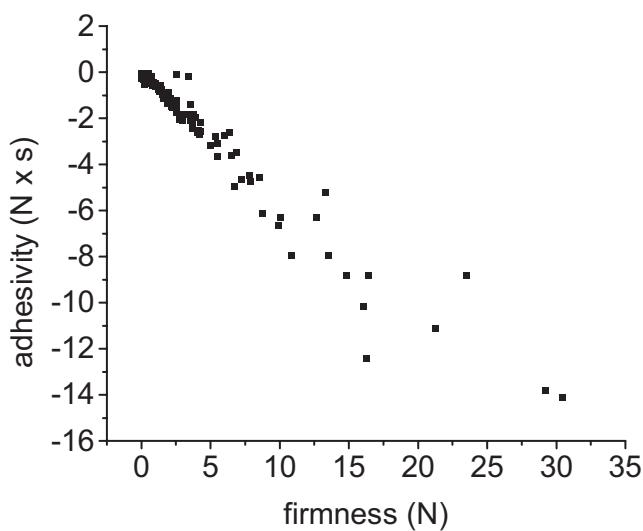
**Figure 5** Absorbance at 660 nm (A660) as a function of the relation [moisture - (fructose/glucose)] of 88 honey samples stored at  $-20^{\circ}\text{C}$ .

forces. The adhesion of a material can be described in terms of the sum of two energy contributions—the surface energy and the cohesive energy. The surface energy depends on the type and strength of bonding between the adhesive and the substrate, while the cohesive energy represents the energy dissipated in viscoelastic and plastic deformation within the adhesive.<sup>[21]</sup> In the case of honey, it is possible that samples that presented a high firmness, due to stronger internal forces, had less possibilities to bind to another material, resulting in lower adhesivity values.





**Figure 6** Firmness of 111 honey samples stored at  $-20^{\circ}\text{C}$  as a function of their moisture content.



**Figure 7** Adhesivity vs. firmness of 119 honey samples stored at  $-20^{\circ}\text{C}$ .

## CONCLUSIONS

Honey stored at freezing temperatures presented Newtonian, pseudoplastic, or thixotropic characteristics, depending on the number, size and disposition of sugar crystals. Crystallization at  $-20^{\circ}\text{C}$  was favored in samples containing higher moisture contents, in contrast to the behavior expected at room temperature. An inverse relationship was observed between adhesivity and firmness of honey.

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