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"THE CORRELATION BETWEEN WATER ACTIVITY AND % MOISTURE IN HONEY: Fundamental aspects and application to Argentine honeys"

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Abstract

Present work examined some fundamental aspects of the relationship between water activity and % moisture in honey. For this purpose a theoretical analysis was made on water activity lowering in sugar solutions and honey; the correlation between water activity and % moisture in Argentine honeys, was then experimentally determined and explained on the basis of above analysis. A very good straight line relationship (correlation coefficient 0.971) was found between both parameters in the range examined (15 % to 21 % moisture), and also the goodness of fit of the regression equation was found to be quite satisfactory.

Previous literature results were compared with present ones.

Keywords: water activity /% moisture / sugars/ glucose/fructose/ honey

1. Introduction

Fermentation of honey is caused by the action of osmotolerant 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 honey is a key factor concerned in spoilage by fermentation. However, it is not the water content but the water activity (a_w) of a food which governs microbial growth (Troller & Christian, 1978). Water activity is a major factor preventing or limiting microbial growth and in several cases a_w is the primary parameter responsible for food stability, modulating microbial response and determining the type of microorganisms encountered in food. Of all other factors affecting microbial growth in food products (temperature, pH, oxygen, nutrient availability, etc) the influence of water activity on vegetative microorganisms and spores is one of the most complex and fascinating and for this reason has been extensively studied by food microbiologists (Scott, 1953; Beuchat, 1981; 1983;1987; Brown, 1974; Christian, 1963). The minimal a_w level for growth emerged as one of the most investigated parameter that determines the water relations of microorganisms in food ; this limiting value defines the level below which a microorganism cannot longer reproduce (Troller & Christian, 1978); osmotolerant yeasts-as it may be found in honey- may grow down to about a_w = 0.61/0.62 (Beuchat, 1983). Knowledge of water activity of honey is also needed to predict moisture exchange with the environment, since water activity is the driving force behind water transfer from/to honey.

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 while small amounts of disaccharides (maltose and sucrose) are also present; other disaccharides and higher sugars (trisaccharides and oligosaccharides) are also present in quite small quantities. Due to the high content of monosacharides (fructose and glucose) and relatively low moisture content, the water activity of honey is usually, but not always, below 0.60 which is enough to inhibit the growth of osmotolerant yeasts (Ruegg & Blanc, 1981; Beckh, Wessel, & Lüllmann, 2004; Zamora & Chirife, 2004).

Honey industry utilizes almost exclusively the moisture content (determined by refractometry) as a criterion of microbial stability in honey; the amount of moisture in honey is a function of the factors involved in ripening, including weather conditions and original moisture of the nectar; also, after extraction of the honey its moisture content may change depending on conditions of storage due to water exchange with the environment. For these reasons the water content of honey varies greatly and it may range somewhere between 13 and 23 % (Beckh, et al., 2004; White, Riethof, Subers & Kushnier, 1962). The determination of moisture in honey is performed by refractometry which although it does not yield exactly the true water content it is a very simple and reproducible method and have been used successfully in routine honey control.

It is the purpose of present paper to examine the correlation between water activity and % moisture in honey. For this purpose a theoretical analysis is made of the above relationship and verified in several honeys from Argentina.

2. Material and methods

2.1. Determination of water activity

The water activity of honeys was determined 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 reliability of this meter is about ± 0.003 a_w (Fontana, 2002). 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).

2.2. Moisture

Moisture content of honey was determined using an Atago refractometer (Atago Co., Ltd., Tokyo, Japan) provided with a temperature correction scale to compensate when the sample temperature was other than 20°C; measurements were made by duplicate and the average used.

2.3. Honey

Honey samples (liquid) were obtained from growers in Provincia of Buenos Aires; others were obtained in retail stores. In some experiments honey was diluted by adding predetermined amounts of distilled water.

3. Results And Discussion

The water activity of honey is mainly determined by the molal concentration of soluble chemical species; thus, substances of relatively high molecular weight or which are present in very small quantities such as, nitrogenous compounds (proteins, enzymes, amino acids), trisaccharides and oligosaccharides, acids, vitamins, flavors and minerals make very little contribution to water activity lowering in honey (Chirife, 1978; Chirife, Ferro Fontan & Benmergui, 1980; Ruegg & Blanc, 1981). Consequently, water activity in honey results mainly from the concentration in the water of honey of the monosaccharides fructose and glucose, and to a lesser extent, to some disaccharides such as sucrose, maltose/isomaltose. For example, for the composition of the “average” USA honeys (White et al., 1962) the molal (moles sugars/1000 g water in honey) concentration of fructose + glucose is 22.4 molal, while that of sucrose + maltose is only 1.46 molal. A review of available data for the sugar composition of honeys from different sources/countries (Spettoli, Bottacin, Pescioa & Girolami, 1982; Mateo & Bosch-Reig, 1998; Mendes, Brojo Proenca, Ferreira & Ferreira, 1998; Mossel, Bhandari, D’Arcy & Caffin, 2003; Mesallam & El-Shaarawy, 1987; White et al., 1962; Oddo & Piro, 2004), revealed that the molal concentration of glucose + fructose ranges between about 19 to 28 molal, while that of sucrose + maltose between less than 0.03 to 3.

As shown by Chirife et al. (1980) the water activity of a multicomponent non-electrolyte solution may be calculated in a first approximation, from the Ross (1975) equation which for honey will read,

$$(a_w)_H = (a_w°)_G \, . \, (a_w°)_F . \, (a_w°)_M \, . \, (a_w°)_S \qquad (1)$$

where $(a_w°)_G$, $(a_w°)_F$, $(a_w°)_M$ and $(a_w°)_S$, are the water activities of binary solutions of glucose, fructose, maltose and sucrose, respectively, at the same molality (moles solute/1000 g water in honey) as in the honey. Favetto and Chirife (1985) have demonstrated that the water activity lowering behavior of glucose and fructose may be considered identical, so eqn. (1) may be simplified to,

$$(a_w)_H = (a_w°)_{F/G} \cdot (a_w°)_M \cdot (a_w°)_S \qquad (2)$$

The thermodynamic properties of binary non-electrolyte solutions have been studied both experimentally and theoretically by many workers in the past 50 years (Robinson & Stokes, 1965; Stokes & Robinson, 1996; Chirife & Ferro Fontán, 1980; Teng & Lenzi, 1974; Chirife, et al., 1980). It has been customary to report the results in terms of the osmotic coefficient, ϕ, and most workers usually also provided theoretical or empirical equations to predict the effect of solute concentration on the osmotic coefficient. The osmotic coefficient is related to water activity through the relationship,

$$a_w = p/p_o = \exp(-\phi\ 0.018\ m\ \upsilon) \qquad (3)$$

where m is molality and υ is number of moles of kinetic units, which for non-electrolytes is equal to 1. Lupin, Boeri & Moschiar (1981), showed that eqn. (1) may be expanded as the series,

$$a_w = 1 - (\phi\ 0.018\ \nu)m + \frac{(\phi 0.018\ \nu)^2 m^2}{2!} - \frac{(\phi 0.018\ \nu)^3\ m^3}{3!} + \ldots\ldots\ldots\ldots\ldots$$

and thus if,

$$(\phi\ 0.018\ \nu) << 1, \tag{4}$$

the following relationship holds true for some given interval of molality,

$$a_w = 1 - K\ m \tag{5}$$

Favetto and Chirife (1985) examined values of osmotic coefficients of various sugars (and also other solutes) and suggested that condition (4) was likely to apply. They showed that for various sugars (and some related compounds) eqn. (5) described satisfactorily the experimental water activity lowering behavior up to a molality corresponding to a water activity of about 0.85.

For very concentrated sugar solutions (as it is the case with honey) we may assume that a form of eqn. (5) stills holds but for small intervals of concentration. Eqn. (5) may be written as,

$$a_w = A - K'.\ [s] \tag{6}$$

where [s] is the solids concentration in g solid/100 g water, and A is a constant not necessarily equal to one. It is to be stressed that this linear correlation is supposed to be valid only for small intervals of solid concentration (condition for which the changes in ϕ are small); also for a mixture of sugars (as is the case in honey) the constant K' would involve a "mixed" osmotic coefficient and an "average" molecular weight of sugar species.

A linear regression analysis was used to test the validity of eqn. (6) as applied to 36 fluid honey samples from Argentina. As shown in Fig. 1, honey closely followed (correlation coefficient, r = 0.985) the linear relationship between water activity and solids content suggested by eqn. (6), the regression line being,

$$a_w = 0.834 - 0.000544 \text{ . g solid/100g water} \qquad (7)$$

A plot of water activity versus % moisture (instead of solids content) should also follow a linear relationship but with positive slope, as shown in Fig. 2. The regression equation for this line is,

$$a_w = 0.262 + 0.0179 \text{ . \% moisture} \qquad (8)$$

and is of practical application since it gives the relationship between "% moisture", -as used by honey industry-, and the parameter water activity. The coefficient of determination, which is a measure of goodness of fit, was found to be, $r^2 = 0.969$ indicating that the regression model fits the data quite well in the range studied (15-21 % moisture); i.e., the estimated values of water activity come close to the observed ones.

Previous literature attempts have been made to correlate water activity and % moisture in honeys (Alcalá & Gómez, 1990; Estupiñán, Sanjuán, Millan & González-Cortés, 1998; Bogdanov, Rieder & Rüegg, 1987; Beckh et al., 2004), although the correlation coefficient and goodness of fit were significantly smaller than those reported here. Beckh et al. (2004) reported a_w and % moisture for a large number of honey samples (liquid, crystalline and partially crystalline) from various countries . Their data for 31 liquid honeys were plotted to test the validity of eqn. (6) and the results are shown in Fig. 3a; they also followed the straight line relationship indicated by eqn. (6). Fig. 3b shows the plot of water activity versus % moisture for the same data ; although a linear relationship between a_w and % moisture is also suggested, (correlation coefficient, r = 0.867) the relatively low value of the determination coefficient ($r^2 = 0.751$) indicates a somewhat more important prediction error as compared with present correlation for Argentine honeys. The regression line of Fig. 3b is given by,

$$a_w = 0.330 + 0.0141 \cdot \%\ \text{moisture} \tag{9}$$

Fig. 4 compares the regression equations for 36 fluid Argentine honeys (eqn. 8) and 31 fluid honeys of various countries (eqn. 9) reported by Beckh et al. (2004). They are similar but not equal since it is clear that the slope of both lines are different. This behavior may be attributed to the sugar profiles of honeys of different botanical source and geographical collection place, studied by Beckh et al. (2004) (their samples were from Spain, Germany, Italy, Australia, Rumania, China, Mexico,Vietnam, etc). As noted before, the behavior described by eqn. (6) is strictly valid for honeys of more or less the same sugar composition (i.e. ratio of fructose-glucose to other sugars) which may have not been the case for honeys studied by Beckh et al. (2004). This may be demonstrated as follows. A sample of Argentine honey was diluted with appropriate amounts of distilled water, and % moisture and water activity determined for each dilution. An almost “perfect” linear relationship (r = 0.997) was obtained between water activity and solids content (Fig. 5a) or moisture content (Fig. 5b). Fig. 6 compares the behavior of two different honey samples of Argentine following dilution with water; in all cases an excellent straight line relationship was observed (correlation coefficients (r) 0.997 to 0.999) between a_w and % moisture, although the regression equations for the two honeys were slightly different,

$$a_w = 0.278 + 0.0174 \cdot \% \text{ moisture}$$

$$a_w = 0.231 + 0.0198 \cdot \% \text{ moisture}$$

and this may be attributed to some differences in sugar composition. This behavior (lines of different slope) resemble to that shown in Fig.4.

Lack of accurate measurement of water activity should have been another reason for relatively poor goodness of fit for literature correlations between water activity and % moisture. Accurate measurements depend not only on the water activity measurement method utilized, but also on standards used for verification and proper temperature control (Fontana, 2002). In the last forty years or so, isopiestic equilibration, freezing point, hair or polymer, electrolytic, capacitance or dew-point hygrometers have been used to measure water activity in foods (Rahman, 1995; Favetto et al., 1983; Aguilera, Chirife, Tapia & Welti-Chanes, 1990).The accuracy of a_w determinations improved through those years up to present times, where for example, chilled mirror dew point instruments are accurate to about ± 0.003 a_w (Fontana, 2002).

4. Conclusions

The use of water activity to predict the storage behavior of honey, instead (or to complement) of moisture %, is recommended ; not only for a better prediction of likehood of fermentation, but also to predict moisture gain or loss when honey is exposed to different ambient relative humidities. It is suggested that correlation between water activity and % moisture must be determined for honeys of different botanical source and geographical collection place, in order to establish the most adequate relationships.

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Figure captions

Figure 1 – Test of eqn. (6) with Argentine fluid honeys (36 samples)

Figure 2 – Correlation between water activity (25 ºC) and % moisture in Argentine fluid honeys (36 samples)

Figure 3 – (a) Test of eqn. (6) with fluid honeys from various countries (data from Beckh et al, 2004) – (b) Correlation between water activity and % moisture in fluid honeys from various countries (data from Beckh et al., 2004)

Figure 4 – Comparison between correlations for Argentine fluid honeys (eqn. 8) and for fluid honeys from various countries (eqn. 9)(Beck et al., 2004)

Figure 5 – Straight line relationships for water activity and solid content (a) or % Moisture (b), for a diluted Argentine honey sample

Figure 6 – Correlations between water activity (at 25 ºC) and % moisture for two different

diluted Argentine honey samples.

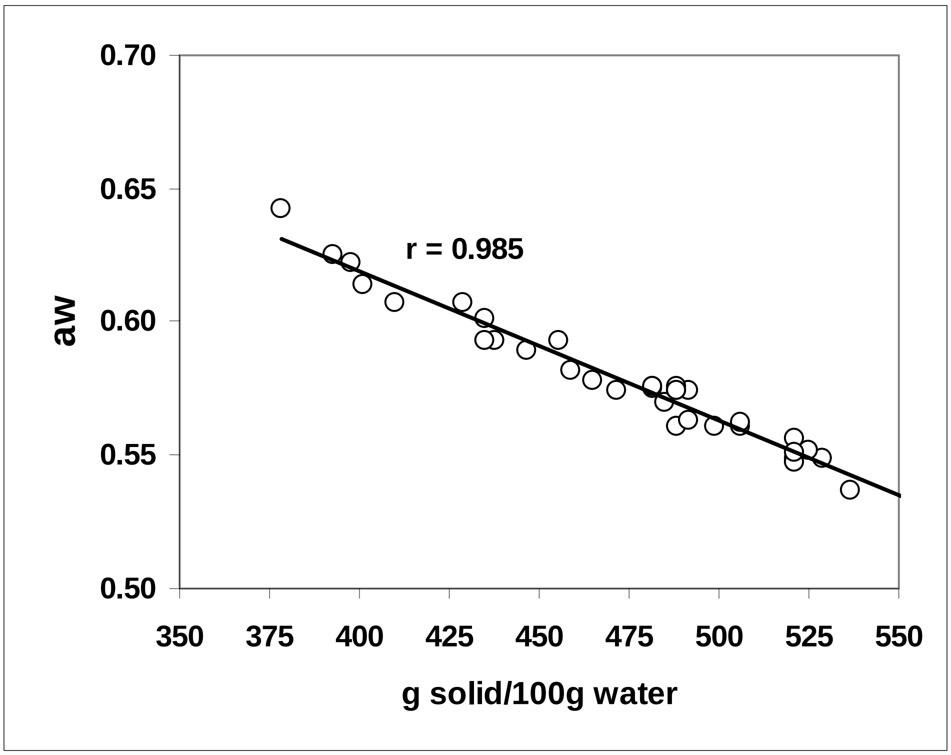

FIG. 1

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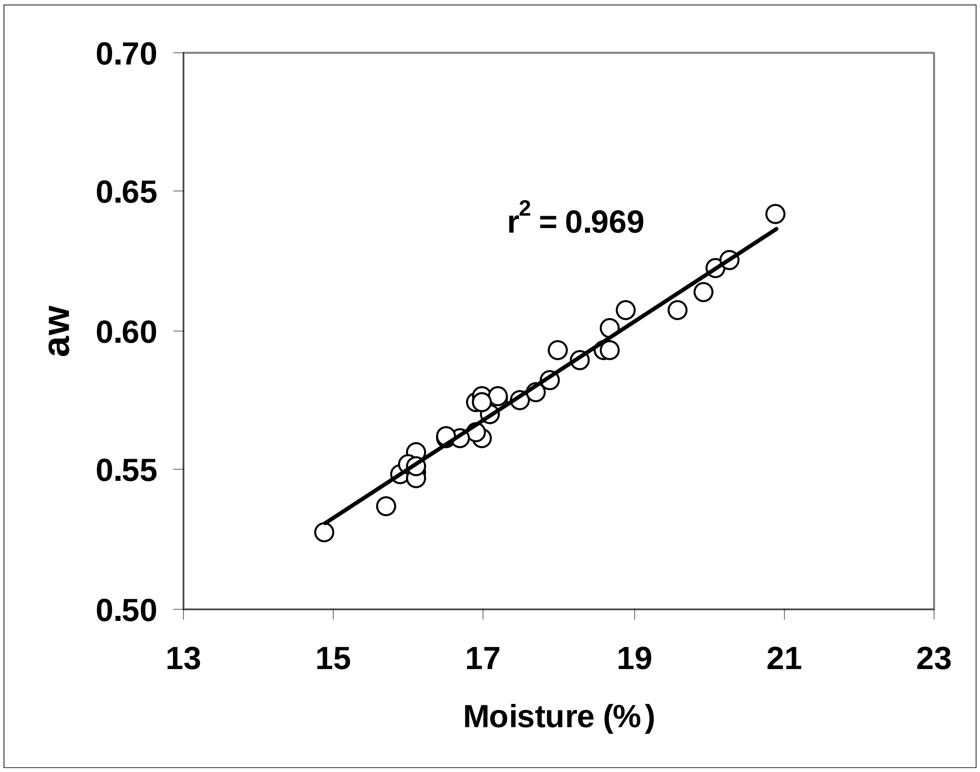

FIG.2

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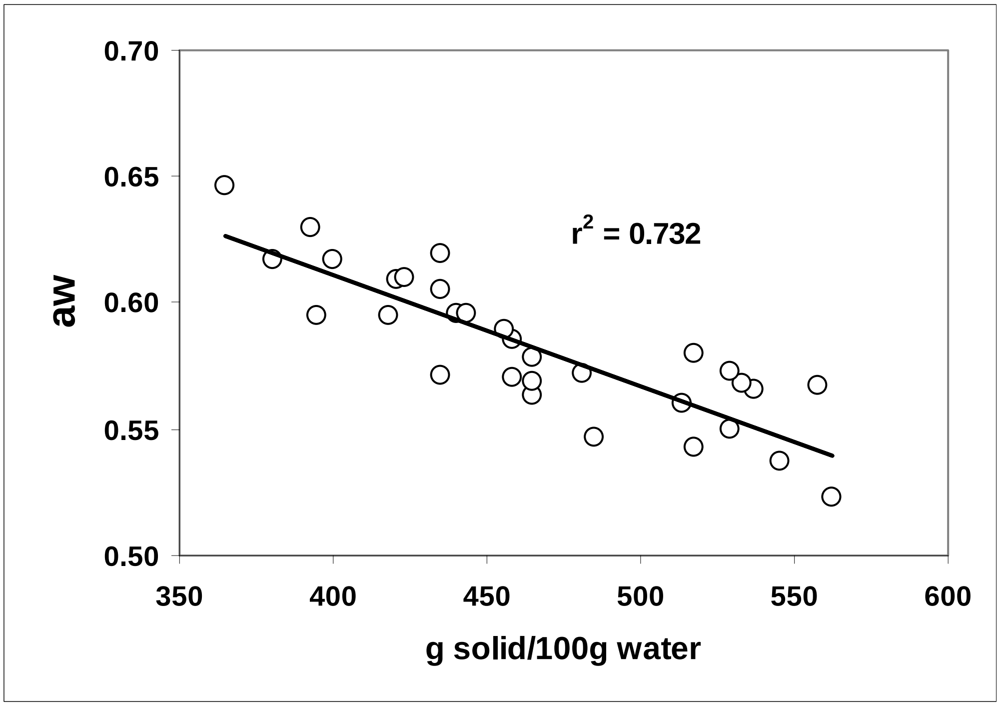

FIG.3a
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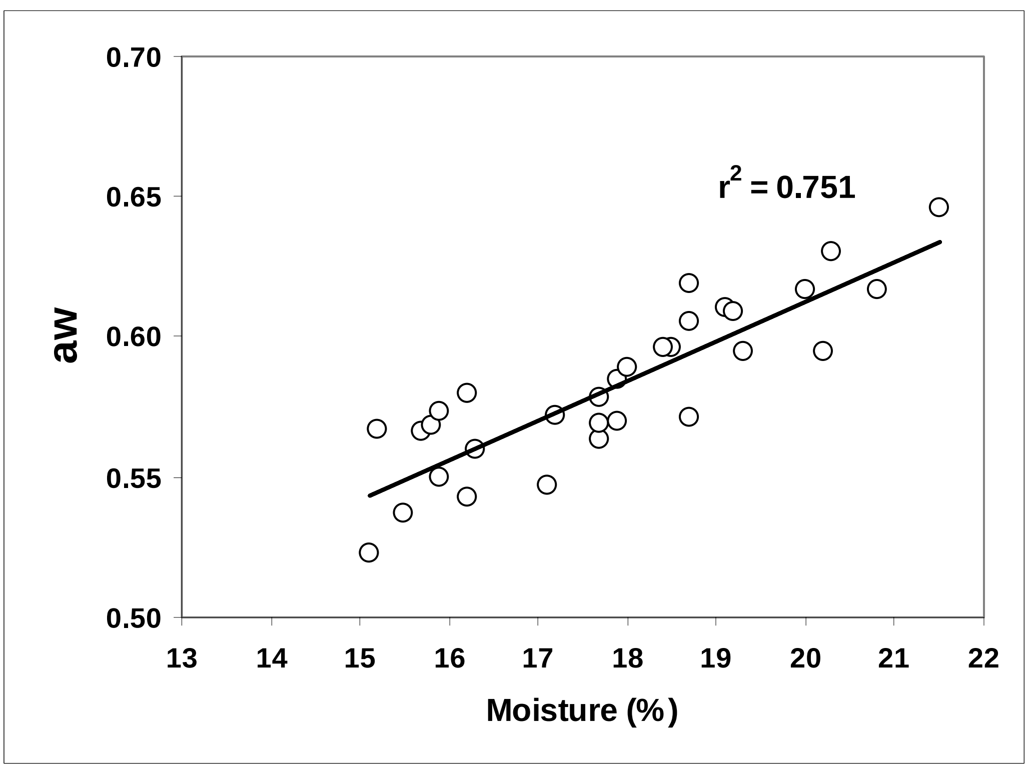

FIG.3b **Jorge Chirife, María Clara Zamora** and **Aldo Motto**

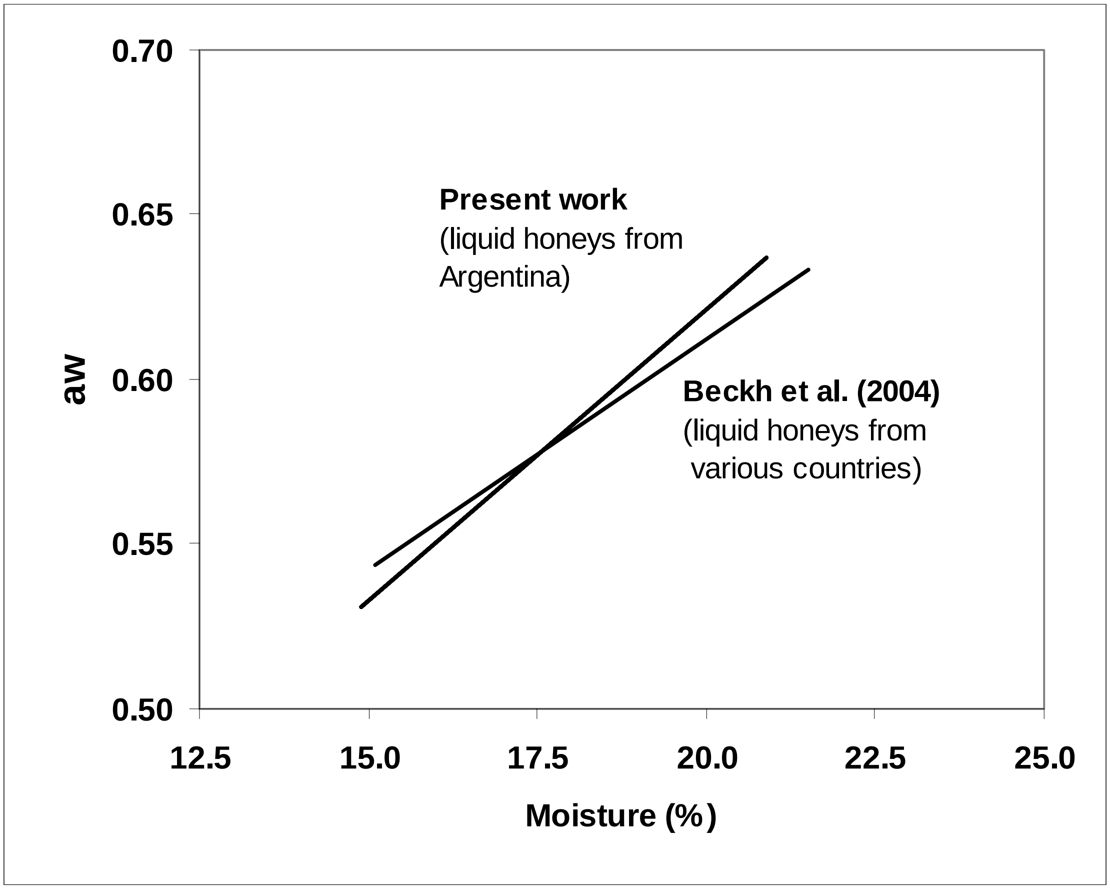

FIG.4

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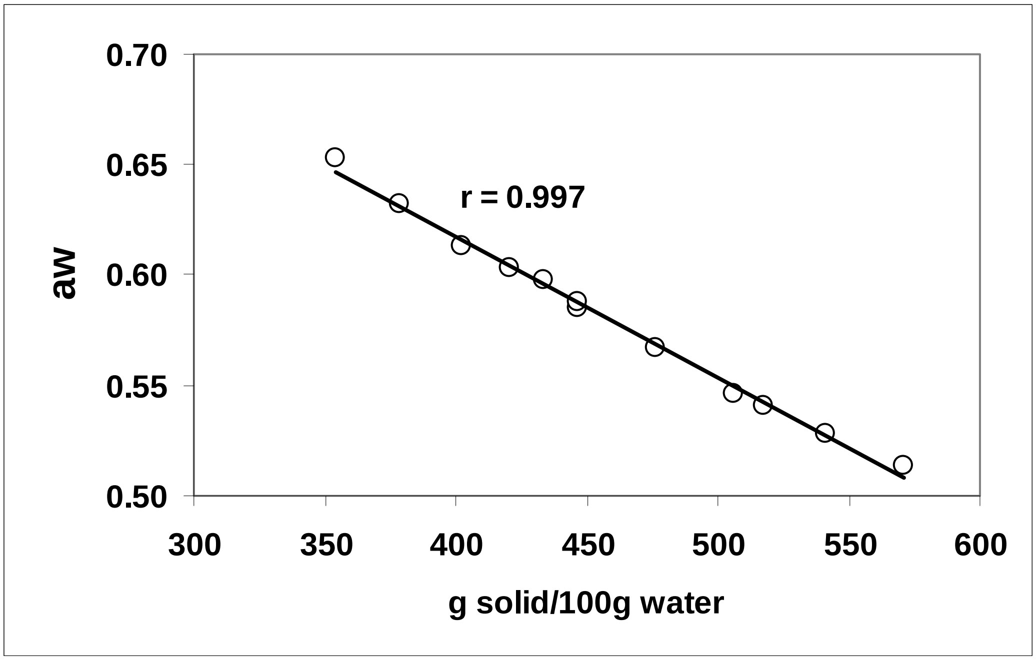

FIG.5a **Jorge Chirife, María Clara Zamora** and **Aldo Motto**

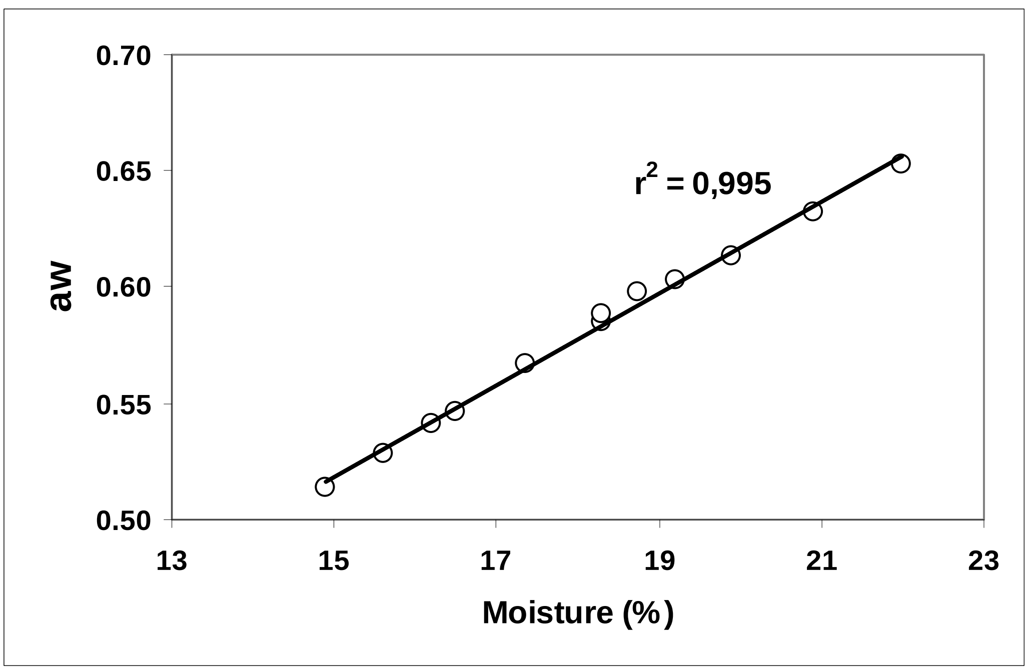

FIG.5b
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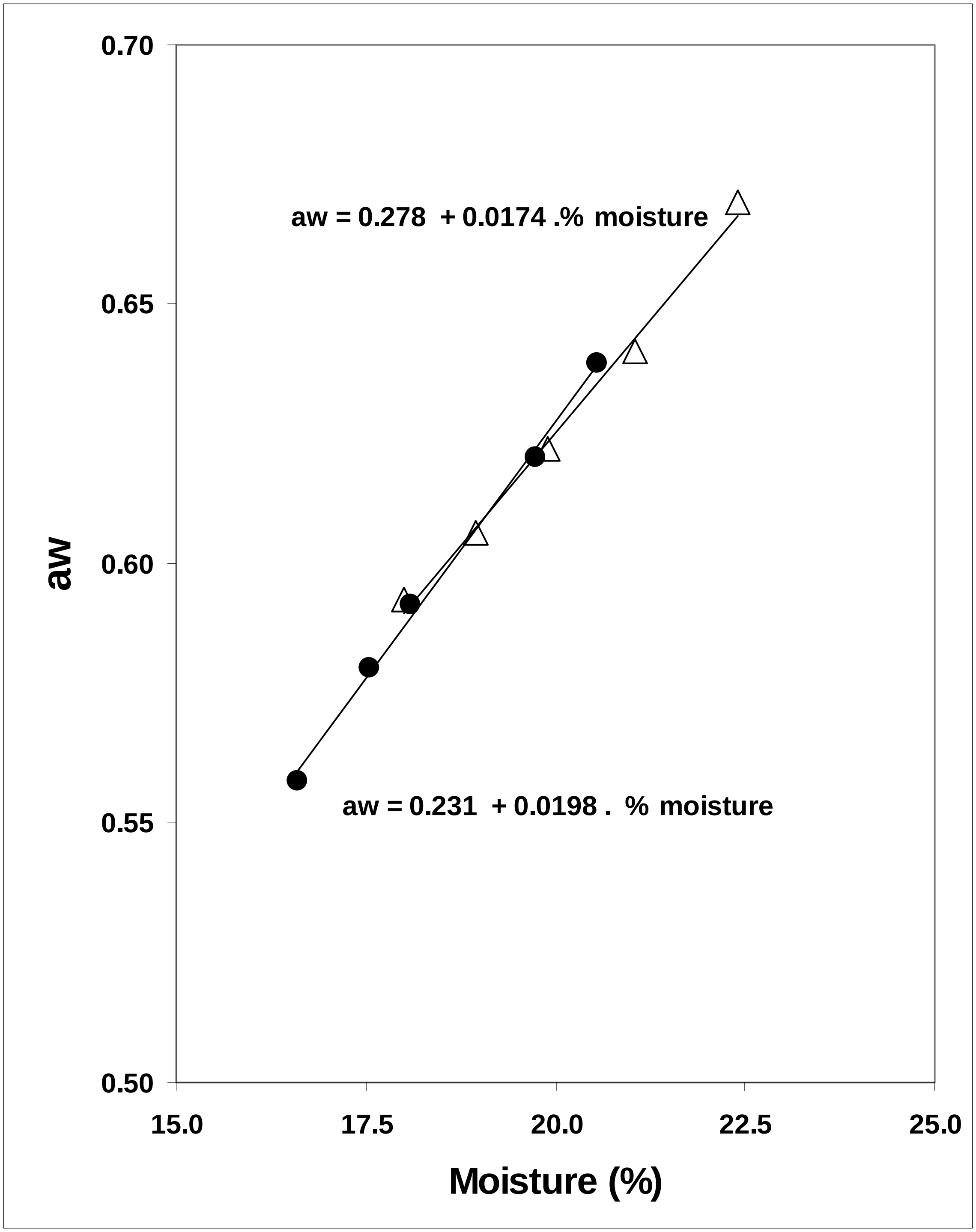

FIG.6

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