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On the nature of the relationship between water activity and % moisture in honey

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"ON THE NATURE OF THE RELATIONSHIP BETWEEN WATER ACTIVITY AND % MOISTURE IN HONEY"

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Abstract

Some theoretical basis of water activity in honey as well as the nature of its correlation with % moisture, are examined. For this purpose, the water activity of high concentrated glucose, fructose and glucose/fructose solutions (many of them supersaturated solutions) was determined and correlated with moisture content. It was found that the a_w of glucose and fructose was identical for practical purposes.

Literature data on the water activity and moisture content of a large number of honey samples (either fluid, crystalline or partially crystalline) from different geographical origin and botanical sources were statistically analyzed, and compared with the water activity of glucose/fructose solutions.

A survey of literature data was performed to obtain and compare regression equations between water activity and % moisture for honeys from different sources.

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

1. Introduction

It is well known that honey fermentation is caused by the action of osmotolerant yeast upon the sugars fructose and glucose resulting in formation of ethyl alcohol and carbon dioxide. The yeast 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 controls microbial growth (Troller, & Christian, 1978 ; Scott, 1953 ; Beuchat, 1987 ; Christian, 1963 ; Chirife, Zamora, & Motto, 2004). The limiting water activity for growth of osmotolerant yeasts (naturally found in honey) is about a_w = 0.61/0.62 (Zamora, & Chirife, 2004) ; 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 (Chirife et al., 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, original moisture of the nectar and others. Also, after extraction of the honey its moisture content may change depending on conditions of storage due to water transfer.

Chirife et al. (2004) recently examined some fundamental aspects of the relationship between water activity and % moisture in honey. They made a theoretical analysis on water activity in sugar solutions and honey, and determined the correlation between water activity and % moisture in 36 liquid samples of Argentine honeys. A very good straight line relationship 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.

It is the purpose of the present paper to further examine the theoretical basis of water activity in honey as well as the nature of its correlation with % moisture. The water activity of high concentrated glucose, fructose, and fructose : glucose (1 : 1) solutions was determined and compared with literature data for the water activity of honeys from different geographical origin and botanical sources.

2. Materials and Methods

2.1. Determination of water activity

The water activity of sugar solutions 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 0.43 – 0.75 (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 stored at 25 °C in an electronic chilling/heating plate for termal equilibration (Decagon Devices, Model 40510, Pullman, Washington, USA).

2.2. Sugars

Glucose and fructose were obtained from Laboratorio Cicarelli, Buenos Aires (Argentina). Concentrated solutions of glucose, fructose, or fructose : glucose (1 : 1) were prepared by adding distilled water to the sugars ; many of these solutions were supersaturated and were prepared by heating the sugar and water in hermetically sealed flasks, and then allowing to cool to room temperature.

3. Results and Discussion

The water activity of honey is determined by the molal concentration (moles/1000 g water) of soluble substances. Sugars represent the largest portion of honey composition and the monosaccharides fructose and glucose are the most abundant, while small amounts of disaccharides (mainly maltose and sucrose) are also present ; other disaccharides higher sugars (trisaccharides and oligosaccharides) are also present in small quantities. Table 1 shows the amount of fructose + glucose as % of total solids (for floral honeys of different origin) calculated from literature references. It can be seen that for almost all samples, fructose + glucose represents more than 80 % of total honey solids.

Since water activity is a colligative property, it depends of the number of moles dissolved in the water of honey; thus Table 2 compares the molal concentration (moles/100 g water) of (fructose + glucose), with that of maltose, sucrose, and other disaccharides, as found in various honeys. It can be seen that values for (fructose + glucose) are much higher than the others, indicating that the monosaccharides glucose and fructose would be main determinants of water activity reduction in honey, while maltose and sucrose (or other disaccharides) are much less important. Others substances of relatively high molecular weight or which are present in very small quantities make very little contribution to reduction of water activity in honey (Chirife, 1978; Chirife, Ferro Fontan, & Benmergui, 1980; Ruegg, & Blanc, 1981).

Favetto, Chirife and Fontán (1982) determined the water activity of fructose and glucose solutions up to 150 g solids/100 g water ; they reported that the water activity of fructose solutions may be considered equal to that of glucose, in that concentration range. However, actual concentrations of fructose and glucose in honey are much higher, usually involving supersaturation for glucose. Analysis of a large amount of literature data indicated that the sum of (fructose + glucose) in honey is in the approximate range of 330 to 527 g/100 g water. Thus, Fig. 1 shows a plot of experimentally determined a_w values versus solids content (280 to 510 g solids/100 g water) for glucose, fructose and the mixture (fructose + glucose) (1 : 1) solutions. The solubility of glucose at 25 ° C is 103.3 g glucose/100 g water and that of fructose is 405 g fructose/100 g water ; thus, all glucose values and a few of fructose corresponded to supersaturated conditions. It can be observed that all data (glucose, fructose, and (fructose + glucose)) fall in the same correlation; and also a linear relationship between a_w and solids content is apparent. The correlation equation is,

$$a_w = 0.890 - 0.0007 \cdot X \qquad (1)$$

where X is g solid/100g water and the correlation coefficient is 0.995.

The linearity of this plot is in agreement with Chirife et al. (2004) who suggested that a linear correlation is expected for relatively small intervals of solid concentration, as is the present case. Fig. 1 also confirms that the a_w of glucose and fructose may be considered identical (for practical purposes) in this high range of solids concentration, typically found in honey.

Fig. 2 shows the same data but plotted as a_w versus moisture content, and are also compared with values measured by Ruegg and Blanc (1981) for a mixture of sugars (48 % fructose + 40 % glucose + 10 % maltose + 2 % sucrose) resembling the composition of honeys. The regression equation for glucose and fructose is

$$a_w = 0.305 + 0.0155 \cdot M \qquad (2)$$

where M is % moisture and the correlation coefficient is 0.996

The similarity between both set of data confirmed that the water activity of honey is largely determined by fructose and glucose.

Beckh, Wessel, and Lüllmann (2004) reported a_w and % moisture for a very large number of honeys involving liquid, crystalline and partially crystalline samples from various botanical sources as well as different countries (i.e. from Mexico, China, Cuba, Argentina, Brazil, Vietnam, El Salvador, Germany, India, etc). Moisture content of honeys ranged between about 15-22 %. Beckh et al. (2004) analyzed separately fluid, partially crystalline and crystalline samples and calculated corresponding regression coefficients for the correlation between water activity and % moisture, which were 0.867, 0.872, and 0.851, for liquid, crystalline and partially crystalline samples, respectively. Although a correlation between water activity and % moisture was demonstrated, the goodness of fit of their correlations was poor, i.e. the estimated values of water activity did not come very close to the observed ones. Beckh et al. (2004) concluded that although moisture content and water activity in honey are effectively related, a mathematical formula cannot express the correlation correctly due to the complex nature of honey.

Although honeys (fluid, partially crystalline and also crystalline) are or may be in a non-equilibrium state because glucose is supersaturated the rate of glucose crystallization is very low (it is inhibited by kinetic factors) and allows measurement of water activity (Zamora & Chirife, 2004). For this reason we re-examined the data of Beckh et al. (2004) ; it was found that no significant differences existed between the correlations for fluid, partially crystalline and crystalline honeys, as indicated by an F value, $F_{2,8}$ = 2.351, and p = 0.176. Fig. 3 shows a plot of all their samples (128 samples ; either fluid, crystalline or partially crystalline ; including 58 samples for which the physical state was not specified) and corresponding regression line. The regression line obtained for fructose and glucose solutions (Fig. 2) is also showed in Fig. 3 for the purposes of comparison. The regression line for all honeys measured by Beckh et al. (2004) is,

$$a_w = 0.342 + 0.014 \text{ H \%} \qquad (3)$$

where H % is the % moisture content. The determination coefficient, is, r^2 = 0.529 and its low value indicates that the correlation has an important prediction error. Nevertheless, the regression line for honeys is not too far from that obtained for pure glucose and fructose solutions, also shown in the same Figure. It is noteworthy that although the goodness of data is relatively poor (ie. R2 = 0.529), the regression line of all honeys is quite similar to that of glucose /fructose (Fig. 3). At a first glance one would attribute the poor fitness of data to the different sugar spectrum of honeys of widely different botanical source and geographical collection place, as used by Beckh et al. (2004). For this reason we analyzed some of their data separately, i.e.

1) honeys from same botanical sources, ("Blute" and "Acazie"), and
2) honeys from same geographical collection place (México and China).

It was found that no significant differences existed between honeys grouped either by botanical source or place of collection. For example, when comparing "Blute", "Acazie", China and México, the F value is $F_{3, 11} = 1.382$, and $p = 0.317$. Moreover, r^2 values for each group did not improve as compared with value found for all honeys ($r^2 = 0.529$), with the exception of Mexican samples. Determination coefficients were, 0.285 for 20 samples of "Blute" honey (moisture range 17.1 - 20.1 %) ; 0.556 for 14 samples of "Acazie" honey (moisture range 16.2-20.8 %), 0.570 for 17 samples of China honey (moisture range 16.2-20.8 %), and 0.785 for 15 samples of Mexican honeys (moisture range 16.5 –21.5 %). Scattering of data (i.e. important prediction error) was still significant. This is in sharp contrast with the data recently reported by Chirife et al. (2004) who determined the correlation between water activity and moisture content in 36 samples of Argentine liquid honeys. In this case the regression model fitted quite well, as indicated by $r^2 = 0.969$ in the moisture range studied (15-21 %). Ruegg and Blanc (1981) measured the a_w and moisture content of various commercially available natural honeys (flower nectar or honeydew) from various countries; these samples were all crystalline but were liquefied at 42 °C before determination of water content and water activity. A regression analysis of their data showed that a straight line correlation between water activity and % moisture was also followed (regression coefficient 0.900) in the range 15-21 % moisture. The determination correlation is $r^2 = 0.811$, which is much better than that obtained from the data of Beckh et al. (2004).

Salamanca, Pérez and Serra (2001) also reported a straight line correlation between water activity and % moisture for Colombian honeys. Table 3 compares various regressions equations for honeys in the practical range 15-21% of moisture. It can be seen that the regression equation obtained with the data of Ruegg and Blanc (2004), Chirife et al. (2004) and Salamanca et al. (2001) were almost identical.

As shown in present paper, compositional factors of different honeys does not seem to justify the dispersion of data reported by Beckh et al. (2004). The determination of moisture content in honeys is a well known and standardized method (refractometric); thus, we are inclined to believe that a lack of accurate measurement of water activity may have been a reason for the poor goodness of fit observed in some cases. 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 the accuracy of a_w determinations improved through years up to present times, where for example, chilled mirror dew point instruments are accurate to about ± 0.003 a_w (Fontana, 2002).

The problem of inaccurate determination of water activity in honey seemed to have been also present in a work reported by Comi, Manzano, Lenardon, Cocolin and Cantoni (2000); they evaluated the physical-chemical parameters (including a_w) influencing yeast fermentation in Italian honeys. Comi et al. (2000) reported that fermentation at 30 °C occurred in honey at an a_w as low as 0.57, being Zygossaccharomyces spp. the most common isolated strain. This result is in open contradiction with a vast amount of literature data (too much to be mentioned here) collected in the last 50 years which showed conclusively that no microbial growth may occur below aw = 0.61. So we may suspect that something was wrong with the determination of water activity.

Unaccounted crystallization of honey may be also another reason for mistakes in the determination of the correlation between water activity and % moisture. Most honeys are non equilibrium systems because they are supersatured in glucose and this sugar may crystallize in the form of glucose monohydrate. This crystallization lowers the solute concentration in the liquid phase and thus increase the water activity (Zamora and Chirife, 2004). A problem may arise if moisture content is measured in the liquid honey but water activity is measured after crystallization occurs. This will lead to a mistake in the evaluation of the correlation a_w-moisture content.

Additional research on the correlation of water activity and % moisture in honeys of different botanical sources and geographical origin, is needed, using reliable devices for measuring water activity, as well as consideration that honey is a non-equilibrium system likely to crystallize.

References

Beckh, G., Wessel, P., & Lüllmann, C. (2004). Naturliche bestandteile des honigs : Hefen und deren stoffwechselprodukte - Teil 2 : Der wassergehalt und die wasseraktivität als qualitätsparameter mit bezug zum hefewachstum. *Deutsche Lebensmittel-Rundschau*, 100, Jahrgang, Helt 1,: 14-17.

Beuchat, L. (1987). Influence of water activity on growth, metabolic activities and survival of yeasts and molds. *J. Food Protection*, 46, 135-141.

Chirife, J. (1978). Prediction of water activity in intermediate moisture foods. *J. Food Technology,* 13, 417-421.

Chirife J., Ferro Fontan C.,& Benmergui, E.A. (1980).The prediction of water activity in aqueous solutions in connection with intermediate moisture foods. 4. a_w prediction in nonelectrolyte solutions. *J. Food Technology* 15, 59-65.

Chirife, J., Zamora, M.C., & Motto, A. (2004). The correlation between water activity and % moisture in honey : Fundamental aspects and application to Argentine honeys. Submitted for publication.

Christian, J.H.B. (1963). Water activity and the growth of microorganisms. *Recent Advances in Food Research*, 3, 248-255.

Comi, G., Manzano, M., Lenardon, M., Cocolin, L., & Cantoni, C. 2000. Parametric he influenzano a'lterazione microbiologica del miele. *Industrie Alimentarie*, 34, 1127-1133.

Favetto, G.J., Chirife, J., & Ferro Fontán, C. (1982). The water activity of fructose solutions in the intermediate moisture range. *Lebensm. Wiss. U. Technologie,* 15, 159-160.

Favetto, G.J., Resnik, S.L., Chirife, J., & Ferro Fontán, C. (1983). Statistical evaluation of water activity measurements obtained with the Vaisala Humicap humidity meter. *J. Food Science*, 487, 534-538.

Fontana, A.J. (2002). Measurement of water activity. Fundamentals of Water Activity, *IFT Continuing Education Committee*, June 14-15, Anaheim, CA, USA.

Joshi, S.R., Pechhacker, H., Willam, A., & von der Ohe, W. (2000). Physico chemical characteristics of *Apis dorsata*, *A. cerana* and *A. mellifera* honey from Chitwan district, Nepal. *Apidologie*, 31, 367-375.

Mateo R., & Bosch-Reig, F. (1998). Classification of Spanish unifloral honey by discriminant analysis of electrical conductivity, color, water content, sugars and pH. *J. Agric. Food Chem.,* 46, 393-400.

Mendes E., Brojo Proenca, E., Ferreira, I.M.P.L.V.O., & Ferreira, M.A. (1998). Quality evaluation of Portuguese honey. *Carbohydrate Polymers*, 37, 219-223.

Messallam, A.S., & El-Shaarawy, M. (1987). Quality attributes of honey in Saudi Arabia. *Food Chemistry,* 25, 1-11.

Mossel, B., Bhandari, B., D'Arcy, B., & Caffin, N. (2003). Determination of viscosity of some Australian honeys based on composition. *Int. J. Food Properties*, 6, 87-97.

Persano Oddo, L., & Piro, R. (2004). European Unifloral honeys: descriptive sheets. *Technical Report* from the International Honey Commission.

Ruegg, M., & Blanc, B. (1981).The water activity of honey and related sugar Solutions. *Lebensm. Wiss. Und Technologie*, 14, 1-6.

Salamanca, G.G. Pérez, F.C., & Serra, B. J.A. (2001). Determinación de la actividad de agua en mieles colombianas de las zonas de Bocayá y Tolima. Apiservices – *Galería Apícola Virtual.*

Scott, W.J. (1953). Water relations of *Staphylococcus aureus* at 30 °C. *Australian Journal of Biological Science*, 6, 549-556.

Snowdon, J.A., & Cliver, D.O. (1996). Microorganisms in honey. *Int. J. Food Microbiology,* 31, 1-26.

Spettoli, P., Bottacin, A., Pescioa, P., & Girolami, V. (1982). *Industrie Alimentari,* September.

Troller, J.A., & Christian, J.H.B. (1978). Water Activity and Food. Academic Press, New York.

White, J.W., Riethof, M.L., Subers, M.H., & Kushnier, I. (1962). Composition of American honey. *Tech. Bull.* U.S. Dep. Agriculture No. 1261.

Zamora, M. C., & Chirife, J. (2004). Determination of water activity change due to crystallization in honeys from Argentina. *Food Control* (in press).

Table 1 – Concentration of fructose + glucose as % of total solids, for various floral honeys (calculated from literature data).

Honey	(Fructose + Glucose), as % total solids
Floral U.S. honey (average 490 samples) [(a)]	**83.9**
Italian unifloral from Erica arborea (average 8 samples) [(b)]	**92.7**
Spanish unifloral : rosemary, citrus, lavender, sunflower, eucalyptus, heather & forest (average from 93 samples) [c]	**81.9**
Australian unifloral : heath, tea tree, yapunyah, yellow box (average from 4 samples) [(d)]	**83.7**
Saudi Arabia (average from 5 samples) [(e)]	**81.9**
Australian multifloral (average from 3 samples) [(f)]	**78.5**
Portugal multifloral (average from 8 samples) [(f)]	**80.7**
European unifloral honeys : brassica, calluna,, Castanea, citrus, eucalyptus, helianyhus, lavandula, rhododendron, rosmarinus, taraxacum, thymus, tilia (average from 4346 samples) [(g)]	**86.4**

Data from :

(a) White et al. (1962)

(b) Spettoli et al. (1982)

(c) Mateo and Bosch-Reig (1998)

(d) Mossel et al. (2003)

(e) Messallam and El-Shaarawy (1987)

(f) Mendes et al. (1998)

(g) Persano Odo and Piro (2004)

Table 2- Moles of sugars per 100 grams of water in honey

Honey	**(Fructose + Glucose)**	**Maltose**	**Sucrose**	**Other disaccharides**	**Reference**
Average U.S. honey	2.24	0.124	0.022		(a)
Spain (rosemary)	1.96	0.060	0.025	0.077	(b)
Spain (citrus)	2.05	0.055	0.068	0.050	(b)
Spain (lavender)	2.27	0.078	0.015	0.095	(b)
Spain (eucalyptus)	2.48	0.090	0.005	0.096	(b)
Australia (multifloral)	2.55	Traces	0.028	Traces	(c)
Greece (multifloral)	2.35	Not detected	Not detected	0.018	(c)
Portugal (rosemary)	2.39	Not detected	0.012		(c)
Nepal (*apis dorsata)*	1.71	0.022	0.0003	0.049 (includes trisaccharides)	(d)

(a) : White et al. (1962)   (b) : Mateo and Bosch-Reig (1998)
(c) : Mendes et al. (1998)   (d) : Joshi et al. (2000)

Table 3. Comparison of regression equations for honeys in the practical range of moisture content.

Regression equation	r^2	Source of data	Comments
a_w= 0.342 + 0.014 H %	0.529	Beckh et al (2004)	Large number of samples of different botanical sources and geographical collection places (fluid, crystallized, partially crystallized)
a_w= 0.271 + 0.0177 H %	0.811	Ruegg and Blanc (1981)	Liquid honeys from various countries
a_w= 0.267 + 0.0177 H %	0.970	Chirife et al. (2004)	Fluid Argentinian honeys
a_w= 0.248 + 0.0175 H %	0.947	Salamanca et al. (2001)	Colombian honeys from Bocayá and Tolima

Legends for Figures

Figure 1 – Water activity of concentrated glucose (supersaturated) and fructose solutions at 25 °C.

Figure 2 – Correlation between water activity and % moisture in glucose/fructose solutions and comparison with literature data for the a_w of a mixture of sugars (fructose, glucose, maltose and sucrose) resembling the composition of honey (Ruegg, & Blanc, 1981).

Figure 3– Literature data for water activity of 128 honey samples (fluid, partially crystalline and crystalline) from different botanical sources and geographical locations (Beckh et al., 2004), as compared with behavior of glucose/fructose solutions.

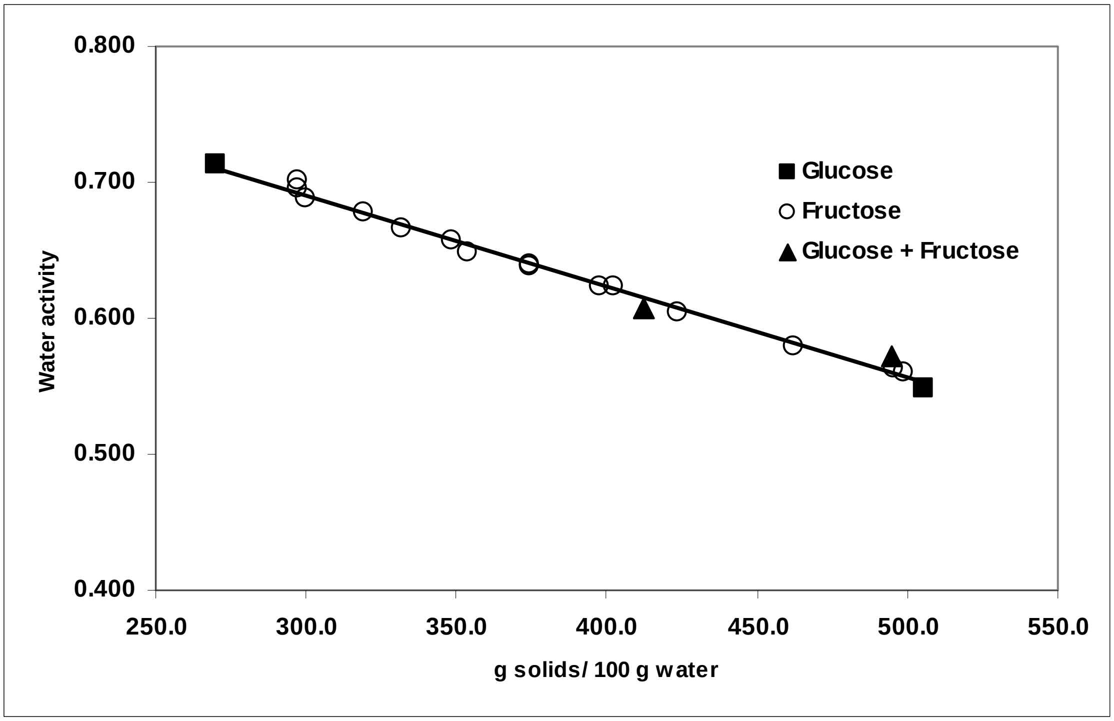

Figure 1

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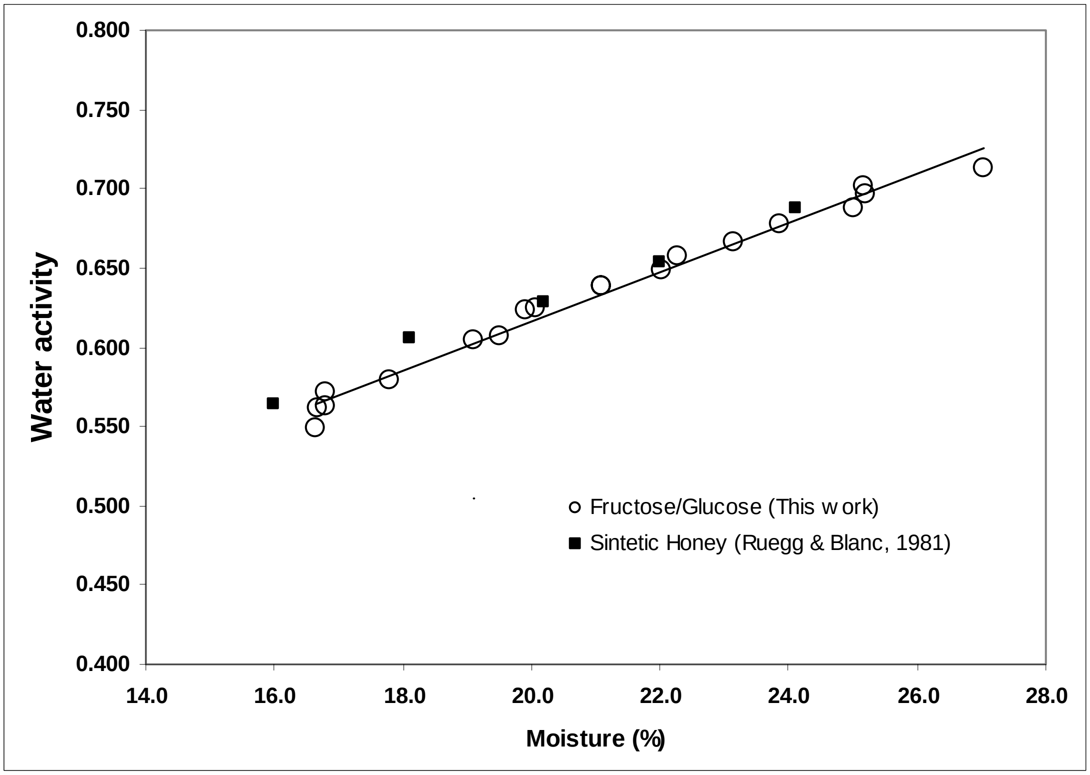

Figure 2

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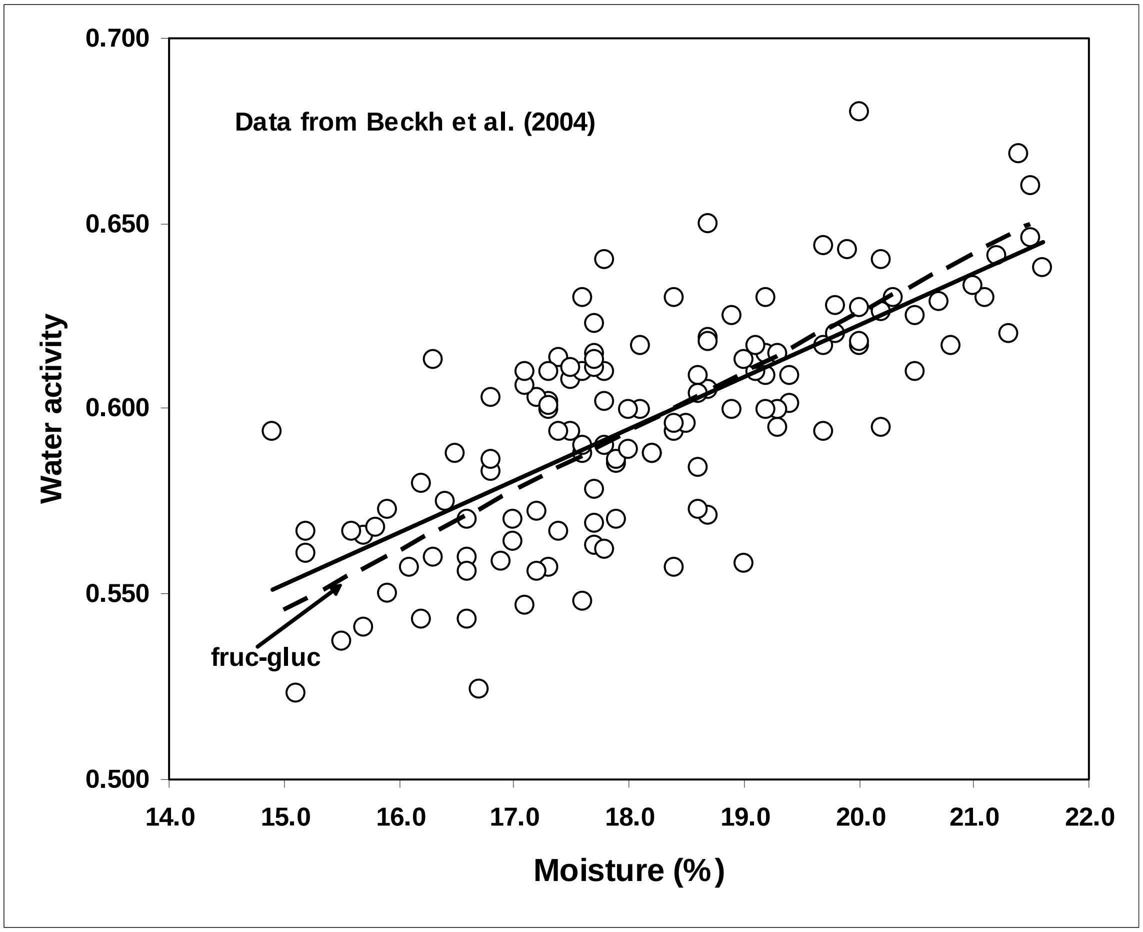

Figure 3

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