Sánchez, V. ; Baeza, R. ; Ciappini, C. ; Zamora, M. C. ; Chirife, J.

Comparison between Karl Fischer and refractometric method for detemination of moisture in honey

Short communication

"Comparison between Karl Fischer and refractometric method for determination of moisture in honey"

V. Sanchez [1], R. Baeza [1], C. Ciappini [2], M.C. Zamora [1, 3, *] and J. Chirife [1]

[1] Facultad de Ciencias Agrarias,
  Pontificia Universidad Católica Argentina,
  Cap. Gral. Ramón Freire 183, C1426AVC, Buenos Aires, Argentina

[2] Universidad del Centro Educativo Latinoamericano
Avenida Pellegrini 1332, Rosario, Santa Fe, Argentina.

[3] Member of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
  Rivadavia 1917, Buenos Aires, Argentina

* Corresponding author : zamoramariacl@gmail.com

Abstract

The aim of this work was to compare refractometric index (RI) and Karl Fischer (KF) titration methods for moisture content measurement in honeys. In addition, the effectiveness of two different solvents (methanol (M) and methanol: formamide in the ratio 1:1 (M+F)) was evaluated.

Results indicated that RI and KF methods yielded similar results for moisture determination in honeys; mainly, when the solvent M+F was used. This solvent mixture (M+F) also allowed a reduction in titration time which may be a potential advantage for measuring moisture content in honey.

Key words : Honey ; Refractometric; Moisture content; Karl Fischer

Introduction

Moisture content is a quality parameter important for honey shelf life and is critical in order to prevent from microbiological spoilage ; moisture also affects some physical properties of honey, such as viscosity and glucose crystallization (Bogdanov et al., 2004; Isengard et al., 2001).

Moisture content of honey is usually determined by an indirect method based on soluble solids content estimation through the refractometric index (RI). Since the composition of honey solids may vary in different honeys, this affects the conversion of RI into moisture content. Thus, moisture determination in honeys by refractometry does not yield exactly the "true" moisture content ; nevertheless it is a simple, fast and reproducibly method and for this reason is successfully used in routine honey control.

Gravimetric methods based on drying (with conventional oven or infrared drying) have been also used to determine moisture content of honey, but the high viscosity of the rubbery matrix formed during drying difficult water diffusion leading to an underestimation of moisture content. Besides, other volatile substances present in honey may evaporate even of those which might be produced by chemical reactions during the process itself (Isendard et al. 2001).

Karl Fischer (KF) titration is known to determine water selectively by a chemical reaction (Scholz; 1984). Despite it is an expensive and time-consuming method, it is considered the most accurate for determining moisture content ; is to be noted that values obtained may somewhat depend on experimental conditions of titration (solvent utilized, temperature).

The aim of this work was to compare RI and KF titration methods for moisture content measurement in honeys. Moreover, in the case of KF the effectiveness of two different solvent systems were also evaluated.

Materials and Methods

Honey

Honey samples of the 2007 harvest were collected and packaged in glass flasks hermetically sealed. Sampling was carried out in honey production and bee-hive product areas of Santa Fe province, Argentina (28º - 35º SL; 58º-62º WL). Twenty two samples of floral origin were used in this study.

Fructose solutions

Model solutions consisting of supersaturated solutions of fructose (Laboratorio Ciccarelli, Buenos Aires, Argentina) in the moisture range of 14.8 - 22.1% (resembling total soluble solids in honeys) were prepared by adding distilled water to fructose. Solutions were heated in sealed flasks to reach complete solubilization ; it was verified that no fructose crystallization from these supersaturated solutions occurred over the time frame of measurements. These model solutions were used because allowed us to know accurately the true moisture content.

Moisture content of honeys

Moisture content was determined either by refractive index (RI) or Karl Fischer (KF) titration. Cristallized or partially crystallized honeys samples were liquefied at about 42-45°C in hermetically sealed glass container before moisture determination.

A digital honey moisture refractometer Pal-22S, Atago (Japan) was used to obtain moisture content (%) ; measurements were made by duplicate at 25 ± 1 °C and the average informed.

KF titration were carried out at 25 ± 1 °C with a Karl-Fischer titrator DL 31 from Mettler Toledo , applying the one-component technique with Hydranal Titrant composite 5 from Riedel-de Haën, Germany. Pure methanol or a methanol : formamide mixture (1:1) were used as solvent and they were purchased from Merck, Darmstadt, Germany. Sample sizes were approximately 100 mg and were analyzed twice each. The standard deviation for KF titration using as solvent methanol or methanol : formamide mixture, was calculated from six replicate measurements performed on a honey sample of about 17 % moisture, and found to be 0.24 and 0.16 (% moisture) respectively.

Determination of water activity

Water activity of honeys samples were also measured in order to test the appropriateness of moisture content, as determined by KF method, to predict water activity of honeys. Water activity was measured 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 maintains a temperature stable sampling environment. The equipment was calibrated with saturated salt solutions in the water activity range of interest (Favetto et al., 1983). For each determination three replicates were obtained and the average reported.

Results and Discussion

As mentioned before, refractive index measurements are frequently used for moisture content determination in honeys. However, it has been reported that Karl Fischer (KF) titration method may give more accurate values (Bogdanov et al., 2004).

The reliability of KF method to accurately determine moisture content in model solutions of fructose of known moisture values, was first evaluated. **Figure 1** shows the correlation between measured (determined by KF ; solvent methanol) and actual moisture values. An excellent linear correlation ($R^2 = 0.996$) was observed. The average percentage error ($\bar{\varepsilon}\%$) from measured and actual data (Figure 1) was calculated using the following equation:

$$\bar{\varepsilon}\% = \frac{\sum |x - x_c|}{n} \bullet 100 \qquad\qquad (1)$$

where

x = measured moisture % (KF method) ,

x_c = calculated moisture % ,

n = number of data

The value of $\bar{\varepsilon}\%$ was 0.56 % indicating that KF yields very accurate moisture values in highly concentrated fructose solutions.

An important aspect in KF titration is the total solubilization and availability of water from the sample. In the case of honey, high viscosity could affect the water transfer to the titration solvent. For this reason two solvent systems were evaluated for moisture determination in honeys, : methanol (M) and methanol : formamide in the ratio 1:1 (M+F), at room temperature. **Table 1** compares moisture content values of honeys obtained either by RI and KF method with different solvent systems. It is to be noted that the use of M+F mixture as solvent for KF method, allowed a notably reduction in titration time, which is a potential advantage. The following observations can be made form the data shown in Table 1, : a) for moisture contents below 17% the values obtained by KF using the solvent mixture (M+F) were somewhat higher than those obtained with methanol ; b) in the whole moisture range (14-22 %) values obtained by KF with (M+F) solvent were in general closer to RI values than those obtained by KF with (M) solvent.

Other authors have used different conditions during KF titrations to optimize the measurement time and water solvation from the sample. Isengard

et al. (2001) reported a shortening in determination times increasing the temperature of titration to 50°C. **Figure 2** shows the correlation between moisture % measured by RI and moisture % measured by KF titration using (M+F) at room temperature ; the data of Isengard and Schultheiß (2003), at 50°C using (M) solvent were also plotted for the purposes of comparison. In both cases, present work and Isengard and Schultheiß (2003), a similar behavior was observed and good linear regressions were obtained, although a higher regression coefficient (R^2 = 0.934 vs. 0.864) was achieved in present work.

Previous literature reports indicated that values of moisture content determined by KF are a little different from those measured by RI. These differences could be attributed to the botanical origin and the nature of dry matter of honey (Isengard et al., 2001; Isengard and Schultheiß, 2003).

In last years water activity was studied as criteria of microbiological stability of honeys as alternative to moisture content (Chirife et al., 2006) and efforts were made to correlate water activity with refractometric moisture content. Eqns. (2) and (3) show linear regression equations between water activity and moisture content (for present honeys) determined either by RI or KF measurements with (M+F) solvent. It can be seen that both equations are almost identical,

$$a_w = 0.2748 + 0.0171 \cdot \text{Moisture \% (KF)} \qquad (R^2 = 0.928) \qquad (2)$$

$$a_w = 0.2702 + 0.0172 \cdot \text{Moisture \% (RI)} \qquad (R^2 = 0.959) \qquad (3)$$

The similarity between eqn. (2) and eqn. (3) confirms that both methods for measuring moisture (RI or KF with (M+F) solvent**)**, can be satisfactorily used to predict water activity from knowledge of moisture content of honey.

References

Bogdanov, S., Ruoff, K., & Persano Oddo, L. (2004). Physico-chemical methods for the characterization of unifloral honeys: a review. *Apidologie, 35,* 4-17.

Chirife, J., Zamora, M.C., & Motto, A. (2006). The correlation between water activity and % moisture in honey: Fundamental aspects and application to Argentine honeys. *Journal of Food Engineering, 72,* 287-292.

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

Isengard, H-D., Schultheiß, D., Radović, B., & Anklam, E. (2001). Alternatives to official analytical methods used for the water determination in honey. *Food Control, 12,* 459-466.

Isengard, H-D., & Schultheiß, D. (2003). Water determination in honey – Karl Fischer titration, an alternative to refractive index measurements?. *Food Chemistry, 82,* 151-154.

Scholz, E. (1984). *Karl Fischer titration*. Berlin: Springer.

Legends for figures

Figure 1 : Comparison of measured (KF method, solvent methanol) and *actual moisture contents in model solutions of fructose.

Figure 2 : Correlation between KF method and RI measurements of moisture content in several honeys : data from present work (○); data from Isengard and Schultheiß (2003) (♦) .
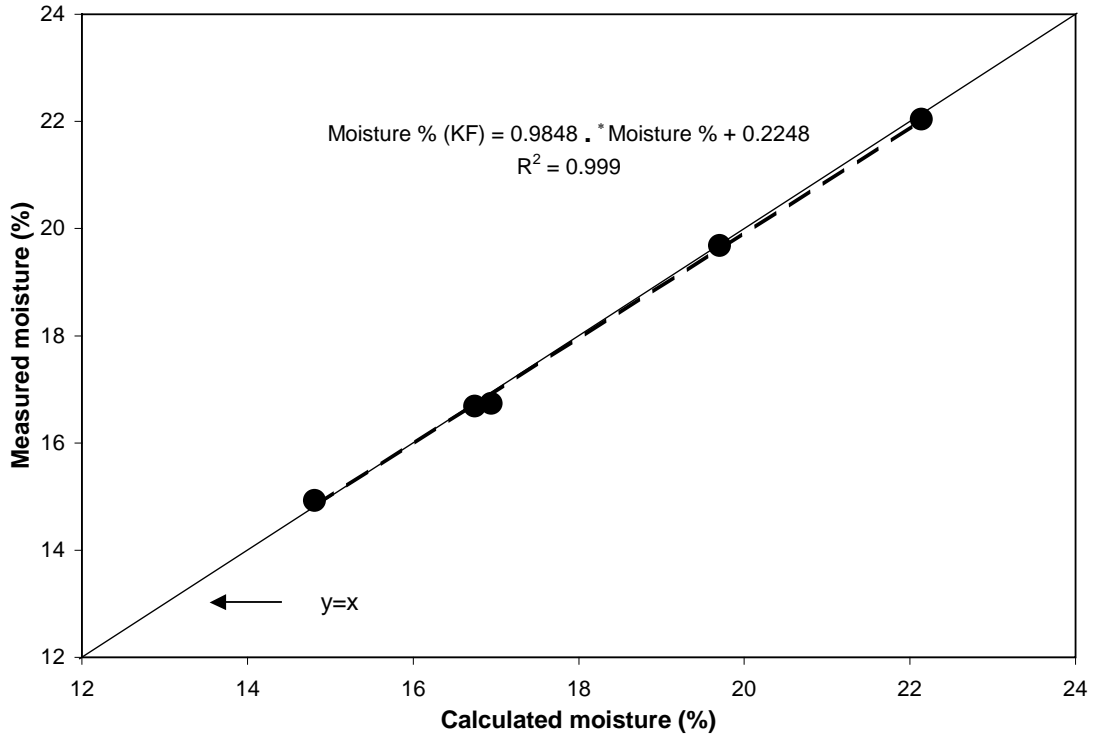
Figure 1

Figure 2

Moisture % (KF) = 0.9848 $\cdot$ * Moisture % + 0.2248
$R^2 = 0.999$

y=x

Isengard and Schultheiß, 2003
y = 1.0531x - 0.816
$R^2 = 0.864$

Present work
KF (M+F)
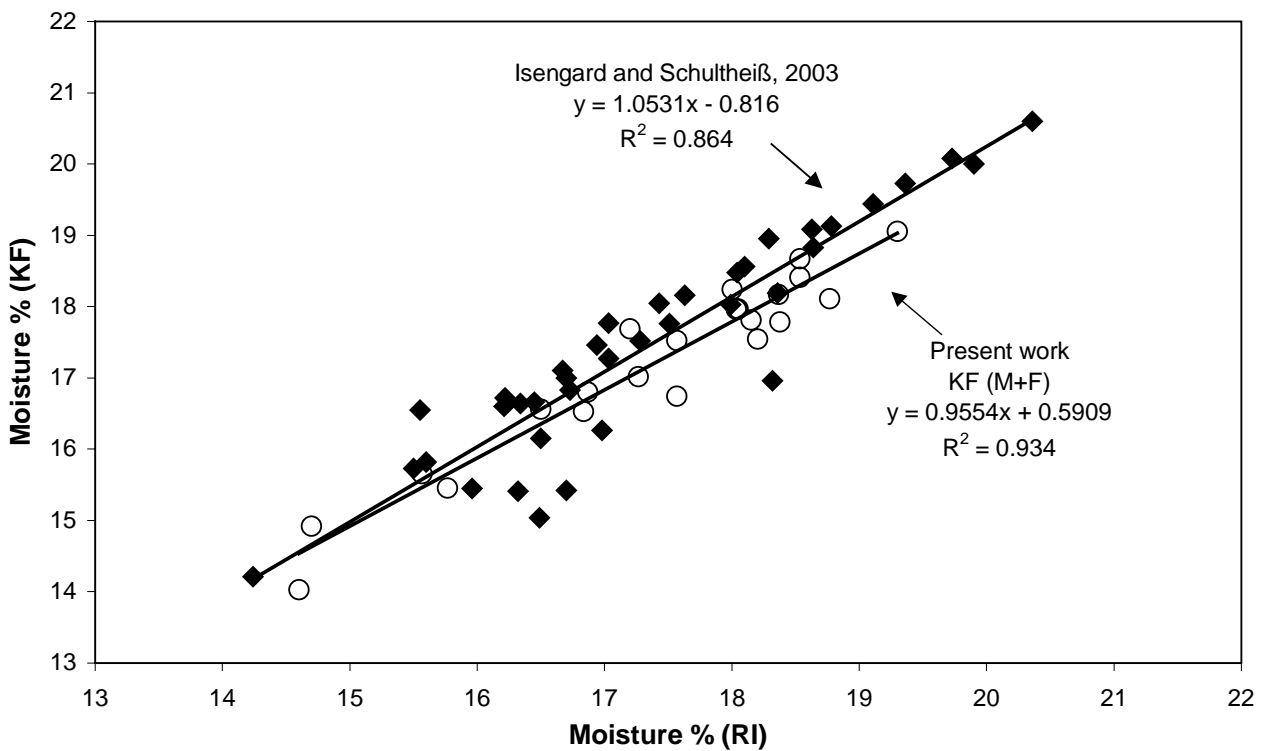y = 0.9554x + 0.5909
$R^2 = 0.934$

Table 1. Moisture content (%) in honeys determined by three methods: a) Karl Fischer titration (KF) using methanol; b) KF titration using methanol : formamide, and c) refractometric index (RI).

Honey Sample	Method		
	KF (methanol)	KF (methanol:formamide)	RI
1	14.07	14.03	14.6
2	14.20	14.92	14.7
3	14.92	15.65	15.6
4	15.10	15.45	15.8
5	15.81	16.56	16.5
6	16.11	16.53	16.8
7	17.52	17.52	17.6
8	16.63	16.74	17.6
9	16.38	16.80	16.9
10	17.09	17.02	17.3
11	17.21	17.69	17.2
12	18.32	18.24	18.0
13	17.99	17.81	18.2
14	17.60	17.96	18.0
15	17.77	17.97	18.1
16	17.79	18.41	18.5
17	18.26	18.68	18.5
18	17.55	17.78	18.4
19	17.87	17.54	18.2
20	17.90	18.11	18.8
21	19.36	18.17	18.4
22	21.12	19.05	19.3