

WATER DETERMINATION IN BEE PRODUCTS USING THE KARL FISCHER TITRATION METHOD

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Received 28 September; accepted 19 November 2009

S u m m a r y

One of the most widely used techniques for water content determination in food is Karl Fischer (KF) titration. Compared to other methods based on loss of weight, the primary advantage of the Karl Fischer titration method is its high selectivity to water. The aim of the study was to develop and validate the Karl Fischer method for moisture determination in pollen loads, royal jelly, bee venom and propolis. The effects of sample weight and mixing/homogenization time were investigated. A study of the main validation parameters (repeatability and reproducibility) of the elaborated methods for the studied bee products was also conducted.

Optimal parameters (sample weight and minimal mixing/homogenization time) for water determination in bee products using the Karl Fischer method were established as follows: sample weight: 0.10 - 0.20 g and time of sample homogenization: 120 s for bee-collected pollen, 0.05 - 0.10 g and 120 s for bee venom, 0.02 - 0.05 g and 180s for royal jelly and 0.20g and 300s for propolis, respectively. The coefficient of variation of the results received for series determinations of water content in each studied bee product with the exception of propolis, conducted at repeatability and reproducibility conditions did not exceed 10%.

Mean values for water content of the bee products were as follow: 6.99% for bee venom, 18.34% for fresh and 6.25% for dried bee-collected pollen, 62.56% for royal jelly and 2.30% for propolis. These results will be used to establish water requirements and will be introduced into the International Honey Commission (IHC) standards.

Keywords: bee-collected pollen, royal jelly, bee venom, propolis, water content, Karl Fischer method.

INTRODUCTION

Moisture determination in bee products is used for a variety of reasons. It is used to reveal the remaining dry matter content and nutritional value determination of that dry matter. Moisture determination is also used for confirmation of storage stability and quality parameter requirements by established standards (Szczęsna et al., 1995; Szczęsna et al., 2002; Szczęsna, 2006a,b,c; Szczęsna, 2007a,b; Campos et al., 2008). There are many methods for water determination in these bee products:

drying at different temperatures (65°C, 90°C, 105°C) under reduced pressure, refractometric analysis, and using the Karl Fischer method (Serra Bonvehi and Casanova, 1987; May et al., 1989; Serra Bonvehi, 1991). All of these methods have been specified by the different error sources derived from applied methodology. Results received for bee-collected pollen by Serra Bonvehi and Casanova (1987) ranged from 4.71% for Karl Fischer titration to 13.39% for drying at 105°C. Drying at over 80°C

resulted in losses of volatile compounds in pollen loads. The moisture content in this product had been overestimated. Refractometric methods of water determination in royal jelly have a high error count. The reason for the high error count is that royal jelly has a high content of water insoluble matter (Serra Bonvehi, 1991).

Karl Fischer titration is an analytical method for the quantitative determination of water in many biological samples. The literature data show that compared to other methods which take into account bee-collected pollen, this method is characterized by higher accuracy and repeatability (Serra Bonvehi and Casanova, 1987). Moreover, when using the Karl Fischer method a smaller sample weight (1 µg - 1000 mg) is required for analysis. This is very important especially in the case of bee venom, because collecting even 1g requires collecting it from many bee colonies (Rybak et al., 1995). Karl Fischer titration is only possible when water in food samples is freely available. Thus, a suitable sample preparation has to be selected and done to release water completely, prior to the KF titration. Although Karl Fischer titration itself is a fast method (1 - 2 min for a single analysis), the sample preparation step is time-consuming, e.g. 1-2 h for performing an external extraction. The use of a high-speed stirrer for internal homogenization, offers a fast, quick and efficient alternative to more traditional sample preparation techniques.

The laboratory of the Department of Bee Products at our Institute was established with a quality system in accordance with PN-EN ISO/IEC 17025: 2005 "General requirements for testing competence and calibration standards in laboratories". In 2006 it was accredited by the Polish Centre for Accreditation. The laboratory tests the

physical properties and main quality parameters for honey. New analytical procedures (methods) for quality control of other bee products should be validated according to the above mentioned standard and quality system document for laboratory (General Procedure PO-03, 2008, E-02: "Validation, quality control of test").

The aim of the study was to develop and validate the Karl-Fischer method for use in determining moisture in pollen loads, royal jelly, bee venom and propolis.

MATERIAL AND METHODS

Chemicals and reagents

Karl Fischer reagents: HYDRANAL® Composite - 5 mg H₂O/ml (Composite 5), HYDRANAL®- Methanol dry (max. 0.01% H₂O) and HYDRANAL®-Sodium-Tartrate-2-hydrate (15.66 ± 0.05% H₂O) were purchased from Riedel-de Haen.

Karl Fischer method

Karl Fischer moisture measurements were done using Mettler Toledo Karl Fischer Titrator DL38 integrated with IKA Homogenizer. In this procedure iodine from HYDRANAL® Composite 5 reacts quantitatively with water from a sample in a solvent containing sulfur dioxide and imidazole, dissolved in diethyleneglycol monoethyl ether (DEGEE). HYDRANAL®- Methanol dry is used as a medium for the titration vessel. Water content was calculated from the consumption of HYDRANAL® Composite 5 and its water equivalent (5 mg H₂O/ml).

Elaboration of the procedures depended on the determination of optimal sample weight and homogenization time for each analysed bee product with the aim of receiving satisfactory results. These parameters were collected and shown in Tab. 1. Water determination was done in different range of sample weight

(0.01 - 0.50 g) for these products and in different homogenization time (60 - 360 s, with 60 s increase). Analyses were done in 7 replicates for each weight and in 7 replicates for each homogenization time.

Validation of the procedure

For validation purposes repeatability and reproducibility of the elaborated methods was established by serial analysis of water determined in each of the studied products. Analyses within each series were conducted in repeatability conditions and analyses between series were conducted in reproducibility conditions. Seven series for pollen loads and three series for other bee products (bee venom, royal jelly, propolis) were done. Received results were evaluated according to General Procedure PO-03, E-02: 2008 "Validation, quality control of test".

HYDRANAL ®-Sodium-Tartrate-2-hydrate ($15.66 \pm 0.05\% \text{H}_2\text{O}$) was used as a control sample for the Karl Fischer method. This sample was analysed for moisture periodically to make sure that the Karl Fischer method was working properly and that everything was under control.

Material

Bee-collected pollen, royal jelly and bee venom samples were collected at apiaries of the Bee Technology Department of Apiculture Division in Puławy, Poland

from 2005 to 2007. Propolis samples were purchased from Polish beekeepers from 2007 to 2008. Collected samples were kept frozen (-21°C) until analyses. Water content was also analyzed in dried bee-collected pollen. The drying process was done at a temperature of about 40°C using a special drying process destined for this product.

RESULTS AND DISCUSSION

The received results show that sample weight and time of sample homogenization had a significant influence on the results of water content in bee pollen, royal jelly, bee venom and propolis. For bee venom, satisfactory results (coefficient of variation 5.3%) were received for 0.05 g sample weight of and 120 s homogenization time (Tab. 2). A lower coefficient of variation (2.4 - 3.0%) was calculated for results received for higher sample weight (0.10 - 0.20 g). For bee-collected pollen, satisfactory results (coefficient of variation 2.8%) were received for sample weight of 0.10 g and homogenization time 120 s. Worse, but still satisfactory results (coefficient of variation 7.3%) were received for a higher sample weight (0.30 g). For royal jelly, satisfactory results (coefficient of variation 5.20%) were received for a sample weight of 0.01 g and

Table 1

Sample weight and mixing/homogenization time

Bee product	Sample weight (g)	Mixing/homogenization time (s)
Bee venom	0.01; 0.02; 0.05; 0.10; 0.20	60; 120; 180; 240; 300
Honeybee-collected pollen	0.05; 0.10; 0.20; 0.30; 0.40	60; 120; 180; 240; 300
Royal jelly	0.01; 0.02; 0.03; 0.04; 0.05; 0.10	60; 120; 180; 240; 300
Propolis	0.10; 0.20; 0.30; 0.40; 0.50	60; 120; 240; 300; 360

homogenization time 180 s. Better results (coefficient of variation 0.9 - 3.0%) were received for a higher sample weight of this product (0.02; 0.03; 0.04 and 0.05 g). For propolis, satisfactory results (coefficient of variation below 10%) were received for sample weight in the range of 0.20 - 0.50 g and homogenization time 300 s.

In the case of homogenization time of the samples during water determination using the Karl Fischer method, for bee venom, satisfactory results (coefficient of variation below 10%) were received for 120 s at least and 0.05 g sample weight (Tab. 3). Further extending of homogenization time (180, 240 and 300 s) gave similar results. For bee-collected pollen, satisfactory results were received

for 120 s and 0.10 g sample weight. Further extending of homogenization time (180, 240 and 300 s) had no influence on the results for water content in this product. For royal jelly, satisfactory results (coefficient of variation 6.3%) were received for 120 s and 0.02 g sample weight and extending the homogenization time to 180 s reduced the coefficient of the results to 1.4%. For propolis, satisfactory results (coefficient of variation 7.2 - 7.5%) were received for 300 - 360 s and 0.20 g sample weight.

Optimal parameters (sample weight and minimal mixing/homogenization time) for water determination in bee products by the Karl Fischer method were shown in Tab. 4. For bee-collected pollen, sample

Table 2
Results for water content in bee products depending on sample weight (n=7)

Bee product (homogenization time)	Sample weight (g)	Water content (%)	
		Mean \pm SD	Coefficient of variation (%)
Bee venom (120 s)	0.01	6.32 \pm 1.05	16.61
	0.02	6.48 \pm 0.98	15.12
	0.05	6.53 \pm 0.35	5.34
	0.10	6.51 \pm 0.16	2.44
	0.20	6.58 \pm 0.20	3.04
Honeybee-collected pollen (120 s)	0.05	14.29 \pm 1.47	10.28
	0.10	14.14 \pm 0.39	2.76
	0.20	14.21 \pm 0.36	2.54
	0.30	14.37 \pm 1.04	7.25
	0.40	14.15 \pm 1.09	7.68
Royal jelly (180 s)	0.01	62.27 \pm 3.23	5.20
	0.02	62.35 \pm 0.54	0.86
	0.03	62.78 \pm 0.78	1.24
	0.04	64.25 \pm 1.95	3.03
	0.05	65.01 \pm 1.68	2.58
	0.10	65.12 \pm 4.59	7.05
Propolis (300 s)	0.10	1.74 \pm 0.22	12.36
	0.20	1.84 \pm 0.07	3.99
	0.30	1.81 \pm 0.09	5.21
	0.40	1.78 \pm 0.08	4.60
	0.50	1.70 \pm 0.14	8.16

Table 3

Results for water content in bee products depending on mixing/homogenization time (n=7)

Bee product (sample weight)	Mixing/homogenization time (s)	Water content (%)	
		Mean \pm SD	Coefficient of variation (%)
Bee venom (0.05 g)	60	6.53 \pm 0.72	11.03
	120	6.42 \pm 0.34	5.30
	180	6.57 \pm 0.35	5.32
	240	6.49 \pm 0.40	5.25
	300	6.45 \pm 0.39	4.78
Honeybee-collected pollen (0.10 g)	60	4.65 \pm 0.47	10.21
	120	4.77 \pm 0.17	3.48
	180	4.63 \pm 0.13	2.88
	240	4.71 \pm 0.16	3.45
	300	4.56 \pm 0.14	2.98
Royal jelly (0.02 g)	60	61.78 \pm 7.62	12.34
	120	62.09 \pm 3.88	6.25
	180	61.76 \pm 0.85	1.37
	240	61.25 \pm 1.26	2.05
	300	62.05 \pm 0.88	1.42
Propolis (0.20 g)	60	1.82 \pm 0.37	20.4
	120	1.75 \pm 0.27	15.6
	240	1.80 \pm 0.20	10.9
	300	1.78 \pm 0.13	7.2
	360	1.86 \pm 0.14	7.5

weight was established at 0.10 - 0.20 g, and time of sample homogenization was set at 120 s. For bee venom these parameters were established as follow: 0.05 - 0.10 g and 120 s, for royal jelly: 0.02 - 0.05 g and 180 s, and for propolis: 0.20 g and 300 s, respectively.

Received results for water determination in bee products (pollen, bee venom, royal jelly and propolis) according to the Karl Fischer method were characterized by satisfactory repeatability and a satisfactory reproducibility. Mean coefficient of variation for series analysis of pollen samples with water content from 3.0 to 22% conducted in repeatability conditions was 2.7% and in reproducibility conditions

- 8.3% (Tab. 5). For series analysis of royal jelly (water content 60-65%) the coefficients of variation for repeatability and reproducibility were - 1.8 and 5.3% respectively, for bee venom (water content 6 - 9%) coefficients were - 3.5 and 6.4% respectively, and for propolis (water content 1.7 - 2.6%) 4.9 and 20% respectively. Serial analysis of reference material (HYDRANAL-Standard Sodium Tartrate-2-Hydrate) show that water content in this material was on the level of 15.79%, with the coefficient of variation of reproducibility 2.6%.

Elaborated procedures were used for water determination in bee product samples collected during the 2005-2008 seasons

Table 4

Optimal parameters (sample weight and minimal mixing/homogenization time) for water determination in bee products using the Karl Fischer method

Bee product	Sample weight (g)	Minimal mixing/homogenization time (s)
Bee venom	0.05 - 0.10	120
Honeybee-collected pollen	0.10 - 0.20	120
Royal jelly	0.02 - 0.05	180
Propolis	0.20 - 0.30	300

Table 5

Repeatability and reproducibility of water determination results in bee products using the Karl Fischer method

Bee product (water content %)	Repeatability		Reproducibility	
	Coefficient of variation (%)		Coefficient of variation (%)	
	From -to	Mean	From -to	Mean
Bee venom (6-9%)	2.44 - 5.34	3.45	2.87 - 9.55	6.38
Honeybee-collected pollen (3-22%)	2.54 - 3.48	2.70	3.06 - 10.25	8.25
Royal jelly (60-65%)	0.86 - 2.58	1.83	2.05 - 8.75	5.25
Propolis (1.7 -2.6%)	1.80 - 8.16	4.90	6.26 - 26.25	20.00

Table 6

Water content determined in bee products using the Karl Fischer method (%)

Bee product (number of samples)	From - to	Mean
Bee venom (16)	5.90 - 8.89	6.99
Honeybee-collected pollen fresh (32)	10.16 - 21.37	18.34
Honeybee-collected pollen dried (32)	2.79 - 8.56	6.25
Royal jelly (5)	60.15 - 65.18	62.56
Propolis (10)	1.70 - 2.60	2.30

(Tab. 6). Water content of bee venom was about 7%, and ranged from 5.90 to 8.89%. Water content in fresh bee-collected pollen was about 18%, and ranged from 10.16 to 21.37%, Pollen dried at a temperature of about 40°C was characterized by a much lower moisture content which ranged from 2.79 to 8.56%, with 6.25% mean value. Water content in royal jelly was 62.56% and in propolis 2.30%.

CONCLUSIONS

1. Elaboration and validation of the procedure for water determination using the Karl Fischer method is an efficient method for testing honeybee - collected pollen, royal jelly, bee venom and propolis.
2. Results of water content for bee pollen, royal jelly, bee venom and propolis will be used to establish requirements of this parameter in these products and will be introduced into the International Honey Commission (IHC) standards.

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OZNACZANIE WODY W PRODUKTACH PSZCZELICH METODĄ MIARECZKOWĄ KARLA FISCHERA

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S t r e s z c z e n i e

Oznaczanie wody w produktach pszczelich bywa rzadko celem samym w sobie. Jest ono przeprowadzane głównie w celu potwierdzenia innych cech badanego produktu, w szczególności: trwałości w trakcie przechowywania, oznaczenia zawartości suchej pozostałości (masy), określenia wartości odżywczej oraz potwierdzenia zgodności parametrów jakościowych produktu z określonymi normami. Spośród wielu metod oznaczania wody w produktach spożywczych, na uwagę zasługuje metoda chemiczna Karla Fischera, charakteryzująca się dużą precyzją i powtarzalnością. Dotychczas była ona w małym zakresie wykorzystana do oznaczania wody w produktach pszczelich.

Celem badań było opracowanie i walidacja procedur badawczych oznaczania wody w obnóżach pyłkowych, mleczku pszczelim, jadzie pszczelim i propolisie z zastosowaniem metody Karla Fischera. Próbkę materiału badawczego pozyskane zostały w latach 2005-2007 w pasiekach Zakładu Technologii Pasiecznych Oddziału Pszczelnictwa ISK w Puławach, metodami zalecanymi do pozyskiwania tych produktów. Badania nad opracowaniem metody wykonano w Zakładzie Produktów Pszczelich na aparacie Karla Fischera firmy Mettler Toledo DL38 zintegrowanym z homogenizatorem IKA Labortechnik. Dobrano optymalne parametry miareczkowania takie jak: wielkość naważki i warunki rozpuszczania próbki (homogenizacji). Walidacja opracowanych procedur badawczych została przeprowadzona na podstawie serii niezależnych pomiarów wykonanych w warunkach powtarzalności i odtwarzalności wewnątrzlaboratoryjnej.

Istotnymi parametrami wpływającymi na wyniki oznaczeń wody w badanych produktach pszczelich metodą Karla Fischera okazały się wielkość naważki i czas homogenizacji próbki. W przypadku pyłku, optymalna wielkość naważki wynosi 0,1 - 0,2 g, a czas mieszania (homogenizacji) 120 s, dla jadu pszczelego parametry te zostały ustalone następująco: naważka - 0,05 - 0,10 g, czas mieszania 120 s, dla mleczka pszczelego - naważka 0,02 - 0,05 g, czas mieszania 180 s i dla propolisu - naważka 0,20 - 0,30 g, czas mieszania 300 s. Opracowane procedury badawcze oznaczania wody w produktach pszczelich (pyłku, jadzie, mleczku i propolisie) charakteryzowały się zadowalającą powtarzalnością i odtwarzalnością. Dla wszystkich produktów pszczelich za wyjątkiem propolisu, parametr ten dla serii badań wykonanych w warunkach powtarzalności i odtwarzalności wewnątrzlaboratoryjnej był mniejszy od 10%.

Opracowane procedury badawcze zostały wykorzystane do oznaczenia zawartości wody w próbkach produktów pszczelich. Średnia zawartość wody w jadzie pszczelim wynosiła 6,99%, w obnóżach pyłkowych świeżych - 18,34%, w obnóżach suszonych - 6,25%, w mleczku pszczelim - 62,56% i 2,30% w propolisie.

Słowa kluczowe: obnóża pyłkowe, mleczko pszczele, jad pszczeli, propolis, zawartość wody, metoda Karla Fischera.