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ScienceDirect

Food Chemistry 106 (2008) 1485–1490

Food Chemistry

www.elsevier.com/locate/foodchem

Influence of the correction for moisture/water content on the quality of the certification of cadmium, copper and lead mass fractions in rice

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Received 26 October 2006; received in revised form 21 February 2007; accepted 21 February 2007

Abstract

The moisture/water content of rice sample was determined by means of three different methods: oven drying, Karl Fischer titration and thermogravimetry. These results were applied for the certification of the Cd, Cu and Pb mass fractions by isotope dilution mass spectrometry, IDMS, in this rice material.

Two series of measurements were carried out in parallel, first on a set of water "saturated" samples, and second on a set of "non-saturated" samples also corrected for progressive water pick-up. The hygroscopicity correction factor at 1 min (corresponding to the addition of the IDMS spike) was conservatively taken as $H_{1\,\mathrm{min}} = 0 \pm 0.70$ ($k = 2$) from 0.25% to 0.35% spread observed for isotherms established for 15 samples. For the first method, the following optimal sequence was established: successive drying sessions of at least 8 h in a ventilated oven at 85 ± 2 °C until constant mass is attained (and no more than 0.001 g difference between successive weighing). Under these conditions, it was found that the apparent moisture/water content measured with the three methods for "non-saturated" samples coincided within one standard deviation. From this agreement it is reasonable to assume that moisture in this rice material was essentially "free" water. These results also showed that the oven drying method has the potential to produce reproducible, and possibly meaningful, data providing that a reasonable temperature of drying was identified.

Beside, it was found that the uncertainty on these corrections (particularly the hygroscopic effect) plays an important role for all investigated elements in "non-saturated" rice samples (up to nearly 60% in the case of Cu, 25% for Cd and 7% for Pb). The contribution of the uncertainty on the dry mass correction was found to be below 1% for the "saturated" samples. However, the principles of arranging water saturation of the samples prior to their processing introduced supplemental important technical complications and risks of sample contamination.

These issues, nearly never addressed in the case of the uncertainty propagation, are particularly important to achieve comparability conditions for analytical results produced from different laboratories.

Keywords: Rice sample; Moisture/water content; Hygroscopicity; ID-ICP-MS; Uncertainty estimation; Oven drying; Karl Fischer titration; Thermogravimetry; Traceability

1. Introduction

Measuring the composition of food samples is important for both toxicological and nutritional reasons. Accuracy of the measurement results is necessary, particularly for trace elements. Many declarations on food composition are based on dry matter and depend, therefore, on the way the water fraction is measured and corrected for.

Knowledge of the water content, one of the main parameters for characterisation of food stability (microbial activity) and quality (sensory properties), is required for legal and labelling reasons (Isengard, 1995).

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0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.02.048

Food materials contain different proportions of "free" water molecules and different forms of "bound" water (chemically or physically bound), capillary, trapped or bulk water. In addition, "free" water may be present under gaseous, liquid or solid forms (Isengard, 1995). The problem is that many of the analytical procedures developed for water content determination do not address necessarily the same water fraction within the same molecular environment. These procedures vary in cost, speed, sensitivity, specificity, ease of operation, etc. Moreover, the choice of an analytical procedure depends on the nature of the food being analysed and the reason for which this information is needed. This means that the claimed moisture/water content of a particular food material is operationally defined and may depend on the method used to carry out the measurement (Isengard, 1995). In this paper it is assumed that the expression "moisture content" covers the relative mass loss observed during drying experiments, which is possibly broader than the "water content" alone (Rückold, Grobecker, & Isengard, 2000).

Furthermore, the uncertainty on the moisture/water content determination maybe significant (Isengard, 2001; Rückold, Grobecker, & Isengard, 2001a) even though it is rarely evaluated and taken into consideration. The Institute for Reference Materials and Measurements (IRMM) certified the Cd, Cu and Pb mass fractions in a rice test material, and these values served as reference for comparison 19 of the International Measurement Evaluation Programme (IMEP) (Vassileva & Quétel, 2004). Cadmium data were also used as the IRMM contribution to the CCQM (Comité Consultatif pour la Quantité de Matière) Key Comparison 24 on the same rice material. CCQM-K24 was organised by IRMM on behalf of the International Bureau of Weights and Measures (BIPM) among 11 National Measurement Institutes. For the purpose of certification a measurement procedure based on isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) applied as a primary method of measurement was developed (Vassileva & Quétel, 2004). The rice powder was observed to be highly hygroscopic and two series of certification were carried out in parallel. Firstly on a set of "saturated" samples (i.e. in equilibrium with the ambient humidity) only corrected for their moisture content, and secondly on a set of "non-saturated" samples also corrected for water pick-up occurring before the gravimetric addition of the IDMS isotopically enriched spikes.

For each of the certified mass fractions in the "saturated" and "non-saturated" samples combined uncertainties associated to the measurement results were evaluated according to ISO guidelines (ISO-GUM, 1995) using the uncertainty propagation procedure.

This paper reports what was observed during experiments carried out to measure the moisture/water content in this rice material, using three different methods. It includes the description of results that served as basis for the requirements made to CCQM-K24 participants concerning the procedure for moisture content determination. Finally, it examines the importance of the correction for moisture or water content on the uncertainty associated to the final certified element amount content values. These issues, nearly never addressed in the case of the uncertainty propagation, are particularly important to achieve comparability conditions between analytical results from different laboratories.

2. Moisture/water content determination – general aspects

Usually the determination of water or moisture in food leads to a discussion about the different forms of water, present in the sample. "The weakness of defined properties, attributed to different physical states of water binding, is best expressed by a list of vocabulary used to describe surface-water interactions. The terms bound water, solid-like water, water of crystallisation, immobilised water, unfreezable water, ice-like water, etc. are examples" (Rückold et al., 2000; Grobecker, Rückold, & Anklam, 1999). Corresponding to these various water fractions are different methods of determination, resulting in different quantitative values.

Only the "free" water is of interest regarding microbial activity but it is difficult to measure it selectively (Isengard, 1995). The temperature at which water evaporates depends on its molecular environment; free water normally evaporates at a lower temperature than bound water. As sample loses water under increasing temperature conditions, by heating samples and measuring the corresponding changes in mass it is often possible to obtain an indication of the amount of water present in different molecular environments (Yazgan, Bernreuther, Ulberth, & Isengard, 2006). Drying food material, for long-term storage purposes for instance, may lead to mass losses that are not due only to the evaporation of the "free" water but also of part of the "crystalline" water as well as of volatile components (alcohols, flavour components, etc.) and water released from decomposition of compounds at high temperature (reducing sugars, carbohydrates, etc.) (AOAC, 1993).

Thus, experimental parameters of drying methods are critical, and the difference in mass after drying is better described as "mass loss on drying" rather than as "water content" (Isengard, 1995). Again, in this paper, relative "mass loss on drying" is also described as "moisture content".

Water activity is defined as the ratio between the water vapours pressure in the sample and the water vapours pressure of pure water at identical temperature. The optimum point for the stability of dehydrated biological products (when the polar groups of a surface are saturated with water on a molecular 1:1 basis) corresponds to a water activity ranging between 0.2 and 0.4 (Rückold, Isengard, Hanss, & Grobecker, 2003; Rückold, Grobecker, & Isengard, 2001b). In the present study, water activity of the rice material following the drying treatment prior to its bottling was much lower, around 0.1, thus explaining the hygroscopic behaviour observed under the laboratory temperature and humidity conditions during preparation of the IDMS blend samples.

3. Experimental

3.1. Rice material

The rice powder used in IMEP-19 originates from rice grown in cadmium contaminated water by the National Metrology Institute of Japan. The purpose was to produce rice contaminated with cadmium at a level close to the upper limit in Japanese regulations.

The test material was prepared at IRMM following standard procedures, including milling, drying, storage under dry conditions, reprocessing into units of 15 g in glass bottles (Aregbe et al., 2003).

Element content certification was carried out on samples (minimum 1 g) prepared in two different ways. The first series included 15 "non-saturated" samples, i.e. processed (addition of aliquots of the isotopically enriched materials for IDMS purposes) immediately after opening of 5 rice bottles (3 replicates per bottle). The second series included 10 "saturated" samples (5 other bottles, 2 replicates per bottle), left at 22.3 °C and 60% relative humidity for 12 h, in accordance with the rules of the CCQM-K24 comparison: "the bottled rice sample should be spread on a tray and exposed to ambient conditions for at least 6 h until equilibrium is attained (successive weights should not differ more than 0.001 g). It is emphasised that any kind of contamination has to be avoided during this procedure. The Cd, Cu and Pb measurements have to be performed on a sub-sample of this equilibrated rice sample."

Three methods of moisture/water content determination were compared, and for both series of samples these experiments were carried out on rice powder originating from the same bottles as those used for the IDMS work.

3.2. Moisture/water content measurements and hygroscopicity evaluation

3.2.1. Karl Fischer titration

The titration is performed in an automatic mode and determines the *total* water content of a sample selectively by means of a two-step chemical reaction. The parameter measured in the Karl Fischer titration method is the amount of iodine reduced in the second step of this reaction. More details can be found in the literature (Isengard, 1995; Rückold et al., 2000; Schmitt & Isengard, 1998).

Water measurements were performed with an automatic Karl Fischer titrator KFD 758 titrino (Metrohm, Herisau, Switzerland), using the two components technique (Hydranal-Titrant 5 and Hydranal-Solvent from Riedel-de Haën, Seelze, Germany) on 0.2 g "non-saturated" samples (5 bottles and 2 replicates per bottle).

3.2.2. Oven drying

This method was mandatory in the case of the certification for the CCQM-K24 comparison (on "saturated" samples). Instructions stipulated that "correction for dry mass should be obtained from a separate portion of the same material (used for element content determination) of minimum mass of 1 g. The material should be dried for a minimum of 8 h in a ventilated oven at a temperature of 85 ± 2 °C. Then weigh and repeat drying until constant mass is attained. Each weighing has to be carried out after the sample reached thermal equilibrium at room temperature in a desiccator (successive weights should not differ more than 0.001 g). The loss of mass corresponds to the correction that should be applied." These rules were established from results obtained during experiments described in this paper. Eventually, they were applied to both series of certifications each time on 10 replicates of 1 g rice samples (5 bottles and 2 replicates per bottle).

3.2.3. Thermogravimetry

Thermogravimetry applies to very small size samples (between 0.035 and 0.055 g) and, like the oven drying method, consists in measuring mass variation as a function of increasing temperature. Thermogravimetry measurements were performed with a thermogravimeter (Setaram, Saluire, France), each time on 3 replicates from 1 bottle. Samples were placed in a crucible suspended from the balance inside the furnace. The series of "saturated" samples and of "non-saturated" samples were heated both at 85 °C, for 18 h and 16 h.

3.2.4. Hygroscopicity evaluation

The hygroscopicity of the "non-saturated" samples was evaluated on rice powder from the same material (5 bottles, 3 sub-samples per bottle) used for the certification by IDMS of the Cd, Cu and Pb mass fractions (Vassileva & Quétel, 2004). Aliquots of ~0.2 g of material were spread homogeneously on 15 separate folded aluminium foils ($d = \sim 5$ cm), and the mass uptake rate was monitored for each sample at regular time intervals during 150 min from the opening of the sample bottles, at room temperature under 60% relative humidity (same environmental conditions as for the samples used to prepare IDMS blends).

These experiments were performed under controlled temperature (±0.3 °C) and hygrometry (±2.5%) conditions.

The resulting isotherms of water pick-up over time permitted the calculation of the rate of water absorption during the preparation of IDMS blend samples.

3.3. Analytical protocol for the IDMS experiments

The analytical protocol that was developed for the measurement of Cd, Cu and Pb mass fractions in the rice material was based on ID-ICP-MS. It is described and discussed in details elsewhere (Vassileva & Quétel, 2004). Briefly, a multiple spiking approach was applied to reduce significantly the number of analytical steps. For the decomposition of the sample, three different microwave assisted digestion procedures were tested and compared. The use of hydrofluoric acid was found necessary to ensure full solubilisation and complete isotopic equilibration. Uncertainty contributions from the correction for sample homogeneity, procedural blank, spike impurities, spectral interferences, instrumental background, dead time effects and, of course, moisture/water content were evaluated.

3.4. Uncertainty estimation

Combined uncertainties attached to the measurement results were evaluated according to the ISO/GUM guide (ISO-GUM, 1995) by applying an uncertainty propagation procedure to individual uncertainty contributions. In practice, a dedicated software program (GUM Workbench, 2001) was used, based on the numerical method of differentiation described by Kragten (1994). All combined uncertainties are expanded with a coverage factor $k = 2$.

4. Results and discussion

4.1. Correction for hygroscopicity

The correction for hygroscopicity was evaluated by interpolating at 1 min (the duration for the preparation of an IDMS blend sample) the isotherms produced preliminarily under temperature and hygrometry controlled conditions [cf. typical curve reported in Fig. 3 in Vassileva and Quétel (2004), and typical regression reported as Eq. (1) in Table 1].

The spread observed for 15 isotherms was 0.25–0.45%, i.e. rather narrow, and the hygroscopicity correction factor was conservatively taken as $H_{1\,\mathrm{min}} = 0 \pm 0.70\%$ ($k = 2$) and combined to correction factor for moisture/water content (Eq. (2) in Table 1, for "non-saturated" rice samples). It was considered that the uncertainty on this factor included allowance also for other smaller, although not investigated, sources of error.

4.2. Comparison of three methods for moisture/water determination in powder rice samples

Three different methods of moisture/water content determination were compared and results are reported in Table 2. Results of "non-saturated" samples are those which were obtained from samples taken immediately after first opening of the bottles.

Table 1
Equations used to correct the Cd, Cu and Pb amount content for moisture/water content and hygroscopicity

(1)	$H = 3\mathrm{E}{-}6K^3 - 0.0008K^2 + 0.0919K + 0.2626$
(2)	$W_{\mathrm{mc}} = (W_{\mathrm{insat}} - H_{1\,\mathrm{min}})$
(3)	$c_{\mathrm{x\_mc}} = c_{\mathrm{x}} \cdot \frac{1}{(1-W_{\mathrm{mc}})}$

Parameters c – amount content (mol Cd, Cu or Pb g^{-1}); W – correction factor (%); H – water uptake rate (%); K – Time (min).
Indexes x – "non-saturated" rice sample; mc – correction for moisture/water content and hygroscopicity; insat – correction for moisture/water content; 1 min – correction for hygroscopicity at 1 min.

As explained before, oven drying addresses the "free" water in priority and, depending on the drying conditions, part of the "crystalline" water, as well as volatile components and even some compounds originating from the partial decomposition of the sample. Therefore, the choice of our experimental parameters required a careful investigation.

Five different temperatures for oven drying were studied, and a test for constancy of mass was performed by weighing the samples every 2 h (Fig. 1). It can be seen that drying of this rice material at temperatures above 95 °C in an oven caused large losses of volatiles, most likely other than water. At 110 °C, destruction of the sample matrix, at least partially, was obvious (apparent stabilisation is reached only after 18 h of drying).

It is relatively common to find 102 ± 2 °C as the recommended temperature for drying (IDF Standard 26A, 1993). However, our results at 85 °C and 95 °C are in much better agreement with Karl Fischer titration results for "non-saturated" samples. Beside, the profile of mass loss on drying was more regular at 85 °C than at 95 °C, with less than 0.15% mass change over 10 h.

Thus, 85 ± 2 °C was prescribed for the CCQM-K24 comparison as the oven temperature for mass loss determination experiments.

Thermogravimetry applies to smaller size samples than the drying oven method, is more complicated to implement, but may provide insight about the nature of the compounds produced during the heating process. Our experiments confirmed that at 85 °C only water was released from the rice samples investigated.

One of the main advantages of the Karl Fischer titration method is its selectivity, because it is based on a specific chemical reaction. However, it addresses the "total" water content and not only the "free" water fraction. Determination with the Karl Fischer titration method takes few minutes. This method is still not available widely – except for some industrial sectors like the food industry. Also, water determinations by Karl Fischer titration for products with high content of proteins, lipids or carbohydrates require different conditions of temperature and solvent mixture, and must be adapted to the specific sample particle size characteristics (Gergen, Radu, Bordean, & Isengard, 2006; Ronkart et al., 2006).

Table 2 shows that the results obtained for "non-saturated" samples with the three methods are similar within one standard deviation. Statistically (t-test) average values from the oven drying method and from the Karl Fischer titration method are identical. From this coincidence it is reasonable to assume that the water in this rice material was essentially "free" water. These results also show that the oven drying method has the potential to produce reproducible, and possibly meaningful, data providing that a reasonable temperature of drying was identified.

Table 2
Comparative study of different techniques for moisture/water determination in rice powder material (results reported with one standard deviation for *'n'* determinations). Sample size is 1 g for the oven drying method and 0.2 g for the thermogravimetry and Karl Fischer titration methods

Sample	Oven drying method 85 °C	Thermogravimetry	Karl Fischer titration
Non-saturated	4.37% ± 0.17%, $n = 10$	4.8% ± 0.5%, $n = 3$	4.53% ± 0.22%, $n = 10$
Saturated	10.74% ± 0.36%, $n = 10$	10.7% ± 1.0%, $n = 3$	–

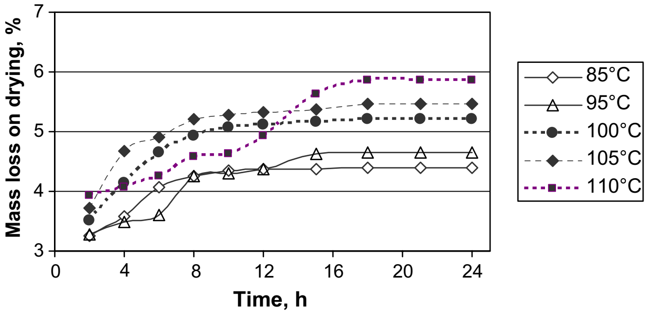

Fig. 1. Mass loss on drying (moisture) of 1 g rice sample at different temperatures.

Another important advantage of the oven drying method is the simplicity of application in nearly every analytical laboratory.

It must be noted that the Karl Fischer titration method (based on the realisation of a chemical reaction independent of the size and configuration of the examined powder sample) has the potential to provide more reproducible results than the drying oven method (possibly dependent on environmental factors such as variations of temperature and air humidity conditions, configuration of the oven, dispersion of the sample powder, etc.). Results from the Karl Fischer titration were then applied to establish the certified values of Cd, Cu and Pb mass fractions in rice for the IMEP-19 exercise.

4.3. "Saturated"/"non-saturated" samples

As expected, mass loss on drying is more than twice larger for the "saturated" than for the "non-saturated" samples. It is obvious that the methods chosen for sample pre-treatment and dry mass determination may influence certified element contents in this rice sample. Thus, explicit methods must be provided to achieve "comparable" results.

Beside, the entire measurement process must be described mathematically to ensure the possibility of propagating individual uncertainty contributions together, and provide, whenever possible, the necessary platform for the establishment of the traceability of the measurement results. The model employed for the certification of the element mass fractions in the rice material is fully described in a separate paper (Vassileva & Quétel, 2004). Correction for moisture/water content, as described here by Eqs. (2) and (3) in Table 1, is a significant part of it. As can be seen from Table 3, the uncertainty on these corrections plays an important role for the three elements in "non-saturated" rice samples, essentially due to the correction for hygroscopicity, which is even the major contributor for Cu (nearly 60%). As explained in Vassileva and Quétel (2004) the variations between elements depended from the relative importance of other typical IDMS related contributors such as the uncertainties on isotopic composition for Cd and the uncertainty on the correction for procedural blank for Pb.

The contribution of the uncertainty on the dry mass correction was found to be below 1% for the "saturated" samples. However, the principle of obtaining water saturation for the samples prior to their processing introduced supplemental important technical complications and risks of sample contamination in comparison with the work on "non-saturated" samples.

Finally, correcting for a characteristic that is operationally defined such as the moisture content in this rice material may lead to an approximation regarding what is actually measured, and there is not necessarily a clear match between the intended measurand and the result reported. In such a case, traceability of the measurement result (to a well identified system of reference) is more

Table 3
Uncertainty contributions from the correction for moisture content and sample hygroscopicity on the determination of the Cd, Cu and Pb amount contents in a rice powder material as the contribution of IRMM to CCQM-K24

	Cd		Cu		Pb	
	Non-saturated	Saturated	Non-saturated	Saturated	Non-saturated	Saturated
Rel U (%), $k = 2$	1.5	1.3	1.0	0.7	1.6	3.1
% contribution to this U estimation						
s.u. on the correction factor for hygroscopicity	24.8	–	59.4	–	6.6	–
s.u. on the correction factor for moisture	0.9	1.1	2.2	3.9	0.7	0.2

Relative expanded ($k = 2$) uncertainty statements (U) on these element content determinations are also given, they correspond to combined uncertainty calculations and are taken from Vassileva and Quétel (2004). This "dry mass" correction is performed using a dry oven method.
s.u.: standard uncertainty.

difficult to establish. This is further amplified if the uncertainty for the correction considered is important as it is the case for the hygroscopicity in the "non-saturated" rice samples.

Acknowledgements

The authors thank B. Dyckmans for the metrological weighing work, Patrick Conneely for Karl Fischer titrations, Sandra Kuxenko for the thermogravimetry measurements and Seyhan Yazgan, who provided the results of the water activity measurements of the rice powder following its preliminary drying treatment.

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