WATER DETERMINATION IN SAMPLES WITH HIGH SUGAR AND PROTEIN CONTENT

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT: The aim of water determination should be detection of water and nothing but the water. Large number of methods use heating where the result shows loss of all volatile compounds and not only water. The result of these techniques is not water content, but the mass loss. One of the best techniques for determination of water content is Karl Fischer titration, based on a chemical reaction selective for water. Determination of water content by heating, in samples that are rich in proteins and sugars is especially hard, because of the Maillard reaction. During the Maillard reaction, water is produced, and it is hard to determine water which is originally from sample and water that is produced by Maillard reaction. In this investigation we used samples of different types of condensed milk - rich in proteins and sugars. Samples were measured in ten probes, and by four methods: Karl Fischer titration with different solvents, Classical Oven, IR drying and Oven Sample Processor. Classical Karl Fischer titration was used as a reference method. The best method was Classical Karl Fischer titration, because of precision, trueness and duration of measurement. Usage of boiling methanol for extraction is not recommended. Due to a small amount of sample, contribution in the amount of water from the Maillard reaction is not significant. The best method for measurement is KF titration.

KEYWORDS: Water; Maillard; Karl Fischer

INTRODUCTION

Water is one of the most important constituents of food. It is present in almost all foods, in a range from extremely low amounts in dried products to very high amounts in beverages. Water content is a parameter that affects many others, both of physical and chemical nature. Amount of water in food is a determinant for its nutritive value, taste, shelf life etc. The aim of water determination should be to detect all water, and nothing but the water. Water occurs in different bonding situations. This has an influence on the separability and the possibility to detect water. Water determination is, for practical reasons a challenge for the analysis as it is certainly the most frequent analysis preformed in foods [1]. It is also a challenge for a second reason. Because of many different methods for water determination, one must ask himself which one yields the correct value [1], [2], [3]. There are three main problems when we want to determine water content in foods:

Heating of a sample could cause loosing of all volatile compounds and not just water.

Classical methods for determination of water content use heating of a sample during a certain period of time, and the result is mass loss before and after heating. [4] It is basically a physical separation of water from a sample. The problem with these methods is that we measure mass loss of every volatile compound (we get a higher result than the correct one). The results of drying techniques should therefore not be termed as „water content". One can use the term „moisture", but the most suitable is „mass loss". Another possibility is to determine water content by a selective chemical reaction. Also, we can use indirect methods which determine property of a sample which depends on water content, such as, density, sound velocity, electrical conductivity etc [5], [6].

Contamination of a sample.

It is very easy to contaminate a sample with water since water is all around us: on our hands, in the air, on the laboratory table, in our breath.

Production of water in samples during heating or storage (Maillard reaction).

The Maillard reaction is a very complex network of chemical reactions which happens in samples which contain reducing sugars and proteins (or amino acids). This type of reaction occurs during heating of samples or during storage. Volatile compounds of low molecular mass, non volatile coloured compounds of intermediate molecular mass and brown substances of high molecular mass can occur as products of these reactions [7, 8]. In some steps of the Maillard reaction water is produced (dehydration) [8].

So, there is a question: how to determine water content that is originally in samples, and not water

that is originally in samples plus water which is formed in the Maillard reaction.

Determination of water content is often considered as an easy task, but if we want to determine only water task is not so easy.

Because it is very difficult to measure amount of water content in samples, the aim of this research is to recommend the best method for determination of water content in samples with high amounts of sugars and proteins. For this investigation we used samples of condensed milk which are very complex and Maillard reaction is easy to occur.

MATERIALS AND METHODS

In this investigation we used different kinds of condensed milk, which have quite high amounts of proteins and sugars. The first type of condensed milk (Milch Mdchen) had 7,6 grams of protein and 55,0 g of sugars per 100 g. The second type (Milblu) had the same amounts of proteins and sugars. The third type (Молоко) had 6,9 g of proteins and 56 g of sugars per 100 g. For determination of water content, we used several different methods of analysis which are used in today's laboratories:

1. Classical Karl-Fischer titration
2. Oven drying
3. Automated Karl Fischer titration
4. IR drying

Karl Fischer titration

Measurements were carried out on Titrando 890 Metrohm (Metrohm, Herisau Schwitzerland). This method uses selective chemical reaction which is selective for water. It is a direct method for determination of water content [9] [10]. This method was reference method in this investigation, since it is known as one of the best methods for determination of water content. The instrument used volumetric Karl-Fischer titration cell with a thermostat. With this instrument we made measurements in two different solvents (Solvent-Riedel de Haën and boiling methanol - Riedel de Haën), and at two different temperatures: room temperature and 50°C. The duration of measurement was 250 s. The mass of sample was between 0.1300 and 0.2000 g. The sample was introduced into the titration cell with the usage of a syringe with a needle. The end point of titration was potentiometric. Measurements were made on every kind of sample in ten repetitions.

Automated Karl Fischer titration

774 Oven Sample Processor (Metrohm, Herisau Schwitzerland) was used for automated determination of water content. In this method there is a combination of vaporisation of water from a sample and Karl Fischer titration [11], [12]. A sample is heated in the oven, and water that is formed, as vapour is transformed by usage of the pump, into the Karl Fischer Cell. It is also a direct method- combined direct method. A device which we used is equipped with the coulometric Karl-Fischer titration cell. The measurement duration was between 65 and 100 min. Stop criteria was absolute drift of 20 µg/min. Sample mass was between 0.1500 and 0.2500 g. The sample was introduced into the vial with the usage of a syringe with a needle, after that the vial was closed. Before the measurement of water content in samples, the „temperature ramping"was made. It is the part of the program in which a sample is heated from 20 to 250°C (1°C/min), in which we can see the temperature at which it is the best to measure the water content. In the samples of condensed milk we got the temperature of 120°C. After determination of the measurement temperature we put three blanks (vials with only air) in the sample changer and samples in ten repetitions.

Oven drying

Oven drying measurements were made on Binder FDL 115 (Binder, Mount Holly, USA). It is a direct method. This method does not measure water as such. The result is a mass loss. The mass los is not only caused bay water, but also by all volatile compounds under the drying conditions. The analysed samples (2.000 – 4.000 g) were weighed into the glass weighing bottles, where were mixed with predried sand, and then dried at 105°C, until the constant mass was reached. These measurements were preformed in five repetitions.

IR dryer

IR drying is rapid method for water determination aimed to determine water content thermogravimetricaly [7]. IR drying measurements were made on Sartorius MA 40 (Sartorius, Göttingen, Germany). IR drying was made at 100°C, duration of the measurement was 60-70 min. Sample mass was between 1.000 and 2.000 g. The sample was introduced in the device on filter paper, with a syringe. Measurements by means of IR dryer were made in five replicates.

RESULTS AND DISCUSSION

In this research an investigation of water content in samples of condensed milk was done. For determination of moisture (water) content in condensed milk several different techniques are used, i.e. Volumetric Karl-Fischer titration with different solvents and at different temperatures, Automatic Karl-Fischer titration, Oven drying and IR Drying. All of these methods are used for moisture content determination

in food samples. Results for different samples are very similar. The differences between methods are significant regarding the optimisation, time of measurement, mass of the sample and standard deviation. All methods are compared with classical Karl Fischer titration which was used as reference. First step before analysis was the optimisation of the method.

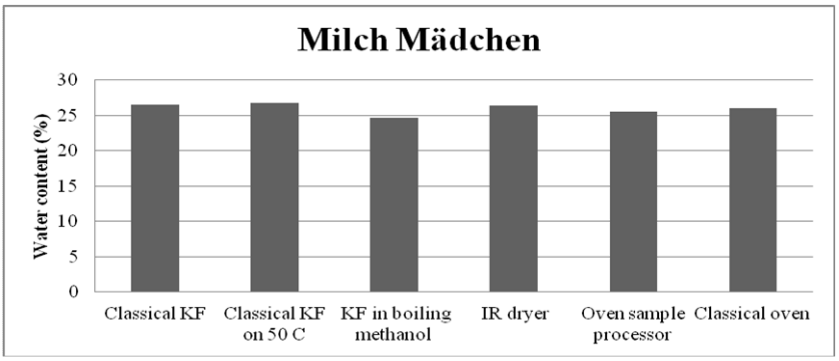

Diagram 1. Water content (mass loss) by different methods in Milch Mädchen

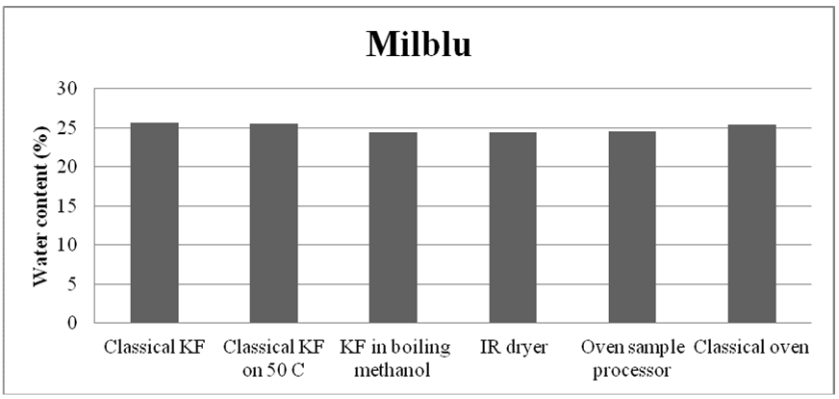

Diagram 2. Water content (mass loss) by different methods in Milblu

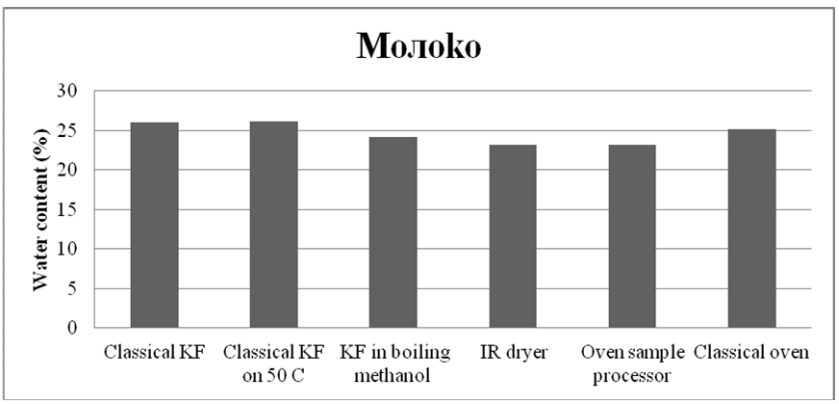

Diagram 3. Water content (mass loss) by different methods in Молоко

Optimisation and application of Karl Fischer titration

Before measurement and extraction of water it was necessary to choose the right solvent for extraction [5]. After several solvents used in testing [13], we chose „Solvent 1"and boiling methanol. Boiling methanol was chosen because of its vapours which can put water from hidden parts of a titration cell back into the titration cell. After solvent selection, we had to choose the extraction time (time at which all water from a sample is extracted). If we choose too short time for extraction, we could get too low results. For standardisation of measurement we used standards with the known amount of water content (10 mg/mL).

For determination of water content we applied two different temperatures: room temperature and 50°C. The second temperature was chosen do see possible water formation during measurement, due to Maillard reaction.

Optimisation and application of Automated Karl Fischer titration

For this method we need to find the best temperature for measurement. The adequate temperature was chosen with "temperature ramping" (1°C/min), with temperature ramping we see the best temperature for measuring. Also, because this method uses heating of a sample and bringing it to the titration cell, we must find the best gas flow rate. For gas carrier we used dried air. The first step in the measurement is to measure amount of water in blanks and then in the standards. As a standard we used lactose with the amount of water of 5.05%. As a blank we used only vial with air.

Optimisation and application of Classical Oven

Classical Oven is a well defined and easy to use method for determination of mass loss. The sand which was used for heating process is pre-dried. The heating was at the standard temperature of 105°C. As a reference material for determination of water content a lactose standard was used with 5.05% of water.

Optimisation and application of IR dryer

IR dryer uses Infra Red radiation for heating of a sample. The drying process is highly dependent on the radiation temperature and distribution of a sample in the sample holder. A sample must be distributed evenly. For distribution of a sample we used a plastic syringe.

On diagrams 1, 2 and 3 are shown the results of measurement of water content by different methods. The results and basic statistical parameters of water determination by different methods in samples of condensed milk are shown in Table 1.

Table 1. Results of water determination gained from different methods

Sample: **Milblu**						
	Classical KF	Classical KF (50 °C)	KF in boiling methanol	Oven sample processor	Classical Oven	IR dryer
Time	250 s	250 s	250 s	65-100 min	6 h	60-70 min
Sample mass	0.02-0.03	0.02-0.03	0.02-0.03	0.01-0.02	2-3	1-2
Maximum	26.33	26.30	25.44	25.11	25.57	24.90
Minimum	24.61	24.05	23.48	22.36	24.94	23.16
Median	25.62	25.75	24.22	24.87	25.41	24.07
Average	25.67	25.54	24.38	24.52	25.30	24.15
STDEV	0.48	0.68	0.71	0.85	0.25	0.67
Sample: **Milch Mädchen**						
	Classical KF	Classical KF (50 °C)	KF in boiling methanol	Oven sample processor	Classical Oven	IR dryer
Time (min.)	180 s	250 s	250 s	65-100 min	6 h	60-70 min
Sample mass (g)	0.02-0.03	0.02-0.03	0.02-0.03	0.01-0.02	2-3	1-2
Maximum	26.80	27.33	25.51	26.19	26.82	27.50
Minimum	26.05	26.33	23.96	24.70	24.20	22.90
Median	26.50	26.67	24.65	25.42	26.11	26.25
Average	26.47	26.73	24.62	25.49	25.69	25.67
STDEV	0.26	0.35	0.49	0.46	1.03	1.73
Sample: **Молоко**						
	Classical KF	Classical KF (50 °C)	KF in boiling methanol	Oven sample processor	Classical Oven	IR dryer
Time (min.)	250 s	250 s	250 s	65-100 min	6 h	60-70 min
Sample mass (g)	0.02-0.03	0.02-0.03	0.02-0.03	0.01-0.02	2-3	1-2
Maximum	26.72	26.76	25.14	23.70	25.74	23.96
Minimum	24.55	25.40	23.19	22.62	24.77	21.68
Median	26.13	26.26	24.39	23.08	25.51	22.72
Average	26.03	26.19	24.22	23.13	25.37	22.84
STDEV	0.64	0.47	0.66	0.36	0.37	0.83

Results gained with all of used methods are similar. The differences in methods are mainly in speed of measurement and precision. The shortest time in measurement was with Karl Fischer titration, and the longest with Classical Oven. Duration of measurement with Classical Karl Fischer titration is around four minutes. Even shorter time is possible if we use higher temperature for analysis, since extraction of water from samples depends on temperature.

Classical Oven has the longest time of measurement. That is the main disadvantage of this method because reaching the constant mass if very time consuming. On the other hand this method has quite high precision and it does not show significant difference from reference method (Classical Karl Fischer).

Karl Fischer in boiling methanol showed lower results from reference method. From this we can conclude that methanol as a solvent is not suitable for extraction of water from condensed milk samples. For purposes of determination of water in condensed milk with Karl Fischer titration it is better to use other solvents, for example: solvent 1, or other mixtures of solvents.

Oven sample processor has one advantage which does not have any of other used methods, and that is sample changer. Disadvantage of this method is many parameters that need to be controlled and time of measurement. It is not possible to have absolutely dried air as a carrier. It is hard to extract all of water from the samples of condensed milk, since during the measurement the crust is formed on the surface of samples.

The mass of the samples was quite small and instrumentation is not precise enough to record this small addition of water because of Maillard reaction. Higher temperature does not give significantly higher amount of water (Maillard reaction).

Precision of methods and comparison to reference method

The precision of Oven drying and Classical Karl-Fischer titration was the best of all tested methods (lowest STDEV).

The next comparison of all above mentioned methods was made by the ANOVA at $p<0.005$ in SPSS statistical program. It was found that differences between Classical KF (which was used as a reference method) and Classical KF in boiling methanol were not significant. Also differences between the reference method and Classical dryer were not significant. The largest difference between the reference method and the other method was found with IR dryer and Automated Karl Fischer titration.

CONCLUSIONS

Water content in samples that contain high amounts of proteins and sugars (condensed milk – Maillard reaction is possible to occur) was determined by several methods.

The results obtained by Classical Karl Fischer titration were used as referential.

The amount of water produced by Maillard reaction during the measurement was not significant (due to small mass of a sample).

For determination of water content in samples of condensed milk we suggest the usage of classical Karl-Fischer titration, because of high precision of the method, low amount of a sample needed and fast measurement.

Instead of classical Karl Fischer titration, one can use Karl Fischer at 50°C, or Classical Oven.

The usage of boiling methanol for determination of water content in samples of condensed milk is not recommended, because it cannot extract all of the water in reasonable time.

In the largest part of samples, Classical Karl Fischer gives lowest, and IR dryer gives the highest standard deviation (precision of method).

Determination of water content in samples that contain proteins and sugars should be made as soon as possible (Maillard reaction).

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REFERENCES

[1] H-D. Isengard, "Water content, one of the most important properties of food" Food Control, Elsevier, Vol. 12, pp. 395-400, 2001.

[2] H. Bünung – Pfaue, Analysis of water in Food by near infrared spectroscopy, Food Chemistry, vol. 82, pp. 107-115, 2003

[3] M. Matlouhi, Water content, water activity, water structure and the stability of foodstuffs, Food Control, vol. 12, pp. 409-417, 2001

[4] H-D. Isengard, "Water Determination in Food", in: Ensuring the Global Food Safety, Cambridge, Elsevier, 2010, pp. 109-123.

[5] H-D. Isengard, Water determination-Scientific and economic dimensions, Food Chemistry, vol. 106, pp. 1393-1398, 2008

[6] M. Adam, P. Dobiáš, P. Bajerová, K. Ventura, Comparison of various methods for determination of water in white yoghurts, Food Chemistry, vol. 115, pp. 1069-1073, 2009

[7] F.J. Morales and M.A.J.S. van Boekel "A study on Advanced Maillard Reaction in heated Casein/Sugar Solutions:

Color formation, Int. Diary Journal, Vol. 8, pp. 907-915, 1998.

[8] M. Namiki "Chemistry of Maillard reaction: recent studies on the browning reaction mechanism and the development of antioxidants and mutagens", Adv. Food Res. Vol. 32, pp. 115-184, 1988.

[9] H-D. Isengard, R. King, C.T. Reh, Proposal of a new reference method to determine the water content of dried dairy products, Food Chemistry, vol. 96, pp. 418-422, 2006.

[10] H-D. Isengard and H. Präger, Water determination in products with high sugar content by infrared drying, Food Chemistry, vol. 82, pp. 161-167,2003

[11] A. Felgner, R. Schlink, P. Kirschenbühler, B. Faas, H-D. Isengard, Automated KarlFischer titration for liquid samples-water determination in edible oils, Food Chemistry, vol. 106, pp. 1379-1384.

[12] V. Kestens, P. Connely, A. Bernreuther, Vaporisation coulometric Karl Fischer titration: A perfect tool for water content determination of difficult matrix reference materials, Food Chemistry, vol. 106, pp. 1454-1459, 2008.

[13] K. Schöffski, New Karl Fischer reagents for the water determination in Food, Food Control, vol. 12, pp. 427-429, 2001.