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Analytical Methods

Comparison of various methods for determination of water in white yoghurts

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ABSTRACT

Water content was determined in 11 samples of white yoghurts. Several techniques, namely Karl Fischer (KF) volumetric titration, near and middle infrared spectroscopy (MIR), oven drying and moisture analyser were used. Optimisations of solvent, titration temperature and extraction time were carried out for the KF titration. Methanol at room temperature with 5 min extraction period was used as final conditions. For the infrared spectroscopy (FTIR) the suitable solvent and the appropriate wavenumbers for water analysis were chosen. The measurement in the near infrared (NIR) region at the wavenumber 10270 cm^{-1} using acetonitrile as solvent and transmission method was evaluated as the best of the FTIR methods. Based on the comparison of individual methods the oven drying at 105 °C seems to be the most suitable, but it is the most time consuming. However, KF volumetric titration and especially FTIR methods appear to be good alternatives to the drying methods.

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1. Introduction

Water content is for a number of reasons one of the most important properties of foodstuffs. Therefore, the determination of water content is very important kind of analysis (Isengard, 2008). Nearly every food product contains water and this parameter affects many others, both of physical and chemical nature. Evaluation of most chemical parameters is based on dry mass and therefore water content must be evaluated. Also, water content affects microorganism growth and enzymatic activity, affecting the stability and shelf-life of foodstuffs. As different methods are available for water determination, the question which one is more appropriate still remains (Mendonça, Franca, & Oliveira, 2007). The problem becomes more difficult due to the facts that water in food is distributed in different bonding states and that both the product itself (dry matrix) and its water content affect method performance (Yazgan, Bernreuther, Ulberth, & Isengard, 2006).

The most frequently used chemical method for the water content determination is volumetric Karl Fischer (KF) titration. This method is together with method of drying within the constant weight often used as the reference method to determine the water content in a whole range of organic and inorganic samples (Kestens, Conneely, & Bernreuther, 2008). KF titration is based on the reaction of water with iodine. The reaction is performed in a methanolic solution (Scholz, 1984). The high selectivity to the water represents the major advantage of the KF titration with respect to the weight loss technique: only the water content is determined, since iodine selectively reacts with water. The KF titration is a fast analysis (in general, a single titration takes only 1–2 min), accurate and precise. This method is ordinarily used for analysis of water content in dried milk samples (Reh, Bhat, & Berrut, 2004; Rückold, Grobecker, & Isengard, 2000).

It is also possible to determine the water content by using the spectral techniques, especially infrared spectroscopy (FTIR). There exist some absorption stretches of the characteristic vibrations in both middle infrared (MIR) and near infrared (NIR) regions just for the water. Mostly it is used NIR spectroscopy. Water content determination by NIR spectroscopy in cheese (Blazquez, Downey, & O'Donnell, 2004; McKenna, 2001) or in butter (Hermida, Gonzalez, Sanchez, & Rodriguez-Otero, 2001) is a very useful technique. The main advantages of NIR spectroscopy for food analysis lie in its speed, no or little sample pre-treatment and the avoidance of the use of chemicals (Osborne, Fearn, & Hindle, 1993).

The aim of this study is to modify and evaluate the KF and FTIR methods (in MIR and NIR regions) for determination of water content in the white yoghurt samples and to compare these methods with drying methods and with each other as well.

2. Materials and methods

2.1. Samples

It was analysed 11 samples of white yoghurts. These were purchased in Czech local markets and were produced from Czech natural sources. These samples were labelled by the numbers 1–11. For all analyses only fresh samples were used.

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Table 1
Comparison of water content determined by KF titration and drying methods.

Sample no.	KF titration		Oven drying		Moisture analyser	
	Water content [% (w/w)]	RSD[a] [%]	Water content [% (w/w)]	RSD[a] [%]	Water content [% (w/w)]	RSD[a] [%]
1	87.3	0.93	85.6	0.08	85.0	0.06
2	83.1	0.56	82.1	0.01	81.5	0.01
3	86.4	0.84	85.1	0.08	84.7	0.09
4	85.9	1.10	84.3	0.01	83.9	0.02
5	84.5	0.97	83.4	0.05	82.1	0.07
6	86.1	1.17	84.8	0.02	84.2	0.08
7	89.8	1.32	88.5	0.07	86.9	0.09
8	86.4	0.63	85.3	0.05	84.9	0.06
9	88.9	0.88	87.4	0.04	87.0	0.10
10	88.2	1.05	87.1	0.01	86.7	0.10
11	81.5	0.56	80.7	0.03	80.0	0.04

[a] Relative standard deviation, $n = 5$.

2.2. Karl Fischer titration

Measurements were carried out on AF8 Orion automatic KF titrator (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a thermostatic titration vessel. An aliquot of 5 mL of fresh sample was placed into the 50 mL graduated flask, weighted, then dissolved in methanol (CombiMethanol for KF volumetric titration - max 0.01% of water; Merck, Darmstadt, Germany) and filled to match mark. Volume 100 μL of this modified sample was inserted into the titration vessel and was titrated with a one-component reagent CombiTitrant 5 (Merck, Darmstadt, Germany). Titration was carried out after 5 min extraction step at laboratory temperature in five replications. Pure methanol (100 μL) was used as a blank sample.

2.3. FTIR spectroscopy

FTIR spectroscope Equinox 55 (Bruker Analytische Messtechnik, Rheinstetten, Germany) was used for water content determination. For MIR technique it was chosen reflection method using attenuated total reflectance (ATR), whereas for NIR measurements was used transmission method carried out in 1 cm quartz cuvette. The sample preparation was the same as for KF titration method, however isocratic acetonitrile (max 0.02% of water; Merck, Darmstadt, Germany) was used as solvent. All experiments were performed in five replications. The water content was evaluated by calibration method.

2.4. Oven drying and moisture analyser

Oven drying was carried out using the APEX AX120 drying oven with forced ventilation (Carbolite, London, UK). Analysed samples (2–3 g) were weighted into the glass weighing bottles (i.d. 5 cm) where were mixed with pre-dried sea sand (1:1) and then dried at 105 °C within the constant weight (for at least three hours). These analyses were performed in five replicates.

Drying in the moisture analyser KERN MLB50-3 (Kern, Balingen, Germany) was also performed. Analysed samples were dried using two halogen quartz glass heaters (200 W each) at the selected temperature 105 °C within the constant weight. The weighing interval was set to 20 s. It was weighted about 1 g of the sample (this value was recommended by the manufacturer). These analyses were performed in five replicates.

3. Results and discussion

3.1. Optimisation and application of Karl Fischer titration

Before temperature and extraction time optimisation it was necessary to choose suitable solvent. For this purpose, several solvents (e.g. methanol, ethanol or acetonitrile) were taken into account. As suitable solvent was selected methanol (CombiMethanol) because of its low water content (max 0.01%). The second reason for the methanol selection was the fact that this solvent is one of the reagents in the KF reaction. After selection of appropriate solvent the extraction temperature was optimised. The main reason for this optimisation was the fact that prior to KF reactions it was necessary to extract the water from sample matrix to the reaction solutions and just this extraction depends on the temperature. For these experiments 100 μL of the sample (No. 1) dissolved in methanol was put into the thermostatic vessel and after short temperature equilibration (30 s) was titrated at 20, 25, 30, 35, 40 and 50 °C. Top temperature was set 50 °C because of the fact that at higher temperatures (near the boiling point) methanol vapour can intrude into the drying tubes of the KF titrator and wash entrapped water from there back into the titration cell. Highest values were reached for 40 °C. But the obtained results at this temperature were far from those obtained by oven drying method

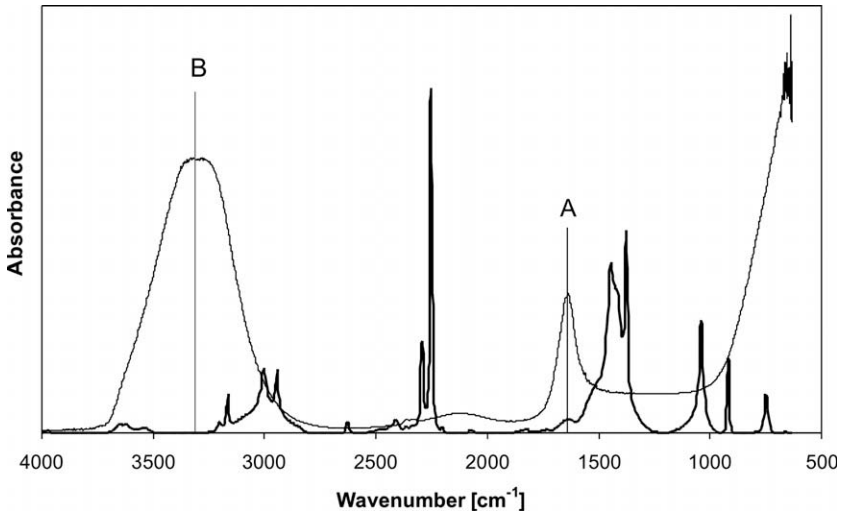

Fig. 1. FTIR spectra of water (thin line) and acetonitrile (thick line) in the middle infrared region (MIR). A and B present the selected wavenumbers for the water content determination.

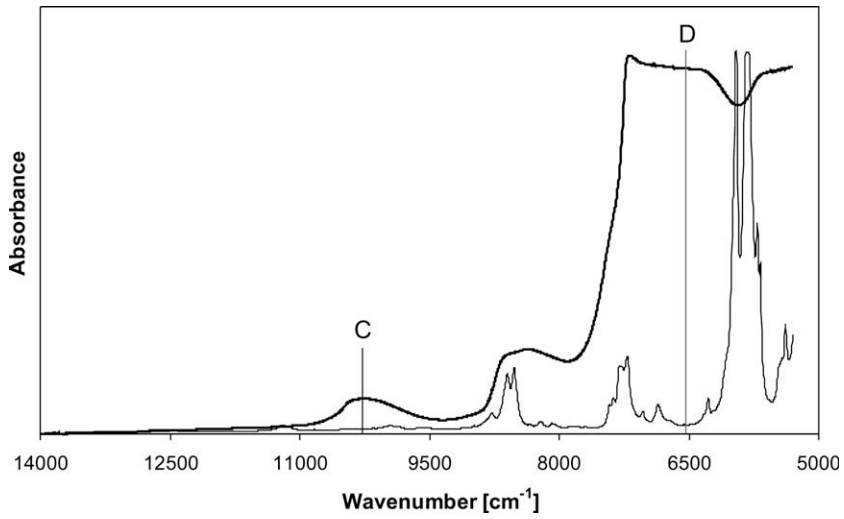

Fig. 2. FTIR spectra of water (thin line) and acetonitrile (thick line) in the near infrared region (NIR). C and D present the selected wavenumbers for the water content determination.

that is usually used as reference method. This could be caused by the side reactions (e.g. formation of ketals producing additional water as one of the reaction products) at higher temperature (Scholz, 1984). According to this the temperature 20 °C was selected as suitable and used for further analyses.

Because of the fact that the sample was not completely dissolved in selected solvent, it was necessary to optimise also extraction time required for transport of the water into the reaction solution. For this purpose the time periods 0.5, 2, 5, 10, 20 and 30 min at optimised temperature were tested. After 5 min time period the water content values were not rising, so this time was chosen as suitable. When shorter extraction time was used the titration end-point was reached worse with lower reproducibility of measurements. On the other hand, at longer extraction time the air moisture could affect the results more and time for sample analysis is wasted as well.

Finally, all 11 samples of white yoghurts were analysed by optimised KF titration method in order to determine the water content. These results are presented in the Table 1 together with the values obtained by drying methods.

3.2. Optimisation and application of FTIR spectroscopy

First of all the appropriate solvent for sample dissolving had to be selected. Unfortunately, it was impossible to use methanol, because this solvent contains -OH groups. From this point of view the isocratic acetonitrile was tested. As shown on the Figs. 1 and 2, the overlay of water and acetonitrile spectra in both MIR and NIR regions is minimal, so this solvent was found to be suitable.

In the case of MIR experiments it was impossible to use KBr cuvettes, because these are easily water soluble. Moreover, it was also not possible to use quartz cuvette because of its strong absorption under the wavenumber 2300 cm^{-1}. So it was necessary to use a reflective ATR method. NIR technique was carried out with 1 cm quartz cuvette that allows easier sample manipulation.

Based on the water and acetonitrile spectra in MIR and NIR (Figs. 1 and 2) the proper wavenumbers for water content determination were evaluated. As suitable wavenumbers were selected 1640 cm^{-1} (label A) and 3310 cm^{-1} (label B) for MIR method and 10270 cm^{-1} (label C) and finally the wavenumber 6530 cm^{-1} (label D) for NIR, respectively. All results were evaluated by the band height (= absorbance).

Five points calibration curves were compiled for all selected regions (A, B, C and D) in the concentration range from 0.02 (pure acetonitrile) to 100% of water. The basic characteristics of these calibrations are summarised in the Table 2. In the case of A and D regions the linearity ranges had to be corrected because at higher water content values the divergence from the Lambert–Beer principle was observed. That's why area A was useful only up to water content 40% and area D only to 20%.

Finally, all 11 samples were analysed by four above-mentioned FTIR methods. All experiments were carried out five times again. Obtained results of water contents together with values declared

Table 2
Calibration characteristics for FTIR methods.

Method	Linearity range			
	[% of water (w/w)[a]]	Wavenumber [cm^{-1}]	Calibration equation	R^2
MIR-A	0.02[b]–40	1640	$y = 0.5659x + 1.0076$	0.9931
MIR-B	0.02–100[c]	3310	$y = 5.2952x - 3.7846$	0.9980
NIR-C	0.02–100	10270	$y = 1.1545x + 14.845$	0.9956
NIR-D	0.02–20	6530	$y = 0.1052x + 0.0727$	0.9936

[a] The mixture of water and acetonitrile.
[b] The pure acetonitrile containing 0.02% of water.
[c] The pure water.

Table 3
Comparison of water content determined by various FTIR methods together with values declared by manufacturers.

Sample no.	Water content [% (w/w)]				
	MIR-A	MIR-B	NIR-C	NIR-D	Declared
1	88.8	83.2	84.0	81.3	Max. 87
2	78.2	79.3	81.6	79.4	Max. 82
3	86.4	79.4	81.1	80.2	Max. 85
4	82.3	78.3	83.6	86.9	Max. 91.8
5	85.1	83.7	81.7	80.7	Undefined
6	80.6	84.1	82.4	85.4	Max. 90
7	90.1	83.6	92.0	86.3	Max. 90
8	85.2	83.3	82.4	81.8	Max. 87.5
9	89.0	86.4	84.2	79.3	Undefined
10	87.0	89.3	86.0	83.5	Max. 87.2
11	79.9	77.7	76.5	81.3	Max. 80
Average RSD[a] [%]	2.57	4.68	3.36	4.14	

[a] Relative standard deviation, $n = 5$.

by manufacturers are summarised in the Table 3. In this table only average RSD values are presented because of the table lucidity. For comparison of all FTIR methods the analysis of variance (ANOVA) was performed. By this statistical analysis the differences at $p < 0.05$ were considered no significant. It was found, that more results with lower RSD values were reached for wavenumber 1640 cm^{-1} (label A), but the linearity for this method is lower (see Table 2). The most appropriate FTIR method seems to be NIR-C for several reasons. First of all no interferences of water were observed in the FTIR spectra of acetonitrile (see Fig. 2). The second reason was good linearity in the whole concentration range. Finally, this method allows easier sample manipulation in comparison with both MIR methods. According to these reasons FTIR–NIR method C was compared with KF and drying methods.

3.3. Application of oven drying and moisture analyser methods

For moisture content determination using the oven drying method it was weighted 2–3 g of yoghurt sample and after mixing with equal amount of pre-dried sea sand this mixture was dried to the constant weight at 105 °C (approx. 3 h).

The same temperature was used for analysis of yoghurt samples using the moisture analyser; however an aliquot of 1 g of sample was weighted, whereas weighing frequency was set at 20 s. From this sample amount it was possible to create thin film which allows faster drying process (approx. 15 min).

All 11 yoghurt samples were measured in triplicate using both above mentioned drying methods. Obtained results are summarised in the Table 1, where is possible to see that in comparison with both FTIR and KF titration method for drying methods very low RSD values were evaluated.

3.4. Methods comparison

There were used several techniques for water content determination in white yoghurts, i.e. KF volumetric titration, FTIR spectroscopy in MIR and NIR regions and, finally, moisture analyser together with oven drying method. All these methods are ordinary used for moisture analysis in different kinds of samples and all of them are suitable also for water content determination in white yoghurts. For comparison of tested methods all 11 samples of white yoghurts were analysed in order to determine the water content. Obtained results are graphically summarised on Fig. 3.

The precision of oven drying method was found to be the best of all tested methods (lowest RSD values). The main disadvantage of this method is long time duration (at least 3 h). Also 3 g of sample amount could be regarded as sample wasting. But still this method is used as standard for many kinds of analyses. Moisture analyser values were slightly lower than those reached by oven drying method; whereas for this method only about 1 g of sample was used and also the measurement time was reduced from 3 h to a few minutes.

KF titration and FTIR methods were much faster than both drying methods mentioned above. One measurement took less then 2 min for both methods. Sample amount was approximately 5 mL, but it should be possible to reduce the sample and solvent amounts. For both methods it was necessary to perform the optimisation of individual experimental conditions. By the KF titration suitable solvent was chosen and then were optimised titration temperature and time. Solvent optimisation was performed also for the FTIR method. Then it was necessary to find suitable wavenumbers for water content determination for both FTIR techniques, i.e. NIR and MIR. On the other hand for both drying methods no organic solvent is necessary.

Finally, the next comparison of all above mentioned methods was performed by the ANOVA at $p < 0.05$. It was found that differences among both drying methods and FTIR method were considered no significant whereas differences between KF method and oven drying method (as reference) were found to be significant. For that reason FTIR method together with drying methods seem to be more suitable for this purpose.

4. Conclusions

Water content in white yoghurts was determined by several methods. Results reached by the oven drying method were used as referential, but this method is quite time consuming. This method could be forced by the moisture analyser, but reached values were about one percent lower than those obtained by oven drying method. Other methods, namely KF titration and FTIR spectroscopy in NIR and MIR regions, had to be optimised. Methanol (Combi-Methanol) was chosen as the suitable solvent for the KF titration method. Titrations were carried out at room temperature and 5 min extraction step. Isocratic acetonitrile was selected as suitable solvent for the FTIR method. Then the suitable wavenumbers were evaluated for water content determination. Those were 1640 cm^{-1}

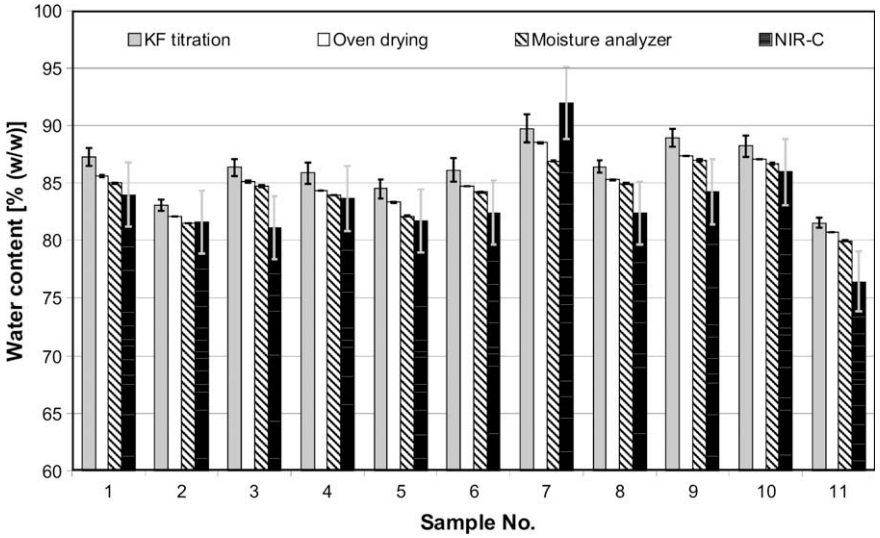

Fig. 3. Comparison of water content in white yoghurts determined by utilised methods.

and 3310 cm^{-1} for MIR method and 10270 cm^{-1} and finally the wavenumber 6530 cm^{-1} for NIR method. The reflective technique with ATR had to be used for MIR; 1 cm quartz cuvette was used for NIR method. Although the best results were obtained by oven drying method, after optimisation performance FTIR method at 10270 cm^{-1} appeared to be good alternative. This method was simple and quite fast with acceptable RSD values. The same could be stated about KF titration method, but by the statistical evaluation (ANOVA) the results obtained by this method were considered as different at $p < 0.05$.

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