A new sample preparation method for the water content determination of inulin by volumetric Karl Fischer (KF) titration was developed and compared to the usual method of introducing the sample directly in the methanol-based working medium, modified or not by formamide (1:3, v/v) in order to increase sample solubility. In the proposed method, inulins were externally prepared by dissolving them in pure formamide (2.5:7.5, w/w). The time of analysis of the liquid/liquid reaction of the new method between the dissolved sample and the reaction medium is about 1–2 min, while the usual KF method is stopped after the 10 min delay time. The developed method permits the determination of water included in the crystals of the sample, confirmed by analysing both crystalline and amorphous inulin samples. Another advantage of this new method is its applicability for the water content determination of other polysaccharides that are not readily soluble in the working medium. Moreover, water content determination can be done by any type of volumetric KF titrator, as this proposed technique is not dependent on any additional tools such as a built-in homogeniser or a heatable titration beaker.

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1. Introduction

Inulin, depicted in Fig. 1, is a natural storage carbohydrate mainly found in chicory roots, Jerusalem artichoke and dahlia tubers. It is a mixture of polysaccharides composed of fructose unit chains (linked by β-(2→1)α-fructosyl-fructose bonds) of various length, terminated generally by a single glucose unit (linked by an α-α-glucopyranosyl bond). Its composition depends on the plant source, harvesting date but also on extraction and post extraction processes [1]. The use of inulin and derivatives in the food industry are in constant increase, and the main benefits and nutritional interests of these products are broadly discussed in the literature [2].

The most stable form for commercial inulin is the powdered form, which has the advantage of facilitating manipulation, storage and transport. Stored at different relative humidities, powder products can physically changed (i.e. crystallisation), drastically modifying their characteristics, such as caking [3,4]. Because of this, the monitoring of water content of inulin during storage and quality control requires appropriate analytical techniques for accurate determination in order to prevent such negative effects. However, no publication about water content determination particularly in inulin is available and an analytical method for this purpose needed to be developed.

In general, moisture is determined by oven drying and does not correctly represent the water content of a sample. Indeed, high temperatures can induce the formation and the release of volatile substances (water and others), while water included in the crystals might or might not be completely detected. The Karl Fischer (KF) titration is a well-established method for the water content determination [5] based on the Karl Fischer reaction [6]. This technique allows the determination of the total water of a sample and is now the most important direct (primary) method for determining water content in foodstuffs. Details for
the KF titration principle were described elsewhere [7]. KF reagents based on methanol are considered toxic. For this reason, Schióffski [8] tested other reagents replacing methanol by ethanol, but they are less adapted for water content determination in polysaccharides. Indeed, to determine the water content of the sample, it must be dissolved in the reaction medium, used as solvent. In some cases, the medium does not completely dissolve the sample. In order to increase solubility, some authors used an internal homogeniser [9–11], worked at elevated temperatures [12–16], modified the polarity of the working medium by adding further solvents to it [17] or pre-extracted the water [18].

These methodologies using the KF titration present some inconveniences as not all titration devices are equipped with an internal homogeniser or a heatable titration vessel, which limits the use of these techniques for wider applications. More so, at high temperature, possible evaporation of sulphur dioxide from the boiling working medium makes a reflux condenser necessary [12]. Pre-extraction of water is not a broadly discussed method in literature. Moreover, the supernatant containing the extracted water does not in all cases include the water of crystallisation, as a residue is still present. The addition of solvent in the reaction cell seems to be the most adequate technique for solubilizing samples in the working medium. However, the combination of some methodologies like the adding of a further solvent (i.e. formamide) in the medium with heating of the system or the utilization of a homogeniser, lead to some side reactions, falsifying the water content determination. Schmitt and Isengard [14] reported that formamide in the medium led to a higher drift because of a side reaction. However, when the drift was taken into account, they found values in accordance with the theoretical value. This phenomenon is known to occur particularly at high temperatures (i.e. 50 °C) and is much less expressed at lower temperatures.

The aim of the present study was to develop a suitable sample preparation for the total determination of water content of inulin, which can be present in an amorphous or crystalline state. For this purpose, inulin samples were analysed by KF titration using the usual method of introducing the powder directly in the methanol base-working medium, modified or not by formamide (1:3, v/v) in order to increase sample solubility. In addition, an alternative methodology based on the introduction of an inulin solution in pure formamide was developed and compared to the standard KF titration.

2. Experimental

2.1. Materials

2.1.1. Inulin samples

The inulin samples used were two industrial spray dried products extracted from chicory roots, one purchased from Orafti (inulin 1) and the other kindly supplied by Warcoing (inulin 2) both located in the Walloon Region (Belgium). They were placed into Petri dishes (six replicates) and stored for 3 weeks in hermetically closed containers containing P2O5 at 20 °C. This storage was done in order to eliminate the free water.

Determination of the molecular mass distribution of the samples was done by High Performance Anion Exchange Chromatography coupled with Pulse Amperometric Detection (HPAEC-PAD) on a Dionex DX500 chromatographic system operating at 1 ml min⁻¹. About 25 μl of 0.8 g l⁻¹ solution were injected. Separation of the various chain lengths was achieved on a Dionex PA100 column as described by Ronkart et al. [4]. The mobile phase consisted of sodium hydroxide (150 mM) elution in isocratic mode, followed by a linear gradient with a solution containing both sodium hydroxide (150 mM) and sodium acetate (1 M). The gradient ended by washing with sodium hydroxide 500 mM. Relative response factors of the various species were taken from Timmermans et al. [19] and Kang [20]. Inulin 1 and 2 had an average degree of polymerisation number (DPn) and weight (DPw) of about 26 and 31 and 23 and 30, respectively.

Determination of crystallinity was done by a PW3710 Philips Analytical X-ray B.V. static Wide Angle X-ray Scattering (WAXS) diffractometer, using a Ni filtered Cù Ka radiation, generated by an anode device operating at 40 kV and 30 mA in conjunction with a proportional detector. The patterns were recorded with a fixed time of 0.4 s per step of 0.02° in the 4 < 2θ < 30° range [4,21]. Fig. 2 shows the amorphous state of inulin 1 as only a flat diffractogram is present, while inulin 2 contains several diffraction peaks superimposed on an amorphous halo, hence confirming the semi-crystalline nature of this sample.
2.1.2. Karl Fischer titration

The KF titration was carried out with a Karl Fischer titrator DL31 from Mettler Toledo using the two-component technique with Hydra-Point Solvent G and Hydra-Point titrant (5 mg H2O/ml), both purchased from J.T. Baker (Deventer, Holland). The polarising current for bipotentiometric end-point determination was 20 μA and the stop voltage 100 mV. The end-point criterion was the drift stabilisation (15 μgH2O min⁻¹) or maximum titration time (10 min). The water content was calculated as described by Scaccia [22], and the measurement was corrected for the baseline drift, defined as the residual or penetrating water that the apparatus removes per minute.

For better sample dissolution, the working medium was modified by adding formamide pro analyse min 99% purchased from Merck (Leuven, Belgium).

2.2. Methods

2.2.1. Experimental precaution

Traces of water can penetrate into the titration vessel from the exterior through tubes and joints. For this reason, before starting experiments, 15 ml of titrating agent were washed through. Then, the exact water equivalent of the titrant component was determined by analysing a water standard reference (apura® Water standard 1% standard for volumetric Karl Fischer Titration, Merck KGaA, Darmstadt, Germany). Before starting an experiment (drift or water equivalent determinations) or introducing the sample into the working medium for water determination, the cell was titrated to dryness. In the course of time, a slight reagent consumption to keep the titration cell dry is always observed. This so-called drift (expressed in μg water min⁻¹) is due to water intruding through the tubes and joints. For precise analyses, especially when the determination times were long, consumption due to the drift was subtracted from the result, taking the titration time into account. Sample analyses were started when the drift was lower than 10 μg water min⁻¹.

2.2.2. KF titration of powdered inulin

About 250 mg of inulin was introduced in the reaction cell. The latter contained either only the methanolic KF working medium or a modified one with additional formamide (1:3, v/v) in order to increase the solubility of these samples.

All mass determinations were carried out by back-weighing, using a Sartorius BP61S balance (±0.0001 g). Thus, the exact sample weight was determined only after adding the sample into the titration cell. Stirring time in the cell before titration was fixed at 20 s. Six analyses were performed for each method and sample, and the respective results were averaged.

2.2.3. KF titration of inulin solution in formamide

About 2.5 g of inulin were dissolved in 7.5 g pure formamide in a 15 ml Falcon® tube and heated at 45 °C for 30 min, then vortexed until complete dissolution. Then, about 1.5 g of this 25% (w/w) solution was injected in the reaction cell using a syringe.

Beforehand, to determine if inulin was totally dissolved in the formamide solution, a solubility test was performed. Inulin 2 (less soluble in formamide than inulin 1) was dissolved in formamide in the 1–35% (w/w) range. These solutions were then centrifuged at 30,000 × g for 1 h and the supernatants were checked by HPAEC-PAD as described above. Results have shown a total solubility up to 25% (w/w) and a slight decrease above this concentration. For this reason, the chosen concentration for sample preparation in formamide was 25% (w/w).

3. Results and discussion

Water content calculated for the inulin samples by volumetric KF titration are summarized in Table 1.

3.1. Analysis of powdered inulin by KF titration without formamide in the working medium

The water content found for inulin 1 and 2 was 1.27 and 1.10 g water/100 g anhydrous inulin, respectively. However, the dissolution of the product was not complete, as the working medium was very cloudy, and the analysis was stopped by the imposed maximum titration time of 10 min and not by the drift criterion. So, the water content was underestimated for both samples. This phenomenon can be related with the way the end-point indication is fixed. Indeed, the volumetric KF titration is based on the following two-step reaction:

\[ \text{R–OH} + \text{SO}_2 + \text{B} \rightarrow \text{BH}^+ + \text{ROSO}_2^- \]
\[ \text{BH}^+ + \text{ROSO}_2^- + \text{I}_2 + \text{H}_2\text{O} + 2\text{B} \rightarrow 3\text{BH}^+ + \text{ROSO}_3^- + 2\text{I}^- \]
\[ \text{R–OH} + \text{SO}_2 + \text{I}_2 + \text{H}_2\text{O} + 3\text{B} \rightarrow \text{BH}^+ + \text{ROSO}_3^- + 2\text{I}^- \]

Table 1

Results of the Karl Fischer titration (g water/100 g anhydrous sample) of inulin, by the conventional KF titration (with or without formamide in the working medium) and for the proposed KF method, all performed at 20 °C

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conventional KF method</th>
<th>Proposed KF method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without formamide a</td>
<td>With formamide a</td>
</tr>
<tr>
<td>Inulin 1</td>
<td>1.3 ± 0.3 (&gt;10 min)</td>
<td>2.1 ± 0.1 (4–5 min)</td>
</tr>
<tr>
<td>Inulin 2</td>
<td>1.1 ± 0.3 (&gt;10 min)</td>
<td>1.8 ± 0.3 (&gt;10 min)</td>
</tr>
</tbody>
</table>

a In parentheses, the titration time in min.
In the first step, an alcohol ROH (usually methanol) is esterified with sulphur dioxide. To obtain a quantitative reaction, the ester is neutralised by a base B (usually imidazole) to yield alkyl sulphite (Eq. (1)). In the second step, alkyl sulphite is oxidised by iodine to give alkyl sulphate in a reaction that requires water. The base, again, provides for a quantitative reaction (Eq. (2))[23].

Two platinum electrodes are submerged in the working medium and are polarized by a constant current. A certain voltage is necessary to maintain this current. When it falls and remains below a certain chosen value for a chosen time, the so-called stop delay time, the analysis is stopped. Another stop criterion is the drift. When the reagent consumption rate reaches the same (or a slightly higher) value as immediately before the start of the analysis, it is assumed that all the water of the sample is detected, and the analysis is stopped. Additionally, it is possible to set a maximum determination time, which must not be exceeded. If the determination is stopped after this maximum time, the analysis is incomplete, since the "real" stop criteria have not been fulfilled. In this case, as the samples were not completely dissolved, water diffusion was low and delayed, so iodine was continually consumed keeping the titration rate above the drift value. Thus, since the measurement was stopped after 10 min and not because water detection was complete, it would have been necessary to increase the maximum titration time to allow the water to reach the working medium completely by diffusion and extraction.

3.2. Analysis of powdered inulin by KF titration with formamide in the working medium

In comparison with the analysis of powdered inulin by KF titration without formamide in the working medium, analysis of inulin 2 was still stopped by the maximum time (10 min), whereas the analysis of inulin 1 was stopped by the drift criterion after 4–5 min. The addition of formamide led to a higher water content estimation. Indeed, 2.07 and 1.83 g water/100 g anhydrous inulin for inulin 1 and 2 were found, instead of 1.27 and 1.10 g water/100 g anhydrous inulin (without formamide). We can correlate this phenomenon with the fact that the addition of formamide to the medium leads to a complete extraction of the water between the amorphous particles of inulin 1, while the extraction of the partially crystalline inulin was not completely achieved.

Although Wünsch and Schöffski [24] claimed that formamide accelerated the iodination of imidazole, leading to slightly over-estimated water content values, we assumed that formamide up to one third by volume did not yield false results. Indeed, the analysis of a 1% water standard before and after the formamide addition in the working medium led to the same mean values even if the addition of formamide to the system delayed the reaching of the drift criterion.

By adding formamide in the methanol-based medium (1:3, v/v), an increase of the water content value is obtained because of a better solubility of the sample.

3.3. Analysis of inulin dissolved in formamide by KF titration

Water content was calculated by subtracting the water content of formamide. The latter was determined before experimentation and was 0.03 g water/100 g formamide.

Inulin 1 analysis led to the same water content determination as the introduction of inulin powder in the titration cell with addition of formamide (2.07 g water/100 g anhydrous inulin), but the analysis time was reduced by at least a factor 2. However, the water content of inulin 2 was higher than that analysed by the introduction of inulin powder in the titration cell. This difference is probably due to the release of water entrapped in the crystals of the semi-crystalline products. So, it can be assumed that the proposed method fully extracts the water content of inulin samples. In addition, the analysis time was only 1–2 min, due to the immediate availability of the water. This shorter analysis time reduces the influence of a possible change of the drift during the analysis. The developed method has a higher precision of the measurements than the methods with incomplete water detection. It is well adapted when the water content of the sample is low.

4. Conclusion

The total water content of inulin has been determined by volumetric Karl Fischer titration, using a complete pre-dissolution of the sample in pure formamide, and was compared to the usual introduction of the sample directly in the methanol-based working medium, modified or not by addition of formamide (1:3, v/v) in order to increase sample solubility. The described method allowed the determination of water included in the crystals, as shown by comparing the results for amorphous and semi-crystalline inulin samples.

The titration time for the proposed method was 1–2 min, while complete water detection was not achieved after 10 min by the standard method. Dissolving the sample beforehand and transferring an aliquot into the titration cell is time consuming, but this is compensated by the shorter titration time. This advantage is even more important, when routine analyses are carried out and several samples can be dissolved simultaneously [18]. Moreover, the proposed method has the advantage of working at ambient temperature and can be applied to every volumetric KF titrator.

This methodology can be applied for the water content determination of other polysaccharides presenting a solubility problem in the working medium.

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