



NMR-based identification of thickeners in membrane-filtered food premixtures

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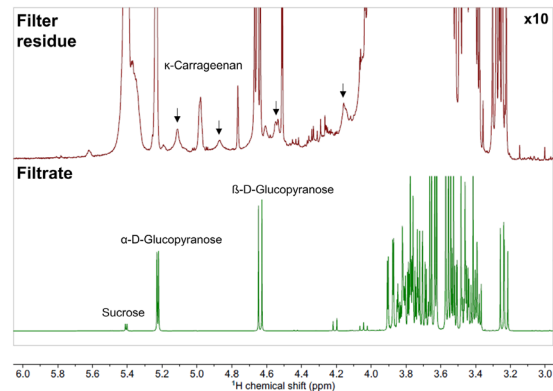
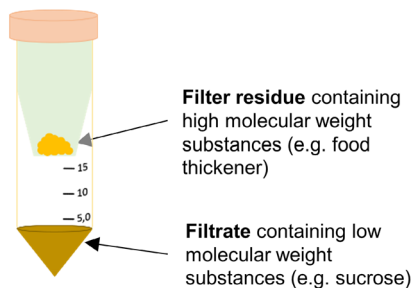
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Abstract

Premixtures for food production are complex mixtures typically containing thickeners due to their water binding capacity. Here, we report an improved protocol for the fast identification of food thickeners by ¹H-NMR spectroscopy. The method is based on four steps: (i) dissolving of the dry premixture in water, (ii) centrifugation of the solution using centrifugal concentrators with a cut-off of 100 kDa, (iii) re-dissolving of the freeze-dried filtrate and the filter residue in small volumes of deuterated water, respectively, and (iv) ¹H-NMR analysis of these fractions focusing on specific marker signals. Using this procedure, the high molecular weight thickeners (above 100 kDa) κ-carrageenan, galactomannans, gum arabic and pectin were unequivocally identified in the NMR spectra of the filter residues from different premixtures, whereas low molecular substances (below 100 kDa) including sucrose and glucose were determined in the filtrates.

Graphical abstract

Centrifugal concentrator
(cut-off 100 kDa)



Keywords Premixtures · Food thickeners · NMR spectroscopy · Centrifugal concentrators

Introduction

In recent years, food fortification using supplements of vitamins and minerals has become an increasing trend in an attempt to provide health benefits for the consumers [1]. In the food industry, the application of formulations or blendings is a simple and cheap technology for the production of foodstuff [2]. Fine powders can be produced using different methods like pressure agglomeration, extrusion agglomeration or steam jet agglomeration [3]. The composition of these so-called premixtures differs and depends on the

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intended use in the food product. For instance, a formulation of different multiple components like soy protein isolates and wheat gluten is often used for the production of meat analogs, whereas sucrose, wheat flour and fats are important for the texture, volume and conservation of bakery products [4, 5]. Final consumers are also able to purchase premixtures like clear cake glaze powder, cake mix, spice mixture or sauce thickener in the supermarket. Further on, thickening agents containing starch or gums are offered for sale to increase the viscosity of liquids or food, which is especially important for persons with dysphagia [6]. All of these mentioned mixtures often contain food thickeners like carrageenan, guar gum or locust bean gum which are considered as food additives [5, 6]. The allowed usage and concentration of food additives in premixtures and food are defined in regulation (EC) No. 1333/2008 [7], whereas the specification—containing definition, purity and identification—of these substances is described in regulation (EU) No. 231/2012 [8]. In general, food thickeners are natural high molecular weight polysaccharides carrying various monosaccharide units [9]. The exact structures and hence the molecular weights depend on their biological source, the extraction method and the ripening point of the raw material [10]. For instance, modifications of pectin like alkylation of carboxyl and hydroxyl groups, amidation, thiolation and sulfation are often used to increase the molecular size [11]. The chemical composition and molecular weight from different food thickeners (locust bean gum, guar gum, κ -carrageenan, pectin and gum arabic) are listed in Table 1.

Because of their thickening, stabilizing and emulsifying characteristics, food thickeners are often used in emulsion food (e.g. dairy products, salad dressing), restructured food and frozen food [12]. In Germany, the compulsory method for the official food control is the so-called §64 LFGB

method (here L 00.00-13), which has to be used in case of legal disputes [13]. This procedure is based on degradation of the polysaccharides and the determination of the resulting sugar units. Further time-consuming methods are described in the literature to analyse food thickeners in food products. Nevertheless, all of these methods are not appropriate for a fast screening, as they include numerous steps [14–16].

In our earlier study [17], a fast determination of some intact thickeners could be achieved by a combination of $^1\text{H-NMR}$ spectroscopy and Diffusion Ordered Spectroscopy (DOSY) without time-consuming sample preparation. However, this rapid procedure was not suitable for all analysed matrices mainly because of the low concentration of thickeners in the food or premixtures under study [17]. Here, we describe a simple addition to our earlier protocol which allowed to concentrate and to detect galactomannans, pectin, κ -carrageenan and gum arabic in premixtures.

Materials and methods

Chemicals

Deuterium oxide 99.9% with 0.03% trimethylsilylpropionic acid (TMSP) was purchased from Deutero GmbH (Kastellaun, Germany). κ -Carrageenan and gum arabic were obtained from Acros organics (New Jersey, USA). Guar gum ~ 5000 cP was obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Locust bean gum was purchased from Vitanatur GmbH (Stockstadt am Rhein, Germany). Apple pectin was obtained from Sigma Aldrich Chemie GmbH (Steinheim, Germany). Vivaspin Turbo 15, Membrane 100.000 MWCO Ultrafiltration Centrifugal

Table 1 Food thickeners with typical molecular weights and chemical composition

Name of thickener	Chemical composition	Molecular weight
Galactomannan locust bean gum (E410)	Mannose and galactose (ratio 4:1) [23]	50–1000 kDa [23]
Galactomannan guar gum (E412)	Mannose and galactose (ratio 2:1) [24]	2000–3000 kDa [24]
κ -carrageenan (E407)	Galactose-4-sulfate, 3,6-anhydrogalactose [25]	200–800 kDa [25]
Pectin (E440)	Three polysaccharide structures isolated from primary cell walls: Homogalacturonan Rhamnogalacturonan I Rhamnogalacturonan II containing galacturonic acid as main chain and rhamnose, arabinose, galactose, xylose as side chains; carboxyl groups can be methylated, hydroxyl groups can be acetylated [26]	50–150 kDa [27]
Gum arabic (E414)	Three fractions: Arabinogalactan (main component), Arabinogalactan-protein complex Glycoprotein containing galactose, rhamnose, arabinose, glucuronic acid and proteins [21, 28]	~380 kDa (from <i>Acacia senegal</i>) ~850 kDa (from <i>Acacia seyal</i>) [21]

Concentrators were obtained from Sartorius Stedim Biontech GmbH (Goettingen, Germany).

Samples

Bakery mix, premixture for compound sauce, premixture for brine supplement, premixture for ice dessert strawberry, gelling agent, standardized thickening agent, first infant milk powder, ice cream mix and instant flavored drink.

Sample preparation

- Preparing of reference thickener samples:
 - o Approx. 10 mg of a reference thickener were dissolved in 1 ml D₂O with 0.03% TMSP and warmed up for 10 min at 80 °C for complete solving while stirring.
- Premixture samples:
 - o 50–300 mg of the premixture were dissolved in 2 ml H₂O and centrifuged with ultrafiltration centrifugal concentrators (cut-off 100 kDa) at 2.000 rpm for 1 h at room temperature. The filter residue was resolved in approx. 3 ml H₂O and quantitatively transferred to a pear-shaped flask. The filter residue and the filtrate were placed in a freezer for 1 h and freeze-dried overnight. The dry samples were dissolved in 1 ml D₂O containing 0.03% TMSP and a volume of 550 µl was transferred to a 5-mm NMR glass tube.
 - o Spiking: Similarly, 50–300 mg of the premixture were dissolved in 2 ml H₂O. After having added 200 µl of the reference thickener sample, the mixture was centrifuged with ultrafiltration centrifugal concentrators (cut off 100 kDa) at 2.000 rpm for 1 h at room temperature. The filter residue was resolved in approx. 3 ml H₂O and quantitatively transferred to a pear-shaped flask. The filter residue and the filtrate were placed in a freezer for 1 h and freeze-dried overnight. The dry samples were dissolved in 1 ml D₂O containing 0.03% TMSP and a volume of 550 µl was transferred to a 5-mm NMR glass tube.

NMR acquisition parameters

All NMR spectra were recorded with a Bruker AVANCE III 400 MHz spectrometer equipped with a BBO probe using TopSpin Version 3.6.1 (Bruker Biospin GmbH, Rheinstetten, Germany). ¹H-NMR spectra were recorded applying the Bruker pulse program “noesygppr1d” for suppression of the HDO signal during the relaxation period applying a narrow saturation pulse with a bandwidth of about 25 Hz. The parameters were number of scans (ns)=512, number of dummy scans (ds)=4, relaxation delay (d1)=4.0 s, temperature (TE)=27 °C, acquisition time (aq)=3.98 s, data points size of fid (td)=65,536, data points size of real spectrum (si)=131,072, spectral width (sw)=20.50 ppm, 90 degree pulse (p1)=7.65 µsec. Prior to Fourier-transformation, the free induction decays (FIDs) were multiplied with a mild Gaussian function (line broadening lb=−0.10; gaussian maximal position gb=1.76). Chemical shifts were reported relative to the internal standard TMSP at 0.00 ppm. Data were processed with MestreNova Version 12.0.0 (Mestrelab Research, Santiago de Compostela, Spain).

Results

Food thickeners are polysaccharides with high molecular weight. In food or food premixtures, they are typically used in low concentrations between 0.1 and 5% [18]. The method presented here enables the simple and fast concentrating of these polysaccharides from food premixtures followed by NMR analysis. By ultrafiltration using centrifugal concentrators with a cut-off of 100 kDa, low molecular constituents like sucrose, glucose and vitamins pass through the filter, whereas substances with high molecular weight (above 100 kDa) remain in the filter and are subsequently dissolved in deuterated water for NMR analysis. Starting from different premixtures (e.g. compound sauce, gelling agent, instant flavored drink), the declared food thickeners could be unequivocally identified in the ¹H-NMR spectra based on their characteristic marker signals (Table 2). This procedure was successful for the identification of the thickeners gum arabic, pectin and κ-carrageenan. In the following, the method is illustrated with the specific example of a

Table 2 Overview of marker signals from food thickeners used for the identification in premixtures [17]

Name of thickener	Chemical shifts δ of marker signals used for the identification
Galactomannans locust bean gum (E410) and guar gum (E412)	4.14 ppm and 5.03 ppm
κ-carrageenan (E407)	4.15 ppm, 4.54 ppm, 4.66 ppm, 4.87 ppm and 5.11 ppm
Pectin (E440)	4.96 ppm and 5.08 ppm
Gum arabic (E414)	1.27 ppm, 4.30 ppm, 4.42 ppm, 4.52 ppm, 5.03 ppm, 5.30 ppm and 5.42 ppm

bakery mix containing guar gum. For other examples, see Supporting information.

Bakery products often contain guar gum as an additive due to its ability to improve mixing tolerance and to prevent syneresis in frozen products [19]. For the NMR-based identification of the galactomannan guar gum, characteristic marker signals at 5.03 ppm (H1 of the galactose unit) and at 4.14 ppm (H2 of the mannose unit) can be used, as previously described [17].

To identify the declared thickener guar gum in a bakery mix, an amount of 100 mg of this premixture was dissolved in 1 ml D₂O and directly measured by ¹H-NMR spectroscopy. In the ¹H-NMR spectrum (Fig. 1B) many signals belonging to ingredients like sucrose, dextrose and malt extract were detected. The characteristic marker signal for galactomannans at 5.03 ppm could hardly be identified due to signal overlap with a sharp doublet signal belonging to another substance. To enable the detection of guar gum in this premixture, 100 mg were dissolved in 2 ml H₂O, centrifuged with ultrafiltration centrifugal concentrators (cut-off 100 kDa) and freeze-dried. The filter residue was then dissolved in 1 ml D₂O and analysed by ¹H-NMR spectroscopy. The spectrum (Fig. 1C) now revealed a broad signal at 5.03 ppm which is a characteristic marker signal for galactomannans like guar gum [17]. Spiking this sample

with standard guar gum indeed led to an increase of the 5.03 ppm signal in the ¹H-NMR spectrum of the filter residue (Fig. 1C). As expected, the ¹H-NMR spectra of the filtrates (Fig. 1D, E) showed many sharp signals belonging to low molecular weight substances (e.g. sucrose). The characteristic NMR signals of the galactomannan guar gum at 5.03 ppm and 4.14 ppm were missing.

Discussion

Centrifugal concentrators are often used for biological sample preparation like desalting or concentration of proteins in cell lysates, serum and tissue homogenates [20]. However, they are also suitable for the separation of high molecular weight food thickeners from substances with molecular weight below 100 kDa. In contrast to the §64 LFGB method L 00.00–13 [13] and previously described methods in the literature [14–16], the sample preparation presented here seems to be especially appropriate for a fast screening of thickeners in food premixtures by means of NMR spectroscopy when the direct identification of these additives is not possible due to their low concentration [17]. We here show with various examples that high molecular weight thickeners predominantly remained in the filter residues as no broad

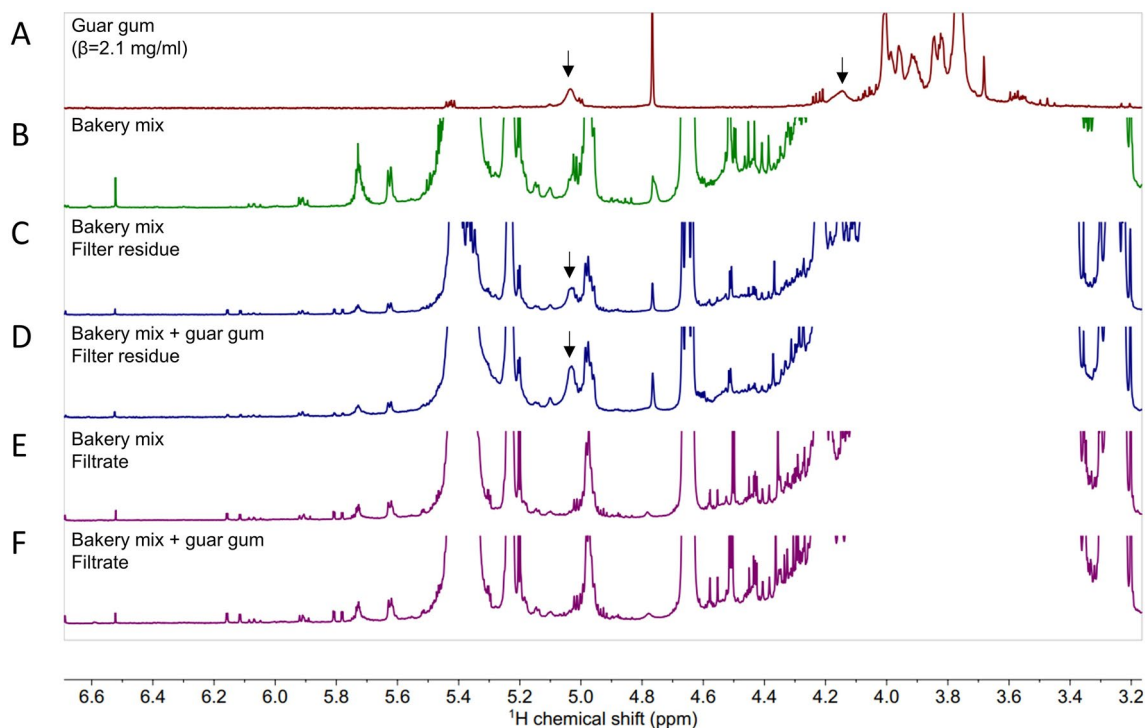


Fig. 1 ¹H-NMR spectra of standard guar gum (A), bakery mix (B), the filter residue of the bakery mix (C), the filter residue of the bakery mix spiked with guar gum (D), the filtrate of the bakery mix (E) and the filtrate of the bakery mix spiked with guar gum (F) in D₂O.

Measurement with ¹H-NMR spectroscopy with water suppression (400 MHz, ns 512, ds 4, TE 27 °C). Guar gum signals at 5.03 ppm and 4.14 ppm are indicated by black arrows

signals were detected in the $^1\text{H-NMR}$ spectra of the filtrates. Although the declared food thickeners were unequivocally identified in the $^1\text{H-NMR}$ spectra of the filter residues, it seemed that the high molecular weight substances were not completely separated from low molecular weight substances as the $^1\text{H-NMR}$ spectra of the filter residues also contained some residual sharp signals albeit at small intensities. It should also be noted that premixtures could contain other ingredients with high molecular weight like starch and maltodextrin or additives like herbs or cocoa powder which could make the separation of the thickeners as well as their NMR detection more difficult. Furthermore, the molecular weight of food thickeners varies depending on seed source, production process and climatic conditions [21, 22]. Using food thickeners with molecular weight smaller 100 kDa for the production of premixtures, the polysaccharides will pass through the filter and no separation will be achieved. In this case, centrifugal filters with a different cut-off (e.g. 30 kDa) have to be used to guaranty a successful thickener concentration. Nevertheless, the application of centrifugal concentrators appeared to be a highly useful tool for concentrating food thickeners from premixtures allowing for their fast identification by NMR spectroscopy.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00217-022-03998-w>.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animals.

Informed consent Informed consent is not applicable.

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