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Original Article

A method for determination of soluble dietary fiber in methylcellulose and hydroxypropyl methylcellulose food gums

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Abstract

A size-exclusion chromatographic approach is proposed as a modification to the currently practiced AOAC International methods, to provide reliable means for the determination of methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) food gums as soluble dietary fiber (SDF). The official enzymic/gravimetric AOAC methods, including the most common method 991.43, do not reflect the SDF status of MC and HPMC, and possibly many other water-soluble cellulosics gums that may either gel or precipitate at high temperatures in 100% aqueous solutions, or may be soluble in approximately 71% ethanol. As referenced within this paper, all of these gums exhibit the characteristics of SDFs, from both the chemical and physiological standpoint. The enzymic digestion principle of the existing AOAC methods is left unchanged in general, with only very minor adjustments introduced. The SDF recovery determined by the modified method is 77% for MC and 90% for HPMC in 0.1% (wt/wt) aqueous solution and 93% and 99%, respectively, in a pound cake food matrix. The method has been adopted *First Action* by AOAC as method 2006.08.
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1. Introduction

There is an ongoing dispute within the scientific community and the regulatory agencies of various governments on the valid official definition of dietary fiber (DF). One of the most commonly known and accepted definitions, from the American Association of Cereal Chemists (AACC), states: "DF is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. DF includes polysaccharides, oligosaccharides, lignin, and associated plant substances. DFs promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" (AACC—Dietary Fiber Definition Committee, 2001). Another criterion used to define these fibers is the detection and determination by officially acknowledged AOAC International (formerly known as Association of Official Analytical Chemists) methods (AOAC International, 2005), including the most popular method 991.43 and its AACC equivalent—method 32-07. Proper fractions collected and measured with these methods can be classified as either insoluble dietary fiber (IDF) or soluble dietary fiber (SDF). The AOAC-method fiber classification is especially important in the United States of America, as it imparts FDA recognition as DF on food nutrition labels. In Europe, other commonly utilized analytical procedures are the Uppsala method (adopted by AOAC as 994.13) and

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the Englyst method (Theander and Westerlund, 1986; Theander et al., 1993; Englyst et al., 1987; Wood et al., 1993).

With multiple obesity-related health problems, any additional source of DF in a regular Western diet would become an undisputable advantage. Many processed foods lack sufficient amounts of fiber; lack of fiber has been reported to be associated with an increased risk of obesity (Alfieri et al., 1995; Van Itallie, 1978). The beneficial role of DF in many aspects of human health has been confirmed by numerous publications, including studies on methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) (Blundell and Burley, 1987; Durrant and Royston, 1978; Pereira and Ludwig, 2001; Brown et al., 1999; Rimm et al., 1996; Pereira et al., 2004; Mancini and Stamler, 2004; Chandalia et al., 2000; Spiller, 1994; Cummings, 1984; Cummings et al., 1976; Cann et al., 1984; Tikhonova et al., 1973; Jenkins et al., 1978; Reppas et al., 1993; Dressman et al., 1993; Swidan et al., 1996; Maki et al., 1999, 2000), and has also been confirmed in experimental animals (Gallaher et al., 1993a, b; Carr et al., 1996; Yokoyama and Shao, 2006). HPMC and MC have been successfully used as food additives with no known adverse effects (Santos and Expert Panel, 1986; McCollister et al., 1973; Nitschke and Fiero, 1977). MC holds GRAS status ("generally regarded as safe"; a United States Food and Drug Administration legal category) and has been widely used in foods since the 1950s. GRAS status is pending for HPMC; however, it has also enjoyed widespread use in the food industry and it can be used in many foods at concentrations sufficient to supply beneficial amounts of SDF.

SDF and IDF are distinguished by their solubility in water. It appears that MC, HPMC and related cellulosics, derived through modification of natural cellulose (Phillips, 2000), could become an important source of SDF in foods (especially "convenience foods" or other processed foods). As such, they should be indicated on food nutrition labels. Prevailing opinion in academics and food industry acknowledges MC and HPMC food gums as part of the SDF family. In fact, the AACC report explains the phrase "analogous carbohydrates" as follows: "Carbohydrate(s) of structure(s) analogous to those of naturally occurring DFs have been shown to demonstrate the physiological properties of the respective materials to which they are analogous. These analogous carbohydrates are produced during food processing, by chemical and/or physical processes affecting the digestibility of starches, or by purposeful synthesis" (AACC—Dietary Fiber Definition Committee, 2001). Further evaluations of this definition were recently published, where HPMC and MC are listed as DF according to the AACC definition (Jones, 2000; DeVries, 2003). In addition, a report from the Institute of Medicine (IOM) states: "There are a number of modified cellulose compounds, such as MC, carboxymethyl cellulose, and derivatives of these, which are soluble […]. Several of these compounds are used as laxative agents and in a variety of food products […]. They have many of the same physiological properties as DF" (IOM, 2001).

There exist a number of polysaccharide fibers which resist digestion in the human gastrointestinal tract but which currently cannot be classified, labeled or marketed as "dietary". This is the case when such polysaccharides bear the physiological properties of DF, but are not properly detected by the current official methods. In other words, although they indeed are DFs in the physiological sense, they are not classified as such in the United States by the FDA because they lack a validated, AOAC-approved analytical method. Many modified cellulosics food gums, including MC and HPMC, are such polysaccharides (being "analogous carbohydrates", according to AACC definition) that fail to be properly determined by the analytical methods currently approved by AOAC (2005) and AACC (2000). This method deficiency has already been reported for some fibers (e.g. fructans such as inulin and oligofructose), by Prosky (2001), McCleary et al. (2000) and FDA scientists—Ku et al. (2001) and Rader and Ku (2001). The IOM has recognized this important issue, as its report states: "All the current methods include all nonstarch polysaccharides that precipitate in 78–80 percent ethanol. Polysaccharides that do not precipitate in ethanol are not included in any of the existing methods. Polysaccharides that are excluded by ethanol precipitation include inulin, other fructans, modified cellulose, and some arabinogalactans" (IOM, 2001). Also, the AACC has acknowledged the lack of official FDA/USDA DF definition and the fact that in lieu of such definition the AOAC methodology is utilized (AACC—Dietary Fiber Definition Committee, 2006). Some of those polysaccharides are now recognized as DFs (for nutritional labeling purposes) after AOAC approval of analytical methods modified to detect them, e.g. AOAC methods 997.08 for fructans, 999.03 for inulin, 2000.11 for polydextrose and 2001.03 for resistant maltodextrin (AOAC International, 2005; Craig et al., 2001; Gordon and Okuma, 2002; Hoebregs, 1997; Dysseler et al., 1999).

The most significant factor that rules out many, if not the majority of modified cellulose fibers from determination as SDF by current enzymic/gravimetric AOAC methods is the solvent and temperature incompatibility. The "golden standard" AOAC method 991.43 (and AACC equivalent method 32-07) utilizes elevated temperatures of 60° and 95 °C for the enzymic reactions, as well as 95% ethanol solutions at 60 °C for precipitation of SDF (added in a 3:1 ratio to the enzymic reaction filtrate, thus resulting in ca. 71% hot ethanol solution), 95% and 78% ethanol and acetone at room temperature to wash the SDF residue. While this procedure is quite successful for the majority of DFs, it is well known that MC, HPMC, and some other cellulosics fibers (such as hydroxypropyl cellulose or methyl ethylcellulose), are soluble in blends of ethanol and water, especially at elevated temperatures (Archer, 1992). Consequently, they will likely remain solvated during the AOAC 991.43 method's step of precipitation

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of SDF with hot ethanol, hence not be retained in the crucible filter. In addition, hot ethanol and acetone are used to wash the SDF precipitate, which will increase the chances of loss. Another very important characteristic of these fibers is that many cellulose-based food gums, including MC and HPMC, thermally gel or precipitate in 100% aqueous solutions when the temperature reaches or exceeds 45 °C (Sarkar, 1979; Sarkar and Walker, 1995; Coffey et al., 1995). Fortunately, since the temperature of a healthy human body does not exceed 40 °C, these cellulosics food gums are either partially or fully hydrated, depending on the method of ingestion (hence understood as SDF). However, when treated by the AOAC 991.43 method's enzymic reaction conditions, namely at 60° and 95 °C in 100% aqueous environment during enzymic digestions and the subsequent filtration steps, they usually appear as not fully hydrated, hence falsifying the outcome of gravimetric analysis (i.e. part of them may be retained in the previous step as IDF). As a result of these method defects, MC and HPMC could not be determined and classified as SDFs.

Clearly, it would be a significant advantage to the scientific community and the food industry, if an analytical method was in place that allows detection and determination of the modified cellulose fibers, yet preserves the fundamental enzymic digestion principle of the existing official AOAC methods. In this paper, we describe the development of a method that has already been optimized for most common MC and HPMC chemistry and viscosity types used in foods (Harfmann et al., 2006). The development and the enhancements were performed on aqueous solutions of MC and HPMC, while a very preliminary quality check was also performed on a prepared food matrix where the MC or HPMC was incorporated as one of the ingredients.

2. Materials and methods

The experiments were conducted utilizing the enzymic digestion procedure of the AOAC method 991.43, however, the fiber detection and determination was modified. Instead of ethanol treatment to precipitate, separate and gravimetrically determine the SDF, detection and determination was performed by a high-performance liquid chromatographic system consisting of: PE-200 LC pump and autosampler (Perkin-Elmer Corporation, Norwalk, CT, USA); differential refractive index (RI) detector ERC-7515A (Shoko America Inc. Colorado Spring, CO, USA); column temperature control device ThermaSphere TS-430 (Phenomenex Inc., Torrance, CA, USA) and ATLAS Chromatography Data System, version 2002-R3 (Thermo Electron Corporation, Waltham, MA, USA).

Three food grade HPMC samples and one food grade MC sample were used in the study (METHOCEL™ brand, manufactured by The Dow Chemical Company, Midland, MI USA). The HPMC samples were USP 2208 type, and USP 2906 type as defined by the US Pharmacopoeia (USP27 NF22 S1 for Hypromellose). The average molecular weights were 776,000, 388,000 and 537,000, with polydispersity of 6.2, 4.1 and 5.1. The MC sample methoxy group substitution was between 27.5% and 31.5%, as defined by US Pharmacopoeia (2002).

Most other reagents were from Sigma-Aldrich (St. Louis, MO, USA), including the enzyme reagent kit for Total DF Assay (10 mL α-Amylase, heat-stable, 10 mL Amyloglucosidase, 500 mg Protease), MES/Tris buffer salts and Celite®. The solvents were of chromatographic purity (JT Baker, Phillipsburg, NJ, USA). Water (18.2 MΩ) was purified using a Milli-Q UV Plus water purification system (Millipore, Bedford, MA, USA). Ethyl alcohol absolute, USP grade, was from AAPER Alcohol and Chemical (Shelbyville, KY, USA).

MC and HPMC are soluble in cold water, but are insoluble in hot water; therefore a technique called "hot/cold dispersion" was utilized to prepare solutions of these analytes. In order to prepare a 1000 ppm (0.1% wt/wt) solution, magnetic stirring was used to disperse 0.5 g of MC or HPMC sample into 250 g of 0.05 M MES/Tris buffer at 95 °C (pH 8.2, measured at room temperature). After the powder was completely dispersed enough additional buffer was added to bring the total solution weight to 500 g. The container was then placed into an ice bath. Dissolution/hydration was achieved through continuous stirring for 1 h at 2 °C. The solutions were then stored in a refrigerator. This procedure allowed 40 mL of the solution to be directly subjected to the enzymic digestion procedure described in AOAC method 991.43. In addition to analyzing 0.1% MC and HPMC solutions, preliminary food matrix testing was also investigated. Pound cakes from a commercially available "ready-to-bake" powder were baked in-house with MC or HPMC added to the baking powder before mixing in other ingredients. In the final baked product, the exact concentration of these added food gums ranged from 2.0% to 2.1% (wt/wt). Apart from the test samples (either 0.1% MC or HPMC solution, or a pound cake), the standard solutions were also analyzed, i.e. 0.1% MC or HPMC solutions undergoing all steps of the procedure (including heated water shaker, pH adjustments), but without actual addition of the enzymes. This way of preparing standard solutions seemed more credible than using simple aqueous reference solutions. Control samples were also analyzed, either as a neat MES/Tris buffer solution or standard pound cake (both without any cellulose gum), subject to the enzymic and chromatographic procedures.

The method uses the enzymic digestion procedure of AOAC 991.43—a portion of the dried, defatted (if necessary) and homogenized sample is subjected to sequential digestion with α-amylase, protease and amyloglucosidase, at the temperatures and durations required by the original method, with a very few minor adjustments (such as reaction vessels, changed from beakers to wide-mouth screw-cap bottles). However, a major modification was to replace gravimetry with size-exclusion

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chromatography (SEC). Before the first filtration to determine IDF (step G of 991.43), an aliquot of the enzyme digestate is taken and filtered through a 0.4 μm PTFE syringe filter into an LC autosampler vial. All residues related to IDF are retained by the filter, but the SDF components pass through and are then analyzed by SEC, where separation of di- and mono-saccharides and other remnants of enzymic digestion from the polysaccharides take place. This procedure only takes into account determination of SDF. The detailed method description is currently available from the authors upon request. Currently, the experimental phases of single-laboratory validation and interlaboratory uniformity study procedure were finished, strictly according to AOAC collaborative study protocol (AOAC International, OMA-2005-Oct-029.R1). The results will be published accordingly, as part of the AOAC official review and approval process (Harfmann et al., in press; Deshmukh et al., 2006).

AOAC Method 991.43 was performed in its entirety on a series of cellulose products at an external contract laboratory (Silliker Laboratories, Chicago Heights, IL, USA). Testing included three MC samples, three HPMC samples (METHOCEL™ brand, The Dow Chemical Company, Midland, MI, USA), one commercially available carboxymethylcellulose—CMC (CMC-7HF™ from Hercules Inc., Wilmington, DE, USA), and two unsubstituted cellulose products: microcrystalline cellulose—MCC (AVICEL™ CL-611 from FMC BioPolymer, Philadelphia, PA, USA), and amorphous cellulose—AC (SOLKAFLOC™ SW-40 from International Fiber Corporation, North Tonawanda, NY, USA). A series of AOAC 991.43 method runs was also conducted in-house for selected MC and HPMC samples. For external analysis, the MC and HPMC was mixed into either a powdered sugar or powdered dry milk at 10% wt/wt ratio. For in-house analysis, ca. 100 mg of exactly weighed commercially available MC/HPMC powder form was used. In both cases, the IDF and SDF steps were performed (steps G and H) and the total DF determination (step F) was skipped.

Several SEC columns were evaluated in the study to find an optimum separation conditions: TSKgel™ GMPWXL, 300 × 7.8 mm (TosoHaas), YMC Pack Diol™ NP-120, 250 × 4.6 mm (Waters Corporation), PolyHYDROXYETHYL A™, 200 × 4.6 mm; 5-μm, 60 Å porosity (PolyLC, Inc.), MACROSPHERE™ GPC 300 (diol) 7-μm, 250 × 4.6 mm (Alltech, Inc.), BioSuite™ 450, 8-μm HR SEC, 300 × 7.8 mm with guard column BioSuite SEC 7.5 × 75 mm (Waters Corporation) and BioSuite™ 250, 4-μm UHR SEC, 300 × 4.6 mm with guard column: Protein-Pak™ 125, 20 × 3.9 mm (Waters Corporation). During the development stage, the following chromatographic conditions were investigated: mobile phase was either pure water or 0.05 M MES/Tris buffer at pH 4.5, degassed with He sparging; column temperatures 15–35 °C, RI detection temperatures: 25–35 °C; flow rates: 0.4–1.0 mL/min; injection volumes: 20–150 μL.

The optimum SEC analysis conditions ultimately employed were: BioSuite™ 250, 4-μm UHR SEC column, 300 × 4.6 mm, Protein-Pak™ 125, 20 × 3.9 mm guard column; 0.05 M MES/Tris pH 4.5 buffer mobile phase, degassed with helium; 25 °C column temperature, 35 °C RI detection temperature; 0.4 mL/min flow rate and 70 μL injection volume.

3. Results and discussion

3.1. Investigation of the official AOAC 991.43 method

The most commonly utilized analytical method for determination of IDFs and SDFs is AOAC method 991.43. MC and HPMC food gums were analyzed by this method to confirm its inappropriateness for this class of SDFs. Precision and recovery values were determined both in-house and through an independent contract laboratory. The results are illustrated in Fig. 1. The recovery is calculated as a ratio of observed vs. expected amount of fiber, expressed as % recovery of DF. It is a sum of average IDF and SDF recoveries of all HPMC and MC samples analyzed in pure powder form as well as mixed into powdered milk and powdered sugar. Overall, the recovery values for MC, and particularly HPMC, are poor, especially for SDF, which is very likely the result of their good solubility in 71% ethanol at higher-than-ambient temperatures. However, IDF recovery for MC is unexpectedly higher that SDF. One possible explanation for this presumably artificially inflated IDF recovery is the fact that the gelation point of MC is usually lower than that of HPMC. Therefore, at 60 °C, the filtration step determining IDF, MC will be relatively more non-hydrated than HPMC. In contrast, the un-derivatized cellulose was recovered at ca. 90–96% as IDF, for both microcrystalline and amorphous form. Although analyzed only once in this study, the un-derivatized cellulose is in fact well recognized as a DF (AACC—Dietary Fiber Definition Committee, 2001) and is expected to be fully recovered. Statistical analysis was only performed on the data for HPMC and MC since less than three analytical runs were made on the other samples. The % relative standard deviation (% RSD) results for MC were 134% for IDF ($n = 5$) and 66% for SDF ($n = 7$), while for HPMC it was 128% ($n = 9$) and 70% ($n = 6$), respectively. Apart from incompatibility with ethanol and temperatures, the current AOAC 991.43 method needed to be modified for MC and HPMC to improve both precision and reproducibility.

Another serious problem was encountered during filtration of the MC and HPMC digestate (steps G and H of AOAC 991.43). These solutions often rapidly clogged the filtration crucible, extending filtration to a very long time. In the most extreme cases,the filtration was not finished after 12 h. These samples were all discarded since evaporation of ethanol from the yet-unfiltered digestate solution was expected to be appreciable. This poor filtration was likely caused by the gelation properties of these food gums

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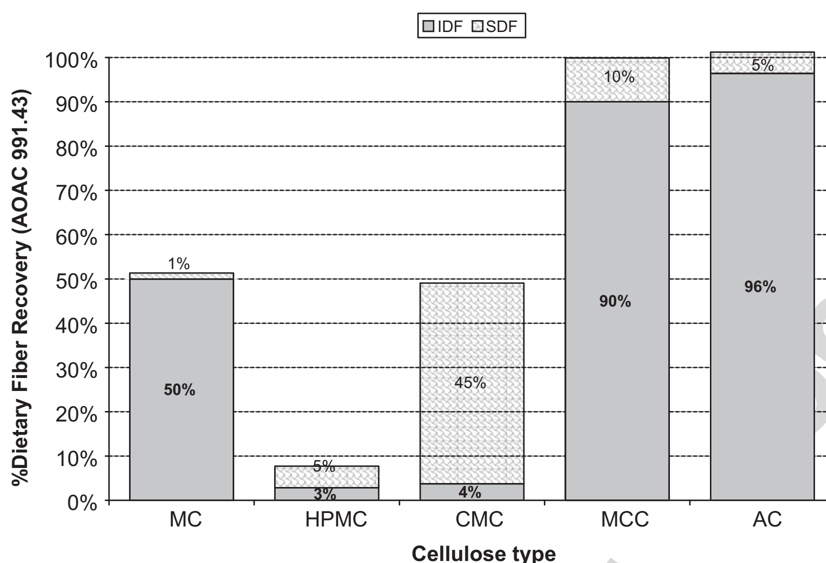

Fig. 1. Averaged % recovery of the various cellulosics fibers, determined as insoluble dietary fiber (IDF) + soluble dietary fiber (SDF), by the enzymic–gravimetric AOAC 991.43 method. Hydroxypropyl methylcellulose (HPMC) and methylcellulose (MC) data are the averages of five or more analyses; CMC is an average of two analyses, while MCC and AC results are a single analysis only.

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at elevated temperatures and/or the extremely high viscosity of HPMC and MC solutions.

To understand the effect of hot 71% ethanol, AOAC method 991.43 was performed using samples of CMC, MC, and HPMC. The method yielded total DF values (IDF + SDF) of 50%, 12%, and 15%, respectively. The 991.43 method discards the resulting alcohol filtrate, but in these studies the filtrate was analyzed and showed 48, 87, and 84 wt% residue (after blank correction) for CMC, MC, and HPMC, respectively. This accounts for nearly all of the modified cellulose in the samples and shows that significant amounts of the test materials are not precipitated by ethanol in the AOAC method.

3.2. Introduction of SEC and related procedure modifications

SEC was previously reported to be successful in characterization of MC and HPMC (Poche et al., 1998; Keary, 2001), including their determination from biological material (Yokoyama et al., 2002). Polymeric and silica-based stationary phases were initially evaluated; however, due to their mechanical stability and durability, the diol-bonded silica-based phases were chosen for further evaluation. While selected porosities provided sufficient size-exclusion capacity, these phases are not retentive for most of the other digestate components, even when using 100% aqueous mobile phase due to their strong hydrophilic properties. The detection technique of choice was RI, and the MES/Tris buffer from the last enzymic digestion step was chosen as the mobile phase since it demonstrated much less "RI disturbance peak" compared to purified water.

Once the gravimetric determination was replaced with SEC, a new test sample preparation procedure needed to be implemented. Significant effort was made to minimize modifications so that the final procedure resembled method 991.43 as closely as possible. For example, the pH change from 8.2 to 4.0–4.7 prior to the last enzymic reaction is accomplished by adding HCl solution. The challenge was to reach the desired pH range for the standard solutions. After adding the amount of HCl recommended in AOAC 991.43, solution pH was usually within the recommended range, but sometimes fell below 4.0. Since MC and HPMC are not stable in solutions below pH 3, the modified procedure requires discarding any standards for which the pH fell below 3.0. Fortunately, the test and blank samples do not have this requirement and so the new procedure is identical to AOAC 991.43. Another example of a modification was to replace the tall-form beakers with safety-coated screw-cap thick-glass bottles. They are safer, and their relatively wide mouth diameter allows for easier use.

A very important issue that concerns many cellulosics fibers, including MC and HPMC, is their proper hydration. The majority of DF polysaccharides (e.g. β-glucans, arabinoxylans, xanthan) dissolve well in water, in particular at high temperatures. This is not the case for many cellulose gums, such as MC, HPMC, HPC, or MEC. These gums either gel or precipitate from aqueous solutions at higher temperatures, but hydrate well at low temperatures. Consequently, MC and HPMC will not re-hydrate in the hot solution conditions employed in AOAC 991.43. This may result in substantial losses during determination of SDF. It is, therefore, critical that proper re-hydration of these cellulose gums take place before filtration through

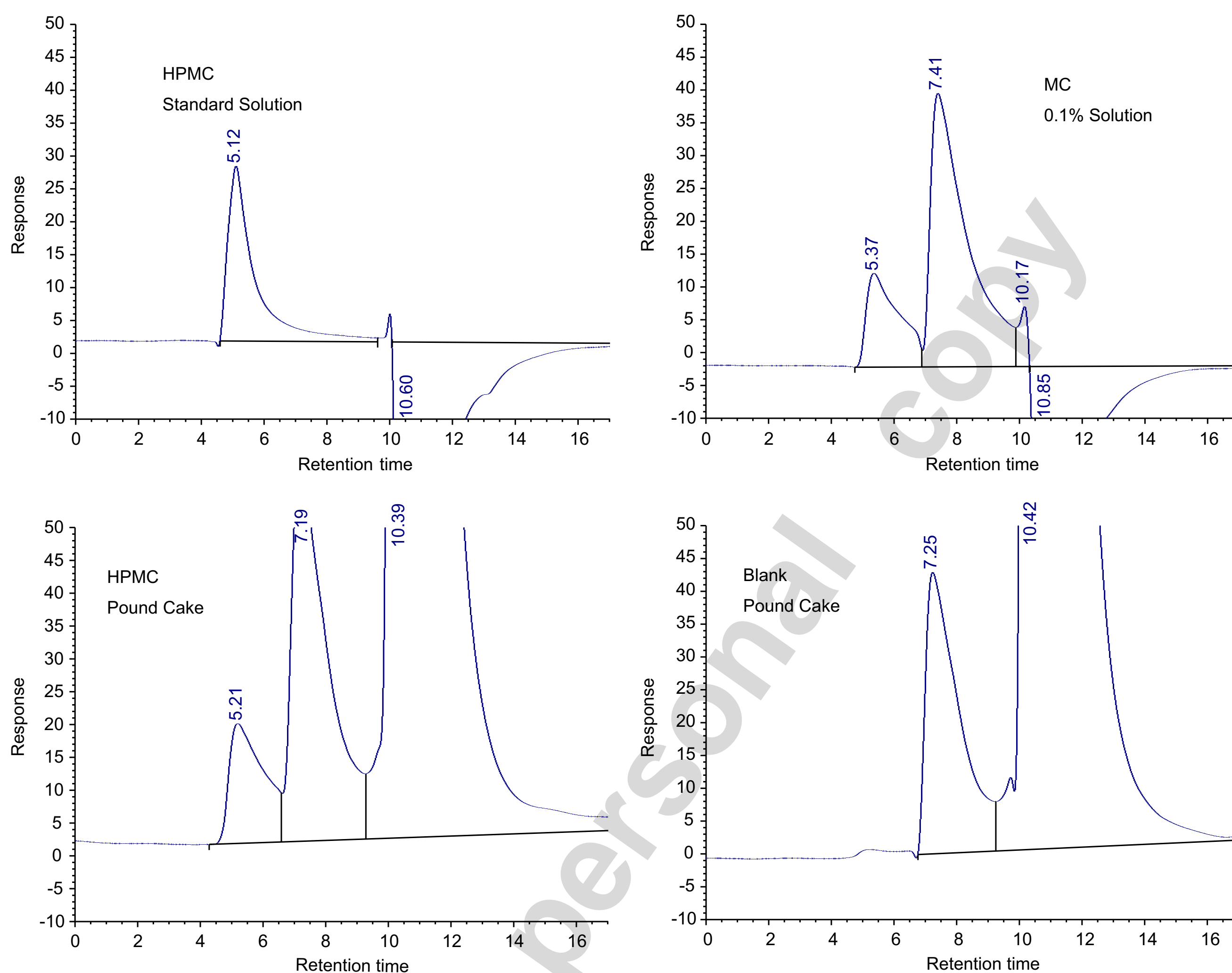

Fig. 2. Representative chromatograms of selected standards, test and blank samples using the optimized enzymic/chromatographic method. See text for details.

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Table 1
Soluble dietary fiber recovery values of methyl cellulose (MC) and hydroxypropyl methylcellulose (HPMC) solutions and as ingredient in a representative food matrix

Cellulose type	Concentration (% wt/wt)	Number of samples	Method runs per sample	Average recovery (%)	Absolute standard deviation (%)
MC in buffer	0.10	1	10	77	7
HPMC in buffer	0.10	3	10	90	7
MC in pound cake	1.76–1.82	1	5	93	1
HPMC in pound cake	1.63–2.14	3	5	99	9

Exact concentration values were calculated in weighed pound cakes after baking and reaching room temperature. In parentheses, the first number signifies number of samples, while the second number is number of analyses per sample.

0.45 μm PTFE syringe filter and subsequent SEC analysis. Several cooling times of the digestate before syringe filtration were investigated, such as 1 h in an ice bath, 12 or 16 h in a refrigerator or 48 h in a circulated air cooling room. In general, when the cooling time was relatively short (1–3 h), the % recovery and precision for SDF was poorer than it was for longer cooling. The most effective rehydration occurred after 48 h of cooling; however, a 16 h cooling period was chosen as it provided very acceptable recoveries without excessively long experimental times.

Every chromatographic stationary phase may have different silica particles size and may be packed in columns that differ in dimensions. This in turn determines the column's sample capacity and the optimum flow rate to

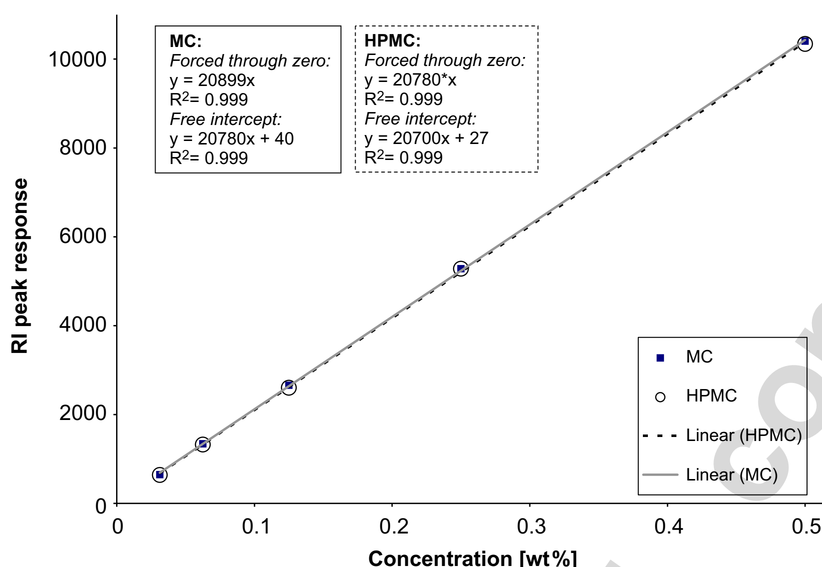

Fig. 3. Plot of refractive index detector response vs. concentration for aqueous solutions of MC and HPMC. Equations are for the best-fit linear regression (with free intercept and forced through zero).

provide the best column efficiency. In this study, all columns had the flow rates adjusted according to their dimensions so that exclusion of MC and HPMC occurred within 10 min and the entire run time was between 15–25 min. The injection volumes were adjusted according to the column capacity for each tested column. An injection volume of 70 μL was selected for the recommended BioSuite 250 column. Flow rate of 0.4 mL/min was selected (due to maximum pressure limits) and the total chromatographic run time was set to 25 min.

Chromatographic column temperature was investigated over the range 15–35 °C. The upper temperature range was limited due to the gelation properties of MC and HPMC. Indeed, somewhat worse resolution and peak profiles were observed for MC type cellulose at 35 °C, hence the column was ultimately thermostated at 25 °C.

The diol-silica-based chromatographic stationary columns were tested with the filtered digestate solutions in order to examine the resolution of the MC and HPMC peaks from enzymes and remnants of the food matrix after the digestion process. In general, a successful separation of the cellulose gums was dependent on silica particles porosity, with the optimum being between 200 and 300 Å; however, column chemistry played a role, too. Most of the phases tested were a monomeric (''brush-type'') diol silicas, with the exception of PolyHYDROXYETHYL-A, which is a unique, silica-bound aspartamide-based polypeptide exposing a polymeric character and is very hydrophilic. This stationary phase is also dynamic in terms of pore structure, and needs to be properly conditioned before and during use. With certain columns, lot-to-lot reproducibility problems were experienced, while with the others the separation was just not satisfactory enough, especially with lower molecular weight soluble cellulose gums. As a result of extensive investigation, an optimum column was found and recommended for the future studies and official AOAC method application: BioSuite 250, 4 μm UHR SEC. Representative chromatograms are presented in Fig. 2. Separation of the enzymes and other digestate remnants is satisfactory and peak profiles for MC and HPMC are acceptable. It has to be noted that a complete separation of MC and HPMC from other components was never achieved on any column; however, the resolution for the BioSuite 250 column was the most acceptable. One can note in Fig. 2 that there is no baseline resolution for HPMC and other digestate components in the pound cake sample, raising a question of whether integration accurately accounts for the entire peak area. However, the average recovery from this actual food matrix was quite acceptable, 93% for MC and 99% for HPMC, as indicated in Table 1.

The results of preliminary SDF determination with the new, modified method are presented in Table 1 for the solutions and pound cake samples. It has to be noted that the recovery values and precision are better for the food sample (pound cake) than for the 0.1% MES/Tris buffer solution. This may be related to multiple factors, such as possible interactions of the analyte with starches and/or proteins, resulting in an easier hydration of MC and HPMC from the food matrix during the cooling procedure.

The HPLC RI detector response was measured as a function of solution concentration for a range of aqueous MC and HPMC solutions. The response was found to be linear for both over the range of 0.3–5 mg/mL. Results are presented in Fig. 3 and also show the best-fit linear regression equations (forced through zero) for MC and for HPMC. Excellent linear correlation was achieved for both and this supported use of a single-point calibration standard, which was used in the AOAC single lab validation process. Although almost identical response is

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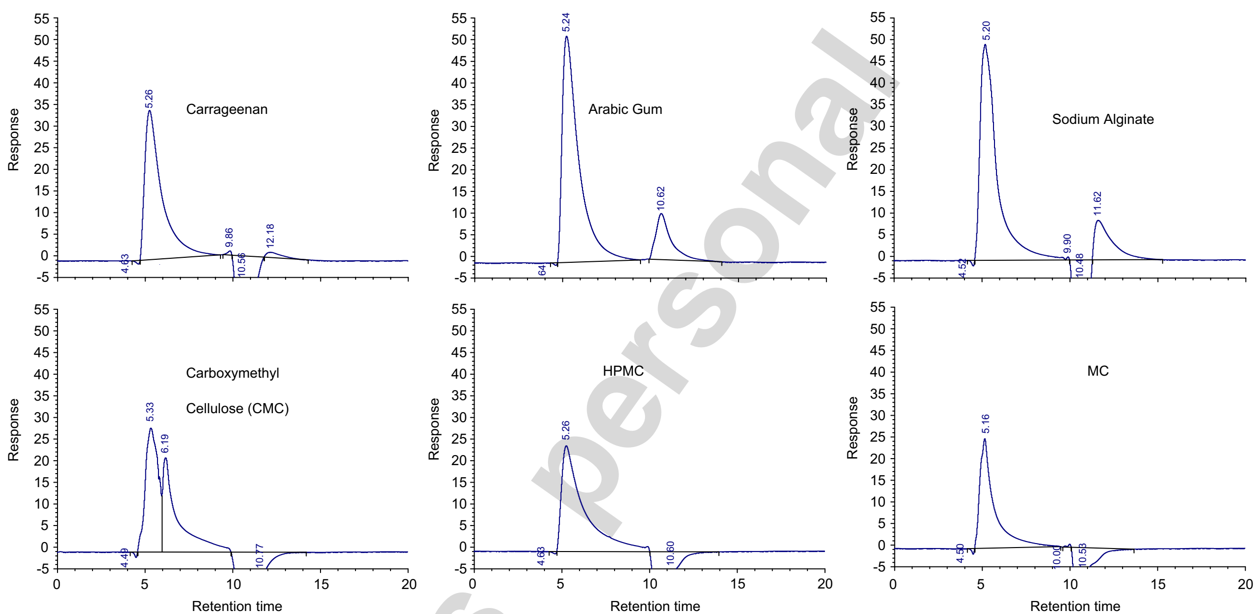

Fig. 4. Differential refractive index response variation of different polysaccharides at exactly same concentration (0.10% wt/wt, in 0.05 M MES/Tris buffer at pH 4.5). Note identical scale for each chromatogram.

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clear for MC and HPMC in Fig. 3, the method is always run using a calibration standard matching the analyte of interest. Given that the density of 0.1% wt/wt MC/HPMC solution is 1.0, one may interpret this as a linearity range from 300 to 5000 ppm. However, it was noted that the SEC analysis of concentrations higher than 10,000 ppm (1% solution) often resulted in column overloading (even at 20–40 μL injection volume) and very poor peak shapes. Also, these higher concentrations can result in high viscosity solutions (e.g. 100,000 cP), which are too viscous for the autosampler to draw into the syringe, making routine method automation more difficult.

Another very important observation made during this study was of the RI detector response for various polysaccharides, including different chemistry types and viscosities of analyzed cellulose ethers. A series of SEC analyses was performed on 1000 ppm solutions of MC, several different HPMC types, and several water-soluble polysaccharides. It was clear from these analyses that each polysaccharide had a different response and thus must have its own calibration standard. This phenomenon is illustrated in Fig. 4. Although the analytical method described in this report applies to water-soluble cellulose derivatives, in particular MC and HPMC, this observation of various RI responses is likely to be important for any future evaluations of a "universal" SDF method using chromatographic determination.

This method is also anticipated to work properly with a variety of matrices, such as other foods and formulations, and it may be suitable for other modified cellulose fibers. It must be noted, however, that while an extensive method validation procedure has been currently accomplished for MC and HPMC types, the method appropriateness for other cellulosics would have to be confirmed through a separate validation procedure.

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References

AACC—Dietary Fiber Definition Committee, 2001. The Definition Of Dietary Fiber; Report Of The Dietary Fiber Definition Committee To The Board Of Directors Of The American Association Of Cereal Chemists. Cereal Foods World, DF DEF 1/10/2001.

AACC—Dietary Fiber Definition Committee, 2006. There is no FDA/USDA definition of dietary fiber. Current FDA rules, appearing in the CFR, pertain to what is measured and reported on the Nutrition Facts panel. The FDA in their rules on health claims refers to dietary fiber provided by whole cereals/grains, fruits and vegetables. In making laws to incorporate the amount of dietary fiber on the food label, the results obtained by approved AOAC methods has become the de facto definition of dietary fiber in the United States. This dietary fiber is the residue resulting after a food is treated with three hydrolytic enzymes, plus the material precipitated in 70% ethanol. Retrieved 2006-05-08 at 〈http://www.aaccnet.org/DietaryFiber/DDF-right_responses.htm〉.

AACC, 2000. Approved Methods of the American Association of Cereal Chemists, 10th ed. St. Paul, MN, USA.

Alfieri, M.A., Pomerleau, J., Grace, D.M., Anderson, L., 1995. Fiber intake of normal weight, moderately obese and severely obese subjects. Obesity Research 3, 541–547.

AOAC International, 2005. Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg, MD, USA.

AOAC, 2005. OMA-2005-Oct-029.R1, Size Exclusion Liquid Chromatographic Method for the Determination of Methylcellulose and Hydroxypropyl Methylcellulose Food Gums as Soluble Dietary Fiber in Food and Food Products: A Collaborative Study Protocol.

Archer, W.L., 1992. Hansen solubility parameters for selected cellulose ether derivatives and their use in the pharmaceutical industry. Drug Development and Industrial Pharmacy 18 (5), 599–616.

Blundell, J.E., Burley, V.J., 1987. Satiation, satiety and the action of fibre on food intake. International Journal of Obesity 11, 9–25.

Brown, L., Rosner, B., Willett, W.W., Sacks, F.M., 1999. Cholesterol-lowering effects of dietary fiber: a meta-analysis. American Journal Clinical Nutrition 69, 30–42.

Cann, P.A., Read, N.W., Holdsworth, C.D., 1984. What is the benefit of coarse wheat bran in patients with irritable bowel syndrome? Gut 25, 168–173.

Carr, T.P., Gallaher, D.D., Yang, C.H., Hassel, C.A., 1996. Increased intestinal contents viscosity reduces cholesterol absorption efficiency in hamsters fed hydroxypropyl methylcellulose. Journal Nutrition 126 (5), 1463–1469.

Chandalia, M., Garg, A., Lutjohann, D., Von Bergmann, K., Grundy, S.M., Brinkley, L.J., 2000. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. New England Journal of Medicine 342, 1392–1398.

Coffey, D.G., Bell, D.A., Henderson, A., 1995. Cellulose and cellulose derivatives. In: Stephen, A.M. (Ed.), Food Polysaccharides and their Applications. Marcel Dekker, New York.

Craig, S.A.S., Holden, J.F., Khaled, M.Y., 2001. Determination of polydextrose in foods by ion chromatography: collaborative study. Journal of AOAC International 84, 472–478.

Cummings, J.H., 1984. Constipation, dietary fibre and the control of large bowel function. Postgraduate Medical Journal 60 (709), 811–819.

Cummings, J.H., Hill, M.J., Jenkins, D.J., Pearson, J.R., Wiggins, H.S., 1976. Changes in fecal composition and colonic function due to cereal fiber. American Journal of Clinical Nutrition 29 (12), 1468–1473.

Deshmukh, B.K., Harfmann, R.G., Coklin, J.R., Turowski, M., Lynch, S.K., 2006. Validation of a size exclusion liquid chromatographic method for determination of methylcellulose and hydroxypropyl methylcellulose food gums as soluble dietary fiber in food and food product. Food Chemistry, doi:10.1016/j.foodchem.2006.10.004.

DeVries, J.W., 2003. On defining dietary fibre. Proceedings of the Nutrition Society 62, 37–43.

Dressman, J.B., Adair, C.H., Barnett, J.L., Berardi, R.R., Dunn-Kucharski, V.A., Jarvenpaa, K.M., Parr, D.D., Sowle, C.A., Swidan, S.Z., Tobey, S.W., 1993. High-molecular-weight hydroxypropylmethylcellulose: a cholesterol-lowering agent. Archives of Internal Medicine 153 (11), 1345–1353.

Durrant, M.L., Royston, P., 1978. The effect of preloads of varying energy density and methylcellulose on hunger, appetite and salivation. Proceedings of the Nutrition Society 37/3, 87A.

Dysseler, P., Hoffem, D., Fockedey, J., Quemener, B., Thibault, J.F., Coussement, P., 1999. Determination of inulin and oligofructose in food products (Modified AOAC Dietary Fiber Method). In: Cho, S.S.,

Author's personal copy

Prosky, L., Dreher, M. (Eds.), Complex Carbohydrates in Foods. Marcel Dekker, New York.

Englyst, H.N., Cummings, J.H., Wood, R., 1987. Determination of dietary fibre in cereals and cereal products—collaborative trials. Part II. Study of modified Englyst procedure. Journal of the Association of Public Analysts 25, 59–71.

Gallaher, D.D., Hassel, C.A., Lee, K.J., 1993a. Relationships between viscosity of hydroxypropyl methylcellulose and plasma cholesterol in hamsters. Journal Nutrition 123 (10), 1732–1738.

Gallaher, D.D., Hassel, C.A., Lee, K.J., Gallaher, C.M., 1993b. Viscosity and fermentability as attributes of dietary fiber responsible for the hypocholesterolemic effect in hamsters. Journal Nutrition 123 (2), 244–252.

Gordon, D.T., Okuma, K., 2002. Determination of total dietary fiber in selected foods containing resistant maltodextrin by enzymic-gravimetric method and liquid chromatography: collaborative study. Journal of AOAC International 85, 435–444.

Harfmann, R.G., Deshmukh, B.K., Turowski, M., Conklin, J.R., Lynch, S.K., 2006. Determination of methylcellulose and hydroxypropyl methylcellulose food gums as soluble dietary fiber in food and food products: a collaborative study. Journal of AOAC International, in press, Official AOAC method number 2006.08.

Hoebregs, H., 1997. Fructans in foods and food products, ion-exchange chromatographic method: collaborative study. Journal of AOAC International 80, 1029–1041.

Institute of Medicine (IOM), 2001. Dietary Reference Intakes: Proposed Definition of Dietary Fiber. National Academy Press, Washington, DC.

Jenkins, D.J.A., Wolever, T.M.S., Leeds, A.R., Gassull, M.A., Haisman, P., Dilawari, J., Goff, D.V., Metz, G.L., Alberti, K.G., 1978. Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. British Medical Journal 1, 1392–1394.

Jones, J.M., 2000. Update on defining dietary fiber. Cereal Foods World 45, 219–220.

Keary, C.M., 2001. Characterization of METHOCEL cellulose ethers by aqueous SEC with multiple detectors. Carbohydrate Polymers 45 (3), 293–303.

Ku, Y., Oles, C.J., Kim, K.W., Rader, J.J., 2001. An approach to modifying AOAC total dietary fiber Method 985.29 to better fit a physiologic definition of dietary fiber. In: AACC Annual Meeting, 14–18 October 2001, Charlotte, North Carolina, USA.

Maki, K.C., Davidson, M.H., Malik, K.C., Albrecht, H.H., O'Mullane, J., Daggy, B.P., 1999. Cholesterol lowering with high-viscosity hydroxypropylmethylcellulose. American Journal of Cardiology 84, 1198–1203.

Maki, K.C., Davidson, M.H., Torri, S., Ingram, K.A., O'Mullane, J., Daggy, B.P., Albrecht, H.H., 2000. High-molecular-weight hydroxypropylmethylcellulose taken with or between meals is hypocholesterolemic in adult men. Journal of Nutrition 130, 1705–1710.

Mancini, M., Stamler, J., 2004. Diet for preventing cardiovascular diseases: light from ancel keys, distinguished centenarian scientist. Nutrition, Metabolism and Cardiovascular Diseases 14 (1), 52–57.

McCleary, B.V., Murphy, A., Mugford, D.C., 2000. Measurement of total fructan in foods by enzymic/spectrophotometric method: collaborative study. Journal of AOAC International 83 (2), 356–364.

McCollister, S.B., Kociba, R.J., McCollister, D.D., 1973. Dietary feeding studies of methylcellulose and hydroxypropylmethylcellulose in rats and dogs. Food and Cosmetics Toxicology 11 (6), 943–953.

Nitschke, K., Fiero, T., 1977. The metabolism of methylcellulose gums. In: Hood, L., Wardrip, E., Bollenback, G. (Eds.), Carbohydrates and Health. AVI Publishing Co., Westport, CT, pp. 57–68.

Pereira, M.A., Ludwig, D.S., 2001. Dietary fiber and body-weight regulation: observations and mechanisms. Pediatric Clinics of North America 48 (4), 969–980.

Pereira, M.A., O'Reilly, E., Augustsson, K., Fraser, G.E., Goldbourt, U., Heitmann, B.L., Hallmans, G., Knekt, P., Liu, S., Pietinen, P., Spiegelman, D., Stevens, J., Virtamo, J., Willett, W.C., Ascherio, A., 2004. Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. Archives of Internal Medicine 164 (4), 370–376.

Phillips, G.O., 2000. Handbook of Hydrocolloids. CRC Press, Boca Raton, FL.

Poche, D.S., Ribes, A.J., Tipton, D.L., 1998. Characterization of cellulose ether: correlation of static light-scattering data to GPC molar mass data based on pullulan standards. Journal of Applied Polymer Science 70 (11), 2197–2210.

Prosky, L., 2001. What is dietary fibre? A new look at the definition. In: McCleary, B.V., Prosky, L. (Eds.), Advanced Dietary Fibre Technology. Iowa State Press/Blackwell Sciences Publishing, Oxford, UK.

Rader, J.J., Ku, Y., 2001. FDA's perspective on recent recommendations for definitions of dietary fiber: potential consequences for health claims and analysis. In: AACC Annual Meeting, 14–18 October 2001, Charlotte, North Carolina, USA.

Reppas, C., Adair, C.H., Barnett, J.L., Berardi, R.R., DuRoss, D., Swidan, S.Z., Thill, P.F., Tobey, S.W., Dressman, J.B., 1993. High viscosity hydroxypropylmethylcellulose reduces postprandial blood glucose concentrations in NIDDM patients. Diabetes Research and Clinical Practice 22 (1), 61–69.

Rimm, E.B., Ascherio, A., Giovannucci, E., Spiegelman, D., Stampfer, M.J., Willett, W.C., 1996. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. Journal of the American Medical Association 275 (6), 447–451.

Santos, E.M., Expert Panel, 1986. Final report on the safety assessment of hydroxyethylcellulose, hydroxypropylcellulose, methylcellulose, hydroxypropyl methylcellulose, and cellulose gum. Journal of the American College of Toxicology 5 (3), 1–59.

Sarkar, N., 1979. Thermal gelation properties of methyl and hydroxypropyl methylcellulose. Journal of Applied Polymer Science 24 (4), 1073–1087.

Sarkar, N., Walker, L.C., 1995. Hydration–dehydration properties of methylcellulose and hydroxypropyl methylcellulose. Carbohydrate Polymers 27 (3), 177–185.

Spiller, R.C., 1994. Pharmacology of dietary fibre. Pharmacology and Therapeutics 62 (3), 407–427.

Swidan, S.Z., Reppas, C., Barnett, J.L., Greenwood, D.E., Tallman, A.M., Tobey, S.W., Dressman, J.B., 1996. Ability of two comestible formulations of hydroxypropylmethylcellulose to lower serum cholesterol concentrations. European Journal of Pharmaceutical Sciences 4 (4), 239–245.

Theander, O., Westerlund, E.A., 1986. Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. Journal of Agricultural and Food Chemistry 34, 330–336.

Theander, O., Westerlund, E.A., Aman, P., 1993. Structure and components of dietary fiber. Cereal Food World 38, 135–141.

Tikhonova, E.P., Berdichevsky, V.K., Paley, A.A., 1973. The utilization of methylcellulose in the dietetic nutrition of patients with adiposity and diabetes mellitus. Voprosy Pitanyia 32 (1), 9–13 (in Russian).

US Pharmacopoeia, USP27 NF22, 2002. cellulose, methyl ether.

Van Itallie, T.B., 1978. Dietary fiber and obesity. American Journal of Clinical Nutrition 31 (Suppl.), S43–S52.

Wood, R., Englyst, H.N., Southgate, D., Cummings, J.H., 1993. Determination of dietary fiber—Collaborative Trial IV. Journal of the Association of Public Analysts 29, 57–141.

Yokoyama, W.H., Knuckles, B.E., Davis, P.A., Daggy, B.P., 2002. Stability of ingested methylcellulose in the rat determined by polymer molar mass measurements by light scattering. Journal of Agricultural and Food Chemistry 50 (26), 7726–7730.

Yokoyama, W.H., Shao, Q., 2006. Soluble fibers prevent insulin resistance in hamsters fed high saturated fat diets. Cereal Foods World 51 (1), 16–18.