Dietary fibre fractions in cereal foods measured by a new integrated AOAC method

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ABSTRACT

The reliable determination of soluble, insoluble and total dietary fibre in baked goods and cereal flours is an important issue for research, nutritional labelling and marketing. We compared total dietary fibre (TDF) contents of selected cereal based foods determined by AOAC Method 991.43 and the new AOAC Method 2009.01. Fifteen bread and bakery products were included in the study. Our results showed that TDF values of cereal products determined by AOAC Method 2009.01 were always significantly higher than those determined by AOAC Method 991.43. This was explained by the inclusion of low molecular weight soluble dietary fibre fractions and resistant starch fractions in the TDF measurement by AOAC 2009.01. This documents that nutritional labelling of cereal products poses the challenge how to update TDF data in nutrient databases in a reasonable time with an acceptable expenditure.

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1. Introduction

Several recent prospective epidemiological studies investigating the influence of dietary habits, environmental and lifestyle factors on the incidence of cancer and other chronic diseases have shown an inverse association between daily dietary fibre consumption and colon cancer risk. Thus, the European Prospective Investigation into Cancer and Nutrition (EPIC) has shown a 40% risk reduction of colorectal cancer (CRC) when consuming more than 30 g of fibre/day (Bingham & Riboli, 2004). The HELGA cohort has shown a significant lower colon cancer risk for men consuming more than 17.8 g/day of cereal fibre (Hansen et al., 2011) and a nested prospective case-control study of colorectal cancer case patients in the UK also reports a significant inverse association between CRC risk and dietary fibre consumption (Dahm et al., 2010). Whole grain based diets are rich in dietary fibre and a regular consumption of whole grain food therefore seems favourable in this respect. In order to eat the recommended 25–30 g/day of fibre consumers need to find reliable labelling of the fibre content on the packages of food. As the term “dietary fibre” comprises of a heterogeneous collection of chemically different plant components having the same characteristic of being indigestible, the measurement of dietary fibre as proposed by internationally acknowledged methods is based on this common feature. Fig. 1 shows different solubility characteristics of dietary fibres present in food.

Based on the dietary fibre definition by Trowell et al. (1976) the Association of Official Analytical Chemists (AOAC) introduced in 1985 AOAC Official Method 985.29 for measurement of TDF in food and subsequently in 1991 an extended and optimised gravimetric method for measurement of TDF in food, AOAC Official Method 991.43. Both methods are based on the enzymatic removal of starch and protein of the samples by amylase and protease at 90 and 60 °C respectively. Insoluble dietary fibres (IDF) are then separated by filtration and high-molecular weight soluble dietary fibres (HMWSDF) precipitated by 78% ethanol and collected by filtration. Both fibre fractions are dried and weighted and collectively give the total dietary fibre content of the sample. For chemically defined dietary fibres (fructans, galacto-oligosaccharides, pectin, resistant starch, resistant maltodextrins), special methods were devised for measurement in different matrices. The inherent problems of AOAC Method 991.43, digestion of starch and protein at non-physiological temperatures, partial hydrolysis of resistant starch and lack of detection of low molecular weight dietary fibre were resolved by McCleary (2007, 2010; McCleary et al., 2010). In this “integrated” method, AOAC Method 2009.01, the sample was incubated first with α-amylase at 37 °C, protein then digested at 60 °C by protease, and insoluble and high molecular weight soluble dietary fibres were precipitated at 78% ethanol and finally determined gravimetrically. Non-digestible oligosaccharides (NDO) were measured in the ethanol filtrate by HPLC. The amount of NDO was calculated from the area under the curve (AUC) of all chromatography fractions with a degree of polymerization DP ≥ 3 relative...
to D-sorbitol as internal standard. This NDO-quantification conforms to the dietary fibre definition adopted by the Codex Alimentarius Commission in 2008 (CODEX, 2008). Fig. 2 depicts the relationship between different fibre fractions. The encircled fractions are those assayed by AOAC Method 991.43, while all fractions can be now be measured by the integrated AOAC Method 2009.01.

We compared the total dietary fibre content of different baked goods by using both AOAC Method 991.43 and AOAC Method 2009.01. The aim of the study was to examine to what extent the TDF content of selected foods is modified when all dietary fibre fractions were assayed in the measurement.

2. Material and methods

2.1. Materials

Fifteen breads and bakery products varying in different TDF composition were obtained from a local supermarket.

2.2. Sample preparation

The pre-crushed bakery products, the rusk sample and the crispbread were finely ground using a laboratory batch mill IKA M20 (IKA Labortechnik, Staufen, Germany). Before milling the soft
bread samples and bagels were dried using an oven with recirculating air (WTB Binder, Tuttlingen, Germany) for 16 h at 40 °C. All milled samples were stored in tightly closed glass bottles at room temperature until analysis. Samples containing more than 10% fat were defatted with petroleum ether as described by AOAC Method 985.29.

2.3. Methods

Protein was determined by Kjeldahl method (N × 6.25) according to ICC standard method No. 104/1, ash was determined according to ICC standard method No. 105/2 (ICC, 2002). Total dietary fibre was quantified according to AOAC Official Methods 991.43 and 2009.01 (AOAC, 2012) using Megazyme assay kits K-TDFR and K-INTDF, respectively (Megazyme International, Bray, Ireland). The Celite filtration of IDF and HMWSDF after precipitation was replaced by paper filters (Whatman® black ribbon 589/1 filter paper; Schleicher & Schuell, Dassel, Germany). This had no influence on the fibre fraction recovery as shown in the second evaluation of the AOAC Method 2009.01 (McCleary et al., 2012). Chromatographic determination of LMWSDF (AOAC 2009.01) was performed using a Waters Sugar-Pak™ guard cartridge and a Waters Sugar-Pak™ column (6.5 × 300 mm; Millipore, Eschborn, Germany). The chromatographic system consisted of Kontron HPLC pumps 422 and 4225, an autosampler 360, column oven 480 (Kontron Instruments, Neufahrn, Germany) and refractive index detector ERC-7515A (ERC Inc., Saitama, Japan). The system was controlled and the chromatograms evaluated by DIONEX Chromolith software 6.30 (DIONEX, Sunnyvale, USA).

Manual deionization was performed using a consecutive array of 2 plastic columns (Alltech, Omni-Lab, Gebräden, Germany) filled with Ambersep 200 (H+) resin and Amberlit e FPA 53 (OH−) resin, respectively (Megazyme International, Bray, Ireland). β-sorbitol was used as internal standard. Complete separation of sugars with DP ≥ 2 and oligosaccharides of DP ≥ 3 was checked by injection of lactose, sucrose or maltose. All sugars were provided by Merck of highest purity grade (Merck, Darmstadt, Germany). All samples were analysed as preset by the applied AOAC methods in duplicate and mean values are shown.

3. Results and discussion

The dietary fibre complex consists of a mixture of different components with different chemical structures of different molecular weights. The analysis of the dietary fibre composition of a food sample is an elaborate and expensive endeavour and needs sophisticated analytical equipment. For nutritional labelling purposes methods for quantification of the non-digestible total dietary fibre content in food were devised more than 20 years ago using human -amylase and protease. The main drawback of the “gold standard” in dietary fibre measurement (“Prosky method”, AOAC Method 985.29/991.43) was, that it assays only 78% ethanol insoluble high-molecular weight polymer fibres but not non-digestible oligosaccharides. These low molecular weight fibres have presumably positive health effects (Gemen, de Vries, & Slavin, 2011).

Thus, any assay of dietary fibre in food or raw material for nutritional labelling should allow also for quantification of the NDO fraction of TDF (van der Kaaij et al., 2009; Nishibata et al., 2009). This can now be accomplished by the integrated method AOAC Method 2009.01 (McCleary et al., 2009, 2012). Also resistant starch can now be completely detected and thus is included in the TDF values measured by this method. Our results of comparative measurements of TDF in cereal and bakery products using both AOAC Methods have demonstrated:

1. The LMWSDF fraction in different types of bread represents a considerable proportion of TDF. The TDF assayed by AOAC Method 2009.01 is for some products (whole meal, rye and products baked at higher temperatures than other breads) significantly higher than the TDF value assayed by AOAC Method 991.43 (Table 1). The strikingly lower values for biscuit cookies and oat short bread are reproducible but cannot be explained yet.

2. The high molecular weight fraction HMWDF (IDF + HMWSDF) assayed by AOAC 2009.01 is of comparable order of magnitude as the TDF fraction assayed by AOAC 991.43.

3. The low molecular weight dietary fibre fractions of TDF (NDO) represent a considerable proportion of the total fibre content of cereal based food (Table 2). The percentage ranges from 62% for biscuit cookies down to 16% for pumpernickel.

Summarising, our study has shown that the amount of total dietary fibre is measurably higher in cereal based food when measured by AOAC Method 2009.01 in comparison to the established AOAC Methods 985.29 and 991.43. This is owed to the fact that 2009.01 now also allows to quantitate the 78% ethanol soluble NDO fibre fraction which 991.43 did not because they were always discarded. Investigations of the molecular weight distribution profiles of the low molecular weight fibre fractions from cereal foods, their molecular structure and last but not least their physiological implications have still to be performed.

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>AOAC 991.43</th>
<th>AOAC 2009.01</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Insoluble DF</td>
<td>Soluble DF</td>
</tr>
<tr>
<td>Crispbread</td>
<td>11.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Rye whole meal bread</td>
<td>6.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Pumpernickel</td>
<td>7.8</td>
<td>2.6</td>
</tr>
<tr>
<td>Rye and wheat bread</td>
<td>4.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Wheat and rye bread</td>
<td>3.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Wheat wholemeal toast</td>
<td>5.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Wheat toast bread</td>
<td>3.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Ciabatta (warmed up)</td>
<td>2.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Rye wheat flat bagel</td>
<td>4.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Butter cookies with inulin</td>
<td>2.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Biscuit cookies</td>
<td>2.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Oat short bread</td>
<td>3.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Rice wafers</td>
<td>2.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Rusk</td>
<td>3.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Salt sticks</td>
<td>3.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

All values are given in % related to 100 g edible part.
effects should ensue now. As pointed out by Gemen et al. recently (Gemen et al., 2011), more investigations into the relation between the health effects (gut health, glucose response) of cereal dietary fibres and their molecular structures is needed.

Comparative measurements of more food items will be necessary to verify whether the presented results reflect a general trend for many foods or are confined to foods of certain plant provenance. More measurements of TDF in various categories of foods by AOAC Method 2009.01 should now be conducted to screen whether a correlation can be found between the “old” and the “new” TDF values. The alternative of determining the TDF content of many products and raw materials anew is not a real one as it must be feared that nobody will be able to conduct or finance such an enormous task in order to update existing nutrient data bases.

**References**


