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ORIGINAL ARTICLE

All-in-one measurement of dietary fibre, including resistant starch, in bread

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Abstract

Introduction In 2008/2009 both Codex and the European Union adopted almost identical definitions of dietary fibre, including all carbohydrate polymers that are not digested or absorbed in the human small intestine. The current method generally used for the analysis of dietary fibre in food products is AOAC Official Method 985.29. This method measures resistant starch and non-digestible oligosaccharides, now officially included in the definition of dietary fibre, only partially. *Objective* Here we present an alternative method for the measurement of total dietary fibre, including resistant starch and non-digestible oligosaccharides. *Result* Employment of this method for the measurement of dietary fibre in different types of bread results in higher dietary fibre values compared to those measured with AOAC method 985.29 with average differences ranging from 0.48% to 0.78%. *Conclusion* Although only proven for bread products in this article the actual levels of dietary fibre, as defined by Codex and European Union, in bread products are higher than levels listed in food composition databases. The same may be true for other food products containing resistant starch and non-digestible oligosaccharides. As shown here for bread, this may result in different claims on food products with regard to fibre content.

Introduction

After many years of debate, definitions of dietary fibre (DF) have been agreed upon in 2008/2009 in Europe and internationally, by the Codex Alimentarius Commission (Codex, 2009). Both definitions include all carbohydrate polymers, which are neither digested nor absorbed in the human small intestine. The European Union (EU) definition includes polymers with a degree of polymerization > 2 whereas Codex leaves the decision for inclusion of polymers with degree of polymerization between 3 and 10 to national authorities.

Codex (2009) and EU (2007) also agree on communication of fibre levels in foods: A claim that a food is a 'source of fibre', and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 3 g of fibre per 100 g, whereas for the claim 'high fibre' at least 6 g/100 g is required. These levels are similar to those allowed in the United States for indicating 'a good source of fibre' or 'high in fibre', respectively.

Discussions on analytical methods for DF, already ongoing in recent years, are now continued in working groups established by Codex and the European Commission. The most commonly used method for analysis of DF is AOAC Official Method of Analysis 985.29 (Horwitz & Latimer, 2005). Analysis of DF for food composition databases has been done largely with this method. However, with AOAC 985.29 resistant starch (RS) is only partially measured, and non-digestible oligosaccharides like resistant maltodextrines, as well as inulins, are measured to a very minor

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extent. Although different AOAC methods exist for the measurement of these fractions, it is not possible to come to a complete quantitative overview of all relevant DFs because the different methods measure partially overlapping fractions. Another complication in analysis of RS is its instability: RS can be formed or disintegrated during food processing and storage, and also by analytical operations.

Therefore EFSA (2007) recommended as follows*: For practical purposes, it would be advisable that analytical methods could actually correspond better to the physiologically RS present in foods and that a single assay could be used to quantify all components of DF.*

A major step forward in using a single assay for quantifying all components of DF is the method proposed by McCleary (2007) (McCleary *et al.* 2009). Regarding RS, McCleary has replaced incubation of samples with thermostable α-amylase enzymes at elevated temperature (as applied in AOAC985.29 and related methods) by incubations at 37 °C with pancreatin, thereby measuring RS more realistically then the AOAC methods.

The McCleary method does not include a mimic of food pre-treatment like boiling or chewing, which may be of large impact on digestibility of many starch containing food products. Also, stomach transit (protease incubation) is only mimicked after the incubation with starch degrading enzymes, and after a boiling step, which does not correspond to physiological conditions.

In order to mimic food pre-treatment we developed a method combining the existing AOAC 2001.03 for the measurement of total DF, the method by McCleary (2007) and the method of Englyst *et al.* (1999) for the measurement of available carbohydrates in food. The Englyst method is based on the use of pancreatic enzymes for mimicking the human digestive tract, and has been well validated with *in vivo* experiments (Englyst *et al.*, 1999; Garsetti *et al.*, 2005). Before analysis, samples are processed in order to achieve optimally 'as eaten' conditions: samples are not ground very finely but homogenized more coarsely and pasta products are boiled before the homogenization process.

Fibre values obtained in the first, orientating, experiments with the TNO total DF method (Sanders & van der Kamp, 2008), turned out to be significantly higher compared with the conventional AOAC 985.29 method with average differences for different types of bread of at least 0.5%; for spaghetti increases of over 1% were found.

Cereal grain-based products, bread, pasta, breakfast cereals etcetera, are often advertized as a source of DF. Bread, for example, is communicated as a source of fibre, and wholemeal bread as being high in fibre. In national food composition databases, current levels of wholemeal bread are between 6% and 7%. However, current fibre levels of white bread are mostly well below 3%; examples (g/100 g): United States – 2.4, the Netherlands – 2.5 and Finland 2.7.

Our initial results, and their relevance for communication to consumers about fibre levels especially for white bread, prompted us to start more detailed studies. In this communication the results obtained with various types of bread are reported.

Materials and methods

Materials

Four types of bread (white, 50% and 100% wholemeal, multi-grain) were kindly provided by Meneba (the Netherlands). Breads had the following composition (in w/w):

White bread: 60.8% white flour, 1.2% yeast, 1.1% salt, 0.6% emulsified fat, 0.5% sucrose, 38.3% water.

Fifty per cent wholemeal: 29.8% white flour, 29.8% wholemeal flour, 1.2% yeast, 1.1% salt, 0.6% emulsified fat, 37.5% water.

Wholemeal: 58.5% wholemeal flour, 1.2% yeast, 1.0% salt, 0.6% emulsified fat, 38.6% water.

Multi-grain: 58.2% 'multi-grain' flour (consisting of wholemeal wheat flour with processed grains of cereals and some rye flour), 1.2% yeast, 1.0% salt, 0.6% emulsified fat, 39% water.

Enzymes and chemicals were from Sigma, unless indicated otherwise.

RS was measured according to AOAC method 2002.02 using Megazyme RS kit.

Methods

Bread sample preparation

All breads were processed directly after baking. Slices of approximately 30 g were made from the middle part of the breads, including the outside crust, and frozen at −20 °C. Slices from the same bread, prepared at the same moment, were used for AOAC 985.29, AOAC 2002.02 and the adjusted TNO total DF method described here.

TNO DF method

Sample preparation

Each bread slice was cut into 25 pieces and mixed with 2.5 mL water per 30 g bread. This mixture was fed through a Solostar II apparatus (Corrupad Korea Co. Ltd., distributor Keimling Naturkost GmbH, Buxtehude, Germany) to mimic chewing. From the resulting mixture, six samples of

1.4 g were taken and used immediately for subsequent analysis.

Enzymatic treatment

Samples were added to 3.5 mL water and homogenized for 5′ with orbital shaking (150 r.p.m.). To each sample, 10 mL of pepsin solution, containing 10 mg pepsin (P7000), 0.25% saturated benzoic acid and 50 mM HCl, was added and samples were incubated at 37 °C for 30 min with orbital motion at 150 r.p.m. Afterwards, 0.7 mL 0.75 M NaOH and 4 mL 0.4 M Na-maleate buffer pH 6 were added. If necessary, pH was adjusted to 6 with 0.75 M NaOH. The enzyme mixture was prepared immediately before use as follows: 0.30 g of pancreatic α-amylase (P-7545, Sigma, St. Louis, MO, USA) was suspended in 110 mL Na-maleate buffer pH 6 containing 4 mM $CaCl_2$ and 0.02% sodium azide. The mixture was stirred for 5 min and centrifuged 5 min at 2000 r.p.m. 0.2 mL amyloglucosidase (Megazyme, amyloglucosidase for total dietary fibre and starch assays, 3.26 U mL^{-1}) was added. To each bread sample, 20 mL of enzyme mixture was added and incubated for 16 h at 37 °C while shaking at 150 r.p.m.

After the incubation, the pH was adjusted to 4.3 ± 0.2 with 2 M HAc. To each sample, 180 mL of 95% EtOH (v/v) was added and precipitation of high-molecular-weight soluble dietary fibre was allowed to form during 16 h.

With each assay, two blank samples were included to compensate for any contribution of reagents and enzymes to the subsequent measurements.

All subsequent steps for measurement of high-molecular-weight soluble dietary fibre, low-molecular-weight resistant maltodextrin (LMWRMD), protein and ash content were performed according to AOAC official method 2001.03

The AOAC 2001.03 method is a combination of AOAC 985.29 for the measurement of DF and a liquid chromatography method for the measurement of LMWRMD.

In short, the samples were sieved through a tarred crucible containing Celite filter and the filter was washed with EtOH and acetone. These samples were dried at 103 °C and weighed, resulting in the weight of the 'residue'. This fraction was subsequently used to determine ash content by heating at 550 °C for 5 h, or protein content using Kjeldahl analysis. The filtrate containing LMWRMD was concentrated using a rotary evaporator and analysed quantitatively using high-performance liquid chromatography. The residue weight, minus protein and ash fraction, plus LMWRMD fraction, represented the total dietary fibre fraction per sample, which was subsequently expressed as a w/w percentage of the original food product.

DF AOAC 985.29

Standard DF measurements were performed according to AOAC 985.29. From each bread, six separate samples were assayed, with each separate sample consisting of three measurements: one for ash determination and two for protein determination.

RS AOAC 2002.02

RS was measured according to AOAC 2002.02 using the Megazyme RS kit (Megazyme, Bray, Ireland). Frozen samples were prepared by crushing. Each bread type was measured in triplicate.

Statistical analyses

For the TNO total DF method, six samples were taken from each bread type. Of these, three were used for determination of nitrogen content in the residue, while three other samples were used for determination of ash in residue. For determination of six values, both ash and N measurements were averaged per bread type, and combined with the individual data on residue weight and LMWRMD content to produce six numbers. The average DF value per bread type was based on the average values of LMWRMD, residue weight, ash and protein content per bread type.

Differences between the TNO total DF method and the AOAC method were tested per bread type using a one-way analysis of variance ($\alpha = 0.05$).

Results

The results of the DF and RS measurements in different bread types are presented in Table 1. For all bread types, the TNO total DFfibre method resulted in significantly higher total DF values compared with the AOAC 985.29 method. The difference was between 0.48% and 0.77% (average difference 0.58%). This difference does not account for the total amount of RS present in the breads, indicating that at least part of the RS fraction has also been included using the AOAC985.29 method, as was reported previously (McCleary, 2003). Within AOAC 985.29, the measurement of LMWRMD was not included: this accounted for between 0.2% and 0.4% of fibre in the breads according to the TNO measurements.

Discussion

The average DF levels in bread as measured with the TNO 'as eaten' method are significantly higher than the values obtained by using the classical AOAC985.29 official method.

Table 1 Total dietary fibre and resistant starch percentage (w/w) of 4 bread types, as measured by AOAC 985.29 and TNO total dietary fibre (TDF) method and AOAC 2002.02

Bread type	Dietary fibre AOAC 985.29	Resistant starch AOAC 2002.02	LMWRMD TNO TDF method	Dietary fibre TNO TDF method
White bread	3.0	1.56	0.31	3.4
	2.9	1.74	0.36	3.3
	3.0	1.74	0.39	3.4
	2.8			3.6
	2.8			3.5
	3.1			3.6
Mean	2.93 ± 0.13	1.68 ± 0.10	0.35 ± 0.04	3.49 ± 0.08
50% wholemeal bread	5.0	1.28	0.23	6.0
	4.6	1.16	0.22	5.7
	4.8	1.10	0.20	6.1
	5.4			5.8
	5.5			5.7
	5.0			5.7
Mean	5.05 ± 0.33	1.18 ± 0.09	0.22 ± 0.02	5.82 ± 0.14
100% wholemeal	6.4	1.29	0.15	6.9
bread	6.3	1.17	0.19	6.9
	6.2	1.17	0.18	6.8
	6.5			6.6
	6.6			7.2
	6.6			7.1
Mean	6.44 ± 0.15	1.21 ± 0.07	0.17 ± 0.02	6.92 ± 0.11
Multi-grain bread	8.3	1.86	0.32	9.0
	8.9	1.98	0.17	9.0
	8.7	2.59	0.14	8.3
	7.9			8.9
	7.8			8.9
	8.4			9.0
Mean	8.32 ± 0.42	2.14 ± 0.39	0.21 ± 0.09	8.84 ± 0.16

LMWRMD, low-molecular-weight resistant maltodextrin.

Comparable differences are found for different types of bread

The higher values measured with the TNO method can be ascribed both to measurement of LMWRMD and the more complete measurement of RS. The measurements were done on fresh bread, frozen shortly after baking. RS values may increase dramatically due to staling when bread is stored at ambient temperature (Pham *et al*., 2005); however, other studies do not show this major increase of RS levels reported by Pham and colleagues (Carcea *et al*., 2009). In any case, in the context of the new definitions of fibre and the communication to consumers, physiologically relevant RS levels should be measured.

We may conclude that actual average fibre levels in bread are about 0.5% higher than values reported in food composition databases. Whether larger differences will be found after staling of breads remains to be investigated.

Measured in this way 'as eaten', average fibre values for white bread increase from approximately 2.5 – the level currently reported in food composition databases – to approximately 3.0 g/100 g – sufficiently high for communicating to consumers that 'white bread is a source of DF'. It should be noted, however, that although the average values of fibre in bread presented in food composition databases may suggest a constant composition, considerable variations between different samples may occur. Levels of fibre in wheat and other grains depend on the variety as well as on environmental conditions (Ward *et al*., 2008), and slight differences in composition may also affect fibre levels.

In this study on bread samples, the obtained values may be similar to those resulting from measurement with the McCleary method, whereas larger differences may be found for products – e.g. pasta, or potatoes – that are cooked before consumption. Such measurements should be done in further studies.

Since cooking is less standardized than the usual sample preparation techniques, the TNO method may be used, after further studies, primarily as a reference method, especially

for products eaten after cooking, rather than as a standard method, whereas the McCleary method has good perspectives for being accepted as a new standard method for DF analysis.

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