codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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**Agenda Item 3** **CX/NFSDU 09/31/3-Rev**
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**JOINT FAO/WHO FOOD STANDARDS PROGRAMME**

**CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES**
**Thirty first Session**

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# LIST OF METHODS FOR DIETARY FIBRE AT STEP 7

*(Prepared by an electronic working group, led by France, with the assistance of Australia, Argentina, Brazil, Canada, Mexico, New-Zealand, Sweden, United Kingdom, United States of America, AAF, AIDGUM, CIAA, ILSI)*

Governments and interested international organizations are invited to submit comments or information on the document below, preferably by an email, to: Mr Georg **Müller,** Federal Ministry of Food, Agriculture and Consumer Protection, Rochusstraße 1, 53123 Bonn, Germany, Fax: +49 (228) 99 529 49 65, e-mail: ccnfsdu@bmelv.bund.de with a copy to: Secretary, Codex Alimentarius Commission, Joint WHO/FAO Food Standards Programme, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy, Fax +39-06-5705-4593, e-mail codex@fao.org **by 15 September 2009.**

## 1.- BACKGROUND[1]

1. At its 32nd h session, the Codex alimentarius Commission adopted the Draft Table (Provisions on Dietary Fibre) including the definition on dietary fibre , forwarded by the 30th session of the Committee.[2].

2. The Committee also recalled that a list of recommended methods has already been drafted by an electronic working group led by the delegations of Sweden and France (see Appendix II of ALINORM 08/31/26), a few years ago. However, as a number of provisions have been newly introduced into the definition for dietary fibre at the 30th session, the Committee noted that the list of methods presented in this Appendix might require an update. The Committee therefore requested the establishment of an Electronic Working Group, led by the Delegation of France, open to all Codex members and observers and working in English only. to:

[1] See ALINORM 09/32/26 – para. 49-54.
[2] See ALINORM 09/32/26 – Appendix II.

• review and update, as appropriate, the list of methods of analysis in Appendix II, taking into account the new provisions in the draft definition of dietary fibre that would require the selection of methods of analysis, and possible information of new available methods;

• consider how the results from different methods specific to different types of dietary fibre could be combined together to arrive at the total dietary fibre content in a food;

• evaluate the performance of methods in measuring different types of dietary fibre;

• make recommendations for methods of analysis for dietary fibre in different food matrices;

• consider the footnote 1 and prepare a recommendation as to its revision with regard to the methods of analysis, if necessary.

3. The French delegation is grateful to acknowledge the inputs received from Australia, Argentina, Brazil, Canada, Mexico, New-Zealand, Sweden, United Kingdom, United States of America, Association des amidonniers et féculiers (AAF), International Association for the development of natural gums (AIDGUM), Confédération des industries agro-alimentaires de l'Union européenne (CIAA), International Life Science Institute (ILSI) as they all have been of great importance for putting together this discussion paper.

4. A wealth of information has been submitted by the participants to the electronic working on methods of analysis for dietary fibres. In order to keep this discussion paper as concise and focused as possible, only information relevant to address the issues mentioned in the terms of reference of the electronic Working Group (see para. 2 above) have been tabulated in the Annex, in order to provide the essential background information.

## 2.- CODEX GENERAL CRITERIA FOR THE SELECTION OF METHODS OF ANALYSIS[3]

5. The Principles of the Codex alimentarius mention methods of analysis and sampling as part of the scope of the Codex alimentarius. The Codex alimentarius Commission requires that "*all methods of analysis and sampling considered necessary should be included*" [in the relevant section of a standard], *either specifically or by reference,* (…), noting that, "*if two or more methods have been proved to be equivalent by the Codex Committee on Methods of Analysis and Sampling, these could be regarded as alternatives*". Furthermore, the Commission has adopted the following principles intended for Committees selecting suitable methods of analysis for testing compliance with food commodity Codex standards:

*"(a) Official methods of analysis elaborated by international organizations occupying themselves with a food or group of foods should be preferred.*

*"(b) Preference should be given to methods of analysis the reliability of which have been established in respect of the following criteria, selected as appropriate: (i) specificity, (ii) accuracy, (iii) precision, repeatability intra-laboratory (within laboratory), reproducibility inter-laboratory (within laboratory and between laboratories), (iv) limit of detection, (v) sensitivity, (vi) practicability and applicability under normal laboratory conditions, (vii) other criteria which may be selected as required.*

*"(c) The method selected should be chosen on the basis of practicability and preference should be given to methods which have applicability for routine use.*

*"(d) All proposed methods of analysis must have direct pertinence to the Codex Standard to which they are directed.*

[3]See Procedural Manual – 18th edition – pp.16 (para. 2), 48, 109 – 110, respectively

*"(e) Methods of analysis which are applicable uniformly to various groups of commodities should be given preference over methods which apply only to individual commodities."*

6. One member's suggestion "*to provide only general guidance on methods of analysis for dietary fibre in the* Codex Guidelines on Nutrition Labelling" appears therefore inconsistent with the procedure established by the Codex alimentarius Commission, the decision by the last session of the Committee to "*update*" the proposed list and the terms of reference of this electronic work group. The fact that the adopted definition leaves to the decision of each Codex member whether to include or not certain types of compounds is irrelevant to its mandate : the task of selecting a method for analyzing a particular class of chemical compounds (which may be included among "dietary fibres" as defined by the Codex alimentarius Commission) is based on its applicability and is independent of another issue, i.e. whether a member of Codex would decide to accept this class of compounds as belonging to the category of "dietary fibres".

7. The Committee has been requested by its sister Committee (CCFL) to provide technical input in order to build the entry for dietary fibres in the "Table of conditions for nutrient contents" (i.e. "*source of*" & " *high in*"), contained in the *Guidelines for the use of nutrition claim* (CAC/GL 23). It is sufficient that the methods of analysis, recommended in this paper, are suitable to check whether a product, bearing the relevant nutrient content claim[4] on its labelling, complies with the provision for this condition in CAC/GL 23.

## 3.- REVIEW OF THE METHODS OF ANALYSIS FOR DIETARY FIBRES

### 3.1- Use of AOAC Official Methods of Analysis

8. Official AOAC methods are widely accepted globally for general labelling of nutrient content in foods as well as for health and nutrition claims. AOAC methods are designed to be accurate for the food matrices studied, cost effective, and reproducible in various analytical environments on which industry relies. They are the most studied and validated methods available for the quantification of food components.. These methods have been scientifically evaluated to become reference methods.

9. No one AOAC validated method can measure all non-digestible carbohydrates in foods. AOAC 991.43 is one of the most widely used 'total' dietary fibre methods. Both this method and AOAC 985.29 will measure insoluble polysaccharides and soluble high molecular weight components i.e. those that are precipitated by alcohol. Neither measures fully the resistant starch fraction, nor do they recover completely non-digestible oligosaccharide components, that Codex members may decide to include in the definition of dietary fibre.

10. Specifically, they quantify only part of the total resistant starch, inulin, polydextrose, fructo-oligosaccharides, resistant maltodextrins, and other resistant oligosaccharides. Furthermore, some oligosaccharides are not measured at all.

11. Due to the complexity of the molecular structure of fibres, additional AOAC methods were subsequently developed to measure specific dietary fibre components in foods (e.g. AOAC 999.03 for fructans).. By focussing on one component these methods result in higher specificity and accuracy needed to detect fibres present in food products. Equally important, these component-specific methods facilitate routine, cost-effective analysis.

[4] "Nutrient content claim is a nutrition claim that describes the level of a nutrient contained in a food." (see section 2.1.1 in CAC/GL 23-1997)

### 3.2- New method with AOAC status pending

12. The lack of a validated procedure to combine AOAC methods[5] to determine total fibre content has repeatedly raised concerns during the lengthy discussion on the definition of dietary fibre.

13. In response to this gap in methodology, a new integrated method of analysis of total dietary fibre has been developed by McCleary (McCleary, 2007) which measures total dietary fibre (including resistant starch), non-digestible oligosaccharides and available carbohydrates. Based principally on existing official AOAC methods (AOAC 991.43, AOAC 2001.03, and AOAC 2002.02) this new integrated method uses conditions similar to those described in AOAC Official Method 2002.02 (resistant starch) and AOAC Official Method 991.43 to quantify high molecular weight resistant polysaccharides. A further process similar to that described in AOAC Official Method 2001.03 allows for the measurement of those non-digestible oligosaccharides ranging from DP 3 upward to the limit of any particular oligosaccharide's solubility in 4 parts alcohol, 1 part water[6].

14. This new integrated method provides a path forward for analysing the full range of dietary fibres included in the scope of the Codex definition. At the time of writing, the collaborative study has been completed and the manuscript[7] has been submitted *to JAOAC Int*. for review. The study directors have recommended that the method be adopted as an Official First Action Method of AOAC International..

### 3.3 -Other methods

15. NSP[8] methods are inappropriate as routine techniques given their inability to support the now agreed upon Codex definition of dietary fibre. Methods measuring NSP alone[9] give lower estimates than methods for total dietary fibre in foods containing resistant starch, resistant oligosaccharides and/or lignin, e.g. whole-grain flour, cereals which have been processed in a way that generates resistant starch (EFSA, 2007).

16. With regard to the updated versions of NSP method (Englyst et al, 1994, Wood et al, 1993, Pendlington et al 1996) including the option for the measurement of Resistant oligosaccharides as their constituent sugars (Quigley et al, 1999) and of Resistant starch by the method (Englyst et al, 1992, 2000, there is yet no publication about protocol and relevant validation data obtained through an FSA (UK)-commissioned project.

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[5] See, for instance the proposal in: ***Comprehensive Measurement of Total Non-Digestible Carbohydrates in Foods by Enzymatic-Gravimetric Method and Liquid Chromatography*** – Toyohide Nishibata, Kouichi Tashiro, Sumiko Kanahori, Machiko Kitagawa, Kazuhiro Okuma, and Dennis T. Gordon : "*The total non-digestible carbohydrate (NDC) in foods was determined by combining, not modifying, AOAC Official Methods 991.43, 2001.03, and 2002.02. (…) The innovative step that was employed and verified in this study was the ability to measure all the NDO in samples using the LC protocol inherent in AOAC Method 2001.03. (…) By combing and expanding on the analytical capabilities of three AOAC methods, a comprehensive protocol was accomplished to measure all NDC in foods, including NDO that previously could not be measured with AOAC Method 991.43*." (J. Ag. Food Chem. – forthcoming).

[6] The ethanol supernatant can contain carbohydrates of much greater than DP 10, particularly when highly branched. It is therefore not a de facto method for DP 3-10. However, the integrated method will quantify DP 3+ accurately after combining the fractions.

[7] ***Determination of Total Dietary Fibre (CODEX Definition) by Enzymatic-Gravimetric Method and Liquid Chromatography: Collaborative Study***. (B.V. McCleary, J.W. DeVries, J.I. Rader, G. Cohen, L. Prosky, D.C. Mugford, M. Champ, & K. Okuma (2009)).

[8] "DF = Dietary Fibre" – "LMW = low molecular weight" — "RS = Resistant Starch"– "RMD = Resistant Maltodextrin" – "TGOS = Trans-galactooligosaccharides.

[9] e.g. Englyst et al, 1992 Eur J Clin Nutr 46, S33-S50. & 2000 *Encyclopedia of Analytical Chemistry*, pp. 4246-4262 - Not an AOAC approved method through collaborative study: reproducible results could not be obtained on unknown samples within and among collaborating laboratories. However, a few countries still use Dr. Englyst's method(s).

## 4.- CONCLUSIONS

### 4.1- Selection of analytical methods for dietary fibres

17. An official control laboratory routinely checking the levels of total fibres in various food the nutritional composition of which is not known, deploying several complementary methods in order to determine an overall content of dietary fibres is obviously not feasible. In this regard, the methods listed in the annex can be divided in three sets :

    - Three general methods: The phosphate buffer method, AOAC 985.29, the organic buffer based method AOAC 991.43, and the gas chromatographic component sugars method AOAC 994.13 give equivalent results. These methods are approved AOAC techniques, used world-wide, and in the majority of cases applicable in routine analysis.

      Methods AOAC 991.43 and AOAC 985.29 (= PROSKY) are similar based on the same principle.

      AOAC Method 991.43 merely provides an alternative in the conduct of the protocol that allows to obtain separately the insoluble fibres (IDF) and soluble fibres precipitation in ethanol (SDF). Total fibres (TDF = IDF + SDF) obtained by the 2 methods take the same classes of polysaccharides. Only the fraction of high molecular weight of Polydextrose is determined by these 2 methods, due to the low average DP of Polydextrose (between 10 and 12).

      Method AOAC 994.13 gives information about the components which build up the carbohydrate polymers, resistant amylase and Klason lignin (the non-carbohydrate part of the dietary fibre). This method analyse each component separately and the total yield is well-correlated to result of the traditional gravimetric AOAC methods (AOAC 985.29 and 991.43). In nutritional and technological studies, when changes in composition and content are followed, this method is highly appreciated. One advantage with this method, compared to the traditional gravimetric AOAC methods, is that fructan (including fructo-oligosaccharides) is completely removed from the analytes. This component can therefore be determined with one of the official AOAC methods for this analyte and added to the dietary fibre value.

    - One low molecular weight soluble dietary fibre method combined with a general method: AOAC 2001.03, in addition to quantitating the dietary fibre of the 3 general methods above, quantifies low molecular weight soluble dietary fibre, i.e. resistant oligosaccharides such as resistant maltodextrins, fructans, *trans* galactooligosaccharides and polydextrose, by utilizing a liquid chromatographic technique to measure the content of resistant soluble polysaccharides not isolated by alcoholic precipitation. This method does not quantitate all the resistant starch in food samples.

    - Seven specific methods: AOAC 992.28 and AOAC 995.16 for *beta*-D-glucans, AOAC 997.08 and 999.03 for fructans, AOAC 2000.11 for Polydextrose, AOAC 2001.02 for *trans* galactooligosaccharides, and 2002.02 for resistant starch. These specific methods allow for the quantification of particular components of dietary fibre. They are mainly based on enzymatic hydrolysis of the polymers followed by colorimetric detection or chromatographic detection of the monosaccharides released. They have a limitation in that generally the type of dietary fibres must be known in advance before analysis.

### 4.2- Potential double accounting or under recovery

18. The definition encompasses a range of different types of carbohydrate polymers which are recovered to varying extents by different analytical methods. This creates potential problems of double accounting when a carbohydrate fraction is partially or completely measured by more than one method. Examples of this are high molecular weight inulin, which in addition to being measured specifically by enzymatic-chemical fructan methods are also partially recovered in the residue of enzymatic-gravimetric methods. The enzymatic-gravimetric methods AOAC 991.43 and 985.29 also recover some but not all resistant starch, which can create a double accounting problem if this data is then combined with that obtained by

a separate specific determination of resistant starch. There is also the potential for obtaining a lower than expected value if there is under recovery of a specific fraction by particular methods.

19. The high degree of specificity associated with most direct chemical methods generally means that the problems of combining results from different methods are diminished.

**4-3 Amendment of footnote 1**

20. Several delegations have suggested amendments to the current footnote 1, which were basically similar in intent. The most concise among the suggested redrafts could be recommended as a revised text replacing the current Footnote 1:

> "[1]*When derived from a plant origin, dietary fibre may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls. These compounds also may be measured by AOAC analytical method(s) for dietary fibre. However, such compounds are not included in the definition of dietary fibre if extracted and re-introduced into a food.*"

**5.- RECOMMENDATIONS**

21. The Committee may wish to consider four recommendations by the electronic working group:

> (i) The eWG suggests to the Committee to forward for adoption to the Codex alimentarius Commission the methods of analysis for dietary fibre listed in the following Table[10], as the most appropriate to quantify dietary fibre in foods that support the definition of dietary fibres, adopted by the Codex alimentarius Commission, in view of amending the current list of Recommended Methods of Analysis and Sampling [CODEX STAN 234] by inserting a new section on "Dietary Fibres" as follows:

| Standard | Provisions | Method | principle | Type |
|---|---|---|---|---|
| Dietary fibres | Traditional dietary fibre based on precipitation from in 4 parts alcohol and 1 part water. Resistant insoluble and soluble polysaccharides, lignin, and plant cell related substances. | AOAC 985.29 | Enzymatic gravimetric | III |
| Dietary fibres | Traditional dietary fibre based on precipitation from in 4 parts alcohol and 1 part water. Resistant insoluble and soluble polysaccharides, lignin, and plant cell related substances | AOAC 991.43 | Enzymatic gravimetric | III |
| Dietary fibres | (1→3)(1→4) *Beta*-D-Glucans | AOAC 992.28 | Enzymatic | III |
| Dietary fibres | Traditional dietary fibre based on precipitation from in 4 parts alcohol and 1 part water, quantiated as component neutral sugars, uronic acids, plus Klason lignin | AOAC 994.13 | Enzymatic chemical | III |
| Dietary fibres | *Beta*-D-Glucans | AOAC 995.16 | Enzymatic | III |
| Dietary fibres | Fructans (oligofructoses, inulin, hydrolyzed inulin, fructooligosaccharides) | AOAC 997.08 | Enzymatic & HPAEC-PAD | III |
| Dietary fibres | Fructans (oligofructoses, inulin, hydrolyzed inulin, | AOAC 999.03 | Enzymatic & colorimetric | III |

[10] The methods are listed according to their AOAC number and the lay-out of table is the same as in CODEX STAN 234.

| Standard | Provisions | Method | principle | Type |
|---|---|---|---|---|
| | fructooligosaccharides) | | | |
| Dietary fibres | Polydextrose | AOAC 2000.11 | HPAEC-PAD | III |
| Dietary fibres | TGOS | AOAC 2001.02 | HPAEC-PAD | III |
| Dietary fibres | Total dietary fibre in foods containing resistant maltodextrin | AOAC 2001.03 | Enzymatic gravimetric and Liquid chromatography | II |
| Dietary fibres | Resistant starch and algal fibre (Recommended for RS3) | AOAC 2002.02 | Enzymatic | type III ( |
| Dietary fibres | Soluble + insoluble polysaccharides + lignin + resistant starch + oligosaccharides | McCleary 2007 | Enzymatic-Gravimetric-High Pressure Liquid Chromatography Method | IV **(pending complete validation, afterward s type II)** |
| Dietary fibres | Insoluble glucans and mannans of yeast cell wall(for yeast cell wall only) | Eurasyp (European association for specialty yeast product)[11] – LM Bonanno. Biospringer- 2004 | Chemical & HPAEC-PAD | IV |
| Dietary fibres | Fructooligosaccharides (Fructo-oligosaccharides with DP<5) | Ouarné et al. 1999 in *Complex Carbohydrates in Foods*. Edited by S. Sungsoo, L. Prosky & M. Dreher. Marcel Dekker Inc, New York | HPAEC-PAD | IV |

(ii) It also suggests assigning Codex types to each method as proposed in rightmost column of the Table above ;

(iii) In addition, the eWG suggests to the Committee to consider the inclusion of the new method of analysis for total dietary fibre (McCleary, 2007), once its AOAC process has been completed (see Table above)

(iv) The eWG does not recommend to include the methods, mentioned in para. 15 and 16 in the list at this stage and it suggests that, in order not to delay the adoption of a list of methods of analysis, the Committee postpone consideration of this issue until after publication of the relevant information and if a formal request for revision of the list, as new work, is put forward by a member in the future.

(v) The eWG suggests amending the footnote appended to the definition as suggested in para. 20 above.

[11] online version method on the website below : http://www.eurasyp.org/public.technique.home.screen.

**Annex**

| Name | Quantified compounds | Reference | Type | Chapter | Performance in measuring different types of dietary fibre? | Performance in different food matrices? | Behaviour at DP 10? | Other relevant characteristics |
|---|---|---|---|---|---|---|---|---|
| AOAC 985.29 | Traditional dietary fibre based on precipitation from in 4 parts alcohol and 1 part water. Resistant insoluble and soluble polysaccharides, lignin, and plant cell related substances. | Prosky et al. 1992 | Enzymatic gravimetric | 45.4.07, 32.1.16, 45.4.08 | Measures insoluble fibres and soluble fibres: measures most, but not all of RS, part of inulin ; part of polydextrose (only the high molecular weight fraction). Doesn't recover non digestible oligosaccharides which are not precipitated in 4 parts alcohol, 1 part water solution. | All type of matrices. Rugged method that has been extensively studied on a wide variety of matrices. | The 4 part alcohol, 1 part water precipitation does not necessarily precipitate fibres based on DP. Inclusion as fibre depends on solution insolubility and not DP. | No special equipment required, can be run in nearly any laboratory worldwide. Elapsed time per sample is roughly two day, however analyst time per sample is < 1 hour. |
| AOAC 991.43 | Traditional dietary fibre based on precipitation from in 4 parts alcohol and 1 part water. Resistant insoluble and soluble polysaccharides, lignin, and plant cell related substances | Lee et al | Enzymatic gravimetric | 32.1.17 | Measures insoluble fibres and soluble fibres: measures most, but not all of RS, part of inulin ; part of polydextrose (only the high molecular weight fraction). Doesn't recover non digestible oligosaccharides which are not precipitated in 4 parts alcohol, 1 part water solution. | All type of matrices. Rugged method that has been extensively studied on a wide variety of matrices. | The 4 part alcohol, 1 part water precipitation does not necessarily precipitate fibres based on DP. Inclusion as fibre depends on solution insolubility and not DP. | No special equipment required, can be run in nearly any laboratory worldwide. Elapsed time per sample is roughly two day, however analyst time per sample is < 1 hour. |

| Name | Quantified compounds | Reference | Type | Chapter | Performance in measuring different types of dietary fibre? | Performance in different food matrices? | Behaviour at DP 10? | Other relevant characteristics |
|---|---|---|---|---|---|---|---|---|
| AOAC 992.28 | (1→3)(1→4) *Beta*-D-Glucans | Zygmunt et al. | Enzymatic | 32.2.06 | Beta-glucans in cereals but not those originated from yeast cell wall. | Rugged method that has been extensively studied on a wide variety of applicable matrices. | No impact on the method because the polysaccharides are hydrolysed | No special equipment. Can be run in most laboratories. |
| AOAC 994.13 | Traditional dietary fibre based on precipitation from in 4 parts alcohol and 1 part water, quantiated as component neutral sugars, uronic acids, plus Klason lignin | Theander et al. | Enzymatic chemical | 45.4.11 | Includes determination of lignin., Measures insoluble fibres and soluble fibres: measures most, but not all of RS, part of inulin ; part of polydextrose (only the high molecular weight fraction). Doesn't recover non digestible oligosaccharides which are not precipitated in 4 parts alcohol, 1 part water solution. | All type of matrices | The 4 part alcohol, 1 part water precipitation does not necessarily precipitate fibres based on DP. Inclusion as fibre depends on solution insolubility and not DP. | Chemical measurement of dried residues in place of gravimetric method as in AOAC 991.43 or AOAC 985.29. Simple and used world-wide. Elapsed time per sample is roughly two day, however analyst time per sample is < 1 hour. |
| AOAC 995.16 | *Beta*-D-Glucans | McCleary & Codd,1991 | Enzymatic | 32 | Beta-glucans in cereals but not those originated from yeast cell wall | Rugged method based on the same principals as 992.28 that has been extensively studied on a wide variety of applicable matrices. | No impact on the method because the polysaccharides are hydrolysed | No special equipment. Can be run in most laboratories. |
| AOAC 997.08 | Fructans (oligofructoses, inulin, hydrolyzed inulin, | Hoebregs, 1997 | Enzymatic & HPAEC-PAD | 45.4.06A | Inulins and fructo-oligosaccharides | Applicable to the determination of fructans in raw commodities and in processed foods. | Not relevant for the method because the polysaccharides are hydrolysed | No interference by the matrices due to the chromatographic technique used (HPAEC-PAD). Used world-wide. Not very simple |

| Name | Quantified compounds | Reference | Type | Chapter | Performance in measuring different types of dietary fibre? | Performance in different food matrices? | Behaviour at DP 10? | Other relevant characteristics |
|---|---|---|---|---|---|---|---|---|
| | fructooligosaccharides) | | | | | | | but precise for fructans content > 1% Specific equipment ( HPAEC-PAD) required. |
| AOAC 999.03 | Fructans (oligofructoses, inulin, hydrolyzed inulin, fructooligosaccharides) | McCleary & Blakeney, 1999 McCleary et al., 2000 | Enzymatic & colorimetric | 45.4.06B | Inulins and fructo-oligosaccharides: fructans are hydrolyzed to fructose after conversion of sucrose to fructose and glucose and the free fructose and glucose converted to sugar alcohols. Fructose hydrolyzed from fructans measured colorimetrically. | Applicable to the determination of fructan in foods. Not applicable to highly depolymerized (either acid or enyzmatically) polyfructoses. | Not relevant for the method because the polysaccharides are hydrolysed: | Matrices can interfere in the colorimetric response and lead to an overestimation of the content in fibres. - Only a few laboratories are applying this method - No specific equipment. Less precise than AOAC 997-08. |
| AOAC 2000.11 | Polydextrose | Craig et al., Journal of AOAC International 84 (2), 472 - 478, 2001 | HPAEC-PAD | 45.6.06C | The method was specifically designed for polydextrose, however, other resistant oligosaccharides may interfere with polydextrose signal on HPAEC-PAD | The method has been validated for a range of solid foods and beverages (Biscuits, drinks, chocolate, marmalades, tea, milk chocolate candy, iced tea, sugar cookie, grape jelly, soft jellied candy, powdered drink mix) | No separation of <DP10 and >DP10 is performed. Method specifically developed for polydextrose (which has an average DP from 10-12) so other polysaccharides with a similar size could interfere (risk of coelution when complex matrices) | The method may be useful in conjunction with other AOAC methods to provide a measure of total dietary fibre (TDF) content. - Use of appropriate standard is essential. Need specific equipment ( HPAEC-PAD) |
| AOAC 2001.02 | TGOS | De Slegte | HPAEC-PAD | 45.4.12 | The method is specific for Trans galacto-oligosaccharides | Biscuits, dairy products, juice, infant formula,… | No impact on the method because the polysaccharides are hydrolysed | The free lactose is taken into account by the protocol: free sugars with lactose content are measured during the first step and sugars released by trans-galactosidase during the |

| Name | Quantified compounds | Reference | Type | Chapter | Performance in measuring different types of dietary fibre? | Performance in different food matrices? | Behaviour at DP 10? | Other relevant characteristics |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | second step . No interference by the matrices due to the chromatographic technique used (HPAEC-PAD) - Precise determination, High sensitivity – Used world-wide.- Not many laboratories use this method. |
| AOAC 2001.03 | Total dietary fibre in foods containing resistant maltodextrin | Gordon et al 2000 | Enzymatic gravimetric and Liquid chromatography | 45.4.13 | Measures insoluble fibres and soluble fibres: measures most, but not all of RS and measures resistant oligosaccharides. | All type of matrices. | All digestion resistant polysaccharides ≥DP3 are considered fibre; no separation of <DP10 and >DP10 is performed | Non carbohydrate compounds (glycoprotein, phenolic compounds, waxes, etc relevant to the CODEX definition) can precipitate – Used world-wide. Less simple to manage compared to the other general methods (due to HPLC) - Especially interesting if the content of low molecular weight soluble dietary fibre (LMWSDF)(resistant oligosaccharides) is high - HPLC analysis of LMWSDF gives a better estimate of the global content of fibres - Not simple to implement in routine laboratories. Time consuming (3 days) - Need specific equipment (HPLC) - **Combination of total DF method (AOAC 991.43; 985.29) and enzymatic LC-determination of resistant maltodextrin in foods; AOAC 2001 03 is the most** |

| Name | Quantified compounds | Reference | Type | Chapter | Performance in measuring different types of dietary fibre? | Performance in different food matrices? | Behaviour at DP 10? | Other relevant characteristics |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | **exhaustive AOAC method to determine the total DF (except RS3).** |
| AOAC 2002.02 | Resistant starch and algal fibre | McCleary & Monaghan, 2002 | Enzymatic | 45.4.15 | Specific method for resistant starch of types RS2 and RS3. if used in combination with AOAC 991.43 can lead to overestimation of RS | Plant and starch materials containing resistant starch (RS) contents ranging from 2.0 to 64% on an "as is" basis. | Inclusion as fibre/RS only depends on alcohol and aqueous KOH solubility and not DP; fibres soluble in ethanol or not soluble in aqueous KOH are not measured. | No specific equipment |
| Awaiting AOAC Validation and Adoption | Soluble + insoluble polysaccharides + lignin + resistant starch + oligosaccharides | McCleary 2007 | Enzymatic-Gravimetric-High Pressure Liquid Chromatography Method | | Total dietary fibre, resistant starch, Non -digestible oligosaccharides. | All food matrices | | Very promising for the determination of total dietary fibres content in food. It takes the advantages of AOAC 991.43, AOAC 2001.03 and AOAC 2002.02. But not completely validated at the time of writing; the protocol presented is long and probably expensive. Need specific equipment (HPLC) |
| Determination of glucan | Insoluble glucans and mannans of yeast cell wall | Eurasyp (European association for specialty | Chemical and HPAEC-PAD | | Carbohydrates (glucans and mannans) only specially from yeast cell wall | No test on other food matrices | Efficient for high DP ~ 10000 | Specially developed for yeast cell wall with a two step acid hydrolysis. Other methods tests for carbohydrates |

| Name | Quantified compounds | Reference | Type | Chapter | Performance in measuring different types of dietary fibre? | Performance in different food matrices? | Behaviour at DP 10? | Other relevant characteristics |
|---|---|---|---|---|---|---|---|---|
| s and mannans of yeast cell wall | | yeast product)[12] – LM Bonanno. Biospringer-2004 | | | | | | described in literature are not convenient for yeast cell wall. The DP of glucans and mannans are very high (10000-100000) These matrices are acid and enzymatic resistant with classic method. |
| | Fructooligosaccharides | Ouarné et al. 1999 in *Complex Carbohydrates in Foods*. Edited by S. Sungsoo, L. Prosky & M. Dreher. Marcel Dekker Inc, New York | HPAEC-PAD | | Method based on use of specific standards ($GF_2$ , $GF_3$ and $GF_{4)}$) | Method tested only on some types of yoghourt and biscuits (Repeatability SD: 1.3 - 1.9%) | Method based on use of specific standards, thus able to distinguish different DP (between 3 and 5). | |

[12] online version method on the website below : http://www.eurasyp.org/public.technique.home.screen