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Dietary fibers – definitions, classifications and analytical methods for the physiological assessment of their content in foods

ABSTRACT
The aim of this study is to present the most contemporary interpretation of the term “dietary fiber” as an ingredient of carbohydrates and its importance in human nutrition. The authors emphasize the “evolution” of the definition for dietary fibers in the years, as well as different approaches in the methods for their determination. The knowledge of the composition of foods rich of dietary fibers and oligosaccharides; and the harmonization of analytical methods together are a prerequisite for their correct identification and determination in the process of completion of the database for chemical composition of Bulgarian foods as well as in exchange of analytical data at regional and international level.

Key words: dietary fiber, oligosaccharides, physiological properties, foods

Introduction
Dietary fibers are nowadays defined as “edible plant and animal material not hydrolyzed by the endogenous enzymes of the human digestive tract” (Commission Directive, 2008). They are assigned to the group of non-starch polysaccharides (including lignin) and Regulation EU № 1169/2011 (Regulation EC, 2011) on food labeling sets the following definition:

“Dietary fibre means carbohydrate polymers with a degree of polymerisation (DP) not lower than 3 which are neither digested nor absorbed in the small intestine.”

The fiber categories contained in foods are defined by the Regulation as:

a). Edible carbohydrate polymers naturally occurring in the food as consumed,

b). Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means, that have beneficial physiological effect proven by generally acknowledged scientific evidence,

c). Edible synthetic carbohydrate polymers, those have beneficial physiological effect proven by generally acknowledged scientific evidence.”

Discussion
The current European legislation has set a value for the energy conversion factors for dietary fibers of about 8 kJ/g (4 kcal), and the recommended daily intake for adult population as set in scientific publications is from 20 to 25 g.

In the earlier periods of progress of the science concerning non-starch polysaccharides the fibers were identified as “soluble” and “insoluble”. Those terms were very valuable for the initial understanding of the physiological properties of dietary fibers, incorporating the simple division into fibers that principally affect the glucose and lipid absorption in the small intestine (the soluble ones) and fibers that slowly and incompletely ferment with a marked effect on gastrointestinal functions (the insoluble ones). This division, though, is not chemically well substantiated but depends more on extraction conditions (Asp, 1992). Besides, the physiological differences are not very essential in most of the soluble dietary fibers that ferment quickly and fully while not all insoluble fibers affect glucose and lipid absorption. From physiological point of view the dietary fibers pass undegraded from the human small intestine to the colon where a part of them could undergo fermentation under the effect of intestinal
microflora. The final result is expressed in variable amounts of short-chain fatty acids and some gases like carbon dioxide, hydrogen and methane (Cummings, 1981; Asp, 1995).

According to their chemical nature, dietary fibers are represented by cellulose, hemi-cellulose, lignins and pectins of the cell walls; resistant starch; some other components as presented in Figure 1.

**Table 1. Main polymers of dietary fibers in important food groups.**

<table>
<thead>
<tr>
<th>Food groups</th>
<th>Available polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td>Cellulose, arabinoxylans, β-D-glucans, other non-cellulose polysaccharides, phenolic esters, lignins</td>
</tr>
<tr>
<td>Fruits and vegetables</td>
<td>Cellulose, pectin substances, xyloglucans, lignins, cutin, waxes</td>
</tr>
<tr>
<td>Seeds</td>
<td>Cellulose, pectin substances, xyloglucans, galactomannans, other non-cellulose polysaccharides</td>
</tr>
</tbody>
</table>

All plant foods contain a mix of various fiber components depending on the plant tissue and its maturity. Table 1 lists some of the predominant components of the fibers in the main food groups.

Oligosaccharides are specially emphasized. Those are carbohydrate biopolymers with low molecular weight, defined as saccharides containing 3-10 and 3-19 sugar molecules (according to different authors). In principle, there is no rational physiological or chemical reason for setting strict limits (Voragen, 1998). At the same time, based on the physiological properties, oligosaccharides can be divided into digestible and non-digestible (degradable and non-degradable). The main categories of non-digestible oligosaccharides, currently existing or being developed as food ingredients, include carbohydrates in which the monosaccharide residue is fructose, galactose, glucose and/or xylose (Table 2).

**Table 2. Non-digestible oligosaccharides with biphydogenous functions, produced for commercial purposes.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular structure a)</th>
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<tbody>
<tr>
<td>Cyclodextrins</td>
<td>(Gu)&lt;sub&gt;n&lt;/sub&gt;</td>
</tr>
<tr>
<td>Fructooligosaccharides</td>
<td>(Fr)&lt;sub&gt;n&lt;/sub&gt; – Gu</td>
</tr>
<tr>
<td>Galactooligosaccharides</td>
<td>(Ga)&lt;sub&gt;n&lt;/sub&gt; – Gu</td>
</tr>
<tr>
<td>Gentiooligosaccharides</td>
<td>(Gu)&lt;sub&gt;n&lt;/sub&gt;</td>
</tr>
<tr>
<td>Glycosylsucrose</td>
<td>(Gu)&lt;sub&gt;n&lt;/sub&gt; – Fr</td>
</tr>
<tr>
<td>Isomaltooligosaccharides</td>
<td>(Gu)&lt;sub&gt;n&lt;/sub&gt;</td>
</tr>
<tr>
<td>Isomaltooligosaccharides</td>
<td>(Gu-Fr)&lt;sub&gt;n&lt;/sub&gt;</td>
</tr>
<tr>
<td>Lactulose</td>
<td>Ga-Fr</td>
</tr>
<tr>
<td>Lactosucrose</td>
<td>Ga-Gu-Fr</td>
</tr>
<tr>
<td>Maltooligosaccharides</td>
<td>(Gu)&lt;sub&gt;n&lt;/sub&gt;</td>
</tr>
<tr>
<td>Raffinose</td>
<td>Ga-Gu-Fr</td>
</tr>
<tr>
<td>Soybean oligosaccharides</td>
<td>(Ga)&lt;sub&gt;n&lt;/sub&gt; – Gu-Fr</td>
</tr>
</tbody>
</table>

Their implementation as food ingredients provokes certain interest because of their numerous health-beneficial physiological properties. Many researchers (Delzenne & Roberfroid, 1994; Voragen, 1998; Sako et al., 1999; Roberfroid & Slavin, 2000; Rivero-Urgell & Santamaria-Orleans, 2001; Bielecka, 2002; Gibson, 2004; Isoulari et al., 2004; Sangeetha et al., 2005) support that the bacterial fermentation of non-digestible oligosaccharides in the appendix, could cause the following health effects:

1. Significant alteration of the intestinal microflora with growth and proliferation of anaerobes, mainly bifidobacteria that inhibit the growth of decay microorganisms living in the colon;

2. Decreased pH in the colon content and feces as a result of the production of short-chain fatty acids. The lower pH values inhibit the growth of some pathogenic bacteria.
stimulating the growth of bifidobacteria and other lactic acid bacteria species;

3. Production of nutrients, such as vitamins (B1, B2, B6, and B12), nicotinic and folic acid;

4. Increased amount of fecal secretion;

5. Correcting effect at obtipation due to the oligosaccharides as fecal bulking with eventual effect on the intestinal motility as well;

6. Effective absorption of the short-chain fatty acids – end products of the fermentation – by the epithelial cells of the colon;

7. Inhibition of the diarrhea syndrome;

8. Protective effect against infections in the gastrointestinal, respiratory and urogenital tract, due to the ability of oligosaccharides to inhibit the adhesion of bacteria on the epithelial surfaces;

9. Increased absorption of various minerals, such as calcium, iron and magnesium;

10. Beneficial effect on the carbohydrate and lipid metabolism leading to reduced blood cholesterol, triglycerides and phospholipids concentrations, thus reducing the risk for obesity and diabetes;

11. Decreased risk for neoplasms, mainly intestinal cancer.

All above mentioned effects are beneficial for the host’s health and that is the reason to consider the non-digestible oligosaccharides as “functional foods” (Roberfroid & Slavin, 2000; Rivero-Urgell & Santamaria-Orleans, 2001), defined as food ingredients that affect a physiological body function in a way to provide a positive effect that could support the health claims (Roberfroid, 1996). In addition, most of the non-digestible oligosaccharides are classified as probiotics because they selectively stimulate the growth and/or metabolic activity of the probiotic bacteria strains with improvement of the colon microflora composition, thus having a beneficial effect on health (Crittenden & Plane, 1996; Voragen, 1998; Roberfroid & Slavin, 2000; Isoulari et al., 2004).

The methodology of the chemical analysis is in close relationship with the definitions and classifications of dietary fibers and is very important for the application and assessment of food composition, including functional foods and food supplements.

The more comprehensive understanding of the nature of the term “dietary fibers” caused the launching of numerous analytical methods. Many of them determine the different fiber components thus outlining scientifically the great variety of dietary fibers definitions. In 1996 Monro and Burlingame (Monro & Burlingame, 1996) stated that at least 15 different methods were implemented to determine the values of dietary fibers, at the same time being listed in the food chemical composition (FCCT) databases of the individual countries.

Three chemical methods have been successfully tested within interlaboratory and collaborative tests and generally approved by organizations as AOAC International and Bureau Communautaire de Reference (BCR) of the EU (FAO/WHO, 1998). The first one is the enzymatic-gravimetric method of Prosky, 2000 (AOAC 985.29) (AOAC, 2000); the second is an enzymatic-chemical method of Englyst and Cummings (Englyst & Cummings, 1988) and the last one – enzymatic chemical method of Theander and Aman (Theander & Aman, 1982).

The presence of this broad spectrum of analytical methods for dietary fibers where each method provides results, differing to a certain extent, concerns not only the values of the dietary fibers in the database, but principally concerns the values of “available carbohydrates by difference” (FAO/WHO, 1998). The particular assessment of their applicability for the goals of the physiology of nutrition and dietetics could be presented as follows:

AOAC (2000) – Prosky method (985.29) or a similar analytical method for determination of total dietary fibers should be used for analysis of standard foods with energy factor of dietary fibers conversion of 8 kJ/g (2 kcal/g). In order to achieve this factor it is assumed that about 70% of the fibers can ferment, having in mind the fact that a part of the energy, produced during fermentation is lost in the form of released gases, and the other part is lost with the feces.

When fibers and oligosaccharides, specially added to the food, are concerned, Prosky’s method should be implemented but with specific energy conversion factor. For example, such a factor varies from 1.3 kJ/g (0.3 kcal/g) for fibers in corn bran to 11 kJ/g (2.6 kcal/g) for fructooligosaccharides.

The concept “soluble vs. insoluble” dietary fibers should not be used for the evaluation of food energy value.

When enriching foods with isolates of dietary fibers, e.g. resistant starch the specific energy conversion factors should be determined.

Currently the terminology in the field of dietary fibers is intertwined with the analytical methods for their determination. The analytical results provided by a particular
method should be recorded in individual columns in FCCT identified by INFOODS tagnames, listing also the used energy conversion factors for dietary fibers (FAO/ WHO, 1998).

The methods for determination of short-chain fibers, respectively oligosaccharides are very complex and specific. Their development and improvement are a current task of modern biochemistry.

Fructooligosaccharides (FOS) are a class of functional foods widely used as prebiotics, low energy sweeteners, for improvement of calcium and magnesium assimilation (www.food-info.net, 1999). That is why the method is a challenge and should be included in modern experimental practice. Its implementation is necessary for the motivation of nutritional claims for functional and dietetic foods and food additives, as well as for scientific and applied research. Here we shall present our experience in the implementation of AOAC Method 999.03 (MEGAZYME, 2008) for the analysis of fructan for determination of FOS and fructan polysaccharides. The method was verified by using referent material of fructan in concentration (25.5 ± 2.4) %. The parameters of the method are listed in Table 3.

Table 3. Verification of AOAC Method 999.03 for determination of fructan using referent fructan material with concentration 25.5 ± 2.4 %.

<table>
<thead>
<tr>
<th>Expanded uncertainty UΔ, %</th>
<th>2.337</th>
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<tbody>
<tr>
<td>Other analytical parameters of the method:</td>
<td></td>
</tr>
<tr>
<td>Analytical recovery, %</td>
<td>100.7</td>
</tr>
<tr>
<td>Accuracy, %</td>
<td>0.75</td>
</tr>
<tr>
<td>Limit of Detection, LOD, %</td>
<td>0.015</td>
</tr>
<tr>
<td>Limit of Quantitation, LOQ, %</td>
<td>0.030</td>
</tr>
<tr>
<td>Precision (r) expressed by SD in repeatability conditions, %</td>
<td>3.69</td>
</tr>
<tr>
<td>Reproducibility (in-lab reproducibility) %</td>
<td>5.85</td>
</tr>
</tbody>
</table>

The obtained experimental expanded uncertainty 2.337 (≈2.3) % is less than the one listed in the certificate of the standard referent material fructan – 2.4%. The confidence interval is within (22.86 ± 28.14) %. The experimentally obtained concentrations of the referent fructan material and the concentrations of fructan in repeatability and reproducibility conditions fall within the broad margins of the confidence interval as presented in Table 3.

The experimental tests of the method for analysis of fructan showed that it was appropriate for detection and quantitative determination of both low and high concentrations of FOS in foods with various compositions. The method accuracy was 0.75 %, and the limit of quantitation – 0.04 %. The method is accessible, with relatively short execution time but this advantage requires very precise adherence to the analytical procedures. The disadvantages of the method refer to small deviations from the procedures, such as temperature, processes exposure and other details.

Conclusion

The appropriate knowledge and implementation of the definitions and methodology of the analyses for determination of dietary fibers and oligosaccharides is particularly important for the performance of the analysts and other specialists, e.g. dieticians, nutritionists, etc. For the tasks of applied dietetics and for the objectivization of the information on the food composition and energy content it is necessary to legalize a referent method for determination of total dietary fibers. At present we propose AOAC (2000) –Prosky’s method (985.29) for this purpose.

In Bulgaria there is ongoing work on filling in the Food Composition Tables of Bulgarian foodstuffs, including their completion with current adequate information on dietary fibers. Foods that are sources of oligosaccharides are of particular interest but in Bulgaria currently there is no laboratory that could purposefully complete such databases. The implementation of such methods in laboratory practice is one of the most important tasks of specialists in food chemistry.

References


