Methodologies for measurement of dietary fiber – methodologic shortcomings in the evaluation of different fiber types in fruit and fruit-derived products
Métodos para determinação de fibra – identificação de lacunas metodológicas existentes na avaliação de diferentes tipos de fibra em fruta e derivados

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Abstract

Dietary fiber (DF) is a nutrient with proven health benefit, whose potential has been made clear by scientific community, particularly in terms of glycemic control, lipid metabolism and intestinal health. Therefore, the reliable determination of DF in foods is essential, not only for dietitians' clinical practice, but also to correctly inform consumers and support food industries on the definition of products' composition. Since DF definition has evolved over the times, several methodologies to measure this nutrient have emerged. The objective of the present work is to comprehend what are the most used methodologies to measure dietary fiber, realizing their accuracy and reliability as well as limitations. Moreover, it is aimed to understand their appliance to fruit and fruit-derived products, achieving shortcomings in fruit fiber quantification in food composition databases. Classical methodologies (AOAC 985.29 and AOAC 991.43) and integrated methodologies (AOAC 2009.01 and AOAC 2011.25) will be discussed, as it will be briefly described the DF health effects.

Keywords: Dietary fiber; Food analysis; Food composition databases; AOAC methodologies

Resumo

A fibra alimentar (FA) é um nutriente com impacto comprovado na saúde, cujo potencial tem vindo a ser defendido pela comunidade científica, particularmente ao nível do controlo glicémico, metabolismo lipídico e melhoria da saúde gastrointestinal. Torna-se, assim, relevante a determinação precisa dos teores de fibra dos alimentos, não só para suportar a prática clínica de nutricionistas e de outros profissionais de saúde, mas também para informar corretamente os consumidores e apoiar a indústria alimentar na definição da composição dos alimentos. Tendo em conta que a definição de FA tem vindo a evoluir ao longo dos anos, diversas metodologias para a sua quantificação nos alimentos têm surgido. O objetivo do presente trabalho, após um breve resumo dos efeitos da FA na saúde, é identificar quais as metodologias mais usadas, compreendendo a sua precisão na deteção de FA. Além disto, é ainda objetivo perceber a aplicação destas técnicas à fruta e derivados, identificando lacunas e fragilidades dos dados de FA em fruta e derivados nas tabelas de composição alimentar. Serão discutidas as metodologias clássicas (AOAC 985.29 e AOAC 991.43) e as metodologias integradas (AOAC 2009.01 e AOAC 2011.25) e apontadas metodologias de utilização emergente.

Palavras chave: Fibra alimentar; Análise alimentar; Tabelas de composição alimentar; Metodologias AOAC

Abbreviations list

AOAC - Association of Official Analytical Chemists

DF – Dietary Fiber

FCDBs – Food Composition Databases

HMWDF – High Molecular Weight Dietary Fiber

IDF – Insoluble Dietary Fiber

LMWDF – Low Molecular Weight Dietary Fiber

SDF – Soluble Dietary Fiber

TDF – Total Dietary Fiber

Summary

Introduction

Knowledge about food composition is essential. It supports dietary treatments of disease and the promotion of healthy eating habits, playing a substantial role in dietitians' practice[1]. For food industries, product reformulation and improvement of nutritional profiles are only possible with the deep knowledge of its composition[1]. Policy makers and researchers, in food and nutrition fields, underpin a part of their work on food composition data[1]. Even consumers' healthy choices are influenced by the food information presented in food labels and/or health and nutritional claims[2]. There is, therefore, a clear necessity of reliability and transparency on the food composition data provided.

In Europe, information about food is regulated according to the Regulation (EU) No. 1169/2011[3]. According to this Regulation, it is not mandatory to declare dietary fiber (DF) in food labels[3]. However, the health benefits of its consumption are well established and widely accepted by scientific community, particularly in terms of glycemic control, lipid metabolism and intestinal health[4]. Recently, DF consumption has also been associated with prebiotic effects and prevention of some types of cancer and cardiovascular disease[5-9]. To achieve these health effects, it is recommended a daily consumption of DF of 25 g/day for adult women and 38 g/day for adult men[10].

Over the years, following scientific progress and updates to DF definition, several methods to measure this nutrient have been developed. However, considering the diversity of available foods, re-analyzing the composition of all foods according to every new finding is a high-cost and a never-ending task[11]. Consequently, DF may appear erroneously estimated on food composition tables,

which has impact on intake quantification. According to Regulation (EC) no. 1924/2006[12], a food may be claimed as "source of fiber" if it contains at least 3 g of fiber per 100 g (or 1,5 g of fiber per 100 kcal) and "high in fiber" if it has a minimum of 6 g of fiber per 100 g (or 3 g of fiber per 100 kcal)[12]. Underestimated quantifications of DF can also limit the use of these claims[13], which, as stated above, have influence on individual dietary choices. Hence, it is crucial to select the most appropriate method to quantify DF, in order to obtain non-biased results[11].

Fruit is one of the main food sources of DF. In addition to vitamins and minerals, this food group is recognized by its fiber content, which is responsible for most of its health benefits[14]. Hence, provision of accurate data about DF content in fruits and fruit-derived products is essential.

The objective of the present work is to explore the most used methodologies to measure DF, achieving evidence on their accuracy and reliability to define their potential and correct application. Focusing on DF of fruit and fruit-derived products, it is aimed to understand which are the best methods for its quantification and, furthermore, to identify gaps in fiber contents presented on food composition tables and databases.

Methodology

This work was based on the analysis of the scientific literature, using *Pubmed* and *Google Scholar* databases to research relevant information. Scientific papers were identified employing the expressions "dietary fiber" and "dietary fibre" combined with "health", "analysis methods", "AOAC 985.29", "AOAC 991.43", "AOAC 2009.01", "AOAC 2011.25", "labelling" or "food composition data". Restrictions to the publication year or idiom were not established.

Abstracts were analyzed and applicable articles were selected. Pertinent bibliography from the selected papers was also reviewed.

Dietary fiber – definition and different fiber types

The term "dietary fiber" was firstly introduced by Hiplsey, in 1953, to describe non-digestible components of plant cell walls, namely, cellulose, hemicellulose and lignin[15]. Over the years, other constituents were added to this definition and, nowadays, it includes carbohydrates that are not digested nor absorbed in human intestine which can be divided in 3 categories: non-starch polysaccharides (>10 monomeric units), non-digestible oligosaccharides (3-10 monomeric units) and resistant starch (>10 monomeric units). Besides this, some definitions also include other "associated substances" that are not carbohydrates (such as lignin) but appear aggregated to cell walls and are quantified as dietary fiber. Additional to these structural and non-digestibility characteristics, to be considered a DF, substances must also provide benefic health effects to humans, specifically related to improvements on colonic function, blood cholesterol or blood glucose. Although there is a general agreement about the concept of "dietary fiber", health and government authorities have not yet agreed upon a consensual definition. Divergences emerge in relation to the inclusion or not of oligosaccharides (carbohydrates' polymerization grade) and "associated substances" as dietary fibers and to the specification of health benefits that fibers should present[4]. Some definitions are presented in Table I, Annex A.

Observing their molecular structure and physicochemical characteristics, DF can be categorized according to several parameters: structure (linear or branched chain), water solubility (soluble or insoluble fiber), molecular weight

(high or low molecular weight), viscosity and fermentability[4, 16]. These aspects are important to define the procedures used to measure DF content of foods and to predict health effects. Table II, Annex B, presents some of the main dietary fibers and their molecular weight and water solubility.

Dietary fiber and health

Health benefits of DF are recognized and its consumption is recommended by nutritional guidelines. In fact, DF is not digested nor absorbed by human organism, but it plays a substantial role in digestion and absorption mechanisms, which provides them important health effects[17].

Insoluble dietary fibers (IDF), since they remain intact across all gastrointestinal tract, are responsible for increase fecal bulking, which stimulates intestinal motility[18]. Indeed, laxation is one of the *major* health benefits of these fibers[18]. Fecal bulking, due to the modifications it causes in food matrix, also seems to diminish bioavailability and bioaccessability of macronutrients[19]. This may cause a reduction in total energy intake[18], that, in part, may contribute to weight control.

Soluble dietary fibers (SDF), as a result of their water-holding capacity, form gels and/or thicken, resulting in increase of food viscosity - a property related with many digestive effects. Viscosity induces gastric distension, which, by itself, leads to feeling of fulness. High viscosity food also contributes to gastric emptying delay. This may improve glycemic control[18] and decreases absorption of triglycerides and cholesterol[7], improving general metabolic control and increasing satiety.

Opposite to IDF, SDF are fermented by gut bacteria in the colon and cecum. This process generates short-chain fatty acids (mainly, butyrate, propionate and

acetate), that are absorbed and have substantial impact in human health, since they affect host metabolism, immune system and cell proliferation[20]. Low DF intakes lead not only to diminished short-chain fatty acids production but also reduce microbiome richness and diversity, which is associated with chronic disease and metabolic dysfunction[20, 21].

DF intake has been associated with diminished risk of several chronic diseases, namely, type 2 diabetes[22] and cardiovascular disease[7]. It has also been linked to lower risk of ovarian[9] and colorectal[6] cancers.

Considering all these health effects, it is important to ensure an adequate intake of DF, following health authorities' recommendations. Therefore, a correct detection of DF content in foods is essential to support not only individual choices, but also to underpin dietitians and nutritionists' clinical practice.

Methodologies for measurement of DF – Codex Alimentarius

Since the DF definition has evolved over the time, several methodologies for the measure of its content in foods have been developed. Codex Alimentarius Commission recommends well-established methods, detailing their particularities: the ones that measure low molecular weight dietary fiber (LMWDF) and high molecular weight dietary fiber (HMWDF), and the ones that distinguish SDF and IDF[23]. Table III, Annex C, presents all the methods approved in Codex Alimentarius, with these particularities pointed.

Association of Official Analytical Chemists (AOAC) 985.29 was the first analytical method for DF measurement accepted as official. Developed by *Prosky et. al*[24], this is an enzymatic-gravimetric method that determines TDF using duplicate samples of dried and fat-extracted (if > 10% fat) foods which are

gelatinized in the presence of α-amylase. Protein and starch are then digested with protease and amyloglucosidase, respectively. The undigested residue is filtered and washed (with 78% ethyl alcohol, 95% ethyl alcohol and acetone) and dried. The samples are weighed, being one analyzed for protein residue and the other for ash residue. According to AOAC 985.29, TDF is achieved subtracting resultant protein and ash to the weight of the previous residue – correction to protein and ash residues[25].

AOAC 991.43 was the subsequent accepted method. It was developed by *Lee et. al*[26] with the introduction of some modifications to the previous one that brought the possibility of measurement of TDF, IDF and SDF with a unique procedure. Measurement of TDF is similar to AOAC 985.25 procedure, except it uses MES-TRIS buffer. To measure IDF, after sample digestion with α-amylase, protease and amyloglucosidase, the residue is filtered (*A*) and washed with hot water (>95ºC), filtered (*B*), dried, and weighed. To quantify SDF, resultant filtrates from the first sample digestion and water washing are combined in a unique solution (*A+B*). This is treated with alcohol 95% which leads to precipitation of SDF, that is, finally, filtrated, dried, and weighed. Similarly to AOAC 985.25, all the values (TDF, SDF and IDF) are corrected for protein and ash residues[27].

These methods were developed before recognition of non-digestible oligosaccharides and resistant starch as DF[28]. Inclusion of these components lead to the development of new specific methods for measurement of fructooligosaccharides (AOAC 997.08 and 999.03), galactooligosaccharides (AOAC 2001.02), resistant maltodextrins (AOAC 2001.03), and resistant starch (A0AC 2002.02). However, the addition of these specific measures to TDF quantification derived from AOAC 985.25 or AOAC 991.43 lead to overestimations, since a portion

of some components (such as some types of resistant starch[29]) are also included in these methods, having a double counting effect[30].

Methodologies AOAC 2009.01 and AOAC 2011.25 (integrated methodologies) were developed to precvent this effect, since they measure all types of DF components in one procedure[30]. These methods aim to overcome other limitations. It was found that, despite the use of alcohol 78% to precipitate SDF, some DF types (LMWDF) remain soluble in alcohol. Therefore, some literature divides classification of SDF in DF soluble in water but insoluble in 78% alcohol and DF soluble in water and soluble in 78% alcohol[31, 32]. Usage of Liquid Chromatography in these methodologies allows the quantification of water and alcohol soluble DF, enabling more accurate measures.

AOAC 2009.01 was the first enzymatic-gravimetric-liquid chromatographic method developed to measure DF. This method was developed to measure TDF (including non-digestible oligosaccharides and resistant starch) and uses the main features of AOAC 985.29, AOAC 991.43, AOAC 2001.03 and AOAC 2002.02. Duplicated test samples are incubated with α-amylase and amyloglucosidase (simultaneously), so that non-resistant starch is solubilized. This reaction occurs through maintaining the incubated samples as a suspension at 37ºC (physiological conditions) for 16h. Non-resistant starch digestion is concluded by pH adjustment and temporary heating. Protein is digested by using protease. Ethanol or industrial methylates spirits is added to precipitate soluble HMWDF, which consequently joins to insoluble dietary fiber. Resultant precipitate is filtered, washed with ethanol and cetone, dried and weighed. Samples are corrected for protein and

ash. Resultant filtrate, which contains alcohol and ethanol soluble LMWDF, is concentrated, deionized and quantified by Liquid Chromatography[33].

AOAC 2011.25 is a method used for measurement of IDF, SDF and TDF. Similarly to AOAC 2009.01, non-resistant starch (using α-amylase and amyloglucosidase) and protein (using protease) are digested. The resultant digested is filtered, corrected for protein and ash residues and weighted to achieve IDF amount. This resultant filtrated is then used to measure SDF. The addition of ethanol to the filtrated leads to the precipitation of water-soluble HMWDF, which is filtered and weighed (after correction to protein and ash). The water-and-ethanol soluble LMWDF fiber remains in the filtrate and is achieved by concentration and deionization of filtrate which is then submitted to Liquid Chromatography[34].

Classical (AOAC 985.29 and AOAC 991.43) versus Integrated Methodologies (AOAC 2009.01 and AOAC 2011.25) and Future Approaches

AOAC 985.29 and AOAC 991.43 classical methods were considered the "gold standard" methodologies for DF for many years and still are commonly used[4, 35]. However, these methods only quantify 78% ethanol insoluble HMWDF, leading to misdetection of non-digestible oligosaccharides and some types of resistant starch[35, 36]. Development of new integrated methodologies to measure TDF, SDF and IDF (AOAC 2009.01 and AOAC 2011.25) has led some authors to compare them with the classical ones, to understand the real differences and potential of methods.

Englyst K. et al. (2013) compared AOAC 991.43 and AOAC 2009.01, testing real food and model foods with added resistant starch, non-starch polysaccharides

and non-digestible oligosaccharides. The majority of samples did not show significant differences between both measures. This was explained by the high content in resistant starch type 3 (being the only resistant starch type present) in samples, which is measured by both methodologies. Some exceptions, however, showed higher DF contents when applied AOAC 2009.01[29].

Hollmann et al. (2013) analyzed TDF in fifteen cereal based products, using both AOAC 991.43 and AOAC 2009.01 methods. Most of the studied foodstuffs (twelve) presented higher values of TDF when assayed by AOAC 2009.01, with some of them being statistically significant. Authors emphasized the amount of LMWDF in cereal products and the considerable proportion it takes in TDF quantifications[35].

Similarly, *Brunt & Sander* (2013) investigate the variances of TDF content in five different types of bread, applying AOAC 985.25 and AOAC 2009.01. Authors found considerable amounts of LMWDF, which led to significative higher values of TDF quantifications with AOAC 2009.01[13].

Tobaruela et al. (2016) used four Brazilian fruits to compare DF quantification by AOAC 991.43 and AOAC 2011.25 methods. Significant differences were found in three fruits, with AOAC 2011.25 assaying higher TDF values. Authors also measured fructan (fructooligosaccharides) content of each fruit (using AOAC 999.03) and found congruency between fructan content and TDF contents measured by AOAC 2011.25. Mature coconuts – the only fruit with no significant differences established - had, in fact, low fructan content (0.06g/100g), while the other fruits presented higher contents (≈ 6-8g/100g)[37]. *Garcia-Amezquita et al.* (2018) also compared these methods using eight fruits and their by-products

(peels). This study also elucidated that the use of integrated methodologies provides significative different data about DF contents, with AOAC 2011.25 providing higher contents of DF in most fruits[38].

Despite the lack of evidence comparing DF contents using conventional and integrated methodologies, there is general agreement that AOAC 985.29 and AOAC 991.43 underestimate DF, since these methodologies do not detect LMWDF. In fact, all these studies found a great coincidence between HMWDF detected by integrated methodologies and TDF values achieved by the classical ones.

Even with AOAC 2009.01 and AOAC 2011.25 being the most accurate and inclusive methods proposed in Codex Alimentarius, they still present some limitations[39].

Tanabe et al. (2014) detected that amyloglucosidase do not hydrolyze some oligosaccharides (such as sucrose and maltose), which are digested by human organism and, thus, are not DF. These findings suggested an overestimation of LMWDF by integrated methodologies. To contradict this effect, authors proposed a replacement of amyloglucosidase with porcine intestinal enzymes[40, 41]. *Brunt & Sander* (2013) also suggested an improvement to AOAC 2009.01 method since they detected that, similarly to oligosaccharides, digestible starch is not totally hydrolyzed by amylase and amyloglucosidase, leading to overestimations in DF content. Authors suggested the introduction of an extra step of hydrolysis with amyloglucosidase, before desalting the solution[13]. Incubation conditions are also a concern presented to these methods, because time of incubation (16h) is not physiological and because it seems to alter sample composition in DF. A new integrated methodology to measure DF, AOAC 2017.16, was developed to

contradict these limitations[42]. However, its application is not much explored in scientific literature yet, reason why it was not included in the present work.

Food Composition Databases and Fruit Fiber Shortcomings

Food composition databases (FCDBs) integrate nutritional composition of foods, usually from a country or region, which provides fundamental information to Nutrition fields and is relevant to dietary intake estimation[43, 44]. Despite food analysis are the best method to determine food components, different sources can provide data included in FCDBs: some inexistent values can be taken from similar foods, others can be appropriated from other FCDB and other values can be presumed via the general knowledge (eg. dietary fiber in meat products presumed zero)[44]. Besides, food analysis for FCDBs is not limited to authorities. Different entities can provide food data, such as, private company analysis, universities, food industries, government laboratories or even scientific literature and food labelling[45].

Hence, quality of data presented in FCDBs is arguable, considering these different methods used to obtain food component values. Furthermore, since foods are biological materials, many factors may affect their nutrient content, causing natural variations[44].

Fruit is one of the food groups known by its fiber content, being an important source of cellulose, hemicellulose and pectins[4]. Moreover, fruits also have in their constitution frutooligosaccharides[46] and fructans[47]. It is known that DF content of fruits depends on the degree of ripening and that it is not equally

distributed in all fruit constituents. Major contents of DF are founded in peels and seeds, but these are most of the times rejected as by-products[38].

Despite this general knowledge about fruit fiber, there is a wide variety of fruits and the exact composition of DF types of each fruit is not defined yet. Therefore, to measure DF in fruit and fruit products, integrated methodologies should be selected, since these are the most inclusive ones[39]. This option is even more accurate, considering the existence of frutooligosaccharides in fruits, which are LMWDF, as stated in Table 2, Annex B. However, values presented in FCDBs commonly accrue from the use of classical methodologies to measure DF[48], mainly AOAC 985.29.

Table IV, Annex D, compares values of TDF per 100 g of edible portion of raw pear, peach and apple and their derived nectars and 100% juices between four different FCDBs. As it is stated, not all the values are presented, due to the impossibility of including all the foods and drinks, which is one of the limitations of FCDBs.

This comparison also elucidates the importance of accuracy in methodologies to measure DF. Taking into account the "3 g of fiber per 100 g" criteria from the Regulation (EC) no. 1924/2006[12], a raw pear, considering USDA and DTU values, would be classified as a "source of fiber", but, according to TCA and FDHA values, this claim could not be applied. Since the thresholds to establish DF nutritional claims are low, small variations on DF content are not negligible. In fact, these 1g fluctuations between different FCDBs may seem a little amount of DF, but they represent a variation of about 30% in DF content of pear.

According to Directive 2012/12/EU[49], a "fruit juice" is the product obtained from the edible part of the fruit, that must only suffer mechanical

processing and cannot be added other components beyond fruit. However, to elucidate this total composition in fruit, general nomenclature given to these products is "100% fruit juices". A "fruit nectar" is obtained with the addition of water and sugars and/or honey and/or sweeteners to fruit juices[49]. The existence of these 3 nomenclatures can cause some confusion about the products included in FCDBs, since common sense does not associate the term "fruit juice" to "100% fruit juices", but to fruit-derived beverages in general. For example, Federal Department of Hold Affair FCDB refers "pear juice" and "apple juice" and do not present values of DF for "100% fruit juice" or "nectar juice" of these fruits. Hence, despite the legal provisions, it is not well understood if this DF content was measured in "fruit nectar" or "100% fruit juice", which can lead to misinterpretations in the real DF content of these beverages.

Information about what methodologies are used to analysis in FCDBs is not clearly provided. Codex Alimentarius refers different methodologies to measure DF, but no Regulation refers what method must be used to measure DF. Considering all the entities that can provide data to include in these databases, variations between DF values may be a consequence of the different (not stated) methodologies used and not necessarily of real disparities in DF contents. A clear reference to what source and methodology provided the values presented in FCDBs would be an approach that would facilitate data quality analysis.

Critical analysis and conclusion

The perfect methodology to measure DF would be the one to apply in any condition, no matter what the type and characteristics of DF of food and that

would precisely quantify its DF content. However, this ideal methodology to measure DF does not exist yet, with methods to quantify DF still evolving (as it is the case of AOAC 2017.16). Hence, the existence of a wide variety of methodologies to measure DF with no specifically recommended ones to apply increases discrepancies in measurements of DF. In fact, since there are proven more accurate methodologies, these, by default, must be the ones selected to measure DF in any case, so that reliable and comparable data would be achieved. It is understandable that the need of advanced equipment, as it is the necessary to perform Liquid Chromatography, may be a limitation to the use of integrated methodologies in comparison with the classical ones.

However, since DF is a nutrient with such health potential, efforts should be done to perform accurate measurements. Individual intake values are only possible to realize with accurate food composition data. Compliance of health recommendations and the correct guidance about the best sources of DF also depend on reliable food analysis and data provided.

 Food's richness in fiber is, in fact, a factor that should be emphasized, not only for individuals or dietitians' clinical practices, but also as a general competitive advantage for food products. Therefore, product reformulations in food industries to increase fiber amount are only possible with precise measurements.

Existing scientific literature that compares methodologies is scarce. Despite it is possible to understand what the most inclusive methodologies are (and extrapolate them as the best ones), this lack of extensive evidence and the contradicting results founded limit the possibility of understanding the real potential of each methodology. Hence, it is required to deepen the scientific

research and comparison about methods to measure DF. Furthermore, a clear definition of the methodology to measure DF would be a path to produce accurate and comparable data, with significant impact on the reduction of shortcomings of food composition evaluation.

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ANNEXES

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Annexes

Annex A

Table I – Dietary Fiber definition according to Codex Alimentarius, European Community, European Food Safety Agency, and Institute of Medicine

Organization	Definition	Components included	Reference
Codex Alimentarius	'Dietary fiber is defined as carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories: - Edible carbohydrate polymers naturally occurring in the food as consumed, - Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities, - Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.' Footnotes (ALINORM 10/33/26, 10/33/REP) a. 'When derived from a plant origin, dietary fiber may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls. These compounds also may be measured by certain analytical method(s) for dietary fiber. However, such compounds are not included in the definition of dietary fiber if extracted and re-introduced into a food. b. Decision on whether to include carbohydrates of 3 to 9 monomeric units should be left up to national authorities.'	DF= NSP + RS + NDO (when MU number 3–10 included in the definition) + lignin and other compounds (when associated with polysaccharides in the plant cell wall) MU ≥10 (general definition) MU ≥3 (upon local approval)	ALINORM 09/32/REP ALINORM 10/33/26, 10/33/REP
European Community	'Fiber' means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: - Edible carbohydrate polymers naturally occurring in the food as consumed; - Edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; - Edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence.' It is said in Article (5) that 'Fiber… has one or more beneficial physiological effects such as: decrease intestinal transit time, increase stool bulk, is fermentable by colonic microflora, reduce blood total cholesterol levels, reduce post-prandial blood glucose, or reduce blood insulin levels' and that 'the definition of fiber should include carbohydrate polymers with one or more beneficial physiological effects'.	DF= NSP + RS + NDO MU ≥3	Directive 2008/100/EC

European Food Safety Agency (EFSA)	Dietary fiber is defined as 'non-digestible carbohydrates plus lignin'. EFSA Panel considers that the main types of DF are: non-starch polysaccharides (NSP) (cellulose, hemicelluloses, pectins, hydrocolloids (i.e. gums, mucilages, β-glucans)), resistant oligosaccharides (fructo-oligosaccharides (FOS), galactooligosaccharides (GOS), other resistant oligosaccharides), resistant starch (consisting of physically enclosed starch, some types of raw starch granules, retrograded amylose, chemically and/or physically modified starches), and lignin associated with the dietary fibre polysaccharides.'	DF= NSP + RS + NDO + lignin (when associated to DF polysaccharides) MU ≥3	EFSA, 2010
Institute of Medicine (USA)	'Dietary fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Added fiber consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans. Total fiber is the sum of dietary fiber and added fiber.' It is indicated in the document that the beneficial physiological effects expected from the added fiber are 'attenuation of postprandial blood glucose concentrations, attenuation of blood cholesterol concentrations and/or improved laxation'.	DF = NDC (intrinsic and intact from plants) + lignin (when associated to DF polysaccharides of plants) = NSP (intrinsic and intact from plants) + RS (intrinsic and intact from plants) + NDO (intrinsic and intact from plants) + lignin (when associated to DF polysaccharides of plants). Added fiber = isolated NDC Total fiber =DF + added fiber MU≥3	IoM, 2005

MU - monomeric unities; **NDC** – non-digestible carbohydrates; **NDO** - non-digestible oligosaccharides; **NSP** - non-starch polysaccharides; **RS** - resistant starch;

Adapted from: Stephen AM, Champ MM, Cloran SJ, Fleith M, van Lieshout L, Mejborn H, et al. Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutr Res Rev. 2017; 30(2):149-90

Annex B

Table II – Main dietary fiber types and their water solubility and molecular weight characteristics.

Dietary fiber types		Water Solubility	Molecular weight
Non-starch polyssacharides	Cellulose	-	High
	Hemicellulose	-	High
	Pectin	+	High
	Gums	+	High
	Mucilages	+	High
	Inulin	+	High
	Fructans	+	High
	Mannans and heteromannans	+/-	High
Non-digestible oligosaccharides	Galactooligosaccharides	+	Low
	Fructooligosaccharides	+	Low
Resistant Starch	Physically innacessible starch	-	High
	Granular starch	-	High
	Gelatinised and retrograd sataches	-	High
	Chemical modified starches	-	High
Associated substances	Lignin	-	
	Waxes	-	
	Chitins	-	

+: water soluble fiber; **-:** water insoluble fiber.
Adapted from: Stephen AM, Champ MM, Cloran SJ, Fleith M, van Lieshout L, Mejborn H, et al. Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutr Res Rev. 2017; 30(2):149-90

Annex C

Table III – AOAC methods approved in Codex Alimentarius and dietary fibers types measured.

AOAC method	HMWDF	LMWDF	SDF	IDF	Observations	
985.29	x					Quantitation lost for inulin, resistant starch, polydextrose and resistant maltodextrins
991.43	x		x	x		
993.21	x				Applicable for food products >10% DF e <2% starch	
994.13	x				Provides sugar residue composition of DF and content of Klason lignin	
991.42				x		
993.19			x			
2001.03	x	x			No resistant starches	
2009.01	x	x				
2011.25	x	x	x	x		
Methods that measure individual specific components						
995.16	(1→3)(1→4) ß-D-glucans					
997.08	Fructans (applicable to added fructans)					
999.03	Fructans (not applicable highly depolymerized fructans)					
2000.11	Polydextrose					
2001.02	Trans-galacto-oligosaccharides					
2002.02	Resistant starch (R3)					

Adapted from: Comission CA. Recomended methods of analysis and sampling (CX 234-1999)

Annex D

Table IV – Fiber content, per 100 g of edible portion, of pear, peach and apple and their respective derived products, based in food composition databases.

	USDA	TCA	DTU	FDHA
Pear, raw	3.1	2.2	3.2	2.3
Pear juice 100%	-	-	-	0*
Pear nectar	0.6	0.6	-	-
Peach, raw	1.5	2.3	3.2	2
Peach juice 100%	-	0.3	-	-
Peach nectar	0.6	0.2	-	-
Apple, raw	2.4	2.1	2.2	2.1
Apple nectar	-	0.5	-	
Apple juice 100%	0.2	0.2	-	0*

*refered as "apple/pear juice"
USDA – United States Department of Agriculture (Adapted from: http://www.ndb.nal.usda.gov/).
TCA – Tabela de Composição de Alimentos. Centro de Segurança Alimentar e Nutrição. Instituto Nacional de Saúde Doutor Ricardo Jorge. (Adapted from: http://www.insa.pt) **DTU** – Technical University of Denmark (Adapted from: http://www.foodcomp.dk). **FDHA** – Federal Department of Home Affairs (Adapted from: http://www.naehrwertdaten.ch).