Developments in Dietary Fibre Estimation Techniques in Food

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Lalitha R Gowda does not have any Conflict of Interest with the Symposium.

Dietary Fibre

- Dietary fibre is a diverse group of carbohydrates found almost exclusively in plants, including non-starch polysaccharides such as cellulose, pectin and lignin.

- Unlike other types of carbohydrate, these are not absorbed in the small intestine to provide energy.

- Some **fibre** can be fermented in the **large intestine** by gut **bacteria**, producing short chain fatty acids and gases (methane, hydrogen and carbon dioxide).

- Dietary fibre provides **2 kcal/8 kJ per gram** on average.

Benefits of Fibre in the diet

Fiber has potential health benefits:

- improved digestion
- improved cholesterol levels
- reduced risk for colon cancer
- helps to reduce the risk of heart disease, diabetes and some cancers
- improves gut health
- slows nutrient absorption and helps people feel fuller longer, which may enhance weight loss.

Definition of Dietary Fibre: Timeline

1970's

Dietary fiber consists of the remnants of edible plant cells, polysaccharides, lignin, and associated substances resistant to digestion by the alimentary enzymes of humans." Trowell

1985

Codex

Dietary fiber consists of carbohydrate polymers with ten or more monomeric units (DP>10), which are not hydrolyzed by the endogenous enzymes in the small intestines of humans.

2009

Codex

Dietary fibre means carbohydrate polymers*with ten or more monomeric units**, which are not hydrolysed 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.

Definition of Dietary Fibre: Food Safety and Standards (Advertising and Claims) Regulations, 2018

“Dietary fiber” means carbohydrate polymers with a degree of polymerization (DP) not lower than three, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and the same consists of one or more of-

(i) edible carbohydrate polymers naturally occurring in the food as consumed;

(ii) carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means;

(iii) synthetic carbohydrate polymers

Total Dietary Fibre

Total Dietary Fiber is composed of two forms: Soluble and Insoluble Fibre. Together they represent the Total Fibre content in foods.

Soluble Fibre dissolves in water to form a thick gel-like substance in the stomach. It is broken down by bacteria in the large intestine and provides some calories.

Insoluble Fibre does not dissolve in water and passes through the gastrointestinal tract relatively intact and, therefore, is not a source of calories

Total Dietary Fibre

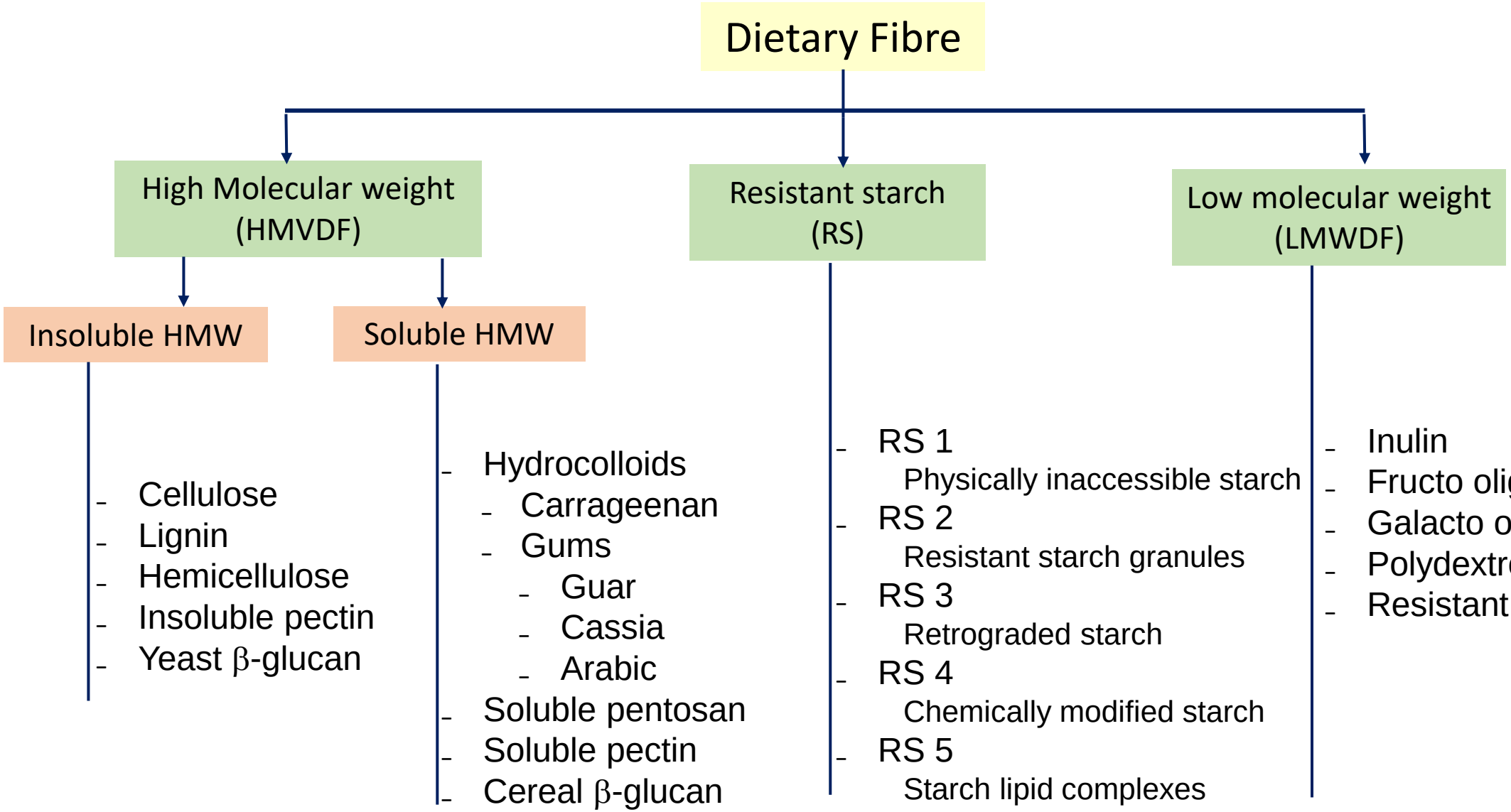
Insoluble HMW	Soluble HMW	Soluble LMW

Fibers are also defined by the degree of polymerization.
High molecular weight (HMW) is considered for molecules with >10 DP
Low molecular weight (LMW) is considered for molecules with <10 DP
Low molecular weight fibers consist mainly of soluble molecules.

Dietary fibre: Soluble and insoluble

Dietary fibres	**Features**	**Sources**
Soluble Fibres		
Pectin	Pectin is a polymer of galacturonic acid monomer units linked via α-(1→4)-glycosidic bond forming a backbone. The average molecular weight of pectin ranges between 50 and 150 kDa.	Fruits, apple, citrus peel, legumes,, root vegetablesetc
Gums	Plants gums are comprised of a rather set number of conceivable sugars, including: l-arabinose (Ara), d-galactose (Gal), d-mannose (Man), and l-rhamnose.	Oatmeal, haricot bean, legumes (Guar, Locust bean)
β-Glucan	A group of β-D-glucose polysaccharides naturally occurring in the cell walls of cereals, bacteria, and fungi.	Oats, barley, seaweed
Mucilages	Are polysaccharides constituted by large molecules of sugars and uronic acids linked by glycosidic bonds.	rhizomes, roots and seed endosperms
Insoluble fibres		
Cellulose	It is the main component of cell walls which is a polymer consisting of glucose monomers in β, 1→4 linksges	Whole grains, bran, peas, root vegetables, beans family of cruciferous, apple
Hemicelluloses	Is one of a number of heteropolymers, such as arabinoxylans, xylan, glucuronoxylan, glucomannan etc present along with cellulose in almost all terrestrial plant cell walls.	Bran, whole grains
Lignin	Comprises of aromatic alcohols and the components of other cell wall	Vegetables, flour

Dietary fibre as ingredients



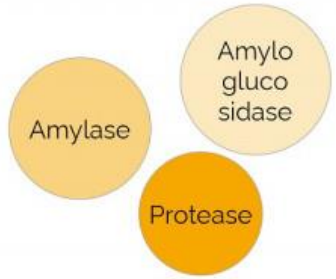
Basic principle of TDF Analysis

Pancreatic α-amylase
Amyloglucosidase
Conditions: 37°C, pH 6.0, 4 h

Protease
Conditions: 60°C, pH 8.2, 30 min

Dried and defatted food sample
(1 g)

enzymes mimicking human digestion



Enzymatic digest
containing simple sugars and non-digestible fraction

precipitation using ethanol and filtration

Non-digestible fraction

Heat-stable α-amylase
Conditions: 98-100°C, pH 8.2, 30 min

Protease
Conditions: 60°C, pH 7.5, 30 min

Amyloglucosidase
Conditions: 60°C, pH 4.5, 30 min

Codex/AOAC/AACCI Methods for the Analysis of Dietary Fiber (DF)

AOAC Method	AACCI Method	Codex Type[a]	What Is Measured[b]
985.29	32-05.01	I	Total HMWDF (IDF + HMWSDF)
991.42	32-20.01	I	IDF in foods
993.19	…	I	HMWSDF in foods
991.43	32-07.01	I	IDF and HMWSDF separately
994.13	32-25.01	I	Total HMWDF; provides sugar composition and Klason lignin
2001.03	32-41.01	I	HMWDF and LMWSDF in foods devoid of resistant starch
993.21	…	I	Total HMWDF in samples with >10% fiber and <2% starch
2009.01	32-45.01	I	HMWDF and LMWSDF in all foods
2011.25	32-50.01	*	IDF, HMWSDF, and LMWSDF in all foods
995.16	32-23.01	II	(1→3)(1→4)-β-Glucan in cereals, feeds, and foods
997.08	32-31.01	II	Fructans and FOS
999.03	32-32.01	III	Fructans and FOS (underestimates highly depolymerized FOS)
2000.11	32-28.01	II	Polydextrose
2001.02	32-33.01	II	Trans galacto-oligosaccharides
2002.02	32-40.01	II	Resistant starch (RS_2 and RS_3)

[a] Asterisk (*) indicates that no decision has yet been made by Codex concerning this method. Method types are defined in Box 2.

[b] HMWDF = higher-molecular-weight DF; IDF = insoluble DF; HMWSDF = higher-molecular-weight soluble DF; LMWSDF = lower-molecular-weight soluble DF; and FOS = fructooligosaccharides.

Codex Alimentarius Methods

Type I (Defining Methods)—"A method which determines a value that can only be arrived at in terms of the method per se and serves by definition as the only method for establishing the accepted value for the item measured."

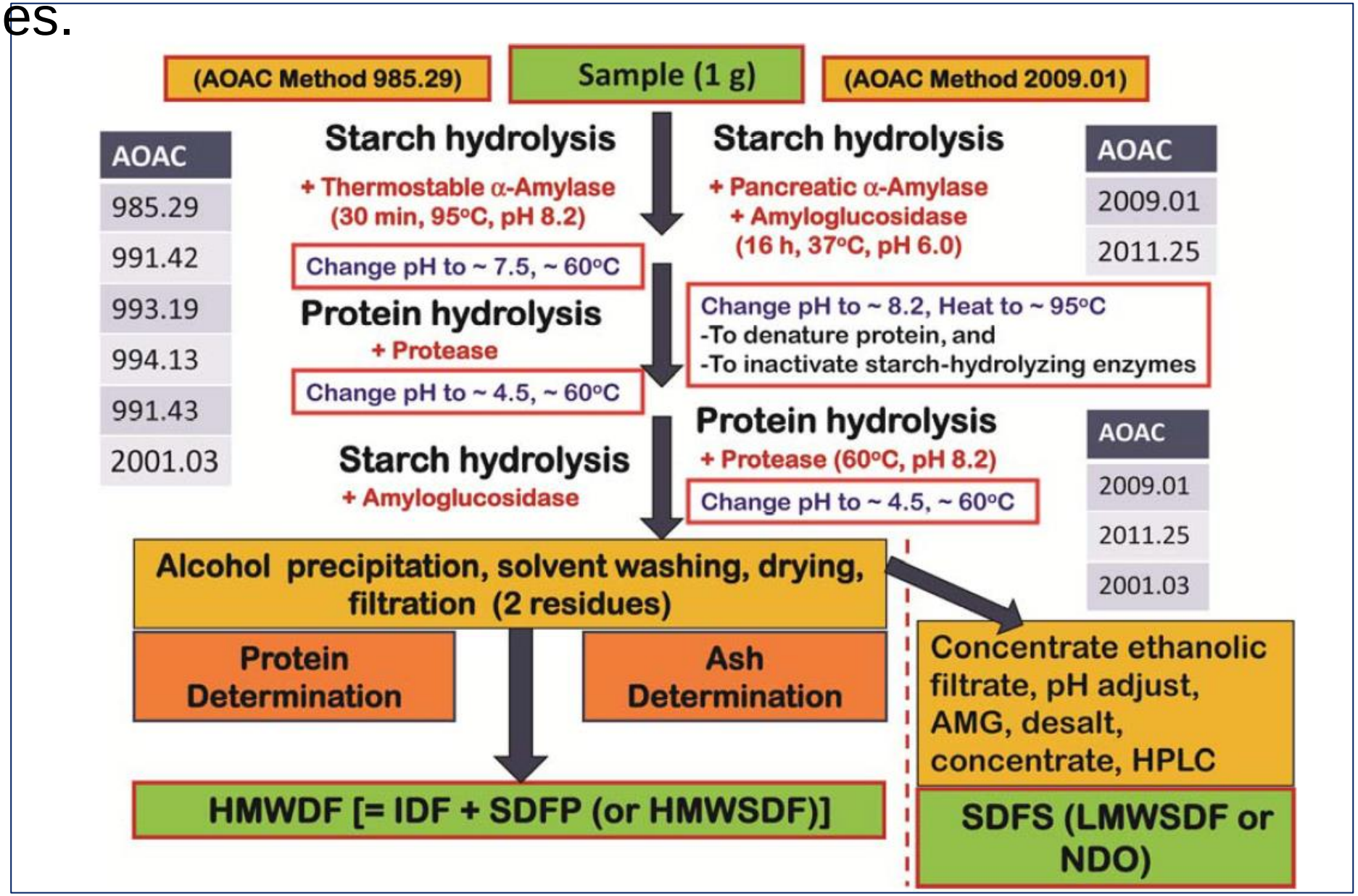
Type II (Reference Methods)—"A Type II method is the one designated Reference Method where Type I methods do not apply. It should be selected from Type III methods (as defined below). It should be recommended for use in cases of dispute and for calibration purposes."

Type III (Alternative Approved Methods)—"A Type III method is one which meets the criteria required by the Codex Committee on Methods of Analysis and Sampling for methods that may be used for control, inspection or regulatory purposes."

Type IV (Tentative Method)—"A Type IV method is one which has been used traditionally or else has been recently introduced but for which the criteria required for acceptance by the Codex Committee on Methods of Analysis and Sampling have not yet been determined."

Codex Alimentarius (1997)

Steps involved in the AOAC dietary fiber Type I methods of analyses.



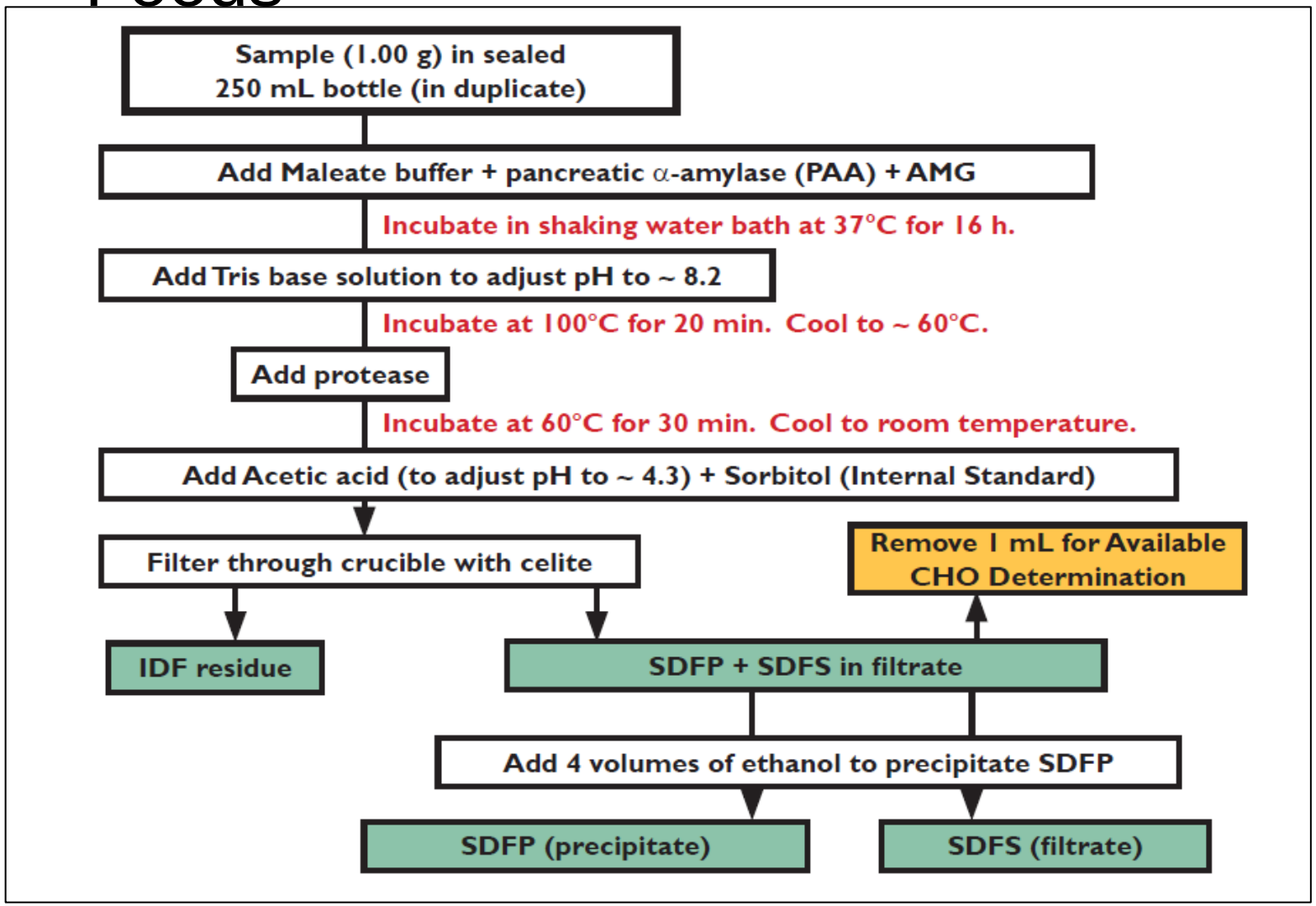
McCleary et al. (2013).. Cereal Chemistry, 90, 396-414.

Measurement of Total Dietary Fibre, Soluble and Insoluble Dietary Fiber

Method No	Method Name	Measures Total Dietary Fiber	Measures Soluble and Insoluble Dietary Fiber
AOAC 985.29	Prosky	✔	✖
AOAC 991.43	Lee	✖	✔
AOAC 2009.01	McCleary	✔	✖
AOAC 2011.25	McCleary	✖	✔
AOAC 2017.06	RINTDF	✔	✖

Modifications to the standard methods (an additional filtration step) exist and these allow the dietary fiber content to be divided into soluble and insoluble dietary fiber (SDF and IDF). In both cases, all other steps in the method remain unchanged.

AOAC 2009.01 (Codex method) – Total Dietary Fiber in Foods



- The method allows the measurement of TDF by summing the quantity of higher molecular weight dietary fiber, which included insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) that precipitates in the presence of 78% aqueous ethanol (SDFP), with SDF that remains soluble in 78% aqueous ethanol (SDFS).

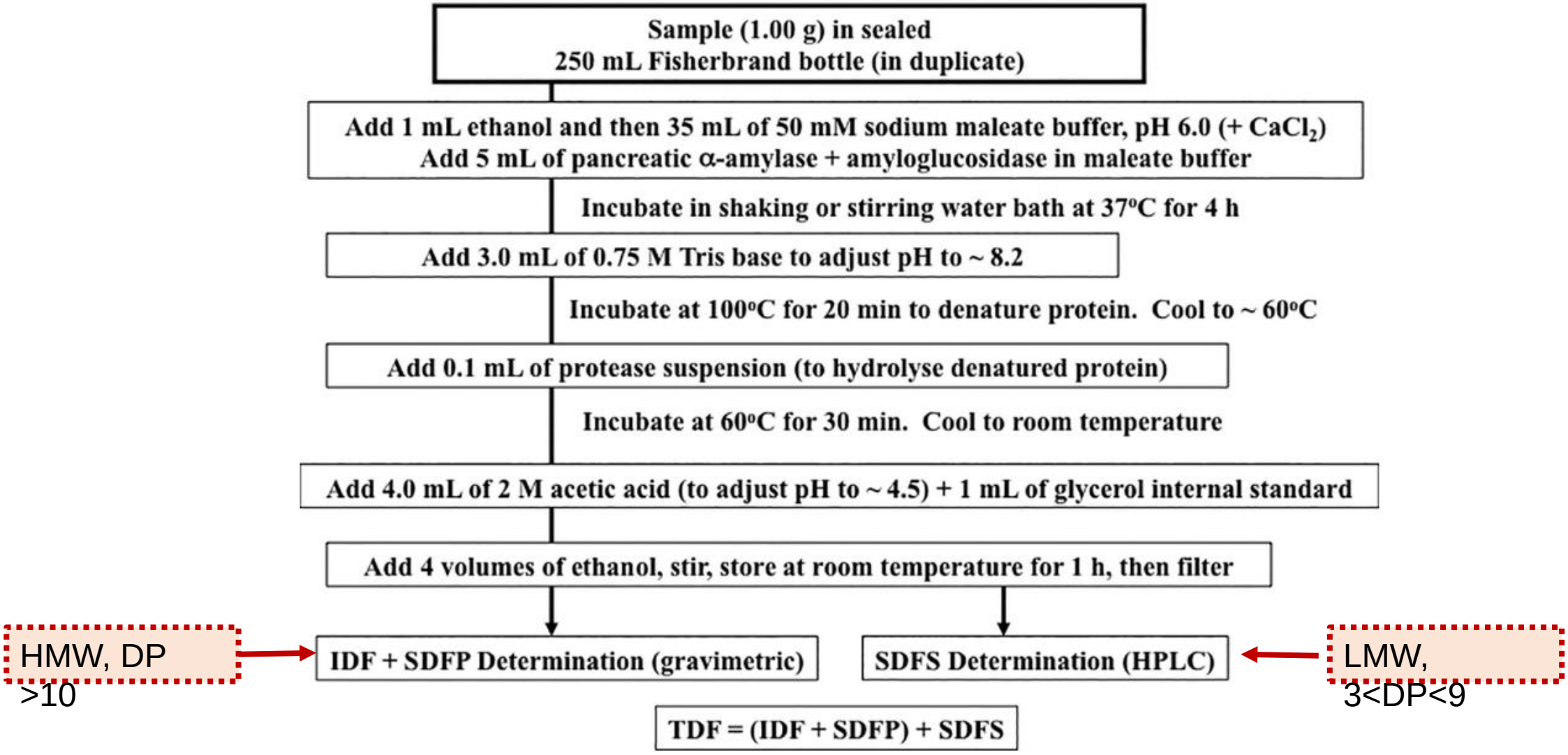
Challenges of AOAC 2009.01 – Total Dietary Fiber in Foods

Several challenges/concerns identified:

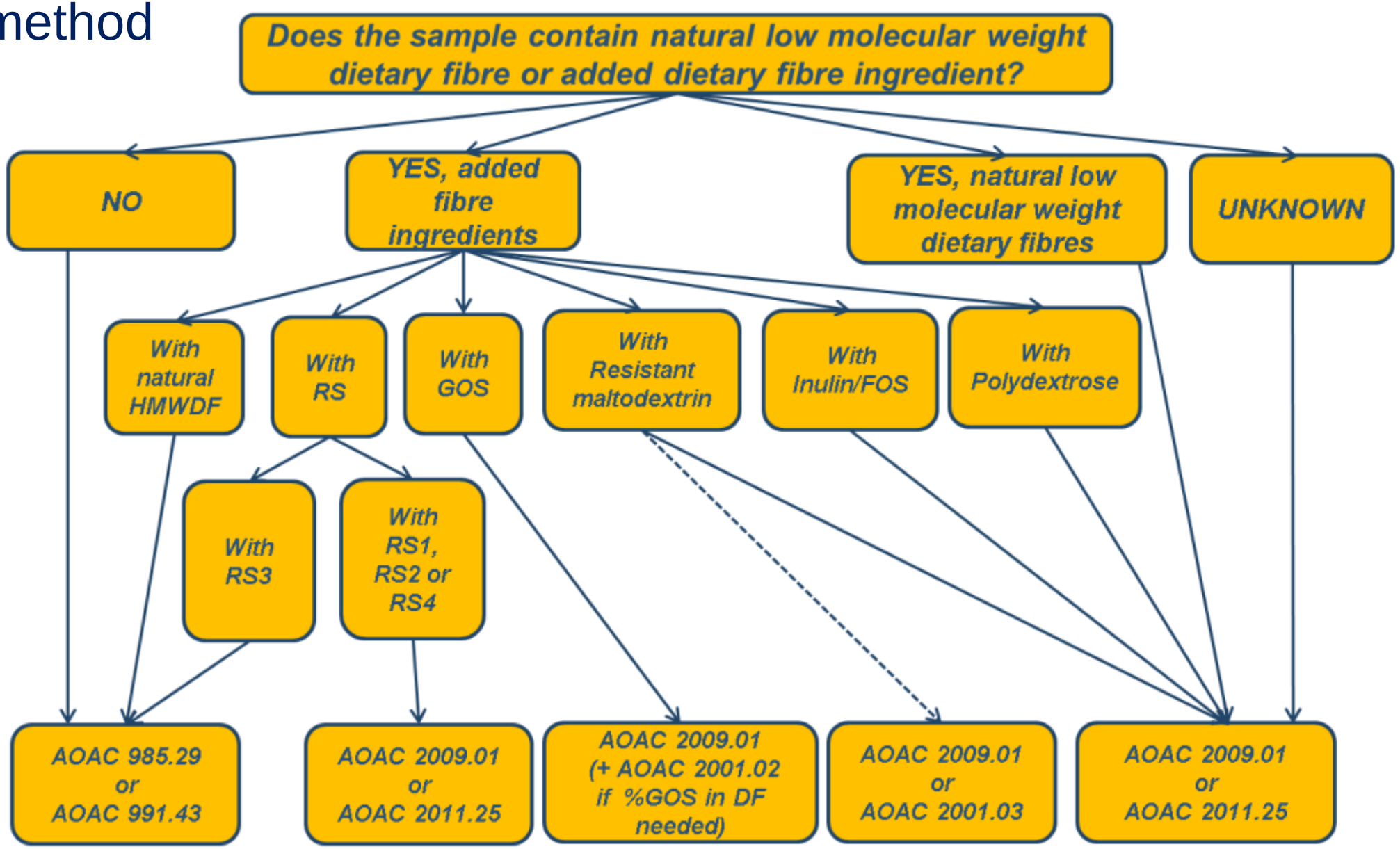
- An incubation time with pancreatic α-amylase plus amyl glucosidase of 16 h has no physiological basis. A more likely residence time for food in the small intestine is 4 ± 1 h
- Under the incubation conditions used, resistant maltodextrins are produced during the hydrolysis of nonresistant starch, and these are incorrectly measured as dietary fiber
- The extended incubation time of samples results in excessive hydrolysis, and thus underestimation, of phosphate cross-linked starch (RS-3)
- The use of the preservative sodium azide is undesirable on the basis of health concerns to analysts

Rapid Integrated Total Dietary Fiber (AOAC 2017.16) was Introduced in 2015. Closely resembles AOAC 2009.01. This method addresses the minor limitations identified in the AOAC 2009.01 and is the only method that accurately measures all components of Total Dietary Fiber (including all forms of resistant starch).

AOAC Official Method 2017.16 Total Dietary Fiber in Foods and Food Ingredients Rapid Integrated Enzymatic-Gravimetric–High Pressure Liquid Chromatography Method
[Applicable to plant material, foods, and food ingredients.]



Decision tree for selecting the appropriate method



https://cdnmedia.eurofins.com/corporate eurofins/media/12146386/dietary_fiber_technical_brochure_v9_2018.pdf

Fiber on food labels: FSSAI Regulations

Food Safety and Standards (Advertising and Claims) Regulations, 2018 states that a product claiming to be a ‘source of fibre’ should contain at least 3 g of dietary fibre per 100 g or 1.5 g per 100 kcal, and for a product which contains at least 6 g of dietary fibre per 100 g or 3 g per 100 kcal , can be labelled as ‘high in’ fibre.

Appropriate method selection: FSSAI regulations

Regulatory Labelling requirement

FSSAI Definition

Carbohydrate polymers with a degree of polymerization (DP) not lower than three, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans.

AOAC 2011.25 TDF/IDF/SF
AOAC 2017.16 TDF/IDF/SF

Manually
Automated DF analyser

Nutrition Facts

Serving Size 100 gm

Amount Per Serving		
Calories 362		
	Daily Value	**%Daily Value***
Total Fat	1.77 g	**2.72%**
Saturated Fat	0.63 g	**3.15%**
Trans Fat	0.1 g	
Polyunsaturated Fat	0.53 g	
Monounsaturated Fat	0.54 g	
Cholesterol	0.001 mg	**0.00%**
Total Carbohydrate	73.27 g	**24.42%**
Dietary Fiber	14.08 g	**56.32%**
Sugars	2.04 g	

Calcium	3.45%	Iron	26.67%
Thiamin	34.00%	Riboflavin	29.41%
Niacin	50.55%	Folate	41.25%

* Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

- AOAC 2017.16, like 2009.01, measures TDF as a summation of higher molecular weight dietary fiber (IDF + SDFP; by gravimetry) and SDFS (by HPLC).
- AOAC 2011.25, a modification of AOAC 2009.01, allows for separate measurements of IDF and SDF (SDFP + SDFS).

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Conclusions

- Several methods are available for the measurement of DF in plant and food products.
- Of these, only AOAC methods 2009.01, 2011.25 and 2017.16 give measurement of all fiber components as defined by Codex Alimentarius.
- A correct measurement of DF is important in relation to correct labelling of the food product and for decisions for granting health claims.
- Analytical laboratories must verify the precision, accuracy and measurement of uncertainty for different food matrices and used certified reference materials.
- There is a need for development of CRMs for TDF.