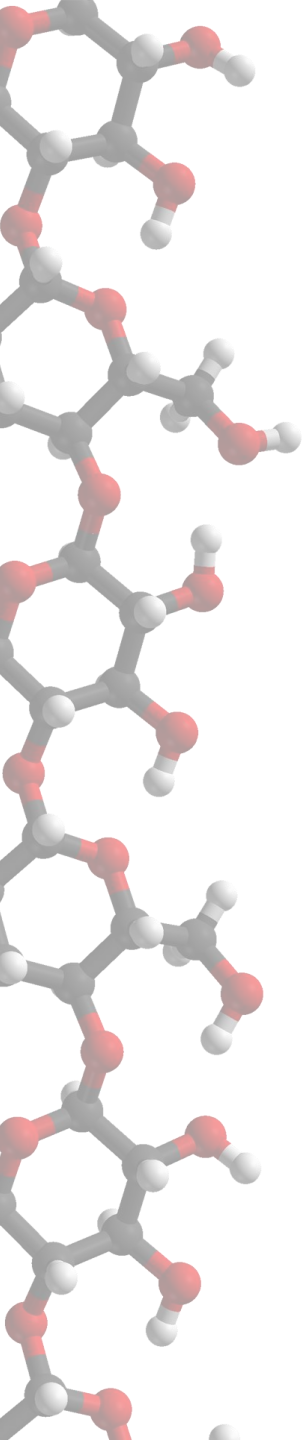


UNIVERSIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS
FBA0201 – Bromatologia Básica

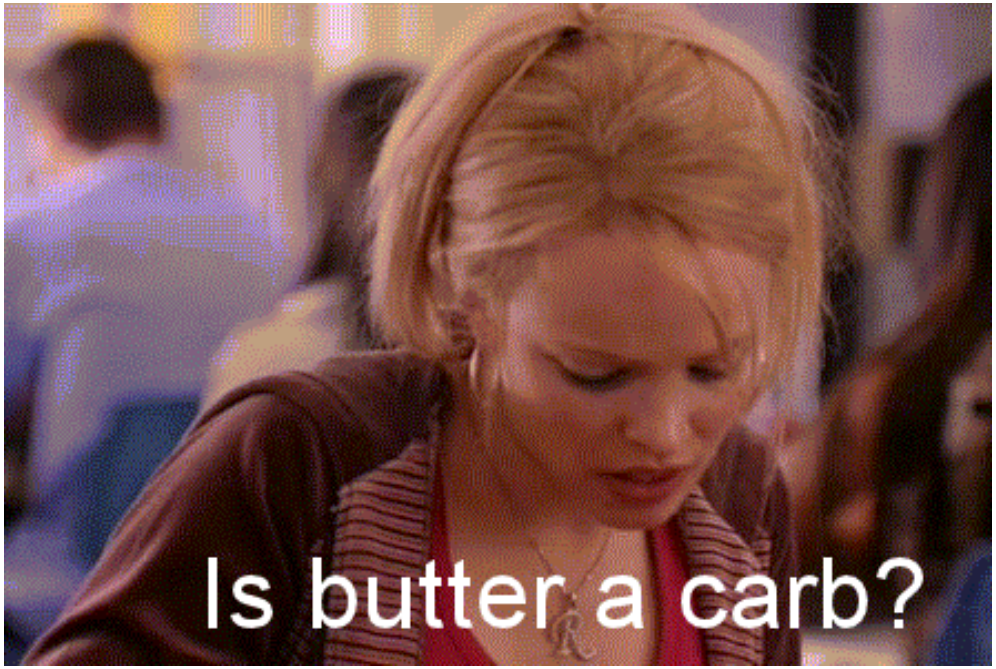
FIBRA ALIMENTAR:
MÉTODOS ANALÍTICOS

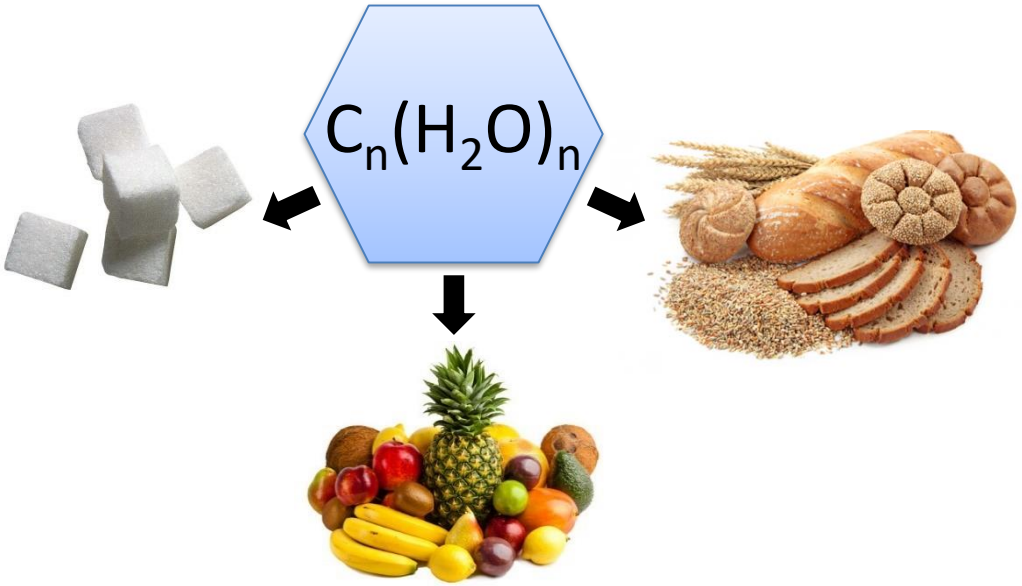
ERIC DE CASTRO TOBARUELA
Farmacêutico – UFC
Mestrado – Ciência dos Alimentos - USP

- Carboidratos
- Fibra Alimentar
  - Contexto histórico
  - Definições
  - Método analíticos
- Tipos de métodos
- Métodos enzímico-gravimétricos
  - Preparo da amostra
  - Princípio dos métodos
  - Cuidados e correções
  - AOAC 991.43 ↔ AOAC 2011.25
- Comparação entre métodos

Considerações finais

O QUE É UM CARBOIDRATO?



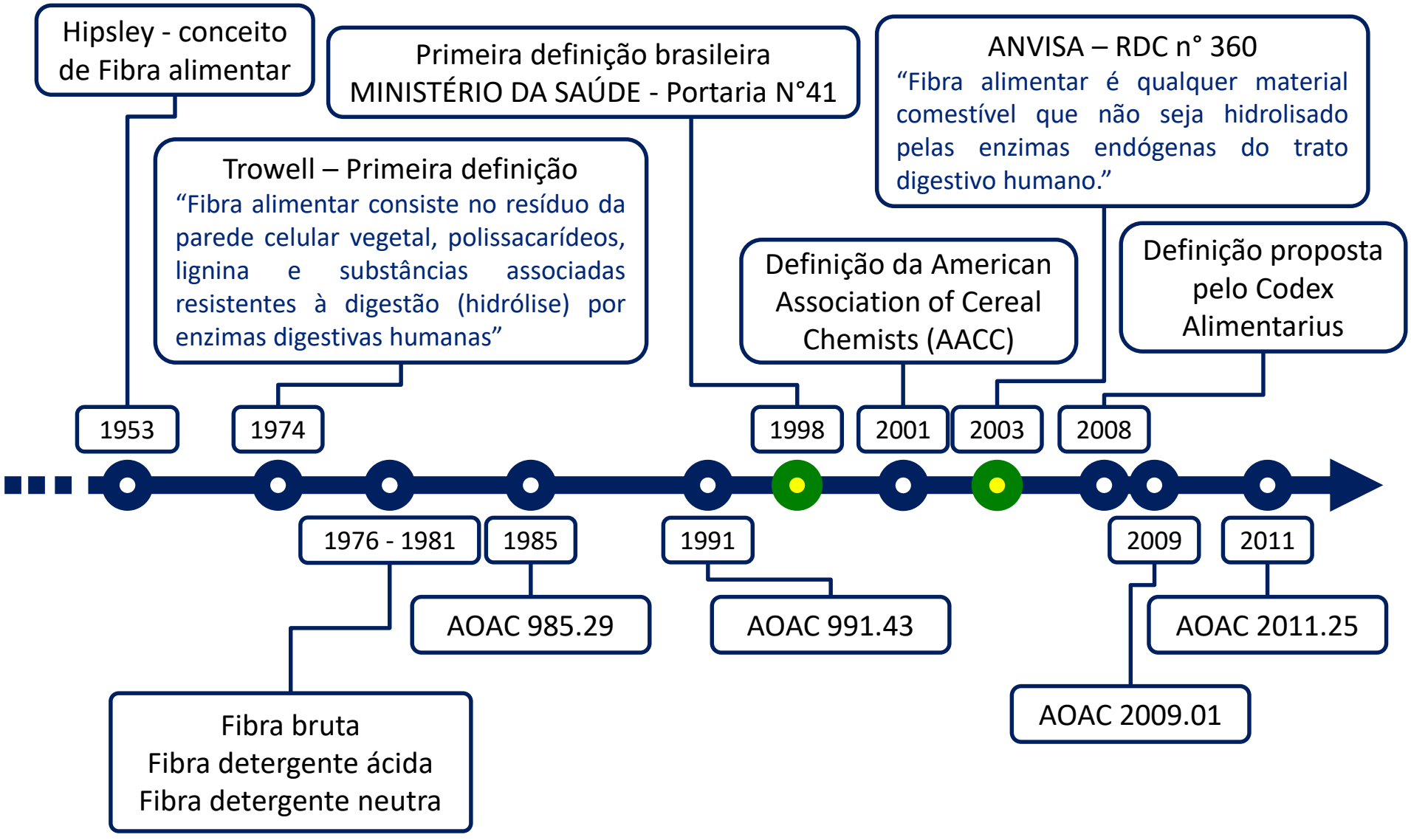


COMPOSIÇÃO CENTESIMAL

CARBOIDRATOS TOTAIS		PROTEÍNAS	LIPÍDIOS	CINZAS	UMIDADE
CHO DISPONÍVEIS	FIBRA ALIMENTAR				

O QUE É FIBRA ALIMENTAR?

TODA FIBRA ALIMENTAR É CARBOIDRATO?


Hipsley - conceito de Fibra alimentar
Primeira definição brasileira MINISTÉRIO DA SAÚDE - Portaria N°41
ANVISA – RDC n° 360
"Fibra alimentar é qualquer material comestível que não seja hidrolisado pelas enzimas endógenas do trato digestivo humano."
Trowell – Primeira definição
"Fibra alimentar consiste no resíduo da parede celular vegetal, polissacarídeos, lignina e substâncias associadas resistentes à digestão (hidrólise) por enzimas digestivas humanas"
Definição da American Association of Cereal Chemists (AACC)
Definição proposta pelo Codex Alimentarius
1953
1974
1998
2001
2003
2008
1976 - 1981
1985
1991
2009
2011
AOAC 985.29
AOAC 991.43
AOAC 2011.25
AOAC 2009.01
Fibra bruta
Fibra detergente ácida
Fibra detergente neutra

2008

CODEX ALIMENTARIUS

1963

Fórum internacional de normatização do comércio de alimentos;

Visa garantir a segurança, a qualidade e a equidade deste comércio;

Implantação de normas, códigos de boas práticas, orientações e outras recomendações relativas a alimentos, produção de alimentos e segurança alimentar.

World Health Organization

http://www.codexalimentarius.org/

2008

CODEX ALIMENTARIUS

1963

Fórum internacional de normatização do comércio de alimentos;

Visa garantir a segurança, a qualidade e a equidade deste comércio;

Implantação de normas, códigos de boas práticas, orientações e outras recomendações relativas a alimentos, produção de alimentos e segurança alimentar.

Comitê Codex sobre Nutrição e Alimentos para Dietas Especiais

http://www.codexalimentarius.org/

codex alimentarius commission

Definition:

Dietary fibre means carbohydrate polymers[1] with ten or more monomeric units[2] , 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

Methods of Analysis for Dietary Fibre

→ To be agreed.

---

[1] When derived from a plant origin, dietary fibre may include fractions of lignin and/or other compounds when associated with polysaccharides in the plant cell walls and if these compounds are quantified by the AOAC gravimetric analytical method for dietary fibre analysis : Fractions of lignin and the other compounds (proteic fractions, phenolic compounds, waxes, saponins, phytates, cutin, phytosterols, etc.) intimately "associated" with plant polysaccharides are often extracted with the polysaccharides in the AOAC 991.43 method. These substances are included in the definition of fibre insofar as they are actually associated with the poly- or oligo-saccharidic fraction of fibre. However, when extracted or even re-introduced into a food containing non digestible polysaccharides, they cannot be defined as dietary fibre. When combined with polysacchrides, these associated substances may provide additional beneficial effects (pending adoption of Section on Methods of Analysis and Sampling).

[2] Decision on whether to include carbohydrates from 3 to 9 monomeric units should be left to national authorities.

serving**

codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION

WORLD HEALTH

Fibra alimentar é constituída de polímeros de carboidratos* com dez* ou mais unidades monoméricas, que não são hidrolisados pelas enzimas endógenas no intestino delgado e que podem pertencer a três categorias:

- Polímeros de carboidratos comestíveis que ocorrem naturalmente nos alimentos na forma como são consumidos;

- Polímeros de carboidratos obtidos de material cru por meio físico, químico ou enzimático e que tenham comprovado efeito fisiológico benéfico sobre a saúde humana, de acordo com evidências científicas propostas e aceitas por autoridades competentes;

- Polímeros de carboidratos sintéticos que tenham comprovado efeito fisiológico benéfico sobre a saúde humana, de acordo com evidências científicas propostas e aceitas por autoridades competentes.

Food Chemistry 140 (2013) 581–585

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Codex dietary fibre definition – Justification for inclusion of carbohydrates from 3 to 9 degrees of polymerisation

Elizabete Wenzel de Menezes *, Eliana Bistriche Giuntini, Milana Cara Tanasov Dan, Fabiana Andréa Hoffmann Sardá, Franco Maria Lajolo

Depto. Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, 05508-000 São Paulo, SP, Brazil

Ausência de embasamento científico, metodológico ou fisiológico que justifique a diferenciação entre carboidratos não disponíveis com GP <10 e GP ≥10;

Harmonização (vários países e entidades já incluíram carboidratos de GP 3-9 na definição de FA);

Existência de métodos analíticos adequados

COMO ANALISAMOS FIBRA ALIMENTAR?

Standard	Provisions	Method	Principle	Type
General methods that do not measure the lower molecular weight fraction (i.e. monomeric units<=9)[2]				
All foods [1]	Dietary fibre based on precipitation in 4 parts alcohol and 1 part water. Resistant insoluble and soluble polysaccharides, lignin, and plant cell wall. [4]	AOAC 985.29	Enzymatic gravimetric	III
All foods [1]	Dietary fibre based on precipitation in 4 parts alcohol and 1 part water. Resistant insoluble and soluble polysaccharides, lignin, and plant cell wall [4].	AOAC 991.43	Enzymatic gravimetric	III
All foods [1]	Dietary fibre based on precipitation in 4 parts alcohol and 1 part water. Resistant insoluble and soluble polysaccharides, lignin, and plant cell wall [4].	AOAC 992.16	Enzymatic gravimetric	III
All foods [1]	Dietary fibre in food and food products with less than 2% starch [4].	AOAC 993.21	Non-enzymatic gravimetric	III
All foods [1]	Dietary fibre based on precipitation in 4 parts alcohol and 1 part water, quantitated as component neutral sugars, uronic acids, plus Klason lignin. [4]	AOAC 994.13	Enzymatic chemical	III

(at Step 8 of the Procedure)

General methods that measure both the higher (monomeric units > 9) and the lower molecular weight fraction (monomeric units <=9) [2]				
All foods [1]	Dietary fibre based on precipitation in 4 parts alcohol and 1 part water. Resistant insoluble and soluble polysaccharides, resistant malto-dextrins, lignin, and plant cell wall. [3]	AOAC 2001.03	Enzymatic gravimetric and Liquid chromatography	III
All foods [1]	Dietary fibre (Soluble + insoluble polysaccharides + lignin + resistant starch + oligosaccharides).	AOAC 2009.01	Enzymatic-Gravimetric-High Pressure Liquid Chromatography Method	III
All foods [1]	Dietary fibre based on precipitation in 4 parts alcohol and 1 part water. Resistant insoluble and soluble polysaccharides, lignin, and plant cell wall [4].	AOAC 992.16	Enzymatic gravimetric	III

Methods that measure individual specific components (monomeric units: the whole range for each type of components is covered)[2]				
All foods [1]	Insoluble dietary fibres in food and food products	AOAC 991.42	Enzymatic gravimetric	III
All foods [1]	(1→3)(1→4) *Beta*-D-Glucans	AOAC 992.28	Enzymatic	III
All foods [1]	Soluble dietary fibres in food and food products	AOAC 993.19	Enzymatic gravimetric	III
All foods [1]	(1→3)(1→4) *Beta*-D-Glucans	AOAC 995.16	Enzymatic	III
All foods [1]	Fructans (oligofructoses, inulin, hydrolyzed inulin, polyfructoses, fructooligosaccharides)	AOAC 997.08	Enzymatic & HPAEC-PAD	III
All foods [1]	Fructans (oligofructoses, inulin, hydrolyzed inulin, polyfructoses, fructooligosaccharides)	AOAC 999.03	Enzymatic & colorimetric	III
All foods [1]	Polydextrose	AOAC 2000.11	HPAEC-PAD	III
All foods [1]	Trans-galacto-oligo saccharides	AOAC 2001.02	HPAEC-PAD	III
All foods [1]	Resistant starch (Recommended for RS2 & RS3)	AOAC 2002.02	Enzymatic	III

Other methods[2]				
All foods	Insoluble glucans and mannans of yeast cell wall (for yeast cell wall only)	Eurasyp (European association for specialty yeast product) – LM Bonanno. Biospringer- 2004 – online version : http://www.eurasyp.org/public.technique.home.screen.	Chemical & HPAEC-PAD	IV
All foods	Fructo-oligosaccharides (monomeric units<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
All foods	Non-starch polysaccharides (NSP) [3]	Englyst H.N, Quigley M.E., Hudson G. (1994) Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic high performance liquid chromatographic or spectrophotometric measurement of constituent sugars – Analyst 119, 1497-1509	Enzymatic Gas-Liquid Chromatography Method	IV

Tipos de métodos

- Gravimétricos

Fibra bruta (FB)

Fibra detergente neutro (FDN) e ácido (FDA)

- Enzímico-gravimétricos

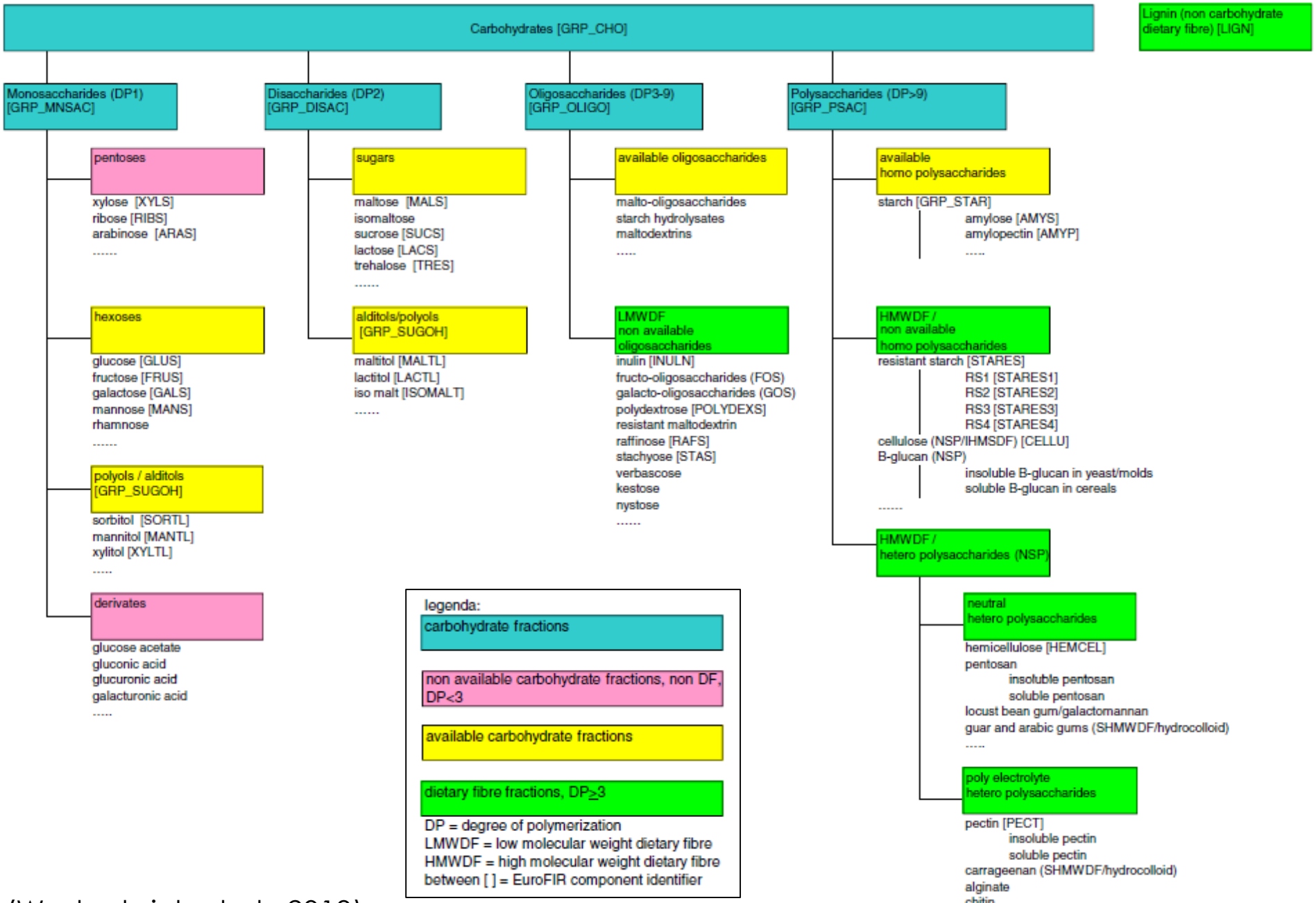
Fibra alimentar total (FAT)

Fibra alimentar solúvel (FAS) e insolúvel (FAI)

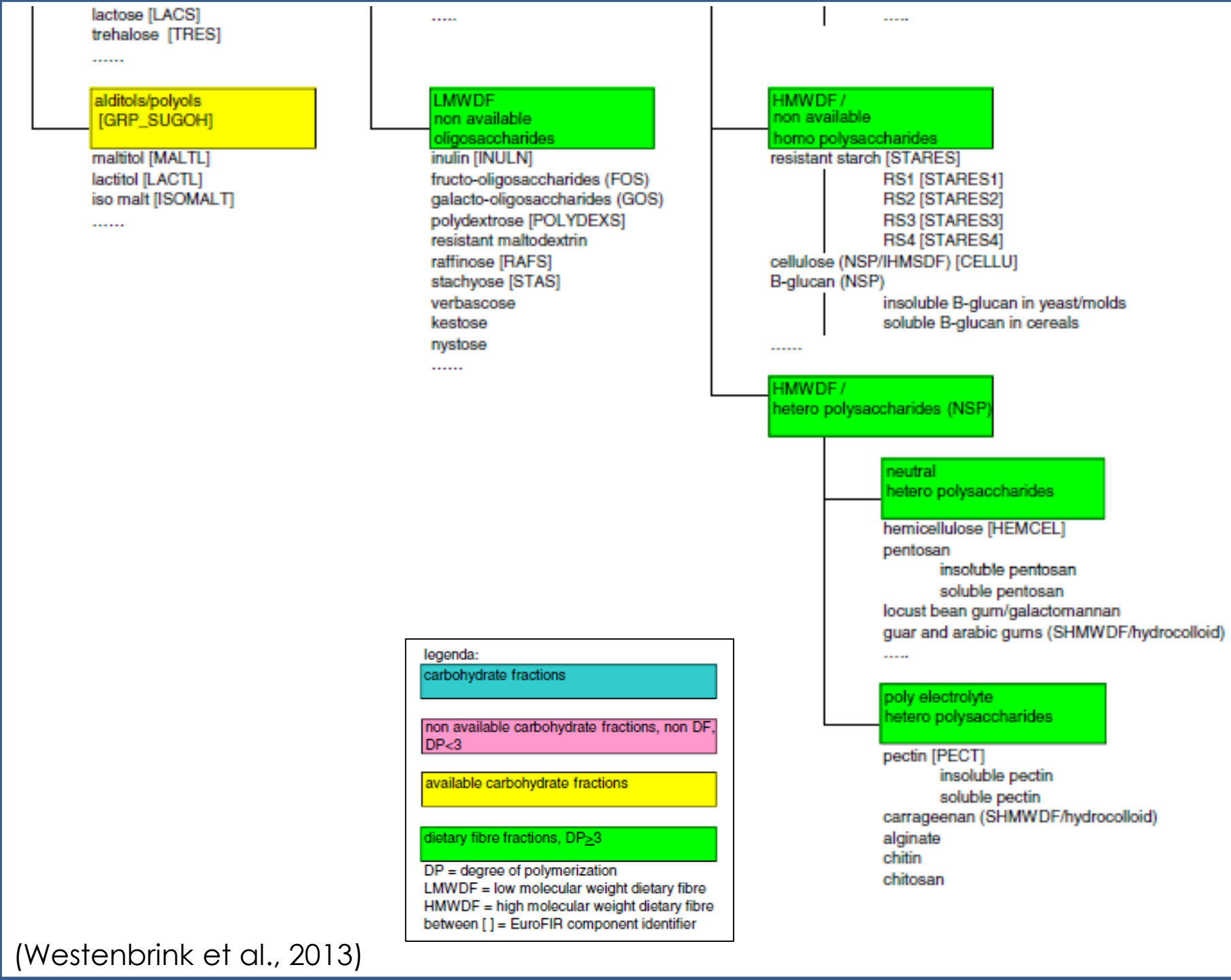
- Enzímico-químicos

Por espectrofotometria

Por cromatografia líquida de alta eficiência (HPLC)

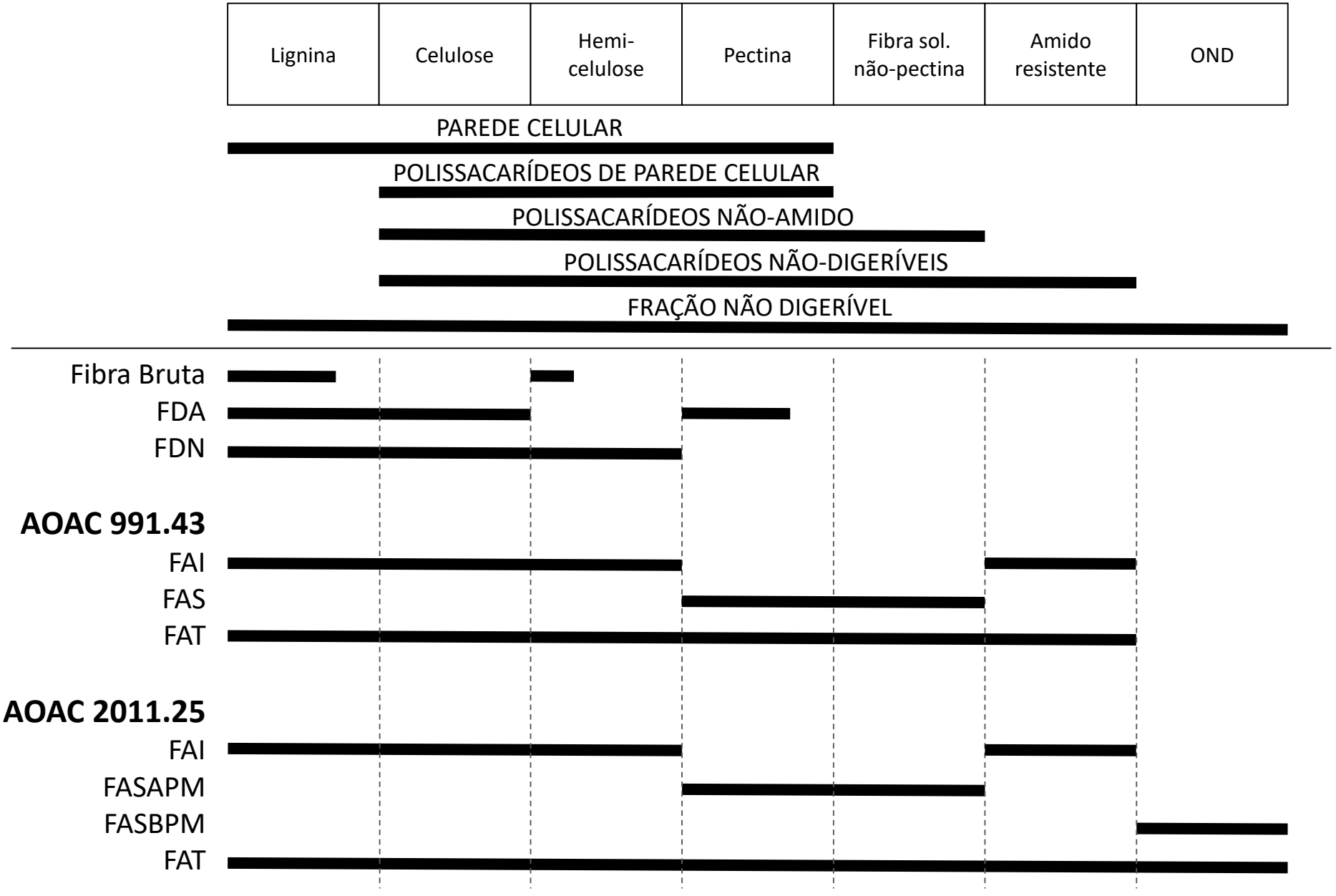


(Westenbrink et al., 2013)



(Westenbrink et al., 2013)

Mas... Qual a diferença?

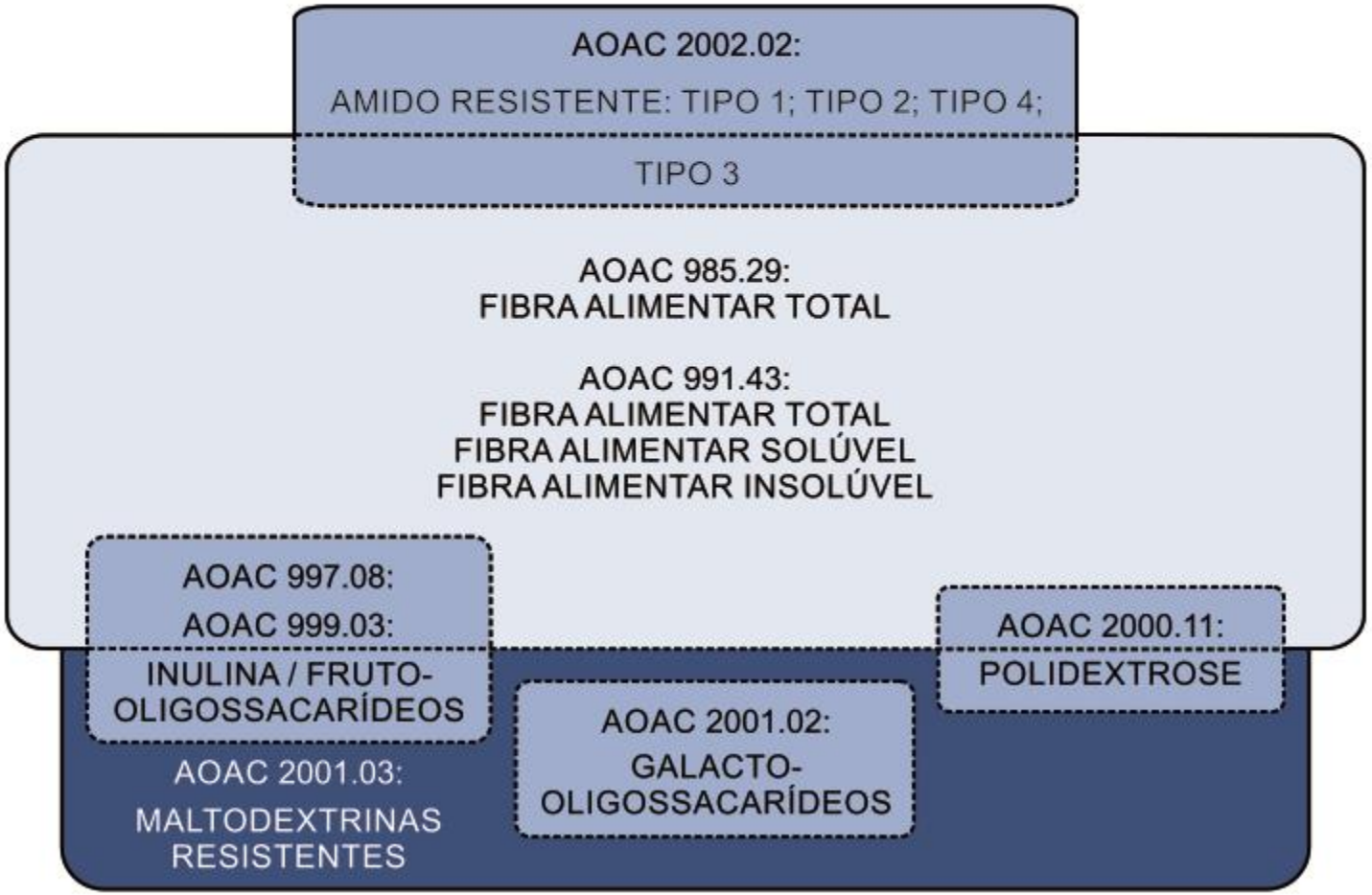


Tipos de métodos

- Gravimétricos
  - Fibra bruta (FB)
  - Fibra detergente neutro (FDN) e ácido (FDA)
- Enzímico-gravimétricos
  - Fibra alimentar total (FAT)
  - Fibra alimentar solúvel (FAS) e insolúvel (FAI)
- Enzímico-químicos
  - Por espectrofotometria
  - Por cromatografia líquida de alta eficiência (HPLC)

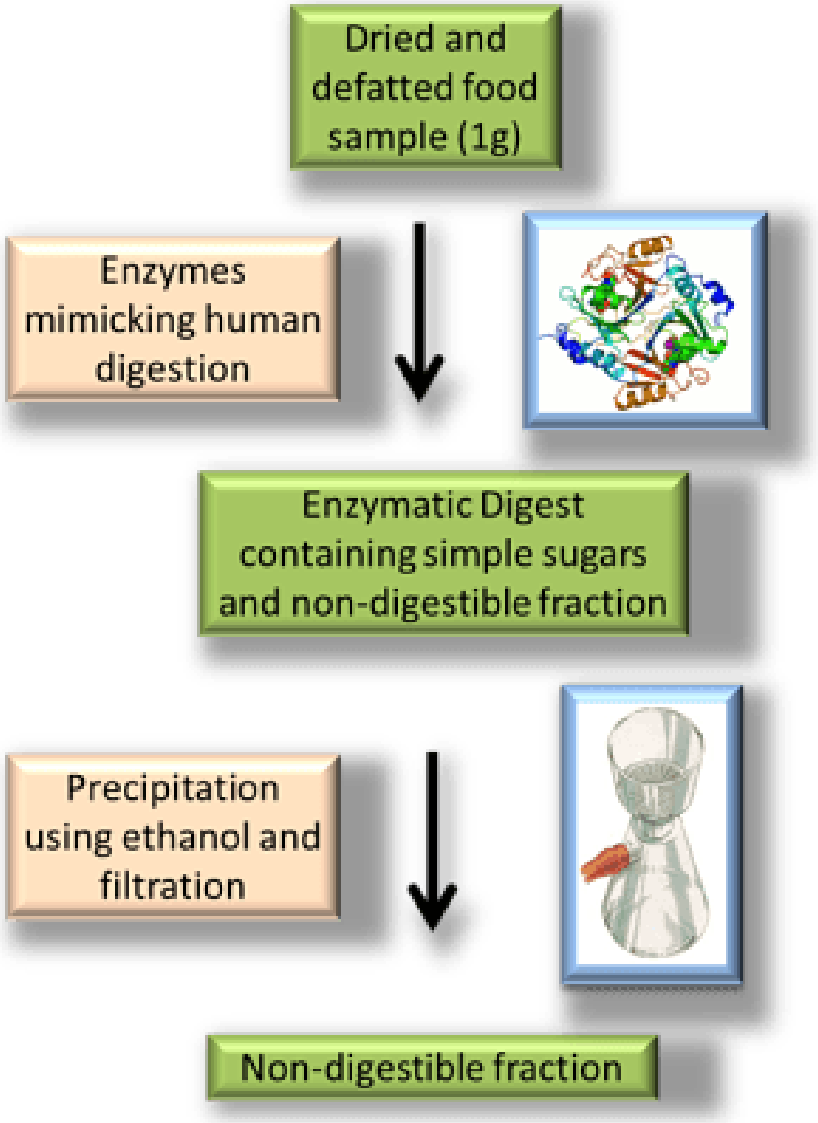
AOAC 2009.01:
FIBRA ALIMENTAR DE ALTO PESO MOLECULAR
FIBRA ALIMENTAR DE BAIXO PESO MOLECULAR
FIBRA ALIMENTAR TOTAL DE ALTO E BAIXO PESO MOLECULAR

AOAC 2011.25:
FIBRA ALIMENTAR SOLÚVEL DE ALTO PESO MOLECULAR
FIBRA ALIMENTAR SOLÚVEL DE BAIXO PESO MOLECULAR
FIBRA ALIMENTAR INSOLÚVEL
FIBRA ALIMENTAR TOTAL DE ALTO E BAIXO PESO MOLECULAR



(Westenbrink et al., 2013)

Métodos enzímico-gravimétricos



(MEGAZYME, 2015)

Preparo da amostra

- ✓ Vidraria

- ✓ Granulometria

60 mesh = 0,250 mm

- ✓ Máximo de 5% de lipídios

Extração em Soxhlet

Cuidados e correções

✓ Enzimas

Devem ser altamente purificadas

✓ Precipitação da fração solúvel

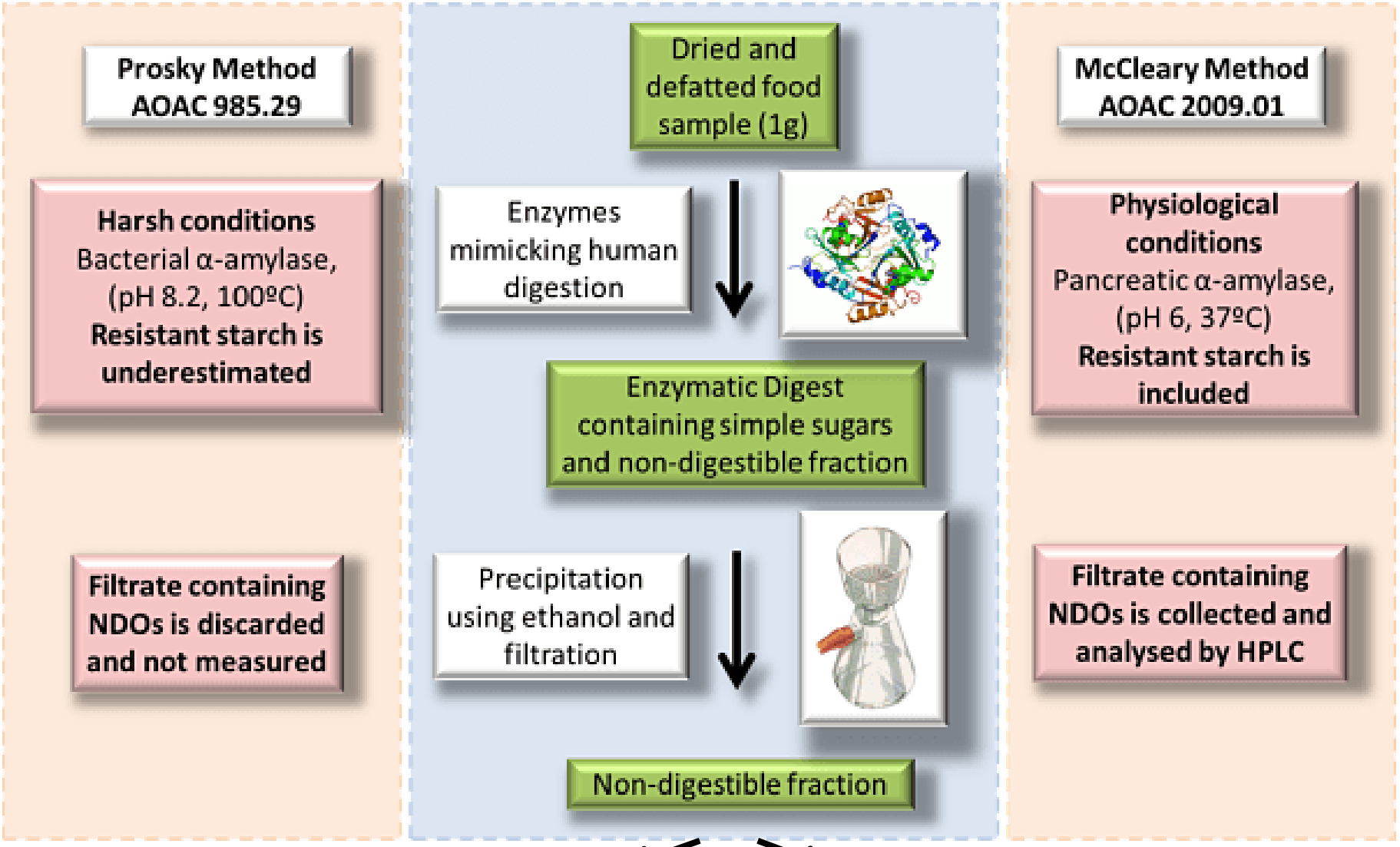
✓ Proteína residual

Deve ser descontada → Análise de proteína no resíduo

✓ Minerais residuais

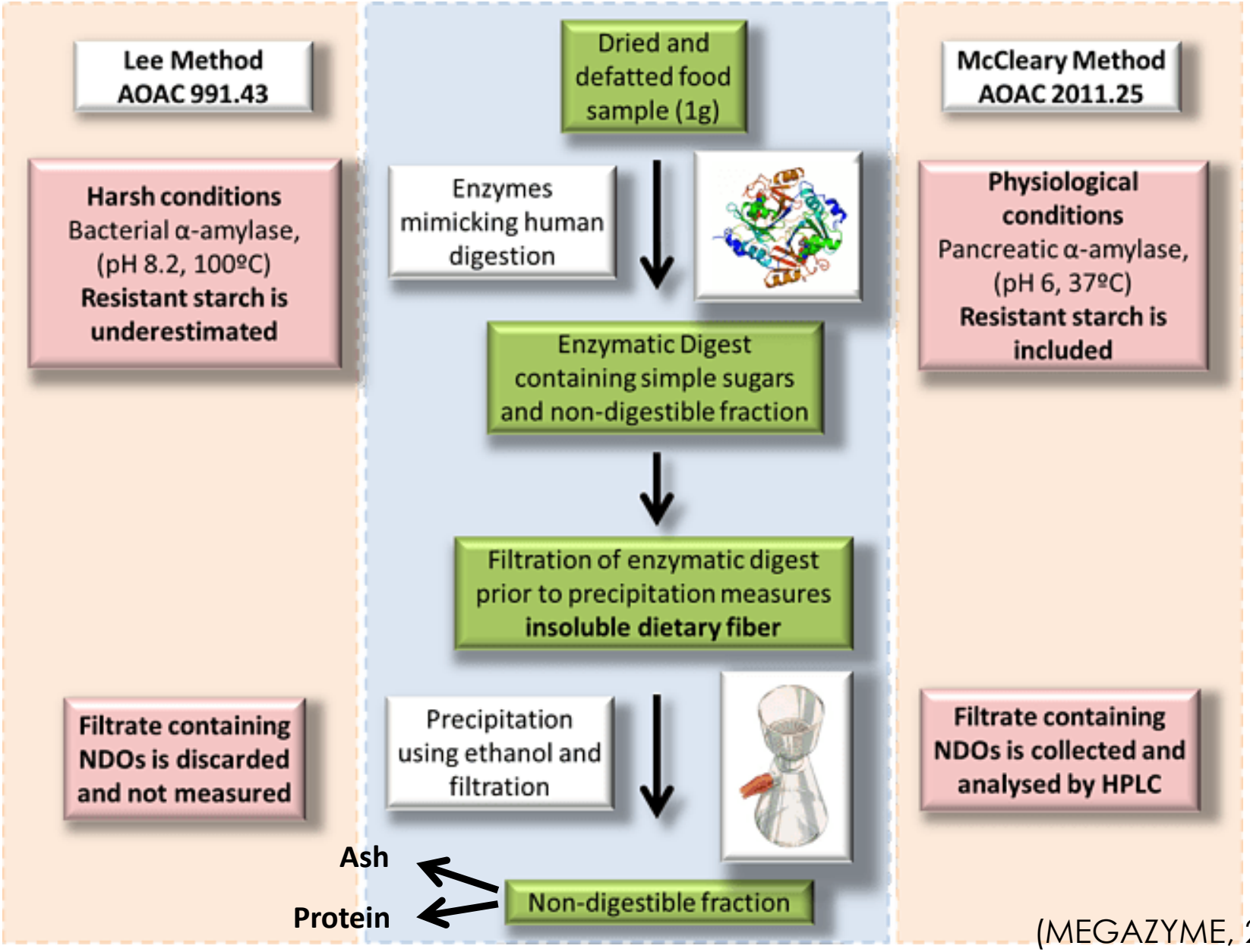
Devem ser descontados → Análise de cinzas no resíduo

AOAC 985.29 ↔ AOAC 2009.01



(MEGAZYME, 2015)

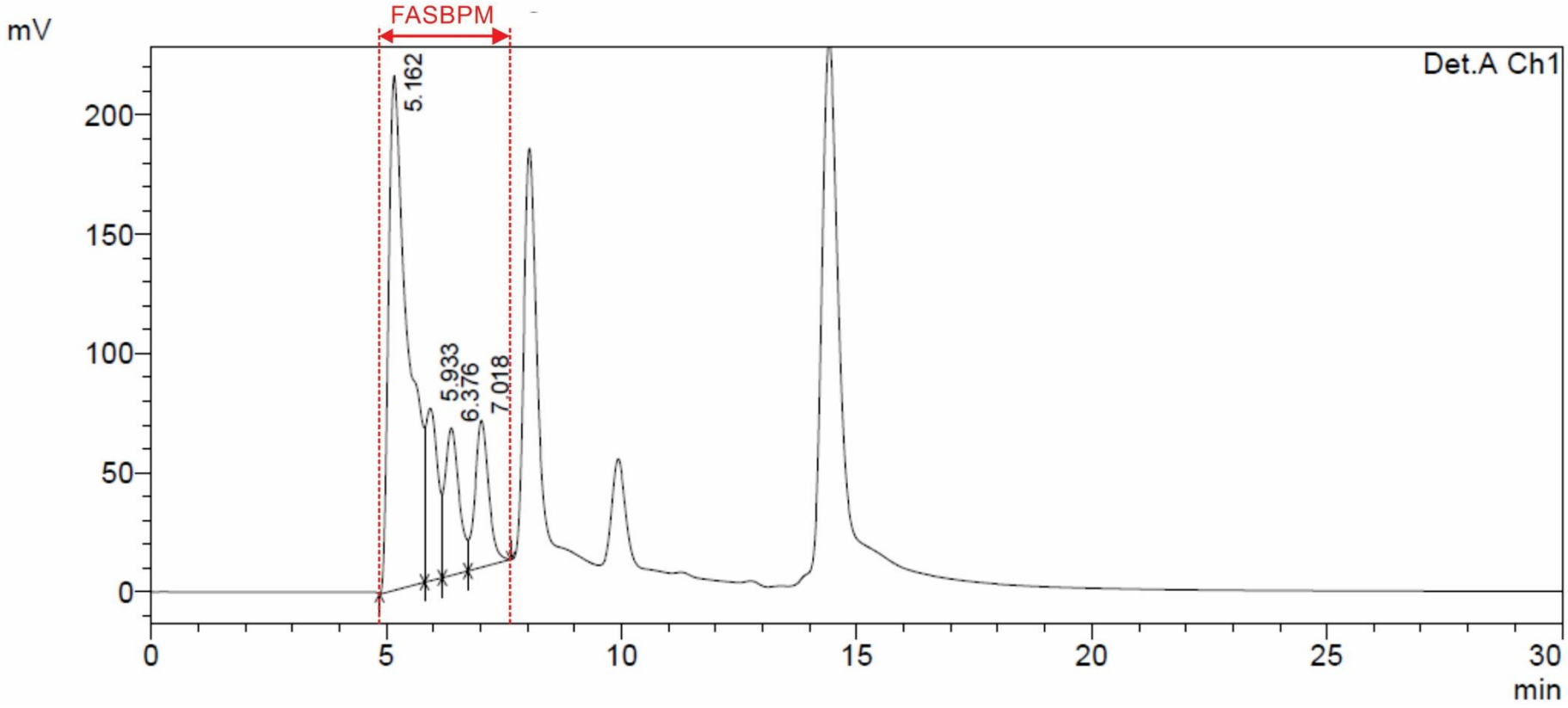
AOAC 991.43 ↔ AOAC 2011.25



(MEGAZYME, 2015)

AOAC 985.29 ↔ AOAC 2009.01

HPLC-RID



AOAC 991.43

α-amilase bacteriana (pH 8,2, 100°C)
Amido resistente é subestimado

Filtrado contendo OND é descartado

Amostra seca e desengordurada (1g)

Enzimas mimetizando a digestão humana

Solução digerida contendo açucares simples e frações não digeríveis

Filtração

Filtrado

Precipitação utilizando etanol e filtração

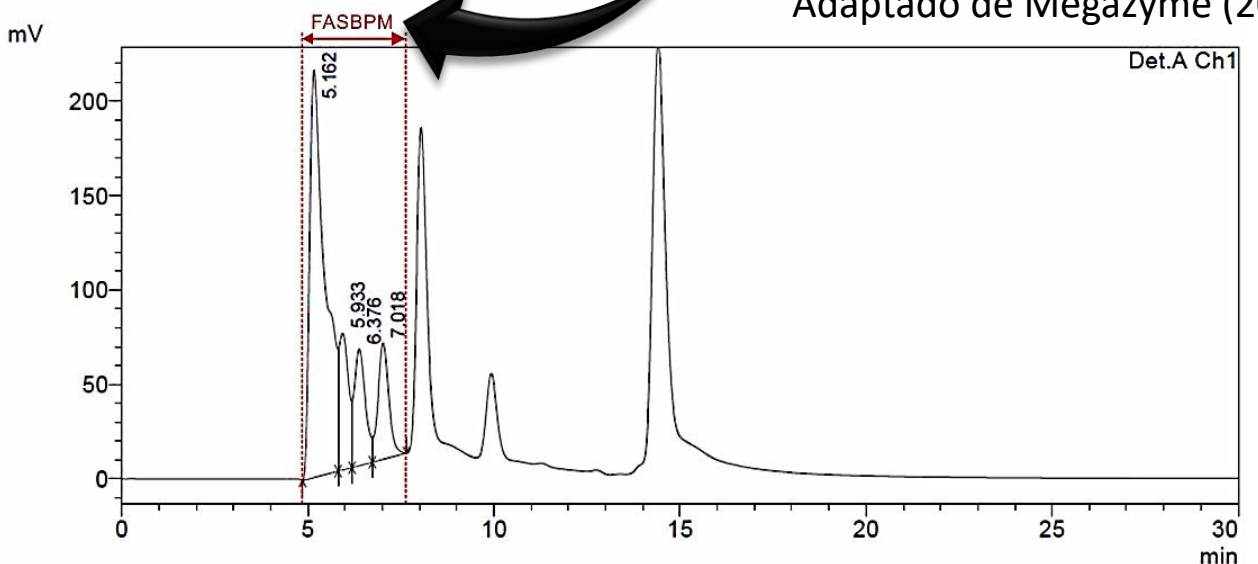
Filtrado

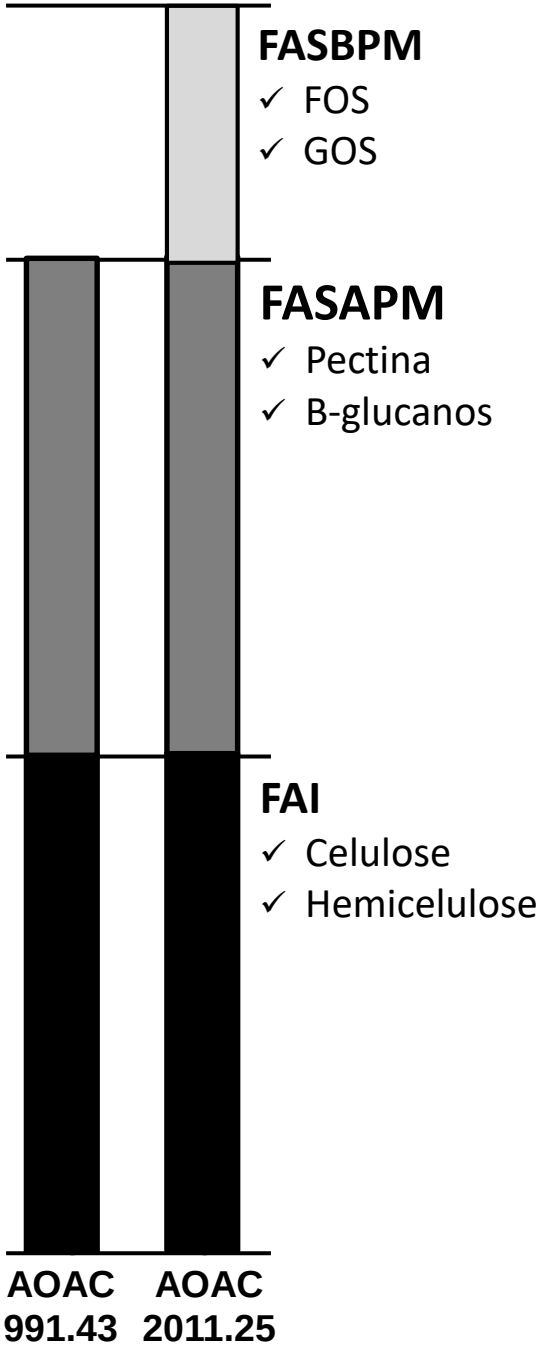
AOAC 2011.25

Condições fisiológicas
α-amilase pancreática (pH 6, 37°C)
Amido resistente é incluído

Filtrado contendo OND é coletado e analisado por HPLC

Adaptado de Megazyme (2015)





Food Chemistry 140 (2013) 586–589

Contents lists available at SciVerse ScienceDirect

Food Chemistry

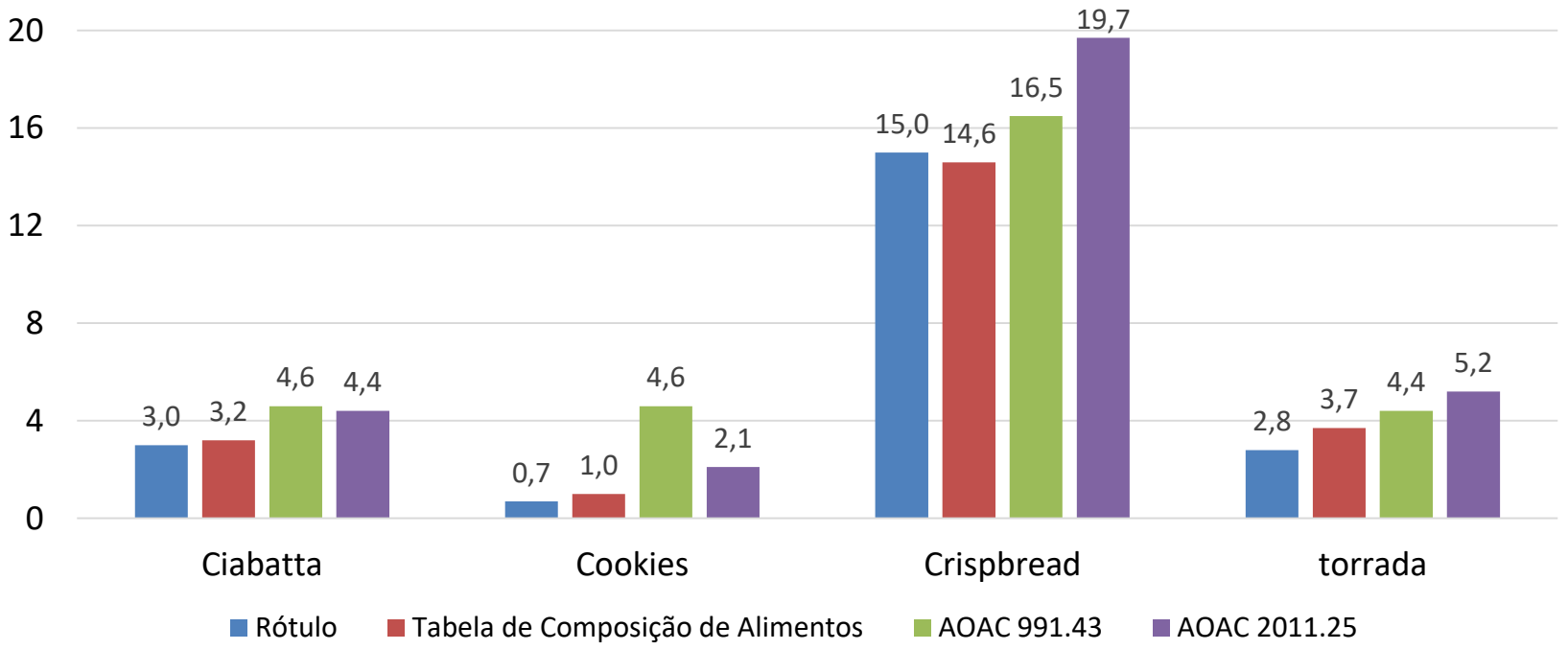
journal homepage: www.elsevier.com/locate/foodchem

Short communication

Dietary fibre fractions in cereal foods measured by a new integrated AOAC method

Juergen Hollmann, Heinz Themeier, Ursula Neese, Meinolf G. Lindhauer*

Max Rubner-Institute (MRI), Federal Research Institute for Nutrition and Food, Department of Safety and Quality of Cereals, D-32756 Detmold, Schuetzenberg 12, Germany



Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Evaluation of methods of analysis for dietary fibre using real foods and model foods

Klaus Englyst [a,*], Michael Quigley [a], Hans Englyst [a], Bhavna Parmar [b], Andrew Damant [b], Selvarani Elahi [c], Paul Lawrance [c]

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Improvement of the AOAC 2009.01 total dietary fibre method for bread and other high starch containing matrices ☆

K. Brunt *, P. Sanders

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Adaptation of the AOAC 2011.25 Integrated Total Dietary Fiber Assay To Determine the Dietary Fiber and Oligosaccharide Content of Dry Edible Beans

Adrienne E. Kleintop,[†] Dimas Echeverria,[†] Leslie A. Brick,[†] Henry J. Thompson,[‡] and Mark A. Brick*,[†]

[†]Department of Soil and Crop Sciences and [‡]Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, Colorado 80523, United States

Food Research International 85 (2016) 144–154

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Dietary fibre: The scientific search for an ideal definition and methodology of analysis, and its physiological importance as a carrier of bioactive compounds

Fernanda Teixeira Macagnan [a,*], Leila Picolli da Silva [b], Luisa Helena Hecktheuer [a]

860 DOI 10.1002/star.201500017 Starc

RESEARCH ARTICLE

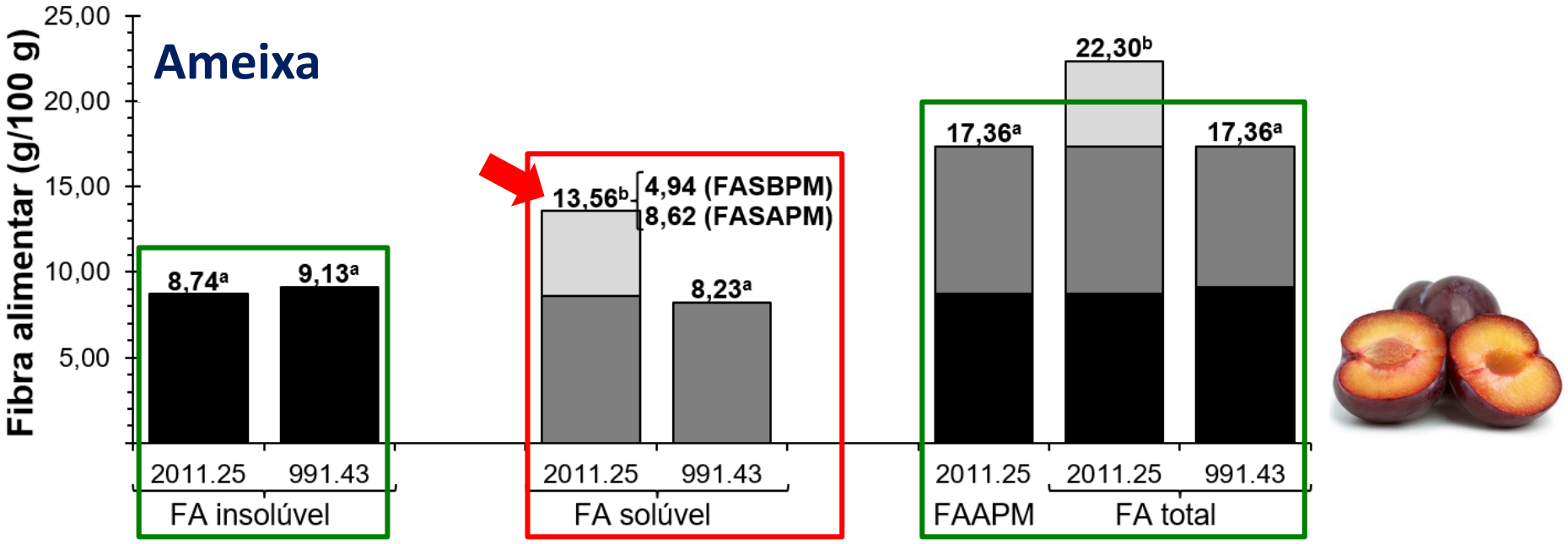
Determination of total dietary fibre and available carbohydrates: A rapid integrated procedure that simulates in vivo digestion

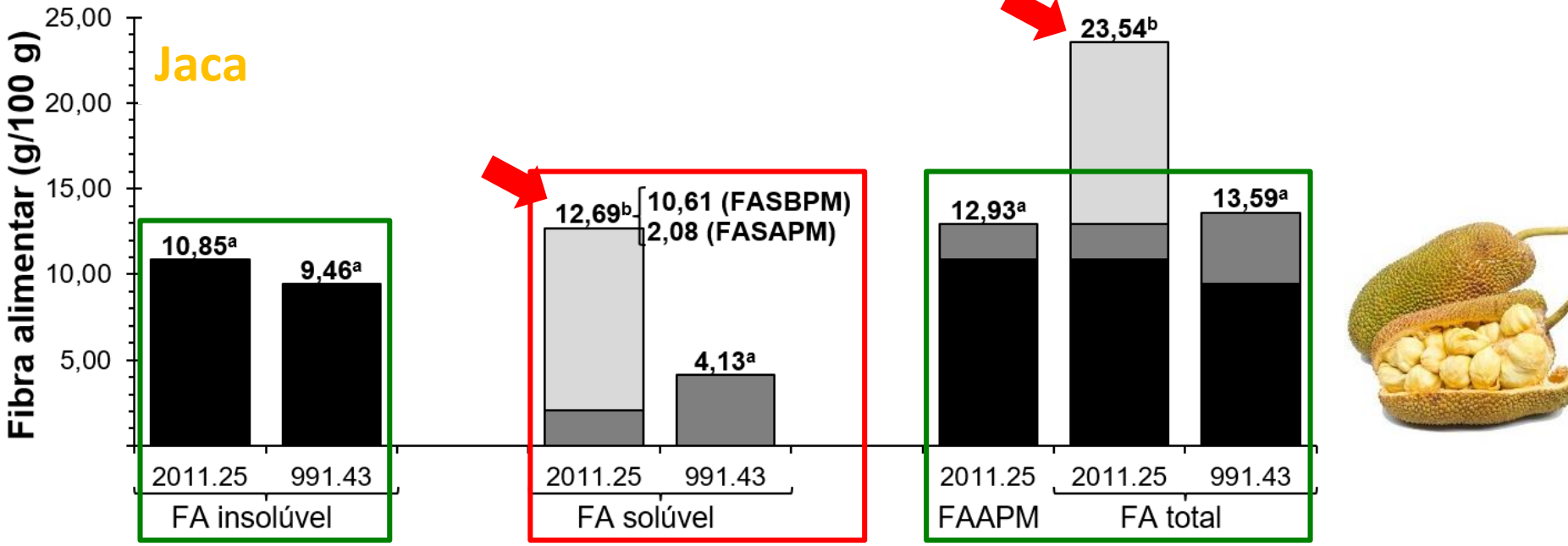
Barry V. McCleary, Naomi Sloane and Anna Draga

FRUTAS BRASILEIRAS

Application of dietary fiber method AOAC 2011.25 in fruit and comparison with AOAC 991.43 method

Eric de C. Tobaruela[1,2], Aline de O. Santos[1,2], Lígia B. de Almeida-Muradian[1], Elias da S. Araujo[1,2], Franco M. Lajolo[1,2], Elizabete W. Menezes[1,2]


Ameixa
Fibra alimentar (g/100 g)
25,00
20,00
15,00
10,00
5,00
8,74a
9,13a
13,56b
4,94 (FASBPM)
8,62 (FASAPM)
8,23a
17,36a
22,30b
17,36a
2011.25
991.43
2011.25
991.43
2011.25
2011.25
991.43
FA insolúvel
FA solúvel
FAAPM
FA total


Jaca
Fibra alimentar (g/100 g)
25,00
20,00
15,00
10,00
5,00
10,85a
9,46a
12,69b
10,61 (FASBPM)
2,08 (FASAPM)
4,13a
12,93a
23,54b
13,59a
2011.25
991.43
2011.25
991.43
2011.25
2011.25
991.43
FA insolúvel
FA solúvel
FAAPM
FA total

Considerações finais

- ✓ Falta de consenso em relação à definição;
- ✓ Posicionamento da ANVISA;

Métodos enzímico-gravimétricos

- ✓ Custo ↑;
- ✓ Conhecimento aprofundado;
- ✓ Mão de obra capacitada.

Considerações finais

Métodos	Alto Peso	Baixo Peso	Separa solúvel de insolúvel	Amido Resistente	Oligossacarídeos não digeríveis
AOAC 985.29	Sim	Não	Não	Somente AR3	Não
AOAC 991.43	Sim	Não	Sim	Somente AR3	Não
AOAC 2009.01	Sim	Sim	Não	Sim	Sim
AOAC 2011.25	Sim	Sim	Sim	Sim	Sim

OBRIGADO!

erictobaruela@usp.br