

Universidade de São Paulo

FBA0201 – Bromatologia Básica

USO DE MÉTODOS OFICIAIS

ERIC DE CASTRO TOBARUELA

Farmacêutico – UFC

Mestrado – Ciência dos Alimentos – USP

Doutorando – Ciência dos Alimentos – USP

TÓPICOS

- ❑ MÉTODOS DE ANÁLISE
- ❑ ORGÃOS DE NORMATIZAÇÃO
 - ADOLFO LUTZ
 - AOAC
- ❑ AOAC OFFICIAL METHODS
- ❑ ESCOLHA DO MÉTODO
- ❑ MÉTODOS UTILIZADOS
- ❑ FATORES QUE DIFICULTAM A ANÁLISE DE ALIMENTOS

- Conjunto de etapas fundamentais que compõem um processo analítico;

MÉTODO ANALÍTICO \neq TÉCNICA ANALÍTICA

- Procedimento pelo qual os dados analíticos são obtidos;
- Instrumentação específica para a obtenção dos dados analíticos;



PORQUE ESCOLHEMOS OS MÉTODOS QUE USAMOS?

Padronização de dados
Confiabilidade do resultado



PORQUE ESCOLHER MÉTODOS “OFICIAIS”?

Estabelecer e aplicar procedimentos que possuem a garantia de serem válidos para os fins desejados.

MÉTODOS DE ANÁLISE

MÉTODOS OFICIAIS

X

MÉTODOS NÃO-OFICIAIS

MÉTODOS CONVENCIONAIS

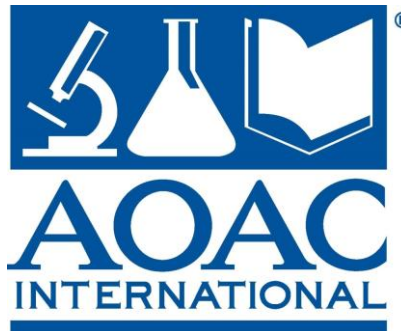
X

MÉTODOS INSTRUMENTAIS

- Métodos rápidos
- Métodos automatizados
- Métodos modificados

MÉTODOS OFICIAIS

- Instituto Adolfo Lutz
- Association of Official Analytical Chemists (AOAC)
- American Association of Cereal Chemists (AACC)
- American Oil Chemists' Society (AOCS)





INSTITUTO ADOLFO LUTZ

Métodos físico-químicos para análise de alimentos

edição **IV**

1ª Edição Digital

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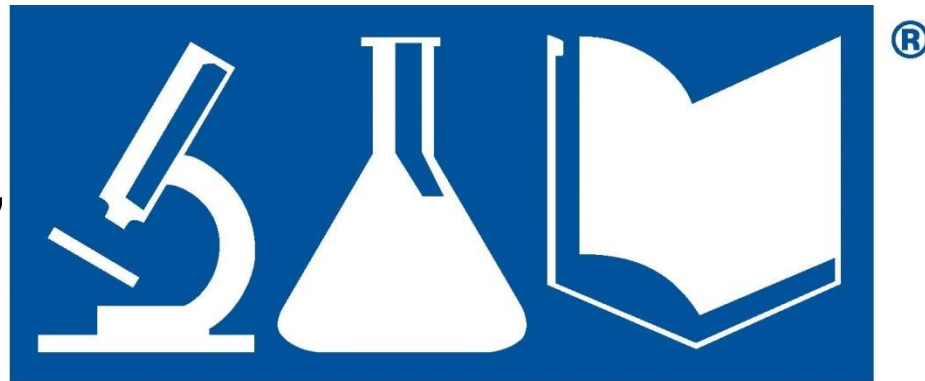
AOAC Official Methods of Analysis

- AOAC INTERNATIONAL ORGANIZATION;

- 125 anos;

- Desenvolvimento

- Validação



dos oficiais;

- É a coleção

métodos c

no mundo

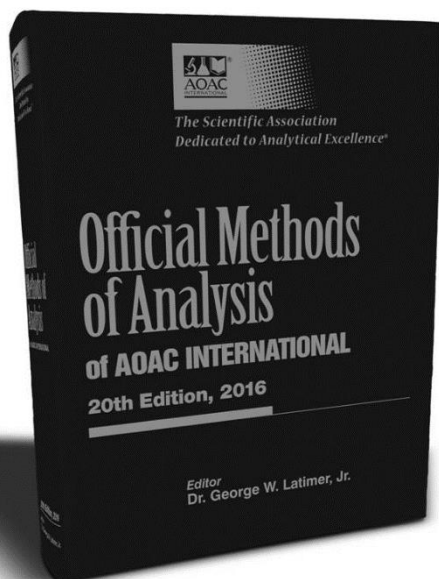
AOAC
INTERNATIONAL

el de

sponíveis

- Referenciado nas normas alimentares do Codex.

AOAC Official Methods of Analysis



Edição Impressa

AOAC INTERNATIONAL
Home Page

Official Methods of Analysis

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Association Preface

In 1885 Harvey W. Wiley, a founder of the Association and its president and secretary, oversaw the publication of the AOAC Methods of Analysis, a 49-page bulletin describing the analysis of fertilizers. [More >>](#)

[AOAC Official Methods](#)

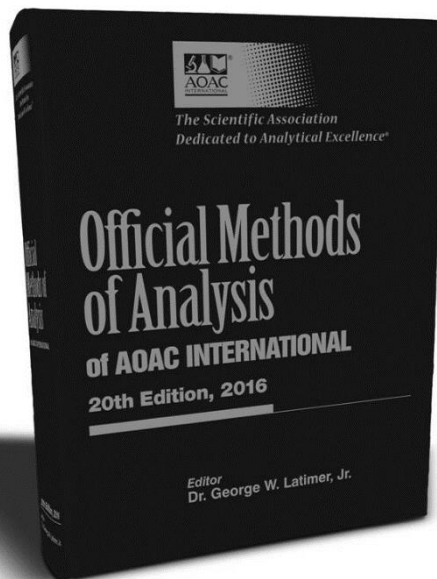
Edição Online

(<http://www.eoma.aoac.org/>)

AOAC Official Methods of Analysis

2939 métodos

71 guias para
avaliação de
desempenho



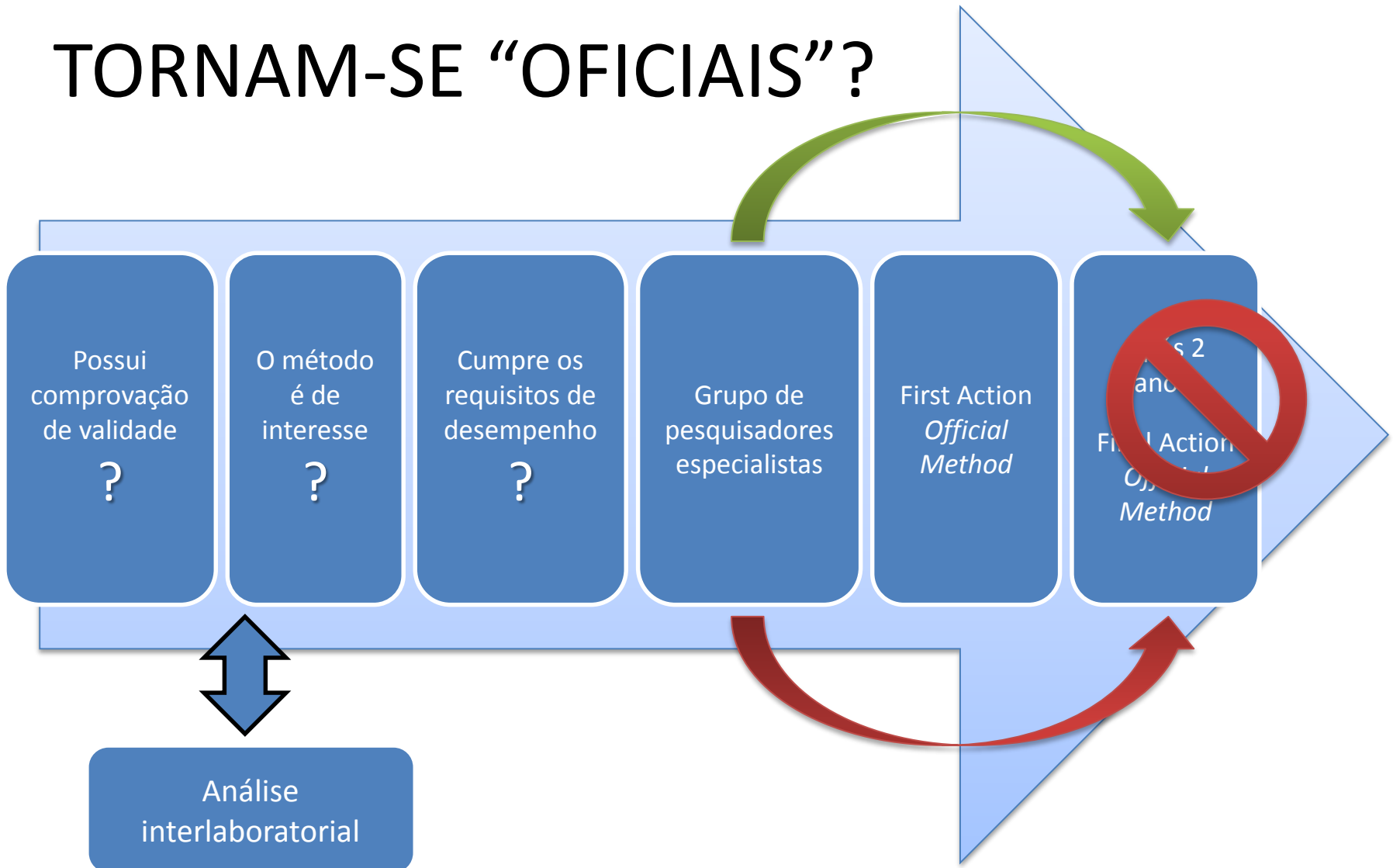
Definições

Segurança no
laboratório

Valores de
referência

Validação

COMO OS MÉTODOS TORNAM-SE “OFICIAIS”?



VALIDAÇÃO



Processo que “garante” que um método analítico pode produzir resultados confiáveis e reprodutíveis e que o mesmo é apropriado para o fim pretendido.

Os parâmetros avaliados são associados às características de desempenho do método, juntamente com robustez, repetibilidade e reprodutibilidade.

PARÂMETROS REQUERIDOS PARA MÉTODOS QUANTITATIVOS

- Intervalo analítico
- Limite de detecção
- Limite de quantificação
- Repetibilidade
- Recuperação
- Reprodutibilidade

4. Method Performance Requirements

Analytical range	0.01–5.0 ^a	
Limit of detection (LOD)	≤0.004 ^a	
Limit of quantitation (LOQ)	≤0.01 ^a	
Repeatability (RSD _r)	0.01 ^a	≤15%
	0.2 ^a	≤7%
	0.5 ^a	
	5.0 ^a	
Recovery	0.01 ^a	90–110%
	0.2 ^a	
	0.5 ^a	
	5.0 ^a	
Reproducibility (RSD _R)	0.3	≤11%
	0.6	
	1.0	
	2.5	
	5.0	
Concentrations apply to (1) "ready-to-feed" liquids "as is"; (2) reconstituted powders (25 g into 200 g water); and (3) liquid concentrates diluted 1:1 by weight.		
^a µg/100 g expressed as cyanocobalamin in reconstituted final product.		

All parameters have a minimum requirement set by the Working Group

<p>Locator number identifies method by chapter, subchapter, and sequence within the subchapter for easy cross referencing and access. 4 = chapter 4; .10 = subchapter 10; .03 = the third method found in Chapter 4, subchapter 10. The locator number is not the permanent number and is included only for convenient accessibility.</p>	<p>4.10.03</p> <p>AOAC Official Method 996.13 Ethoxyquin in Feeds Liquid Chromatographic Method First Action 1996 Final Action 1997</p> <p>(Applicable for determination of 0.5–300 µg/g ethoxyquin in dry extruded pet food or meat meal.)</p> <p>See Table 996.13 for the results of the interlaboratory study supporting acceptance of the method.</p> <p>A. Principle Ethoxyquin is extracted with acetonitrile. Extract is analyzed by isocratic liquid chromatography with fluorescence detection.</p> <p>B. Apparatus (a) <i>Liquid chromatograph (LC)</i>.—Generating 1500 ± 200 psi; with peak area integrator (manual or computer), isocratic LC pump, and column heater. Operating conditions: injection volume, 20 µL; flow rate, 1.3 mL/min; temperature, 35°C; fluorescence detector output, analog to digital conversion; detector settings: excitation, 360 nm; emission, 432 nm. (b) <i>LC column</i>.—250 × 4.6 mm id, C₁₈ octadecylsilane, 5 µm spherical, 100 Å pore size.</p> <p>C. Reagents (a) <i>Water</i>.—LC grade. (b) <i>Acetonitrile</i>.—LC grade.</p> <p>D. Preparation of Standard Solutions (a) <i>Ethoxyquin standard stock solution</i>.—400 µg/mL. Weigh the equivalent of 0.1000 g liquid ethoxyquin into 250 mL amber volumetric flask and dilute to volume with acetonitrile. (Note: Amount of ethoxyquin needed for preparation of stock solution is based on purity of liquid, e.g., for purity of 93.5%, amount of liquid ethoxyquin = 0.100/0.935 = 0.1070 g.)</p> <p>H. Calculations Calculate concentration of ethoxyquin, µg/g or ppm, in test sample from calibration curve (using linear regression with line forced through zero intercept) as follows:</p>	<p>Permanent number identifies method by year of adoption or first appearance in <i>Official Methods of Analysis of AOAC INTERNATIONAL</i>. 996 = First Action 1996; .13 = sequence of adoption in 1996.</p> <p>Title may include analyte and matrix, type of method, and official status.</p> <p>Applicability statement addresses utility and limitations on use of method or other information.</p> <p>Specifications for necessary laboratory apparatus and reagent preparations. See also <i>Definition of Terms and Explanatory Notes</i>.</p> <p>Method may be divided into several descriptive sections.</p> <p>References direct the user to the published collaborative study and any subsequent revisions in the method. Other informative references may be included.</p>
<p>Chemical names of pesticides and drugs are given at end of pertinent chapter.</p>	<p>Ethoxyquin in Feeds Liquid Chromatographic Method</p>	<p>Title may include analyte and matrix, type of method, and official status.</p>
<p>Calculation symbols are identified and show correct units.</p>	$\text{Ethoxyquin, } \mu\text{g/g or ppm} = \frac{C \times 1.5 \times F}{W}$ <p>where C = ethoxyquin concentration from LC calibration curve, µg/mL; 1.5 = volume of acetonitrile added to test solution, mL; F = dilution factor; W = weight of test portion, g.</p> <p>Reference: <i>J. AOAC Int.</i> 80, 725(1997).</p>	<p>Method may be divided into several descriptive sections.</p>
<p>Chemical Abstracts Service Registry Number. A unique identifier that may be used to search a number of data-retrieval systems.</p>	<p>CAS-91-53-2 (ethoxyquin) 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline</p> <p>Revised: March 1998</p>	<p>References direct the user to the published collaborative study and any subsequent revisions in the method. Other informative references may be included.</p>

- LOCALIZADOR
- IDENTIFICADOR
- APLICAÇÃO
- PRINCÍPIO ANALÍTICO
- PRINCÍPIO DO MÉTODO
- MATERIAIS
- REAGENTES
- PREPARO DE SOLUÇÕES
- PADRÃO
- CÁLCULOS
- REFERÊNCIA
- CAS
- (REGISTRO QUÍMICO DO COMPOSTO)
- REVISÃO

Determination of Insoluble, Soluble, and Total Dietary Fiber (CODEX Definition) by Enzymatic-Gravimetric Method and Liquid Chromatography: Collaborative Study

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A method for the determination of insoluble (IDF), soluble (SDF), and total dietary fiber (TDF), as defined by the CODEX Alimentarius, was validated

and gravimetric procedures of AOAC 985.29 (and its extensions 991.42 and 993.19) and 991.43 results in quantitation of IDF and soluble dietary

AOAC OFFICIAL METHOD

AOAC Official Method 2011.25

Insoluble, Soluble, and Total Dietary Fiber in Foods

Enzymatic-Gravimetric-Liquid Chromatography First Action 2011

[Applicable to plant material, foods, and food ingredients consistent with CODEX Alimentarius Commission Definition adopted in 2009 and modified slightly in 2010 (ALINORM 09/32/REP and ALINORM 10/33/REP, respectively), including naturally occurring, isolated, modified, and synthetic polymers meeting that definition.]

See Tables 2011.25A–H for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

A method is described for the measurement of insoluble, soluble, and total dietary fiber (IDF, SDF, and TDF, respectively), inclusive of the resistant starch (RS) and the water:alcohol soluble nondigestible oligosaccharides and polysaccharides of DP ≥ 3 . The method combines the key attributes of AOAC *Official Methods of Analysis*SM 985.29 (and its extensions, 991.42 and 993.19), 991.43, 2001.03, and 2002.02. Duplicate test portions are incubated with pancreatic α -amylase and amyloglucosidase (AMG) for 16 h at 37°C in sealed 250 mL bottles while mixing with sufficient vigor to maintain continuous suspension. During this step, nonresistant starch is solubilized and hydrolyzed to glucose and maltose by the combined action of the two enzymes. The reaction is terminated by pH adjustment and temporary heating. Protein in the sample is then digested with protease. For the measurement of IDF, the digestate is filtered and the IDF is determined gravimetrically after correction for any protein or ash in the residue. For the measurement of the water soluble, but water:alcohol insoluble dietary fiber (SDFP), ethanol is added to the filtrate of the IDF; the precipitated SDFP is captured by filtration and determined gravimetrically after correction for any protein or ash in the precipitate. Nonprecipitable, water:alcohol soluble dietary fiber (SDFS) in the filtrate is recovered

polypropylene caps.

(c) *Fritted crucible*.—Gooch, fritted disk, Pyrex® 50 mL, pore size, coarse, ASTM 40–60 μm (Corning No. 32940-50C® or equivalent; <http://www.labplanet.com/coming-crucible-gooch-high-c-50-ml-32940-50c.html>). Prepare four for each sample as follows: Ash overnight at 525°C in muffle furnace. Cool furnace to 130°C before removing crucibles to minimize breakage. Remove any residual Celite and ash material by using a vacuum. Soak in 2% cleaning solution, C(i), at room temperature for 1 h. Rinse crucibles with water and deionized water. For final rinse, use 15 mL acetone and air dry. Add approximately 1.0 g Celite to dried crucibles and dry at 130°C to constant weight. Cool crucible in desiccators for approximately 1 h and record mass of crucible containing Celite.

(d) *Filtering flask*.—Heavy-walled, 1 L with side arm.

(e) *Rubber ring adaptors*.—For use to join crucibles with filtering flasks.

(f) *Vacuum source*.—Vacuum pump or aspirator with regulator capable of regulating vacuum.

(g) *Water bath(s)*.—Rotary motion, large-capacity (20–24 L) with covers; capable of maintaining temperature of $37 \pm 1^\circ\text{C}$ and $60 \pm 1^\circ\text{C}$; equipped with automatic timers for on-off operation or equivalent (e.g., Grant® OLS 200 shaking incubation bath; <http://www.bioresearchonline.com/product.mvc/Grant-OLS200-Orbital-Shaking-Water-Bath-0001>). Ensure that shaking action/sample agitation in water bath used is sufficient to maintain sample solids in suspension and no residue build-up or rings of sample material form in the digestion bottle during the enzymatic digestions. A linear motion (back and forth shaker) can be used if the bottles are placed at 45° to ensure adequate agitation (if the bottles are vertical or horizontal there will not be sufficient agitation to ensure that the sample remains suspended). Alternatively, a 2 mag Mixdrive 15 submersible magnetic stirrer apparatus (http://www.2mag.de/english/stirrer/multiple/stirrer_multiple_04_mixdrive6_15.html) can be used in a water bath maintained at $37 \pm 1^\circ\text{C}$ with a Julabo

Megazyme

MEGAZIME KIT

INTEGRATED TOTAL DIETARY FIBRE ASSAY PROCEDURE

INCLUDING
RESISTANT STARCH
AND NON-DIGESTIBLE
OLIGOSACCHARIDES

K-INTDF 06/13

AOAC Method 2009.01 & 2011.25
&
AACC Method 32-45.01 & 32-50.01
(100 Assays per Kit)

[UPDATED FORMAT, 2013]

1976³ to:

“Dietary fibre consists of the remnants of edible plant cells, polysaccharides, lignin, and associated substances resistant to digestion by the alimentary enzymes of humans.”

This definition defines a macro constituent of foods which includes hemicellulose, lignin, gums, modified celluloses, mucilages, oligosaccharides, pectins and associated minor substances such as waxes, cutin, and

On the basis of this definition³, appropriate methodology for measurement of DF was developed by a consortium of researchers in Europe and USA. This led to AOAC Official Method 985.2 (Prosky method),^{4,5} and to subsequent modifications of this method including AOAC Official Method 991.43⁴ in which the buffers were changed. The aim of these methods was to give an accurate measurement of the content of total dietary fibre in plant products and food materials. More specifically, the methodology aimed at hydrolysing and removing starch and protein. Fats were removed by the solvents employed to recover the non-hydrolysed material. From the outset, it was realised that all protein was not hydrolysed so each sample was then analysed in duplicate and residues reweighed and weighed. One of these residues is analysed for ash content and the other for protein. These weights are subtracted from the average of the residue weights. It was also realised that, in the analytical procedure, starch also was not completely hydrolysed and removed. This in turn led to the discovery of so-called “resistant starch (RS)”. The question then was, “*should RS be measured and added to the total dietary fibre value, or should it be analytically removed and ignored?*” Since RS escapes digestion in the human small intestine the general consensus is that it should be accurately measured and included. Research in the 1990’s showed that AOAC Official Method 991.43 underestimates RS, so alternative methods for measurement of this component were developed and evaluated. While most of these new methods gave similar results for a range of RS containing samples, none of the methods survived the

codex alimentarius commission

I. LIST OF METHODS FOR DIETARY FIBRE

(at Step 8 of the Procedure)

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
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	AOAC 2001.03	Enzymatic gravimetric and Liquid chromatography	III

CODEX ALIMENTARIUS

TIPO I

Métodos de definição

Método que determina um valor que só pode ser obtido pela utilização do mesmo. Por definição, deve ser o único método para determinação do valor aceito para o parâmetro medido.

TIPO II

Métodos de referência

Métodos de referência utilizados nos casos em que os métodos do Tipo I não se aplicam. Recomendados para fins de calibração.

TIPO III

Métodos alternativos aprovados

Métodos que satisfazem aos critérios exigidos pelo Comitê do Codex. Métodos utilizados para controle, inspeção ou outros propósitos regulatórios.

TIPO IV

Métodos de tentativa

Métodos tradicionalmente utilizados ou recentemente introduzidos que não foram avaliados pelo Comitê do Codex.

ESCOLHA DO MÉTODO

Matriz

- Simples?
- Complexa?
- Interferentes?

Componente

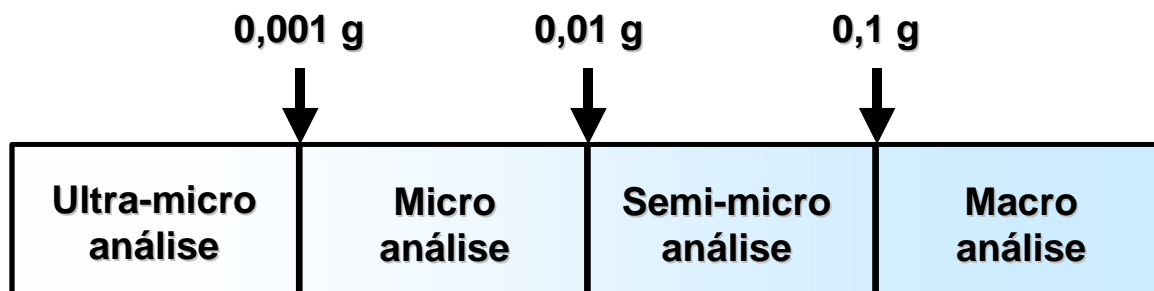
- Específico?
- Grupo?

Maiores: >1%

Menores: 0,01 – 1%

Micro: <0,01%

Traço: (ppm e ppb)



ESCOLHA DO MÉTODO

- Finalidade
- Quantidade de amostra disponíveis
- Quantidade do componente analisado
- Exatidão requerida
- Composição química da matriz
- Recursos disponíveis
- Número de amostras



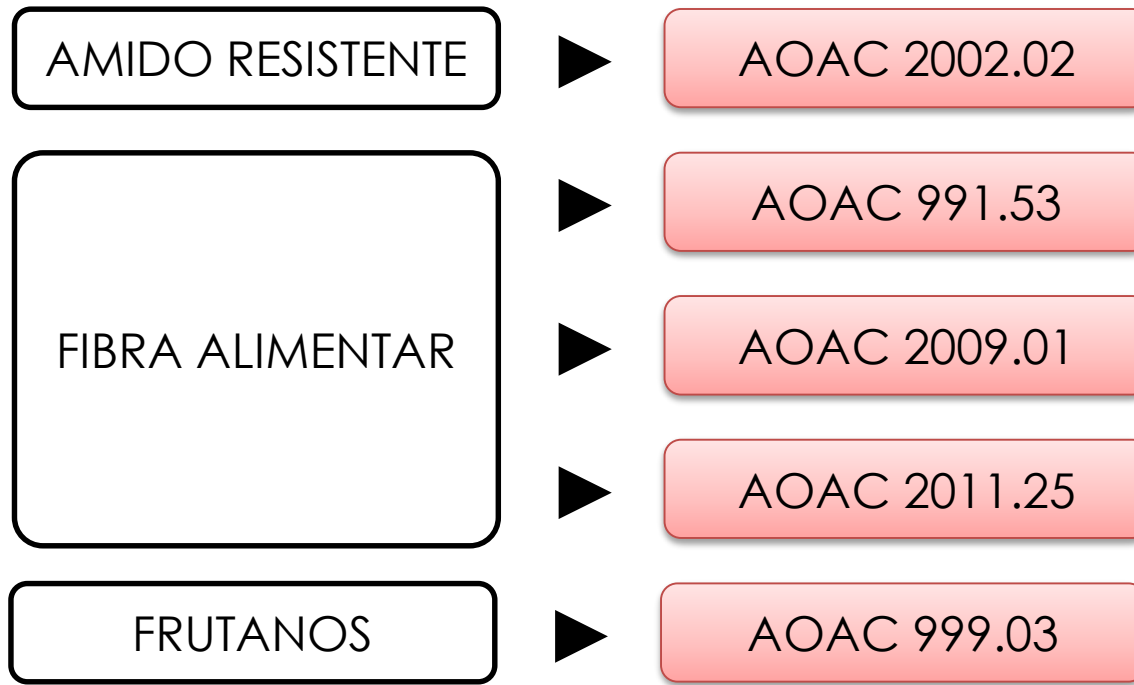
MÉTODOS AOAC ADOTADOS

Composição Centesimal

	Geral	Frutas
UMIDADE	AOAC 925.45b	AOAC 934.06
PROTEÍNAS	AOAC 960.52	AOAC 960.52
LIPÍDIOS	AOAC 920.39	AOAC 920.39
CINZAS	AOAC 923.03	AOAC 940.26

MÉTODOS AOAC ADOTADOS

Carboidratos



FATORES QUE DIFICULTAM A ANÁLISE DE ALIMENTOS

- Complexidade das amostras
- Número de substâncias presentes
- Distribuição não uniforme
- Perecibilidade
- Variabilidade de amostras do mesmo alimento

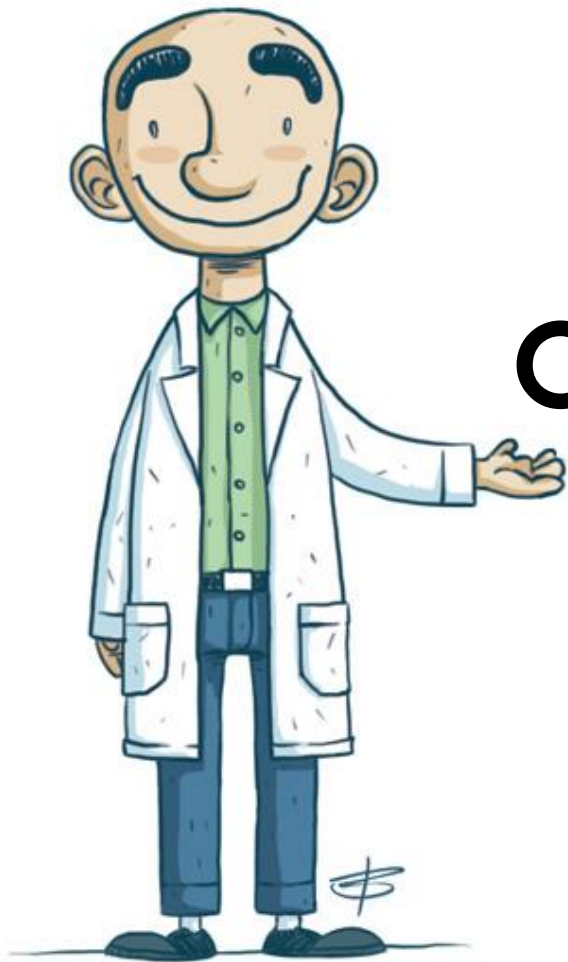
SEGURANÇA NO LABORATÓRIO



EQUIPAMENTOS DE PROTEÇÃO







OBRIGADO!

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ORIENTAÇÕES PARA SEMINÁRIOS

Slide Introdutório

Agenda

Introdução

Slides intermediários

Conclusões/considerações finais

Referências

Cabeçalho
Título do seminário
Nome dos alunos

Temas abordados

Breve introdução ao tema

Objetivos

Desenvolvimento do tema

Aprendizado obtido
Comentários
Etc

Slides



A metamorfose

- Normalmente as metamorfoses habitam ou de hábitos podendo,
- Alguns exemplos de metamorfoses são alguns tipos de pássaros, alguns tipos de peixes, alguns tipos de libélulas são insetos aquáticos de voadores na idade adulta. As rãs girino aquático até um anfíbio (fases de metamorfose em invertebrados aquáticos que nascem fixando-se em seguida a um substrato de locomoção, como é o caso dos urânios, na fase larval têm peças bucais mas depois de voadores com essas peças especializadas).
- O tipo de metamorfose sem que haja mudanças físicas até ao estado adulto é ilustrado por muitas espécies de metamorfoses físicas até ao estado adulto. O habitat do animal se mantém inalterado. A metamorfose segue uma série de etapas. A espécie em questão ao longo da vida sabe que é uma formulação incorreta.



Escolhas alimentares

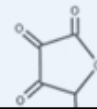
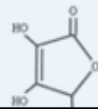
- Normalmente
- Alguns exemplos
- O tipo de metamorfose
- Sem que haja mudanças
- Há décadas



Slides



Ácido ascórbico Vitamina C



Sensibilidade	Estabilidade	F
Temp. elevadas Soluções alcalinas		Ácido
Temp. elevadas Soluções alcalinas		Ácido dehid
		Ácido

Preparo da amostra

Método fluorimétrico: ácido ascórbico + ácido dehidroascórbico + o-fenilenodiamina = derivado fluorescente

Método espectrofotométrico: ácido ascórbico + Cu+2 num meio bifásico = cuproína (em meio ácido)

CONCLUSÕES

Vitaminas hidrossolúveis => baixa quantidade

Matrizes complexas

Presentes em mais de uma forma

Método microbiológico => AOAC

HPLC => muito utilizado

[Texto]

Solução de dúvidas

Outubro

4/10	Definição e métodos AOAC de fibra alimentar Determinação de fibra alimentar	Prof. Eduardo MSc Eric Tobaruela
11/10	Determinação de fibra alimentar	Prof. Eduardo
18/10	Determinação de fibra alimentar Solução de dúvidas - Seminários	Prof. Eduardo Monitores
25/10	Teoria da análise de vitaminas e minerais	Prof. Eduardo

Novembro

1/11	Entrega e apresentação de seminários (grupos 1 a 6)	Profa. Elizabete
8/11	Entrega e apresentação de seminários (grupos 7 a 10) Avaliação dos resultados de composição teórica e química	Profa. Elizabete
22/11	Prova (peso 2)	Monitores

Observações:

1. A média ponderada das avaliações corresponde a 80% da nota final
Avaliação 1 (peso 1) e avaliação 2 (peso 2)
2. A média dos relatórios e seminários corresponde a 20% da nota final
Relatórios de aulas práticas (peso 1) e seminários (peso 2)

OBRIGADO!



1ª JORNADA

CIENCIALIMENTA

05 e 06 de setembro de 2016
Faculdade de Ciências Farmacêuticos - USP

TEMA I

Qualidade dos alimentos:
do genótipo ao fenótipo

TEMA II

Alimentos e sensações

TEMA III

O microbioma humano

TEMA IV

Alergia e intolerância alimentar

**INSCRIÇÕES
GRATUITAS!**

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Público alvo:
Graduandos de Farmácia e Nutrição

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