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USP
Food Chemicals Codex

PhD & MBA @
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Certified Food Scientist

Focus

Analytical techniques, adulteration detection, non-targeted methods, & analytical materials

International Collaboration to Establish Standards that Combat Food Fraud and Protect Food Integrity

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Agenda

- Introduction to the *FCC* and background
- Collaboration on standard development

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Food Chemicals Codex

A compendium of internationally recognized standards for the identity and purity of food ingredients.

Created by the US-FDA and the US Institute of Medicine in 1966

Currently published by USP, a non-profit organization

>1250 standards for additives, ingredients, and other food chemicals

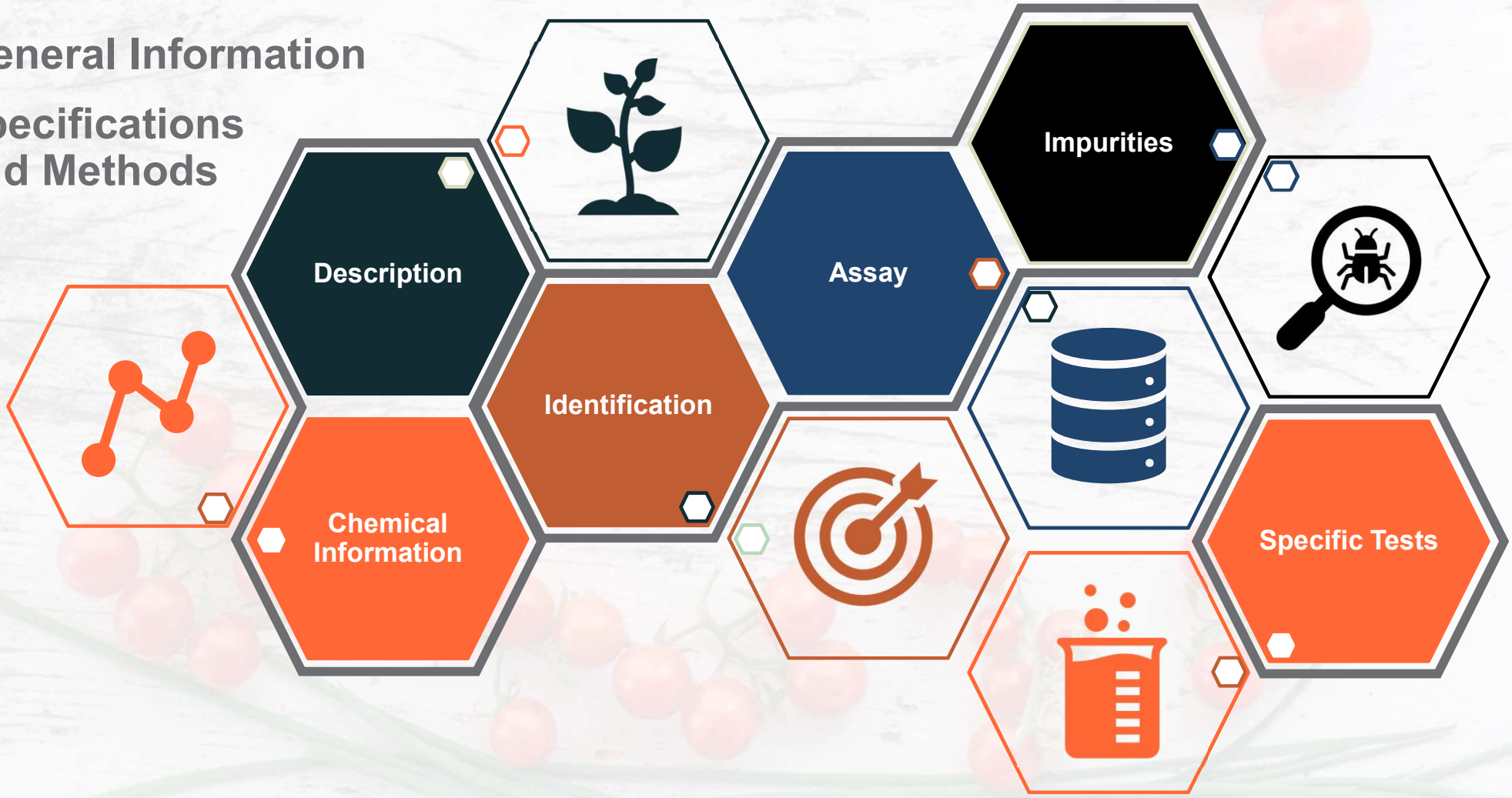
Standards are developed by expert volunteers

A fully independent source of food ingredient standards

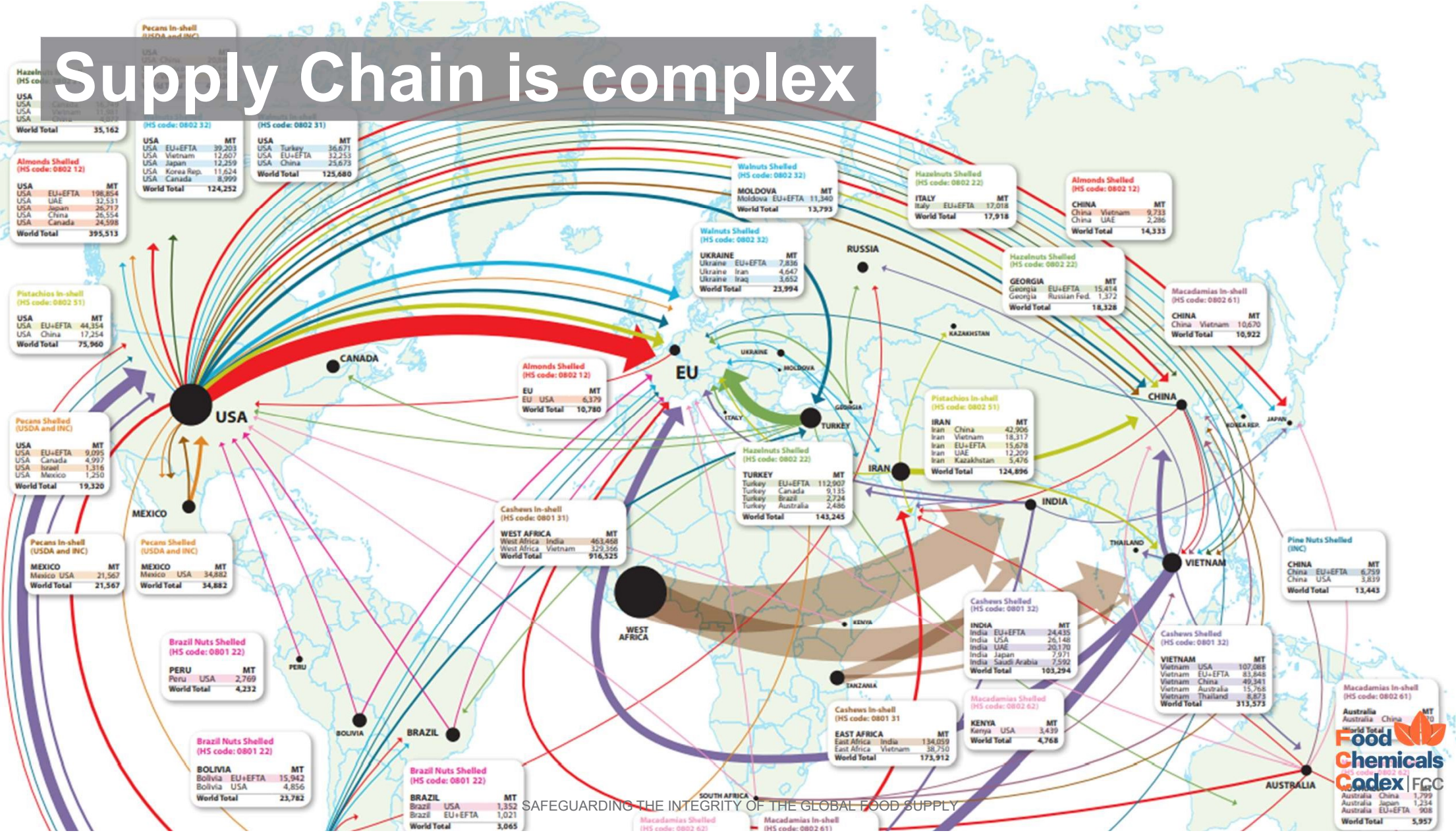
The anatomy of an FCC standard

General Information

Specifications and Methods



Supply Chain is complex



SAFEGUARDING THE INTEGRITY OF THE GLOBAL FOOD SUPPLY

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Supply Chain is complex

- Producing foods that are safe, nutritious, and honest requires control of, and communication along, the whole supply chain
- Food supply chains are complex, non-linear, and subject to sudden disruption
- This creates multiple opportunities for misunderstanding, miscommunication, mishandling, fraud/economically motivated adulteration

Food Fraud

Food Fraud or Economically Motivated Adulteration (EMA)

> “The fraudulent addition of nonauthentic substances or removal or replacement of authentic substances without the purchaser's knowledge for the economic gain of the seller.”
>
> -FCC Appendix XVIII

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Challenges

Lack of supply chain control

Insufficient analytical testing

- Not enough testing
- Lack of sophisticated methods used

Collaboration on Standard Development

- Through Horizon 2020, USP-FCC was able to expand international collaboration on developing FCC standard methods to combat food fraud.

- Experts from members of Horizon 2020 participated in the planning, method development and validation, review and recommendation, and balloting of the standards.

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Collaboration on Standard Development

Horizon 2020 members that participated in standard development as USP volunteers

Wu Yongning

FIEC Member (2015-2020)

Chief Scientist, China National Center for Food Safety Risk Assessment (CFSA)

Director, NHC Key Lab of Food Safety Risk Assessment

Wu Di

DP EP Member

Newton International Fellow of Royal Society, Institute for Global Food Security, Queen's University, Belfast

Huang Xiaoping

DP EP Member

Senior Specialist
Risk Assessment
Yili Group
China

Zhu Wei

FIEC Member (2020-2025)

DP EP Member

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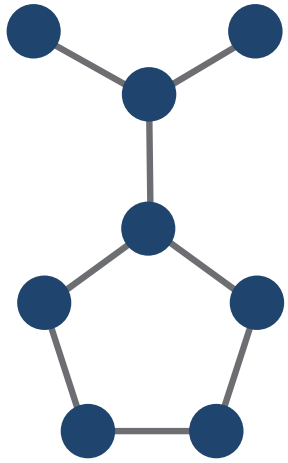
Amino Acid Profile for Skim Milk Powder



Amino acid profile

Principle

- Amino acids are the building blocks of proteins; the proportion of different amino acids varies between different proteins and can be considered a characteristic of a given protein.
- Amino acid profile testing can be a useful screening tool to help substantiate the identity of a protein-rich ingredient.
- Typical total protein analysis by nitrogen determination methods would not detect adulteration on addition of or substitution with foreign proteins .

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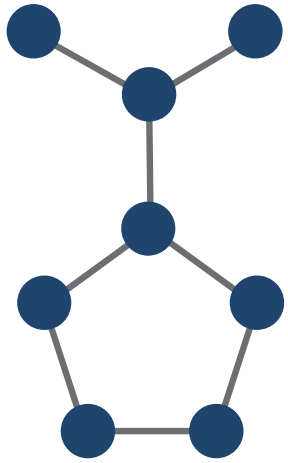
Amino Acid Profile for Skim Milk Powder



Amino acid profile

Hydrolyzed in 6 M HCl by rapid microwave method

↓

6-aminoquinolyl-*N*-hydroxy succinimidyl carbamate (AQC) derivatization

↓

UHPLC(C18)

↓

UV 260nm

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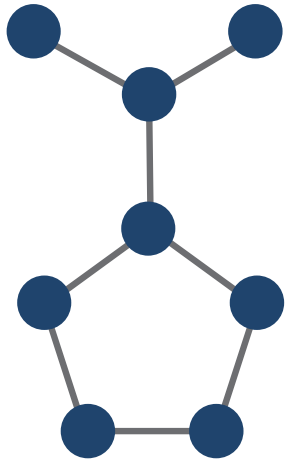
BASED ON AOAC 2018.06

Amino Acid Profile for Skim Milk Powder



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↓

UHPLC(C18)

↓

UV 260nm

↓

Comparison of percentage of the total amount of amino acids between sample and typical range to detect potential adulteration

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BASED ON AOAC 2018.06

Amino Acid Profile-Development

- The typical ranges of amino acids are based on data of 39 diverse bovine-based samples of skim milk powder and nonfat dry milk samples:
  - different geographic origins (US, NZ, India, Ireland, Denmark, Argentina, etc.
  - different processing conditions

- Five collaborative labs in US and China participated in this study.

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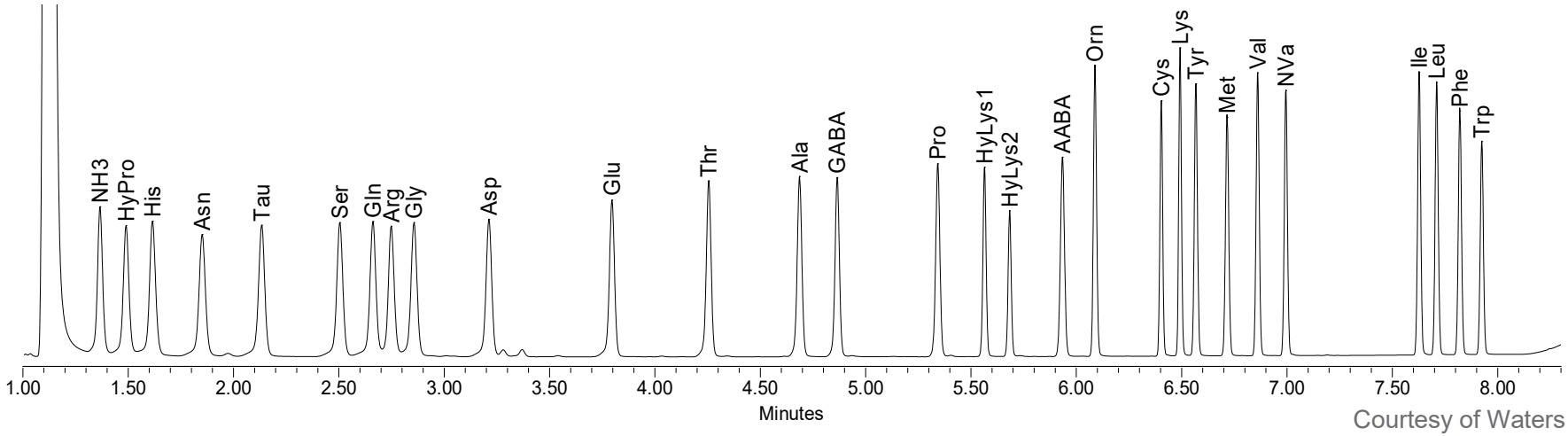
Amino Acid Profile-Development



Courtesy of Waters

Based on performance: 15 amino acids were selected: l-alanine, l-arginine, l-aspartic acid, l-glutamic acid, glycine, l-histidine, l-isoleucine, l-leucine, l-lysine · HCl, l-phenylalanine, l-proline, l-serine, l-threonine, l-tyrosine, l-valine

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Amino Acid Profile-Development

Calculate the amount of each individual amino acid in the sample taken (on the as-is basis) as the percentage of the total amount of amino acids:

$$\text{Result} = (P_U/\sum P) \times 100$$

P_U = percentage of amino acids on an as-is basis (obtained above)

$\sum P$ = sum of percentage of 15 amino acids on an as-is basis (P_U)

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Amino Acid Profile-Development

Amino Acid	Minimum Limit	Maximum Limit
Alanine	2.97%	3.62%
Arginine	2.65%	3.98%
Aspartic acid	7.10%	9.86%
Glutamic acid	19.27%	23.98%
Glycine	1.70%	2.31%
Histidine	1.75%	3.73%
Hydroxyproline	Absent	Absent
Isoleucine	4.30%	5.52%
Leucine	9.51%	10.27%
Lysine	6.57%	9.53%
Phenylalanine	3.84%	6.03%
Proline	9.69%	10.75%
Serine	4.90%	5.87%
Threonine	4.02%	4.46%
Tyrosine	3.78%	6.00%
Valine	5.20%	6.83%

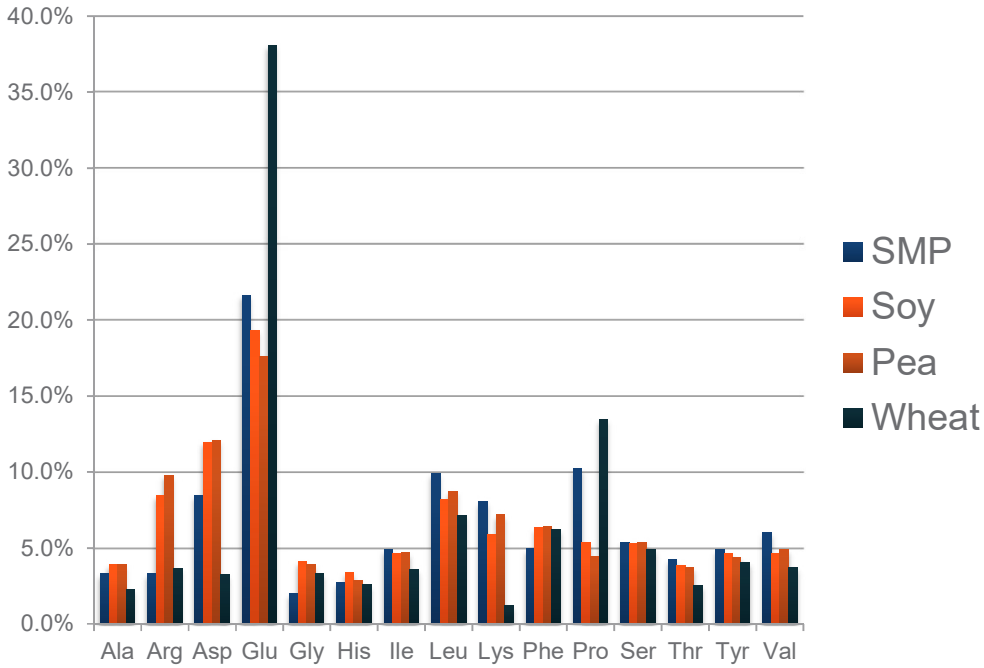
FCC Appendix XVI

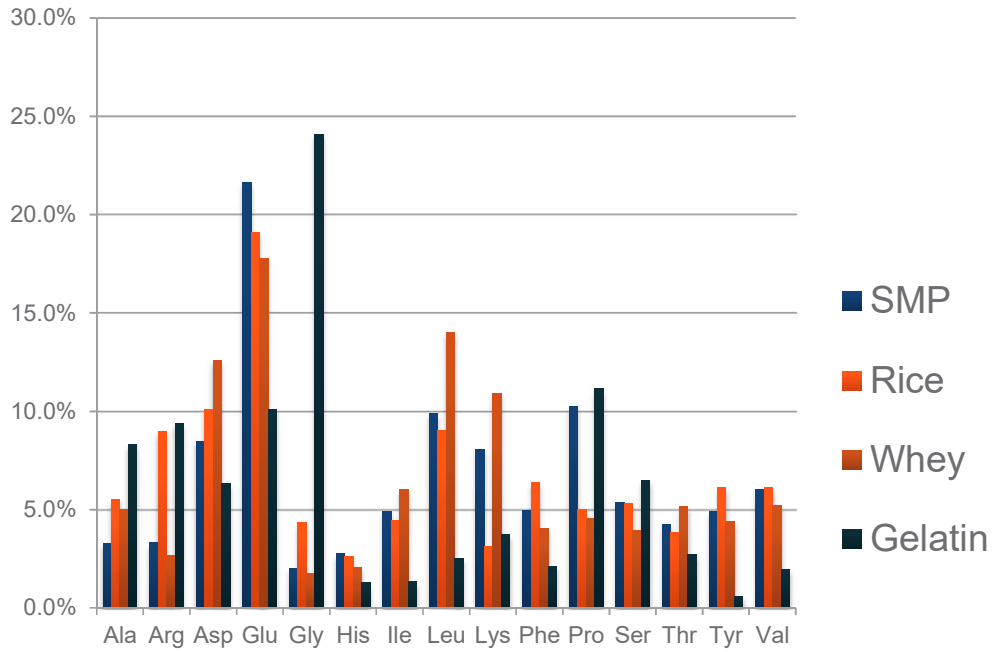
- The limits were calculated as mean ± k*SD, where mean is the grand mean of all results, SD is the root-sum of all variance components, and k is obtained as

$$k = t_{0.95,df} \cdot \sqrt{1 + \frac{1}{df + 1}}$$

Where $t_{0.95, df}$ is the 95th percentile of the Student's t-distribution having df degrees of freedom.

Amino Acid Profile-Development





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Amino Acid Profile-Development

Adulterant Name and abbreviation	Significant Adulterant Spike Levels (%)
Melamine (M)	No significance at 0.16%
Slightly hydrolyzed soy protein isolate (S)	1.0 (Gly*)
Pea protein isolate (P)	1.0 (Arg, Gly, Lys, Phe, Tyr)
L-arginine (A)	0.10(Arg)
Hydrolyzed wheat protein isolate (Wt)	No significance at 2.0%
Rice protein isolate (R)	2.0 (Gly)
Whey protein isolate (Wy)	1.5 (Leu)
High MW fish gelatin (G)	0.30 (Gly)

Peptide Identification of Dietary Proteins

Principle

- Protein digested by a specific protease generates peptides which are often characteristic of the protein used in the digestion. Peptides generated in this way can be detected and quantitated by a liquid chromatography-mass spectrometry (LC/MS) system to provide species information on the tested protein ingredients. This can be a useful diagnostic tool to help substantiate the identity of a protein ingredient.

- If expected peptides are missing, or unexpected peptides are present, an investigation into mislabeling or an intentional adulteration should be performed.

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Peptide Identification of Dietary Proteins

Extraction with 5.0 M Urea in 50 mM Tris buffer

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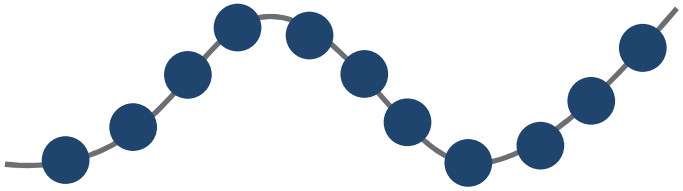
Reducing with 1 M DTT & Alkylation in 0.5 M Iodoacetamide

↓

Trypsin digestion

↓

LC-MS/MS (MRM)



Signature Peptides

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Peptide Identification-Development

Protein Analyte	Peptide	Q1 (m/z)	Q3 (m/z)	Collision Energy
Casein (α-S1-casein)	FFVAPFPEVFGK	692.9	920.5	29
	YLGYLEQLLR	634.4	991.6	33
	HQGLPQEVLNENLLR	880.5	1324.7	51
Whey (β-lactoglobulin)	VYVEELKPTPEGDLEILLQK	1157.1	1453	60
	VLVLDTDYK	533.3	853.5	23
	LIVTQTMK	467.3	707.4	21
Pea (vicilin)	EGSLLLPHYNSR	693.4	773.6	40
	GDFELVGQR	510.8	572.5	26
Rice (glutelin)	ALPNDVLANAYR	658.9	566.8	26
	LQAFEPIR	487.7	732.5	23
	GDEFGAFTPIQYK	736.9	1024.8	33
Soy (glycinin G1)	VLIVPQNFVVAAR	713.4	1001.6	33
	VFDGELQEGR	575.3	903.4	29
	LNALKPDNR	520.8	629.3	32

Peptide Identification-Development

System suitability solution 1: Prepare a protein mixture with approximately 20% each of pea protein concentrate, rice protein concentrate, soy protein isolate, and milk protein concentrate (whey and casein) on the protein basis calculated by Nitrogen Determination, Appendix IIIC. Using this protein mixture, prepare System suitabilty solution 1 according to the directions for the Sample solution.

System suitability solution 2: Spike 0.1% each of pea protein concentrate, rice protein concentrate, soy protein isolate, and milk protein concentrate (whey and casein) on the protein basis calculated by Nitrogen Determination, Appendix IIIC into a negative control matrix (branched-chain amino acids). Using this protein and amino acid mixture, prepare System suitability solution 2 according to the directions for the Sample solution.

Peptide Identification-Development

Tested Sample

VS

System Suitability
Solution 2 (0.1%)

The protein is confirmed as present in the sample if its peak area ratio is higher than that of System suitability solution 2 for all peptide transitions described.

This method is estimated to detect proteins as adulterants in authentic samples at a level of 0.1%.

Publication timeline

June FCC forum (June 30th 2020-Sep 30th 2020)
FCC 12 2S (Mar 1st, 2021)

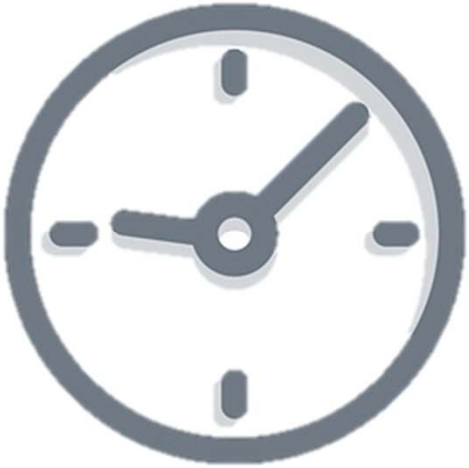
- Amino Acids Profile for SMP/NFDM
- Peptide Mapping ID

Dec FCC forum (Dec 30th 2020-March 31st 2021)
FCC 12 3S (Sept 1st, 2021)

- Intact Protein MS
- Capillary Gel-Electrophoresis
- Soy leghemoglobin

June FCC forum (June 30th 2021-Sep 30th 2021)
FCC 13 (Mar 1st, 2022, expected)

- Hemp Seed Protein



FCC Analytical Material Description

FCC Analytical Materials

FCC Analytical Materials are fit for purpose materials designed specifically for the food industry and can be used for method development, method verification, method transfer, method lifecycle management, method validation or for other purposes

Examples of FCC FAMs (in progress/released)

- Gluten in oat flour (released)

  7 individual packs (rye and wheat gluten 0, 10, 20 mg/kg in oat flour), 50 g each;

  Concentration based on gravimetric addition.

- Whey protein (in progress)

  4 individual packs (1 whey protein concentrate, 3 whey protein isolates), 10 g each;

  Identification based on 4 different state-of-the-art methods;

  Validated methods and data are included.

Other Analytical Materials

Reference materials both authentic and spiked with adulterants could be useful in developing and validating methods including non-targeted methods

- Skim Milk Powder (20 g)
- Skim Milk Powder with **Maltodextrin** Level A (5 g)
- Skim Milk Powder with **Maltodextrin** Level B (5 g)
- Skim Milk Powder with **Maltodextrin** Level C (5 g)
- Skim Milk Powder with **Melamine** - Level A (20 g)
- Skim Milk Powder with **Melamine** - Level C (20 g)
- Skim Milk Powder with **Melamine** - Level D (20 g)
- Skim Milk Powder with **Melamine** - Level E (20 g)

Summary

- Through international collaboration, USP-FCC develops methods, specifications, and materials to help guard against food fraud
- *USP-FCC* provides various analytical materials including matrix-based reference materials for method development and validation.

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