Alternative Proteins Safety Perspectives

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Conflict of interest statement

I am a full-time employee of Abbott Nutrition

The views and opinions expressed in this presentation are those of myself and do not necessarily reflect the position of Abbott Nutrition

Food ingredient safety assessments

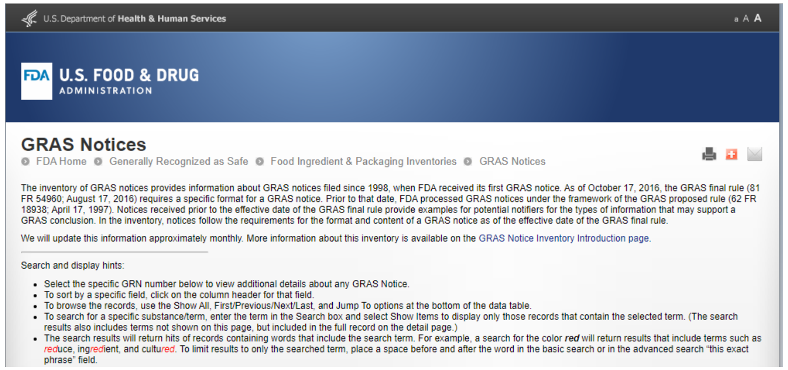
A new or updated safety assessment of a food ingredient is conducted when the food ingredient:

- Has never been consumed as food,
- Is produced through a new manufacturing process,
- Is being added to new foods, or
- Is being added to existing foods in higher amounts

The world of alternative proteins

Isolates from traditional foods

- Legumes: Pea, Fava, Mung bean, Soy
- Cereal grains: Oat, Rice, Barley, Canola
- Milk: Cow, Goat
- Other: Potato, Almond, Hemp, Fungal



U.S. Department of **Health & Human Services**

U.S. FOOD & DRUG ADMINISTRATION

GRAS Notices

FDA Home › Generally Recognized as Safe › Food Ingredient & Packaging Inventories › GRAS Notices

The inventory of GRAS notices provides information about GRAS notices filed since 1998, when FDA received its first GRAS notice. As of October 17, 2016, the GRAS final rule (81 FR 54960; August 17, 2016) requires a specific format for a GRAS notice. Prior to that date, FDA processed GRAS notices under the framework of the GRAS proposed rule (62 FR 18938; April 17, 1997). Notices received prior to the effective date of the GRAS final rule provide examples for potential notifiers for the types of information that may support a GRAS conclusion. In the inventory, notices follow the requirements for the format and content of a GRAS notice as of the effective date of the GRAS final rule.

We will update this information approximately monthly. More information about this inventory is available on the GRAS Notice Inventory Introduction page.

Search and display hints:

- Select the specific GRN number below to view additional details about any GRAS Notice.
- To sort by a specific field, click on the column header for that field.
- To browse the records, use the Show All, First/Previous/Next/Last, and Jump To options at the bottom of the data table.
- To search for a specific substance/term, enter the term in the Search box and select Show Items to display only those records that contain the selected term. (The search results also includes terms not shown on this page, but included in the full record on the detail page.)
- The search results will return hits of records containing words that include the search term. For example, a search for the color ***red*** will return results that include terms such as *red*uce, ing*red*ient, and cultu*red*. To limit results to only the searched term, place a space before and after the word in the basic search or in the advanced search "this exact phrase" field.

Precision fermentation

- Fungal-produced beta-lactoglobulin
- Yeast-produced beta-lactoglobulin
- Yeast-produced ovomucoid

What triggers a safety assessment?

- New food
- New process
- New consumption pattern

Food ingredient safety assessment

What is it?

- How is it manufactured?
- What is the specification?

How much will people consume?

- What foods will it be used in?
- How much of those foods do people eat?

Will amounts consumed be safe?

- What safety information already exists?
- Are additional studies needed?

These questions are the same for all food ingredients, not just alternative proteins

The history of fermentation in food production

Microorganisms have been used in food production since the dawn of civilization (e.g. bread, yogurt, cheese, beer)

The development of precision fermentation is an evolution of this practice, in which the microorganisms are precisely modified to efficiently produce food substances

Precision fermentation: Success story

Riboflavin (Vitamin B_2)

- First commercial production used chemical synthesis (1930s)
- Commercial production with non-modified microorganisms was explored in the 1960s, but was abandoned because it was not competitive with the chemical synthesized form
- In the 1990s, the first riboflavin was produced by a genetically modified microorganism (GMM) on a commercial scale
- By 2015, practically 100% of the supply of riboflavin was produced by GMMs

- Schwechheimer et al., (2016) Appl Microbiol Biotechnol 100: 2107-19
- Liu et al., (2020) Microb Cell Fact 19: 31
- Revuelta et al., (2017) J Ind Microbiol Biotechnol 44: 659-665

What is it? Specifications

Purity

- Nutritive content: protein, fat, carbohydrate
- Other desirable content: fiber, minerals, amino acids

Impurities

- Not readily avoidable substances like heavy metals
- Microbiological contamination
- Substances from the manufacturing process
- Other factors specific to the commodity:
  - Fava bean (GRN 879) includes alkaloid glycosides
  - Potato protein (GRN 447) includes glycoalkaloids

Establishing a specification is critical to evaluating the safety of a food ingredient

What is it?
Alternative protein examples

Parameter	Specification
Proximate analysis	
Moisture (%)	<7
Protein (%)[a]	>80
Fat (%)	3 to 5
Ash (%)	<8
Carbohydrate (%)	<10
Microbiological	
Aerobic plate count (CFU/g)	<100,000
Listeria spp.	Negative
Salmonella spp.	Negative
Escherichia coli	Negative
Heavy metals	
Arsenic (ppm)	≤0.05
Cadmium (ppm)	≤0.05
Lead (ppm)	≤0.05
Mercury (ppm)	≤0.025

GRN 684: Mung bean protein isolate

Chemical Properties (in powder as is)	Specification
Protein	> 75%
Moisture	Maximum 10.0%
Carbohydrate	Maximum 20%
Ash	Maximum 2.0%
Fat by Acid Hydrolysis	< 0.1%
Hg	< 1 ppm
Pb	< 1 ppm
As	< 1 ppm
Cd	< 1 ppm

Microbial Properties (in powder as is)	Specification
Standard Plate Count	< 10000 CFU/g
Yeast & Mold	< 100 CFU/g
Salmonella	Not Detected / 25g
E. coli	Not Detected / 25g
Total coliform	≤ 30 CFU/g

GRN 967: Soluble egg-white protein

Specifications are similar for proteins isolated from traditional foods and precision fermentation

How much will people consume?

<u>Isolates from traditional foods *and* precision fermentation</u>

- They serve as an alternative source of protein
- Using these proteins in a variety of foods would not result in an increase in overall protein consumption of protein
- Their use would not increase protein intake above the Recommended Dietary Allowance (RDA)*

*The RDA is a value “sufficient to meet the nutrient requirements” and is not a maximum/safety value

Will amounts consumed be safe?

1. Is the alternative protein different from proteins that have been traditionally consumed?
2. If so, how do we demonstrate that these differences do not pose any incremental safety concerns?

= OR? ≠

Will amounts consumed be safe?

<u>Hypothesis-driven safety assessments</u>

- Review available information to identify potential risks associated of the traditional protein
- Determine whether there are significant differences with the alternative protein versus the traditional protein either in production or composition
- Assess whether available information supports the safety of the traditional protein or whether additional safety studies are necessary

Identify potential differences: Digestibility of alternative proteins

An example of a difference would be protein digestibility

Both *in vitro* and *in vivo* digestibility methods exist to evaluate the digestibility of proteins:

Ingredient	Potato Protein		Soy Flour	
	Raw	Heated	Raw	Heated
Gain in Weight	103±15[c]	146±16[a]	72±12[c]	119±3[b]
Food Consumption, g	369±27[b]	419±27[a]	316±43[c]	425±28[a]
PER	2.16±0.23b	2.71±0.17a	2.21±0.18b	2.74±0.16a
Protein Digestibility[1] (Apparent %)	76.7±2.9[b]	84.5±1.6[a]	63.4±4.7[c]	76.7±3.1[b]
Size of Pancreas (% of body weight)	0.47±0.03[a]	0.34±0.04[b]	0.450.04[a]	0.35±0.04[b]
PABA recovery	41.5±7.0[a]	30.5±7.3[b]	42.1±7.9[a]	30.6±9.7[b]

Adapted from Lee *et al.* (1985)

[a-c] Mean values (± S.E) of 8 animals during an experimental period of 5 weeks. Mean values with unlike superscripts in the same row were significantly (P<0.05) different

[1] Protein digestibility (apparent) = N intake – N feces/Nitrogen intake X 100

GRN 447: Potato protein isolates

Table 2: Comparison of OVD and NSEWP in-vitro digestibility

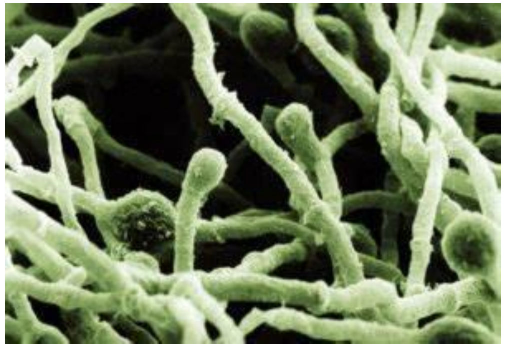
Product	In-vitro digestibility
SOL19303	93%
SOL19317	93%
SOL19351	93%
Native chicken OVD	92%

GRN 967: Soluble egg-white protein

Hypothesis-driven safety assessments

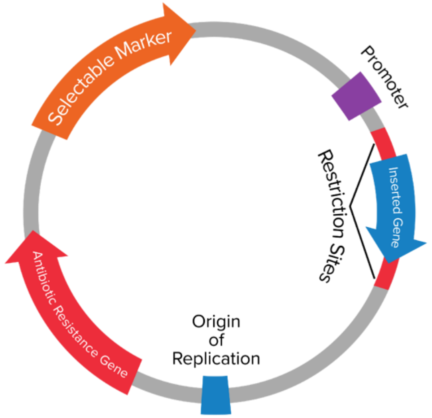
Precision fermentation

- Are there safety concerns about the production microorganism?
    - Pathogenic: Infects an organism (*Listeria monocytogenes*)
    - Toxigenic: Produces a toxin that harms the organism (*Clostridium botulinum*)
- Are there safety concerns about modifications to the production organism?



Trichoderma reesei
Long history of use in food (17+ GRAS notices)

List of genes incorporated into the host organism



Hypothesis-driven safety assessments

Additional safety studies may be necessary, such as:

- Assessment of general toxicity (e.g. 90-day rat study)
- Assessment of genotoxicity (e.g. bacterial reverse mutation, and micronucleus assay)

Some challenges exist for studies of alternative proteins, such as maintaining nutritional balance in animal diets*

*Standard rat diets are composed of ~20% protein

A lesson from enzymes

Many food enzymes are produced using precision fermentation, making these a common alternative protein

A review of over 200 genotoxicity and general toxicity studies on enzymes showed an absence of any adverse effects

This is expected, as proteins (including enzymes) are readily digested into amino acids by mammals after consumption

These studies are unlikely to provide any value in evaluating the safety of the majority of alternative proteins

Ladics and Sewalt (2018) Regul Toxicol Pharmacol 98: 151-154

The future is here, and it’s global!

In November 2022, The Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) are holding an expert consultation as a first step in developing guidance on the safety assessment of alternative proteins

Thank you

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