2021 Protein Trends & Technologies

Post-Webinar Magazine

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Overcoming Technical Issues: Formulating with Protein Ingredients

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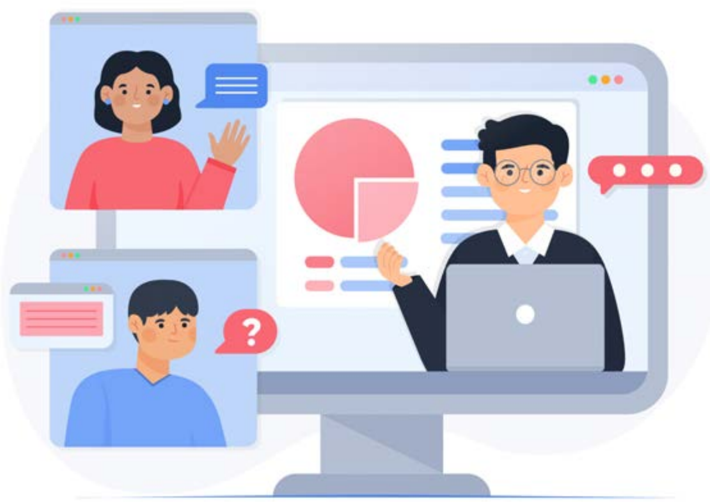
Overcoming Technical Issues: Formulating with Protein Ingredients

2020 became a transformative year for organizations whose operations were based on in-person interactions. As the Covid-19 pandemic felled conferences and expositions one by one, the food industry turned to digital alternatives.



For Global Food Forums, the cancellation of its 2020 and 2021 in-person events provided a new opportunity. In the spring of 2021, Global Food Forums developed its own Premium Protein Webinar titled "Overcoming Technical Issues: Formulating with Protein Ingredients," in addition to hosting ingredient vendor webinars.

This publication is primarily based on three presentations given during the Premium Protein Webinar. When working with protein ingredients, product formulators strive to optimize a product's texture, flavor and nutrition (i.e., protein quality). The webinar started with a short introduction on several industry presentations then delivered insights into each of these technical challenges.

As for those of you who miss the opportunity to share a glass of wine with an old friend—or to meet new ones—and discuss industry happenings in an energetic, in-person crowd setting, we hope to see you on October 25-26, 2022. (See page 26 of this issue.)

See https://globalfoodforums.com/global-food-forums-webinars and scroll down find access to the PowerPoints and videos of speakers at the 2021 Premium Protein Webinar: "Overcoming Technical Issues: Formulating with Protein Ingredients."

Warm regards,
Claudia O'Donnell & Peter Havens
Co-owners, Global Food Forums, Inc.

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THE MARCH FROM ITS ORIGINS in nature to final consumption is long for a protein ingredient. Found in countless environmental niches, a protein must be selected, harvested, processed and delivered to an end-user, whether animal or human. What makes one protein more desirable for commercial development than another is determined by a complex and ever-changing set of factors. Examples such as raw material reliability, cost at each transactional step, regulatory restrictions and consumer concerns—including social and environmental impact and safety and allergenicity—are just a few.

As proteins are refined into ingredients for formulated products, their functional, flavor and nutritional quality become increasingly important. Much research by industry and academia has been spent on improving and fine-tuning these attributes.

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Global Food Forum's webinar, "Overcoming Technical Issues: Formulating with Protein Ingredients," addressed texture, flavor and protein nutritional quality challenges.

A World of Proteins & A Need

THE U.N.'S DEPT. OF ECONOMIC AND SOCIAL AFFAIRS POPULATION DYNAMICS website puts the world's total population in 2020 just shy of 8 billion people. In 2019, it was estimated by a 95% probability that it will grow to some 9.4B to roughly 10.1B by 2050. (See population projections at https://bit.ly/3exUoe5). The race is on to efficiently feed humanity—ideally with foods they enjoy.

Despite proteins being the most expensive of the three macronutrients—followed by lipids and carbohydrates (Traverso, S. and Schiavo, *S. World Development*. 2020/ https://bit.ly/3vN6FRu)—they are also ubiquitous and plentiful. For example, recent research reconfirmed the belief that RuBiCos, a plant enzyme, is the most abundant protein on the globe (Yinon M. Bar-On, Y.M. and Milo, R., *Proc Natl Acad Sci USA*. 2019/ https://bit.ly/33qvBly). More than 90% of RuBiCos is found in land plant leaves and is responsible for most of Earth's global carbon fixation. The study estimated RuBiCos' total mass about 0.7 Gt (Gigatons = 10^{15} g), or some 1.4 trillion metric pounds of protein. In comparison, the authors calculated the most abundant animal protein, collagen, has a global mass of some 0.05 Gt.

Humans have long utilized proteins in forms such as fungi, algae, plants, insects and other animals. Driven by the coming global need, as well as significant business opportunities—and supported by accelerating technical capabilities—resources are being invested into specializing current proteins and developing new sources, such as single-cell proteins, cultured (lab-grown) meats and other meat alternatives (the latter primarily as finished consumer products).

These advances are broad and fascinating. For some, such as Global Food Forums' staff and customers, protein ingredient development and use are the most interesting segments. Optimizing raw protein materials into components specialized for formulated products usually adds the most value per pound. Efficient use of these fine-tuned ingredients better enables a finished product to be tailored for consumers' individual interests and needs.

In March 2021, Global Food Forums hosted its 2021 Premium Protein Webinar titled "Overcoming Technical Issues: Formulating with Protein Ingredients." Two of the three key presenters focused on improving the texture-modifying and nutrition-enhancing benefits of protein addition. The third delved into research needs and what can and cannot be done in efforts to improve the flavor of protein-containing products.

The webinar series, just under two hours, began with a brief introduction that presented a few industry statistics; addressed a question occasionally asked of Global Food Forums on protein concentrates and isolates; and offered a few interesting news items relevant to proteins. – *Claudia D. O'Donnell*

Emerging Protein Trends

THE WEBINAR SERIES began with a brief introduction titled "Emerging Protein Trends & Interesting News Bites" by Claudia O'Donnell, Co-owner and Co-founder of Global Food Forums, Inc. She kept her presentation to some 10 minutes, which did not allow time for a Q&A period. She explained, however, that she worked to compensate for this with robust references and links in the downloadable PowerPoint (and throughout this magazine summary) to supporting websites with more details.

How Much Protein in Concentrates & Isolates?*

This chart is a work in progress. All percents on a dry weight basis.

PROTEIN SOURCE	FLOUR	CONCENTRATE	ISOLATE	REFERENCE
Whey	—	≥25%	≥90%	ADIP (link to other dairy ingredients industry standard)
Whey Protein Concentrate	—	≥25%	—	US FDA
Soy	≥50% to <65%	≥65% to <90%	≥90%	Codex STAN 175-1989, modified
Vegetable Protein Products (VPP)		≥40% for VPP products		Codex STAN 175-1989, modified 2019
Pea		50-60%	≥80%	No known U.S. regulations. Stated values are reported general industry standards. to be confirmed. See Codex
Chickpea		50-60%	≥80%	No known U.S. regulations. Stated values are reported general industry standards. to be confirmed. See Codex
Vital & Devitalized Wheat Gluten		≥80%		Codex STAN 163-1987, Rev. 1-2001
More to come?				

SOURCE: CLAUDIA D. O'DONNELL, GLOBAL FOOD FORUMS 2021

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Little regulatory harmony currently exists for labeling the amount of protein in protein ingredients. *NOTE: This information should not be construed as legal advice. Please check with a legal expert in these areas.*

O'Donnell noted that the Covid pandemic has had a tremendous impact on the food industry. In the area of product development, R&D staffs whose work involve on-site lab work and pilot plant scale-ups had to adjust to work-from-home schedules. Consumer sensory testing or obtaining vendor ingredient samples (sometimes delivered from the trunk of a salesperson's car) also created challenges.

She turned to Mintel to ask if new food product launches had declined. Mintel's Global New Products Database, which tracks all types (including new packaging and line extensions), shows it varies by country. Brazil and the U.S. had a small increase in the percentage of new product launches in 2020 over 2019. In contrast, countries like France, Spain, Germany, Italy and the UK had declines in new product introductions in that period.

The pandemic has increased interest in immunity products. This has created some opportunities for protein and protein-related products. Most importantly, proteins play a part in healthy diets overall, a key element to resisting disease, O'Donnell said. Secondly, fermented and probiotic-containing or fortified protein-based foods, such as yogurt or high-protein bars, continue to enjoy a reputation for immunity support. Thirdly, supplements, such as collagen peptides, amino acids and whey proteins, with their higher level of branch-chained amino acids, have been touted by companies. There is some research on this, particularly their importance for those who are malnourished or extreme athletes, i.e., marathon runners. (Just two examples include Peng Li, et. al., *Br J Nutr.*, 2007/ https://bit.ly/3hbTXYy) and Cruzat, VF, et al., J *Int Soc Sports Nutr.* 2014/ https://bit.ly/3hhzEIW.)

Cow-derived lactoferrin, an FDA-GRAS protein with anti-microbial properties, has also been suggested for protection against Covid-19. (Raymond, C, et. al., *Int J Antimicrob Agents.* 2020/ https://bit.ly/3vQM7aU.)

Plant- and Animal-Sourced Proteins and Sustainability

Much attention has been paid to consumer interest in plant proteins. Some hypothesize that ramifications from the pandemic also may have encouraged plant-based diets. (Attwood, S. and Hajat, C., *Public Health Nutr.* 2020/ https://bit.ly/2Sr8Zz6.) Food manufacturers have certainly taken notice. Innova Market Insights observed +20% average annual growth from 2015 to 2019 in new food and beverage launches containing alternative protein ingredients that also made high-protein claims. Ingredient examples include pea protein isolate and chickpea concentrate, as well as hemp, sunflower, faba, rice, potato, pumpkin and wheat proteins, among others.

Supporters of plants as sources of protein point to benefits such as environmental sustainability, animal welfare and human health benefits, among others. However, a key reason animal proteins, particularly from dairy, are used in food formulations is due to their unique functional properties, taste and high-quality nutrition.

Aquafaba, the viscous liquid leftover after the chickpea cooking process, is not only a viable protein source with functional properties, but is also considered a sustainable, up-cycling product.

The latter enables label marketing claims of high protein content and a more desirable Nutrition Facts panel. As such, research is now underway to help plant proteins and protein blends to match animal protein gold standards in character and performance—and to develop unique new capabilities.

In turn, the animal protein industry also is striving to improve its position in the marketplace. For example, 2050 Environmental Stewardship Goals, set out by the Innovation Center for U.S. Dairy, have set goals by that year for the dairy industry to become carbon-neutral or better, and to optimize water use while maximizing recycling and improving water quality through the utilization of manure and nutrients. The organization reports that, due to recent changes in farming practices, "producing a gallon of milk in 2017 required 30% less water and 21% less land and had a 19% smaller carbon footprint than it did in 2007." (The Innovation Center for U.S. Dairy, accessed 2020. https://bit.ly/3f2hJ6C.)

Sustainability is not an easily defined concept; much depends on what metrics are being used. Life Cycle Assessment (LCA) is a tool that evaluates a product's environmental impact from material extraction through disposal. One recent paper updated LCA methodology to take into consideration the nutritional quality and quantity of the protein in a food, based on its Digestible Indispensable Amino Acid Score (DIAAS) and food serving size, based on the FDA's Reference Amounts Customarily Consumed (RACC). The paper then ranked a selection of foods by their "global warming potential" per serving.

By this methodology, peanuts, whey (protein powder), soy protein isolate and tuna had the lowest global warming potential per serving; salmon and peanut butter were ranked in the middle of the list. Some unexpected foods ended up with the greatest global warming potential. (A. Berardy, et. al., *Sustainability*. 2019/ https://bit.ly/3h8hOs0). (*For more discussion and clarification of calculations of whey protein for this study, see Slide 7 of O'Donnell's PowerPoint presentation at https://bit.ly/3euouPB.*)

How Much Protein in Concentrates & Isolates?

O'Donnell also took the opportunity to address a question occasionally posed to the Global Food Forums' staff, which is whether there are regulations governing the protein content of plant ingredients labeled as "protein concentrates" and "protein isolates."

The answer is "it depends." The U.S. food industry and Codex have established ranges for soy protein flour, concentrates and isolates. However, for other sources, currently only Codex requires "Vegetable Protein Products" to contain 40% or more of protein.

In the U.S., labeling for whey protein powders has some interesting flexibility. Without an upper limit for whey protein concentrate (WPC), theoretically, isolates (WPI) also can be labeled as concentrates. Some companies have chosen this option, since they felt consumers would perceive it to be a "more natural" component, O'Donnell reported. (See chart "How Much Protein is in Concentrates & Isolates" with links to regulations at https://bit.ly/2Q6nxn.)

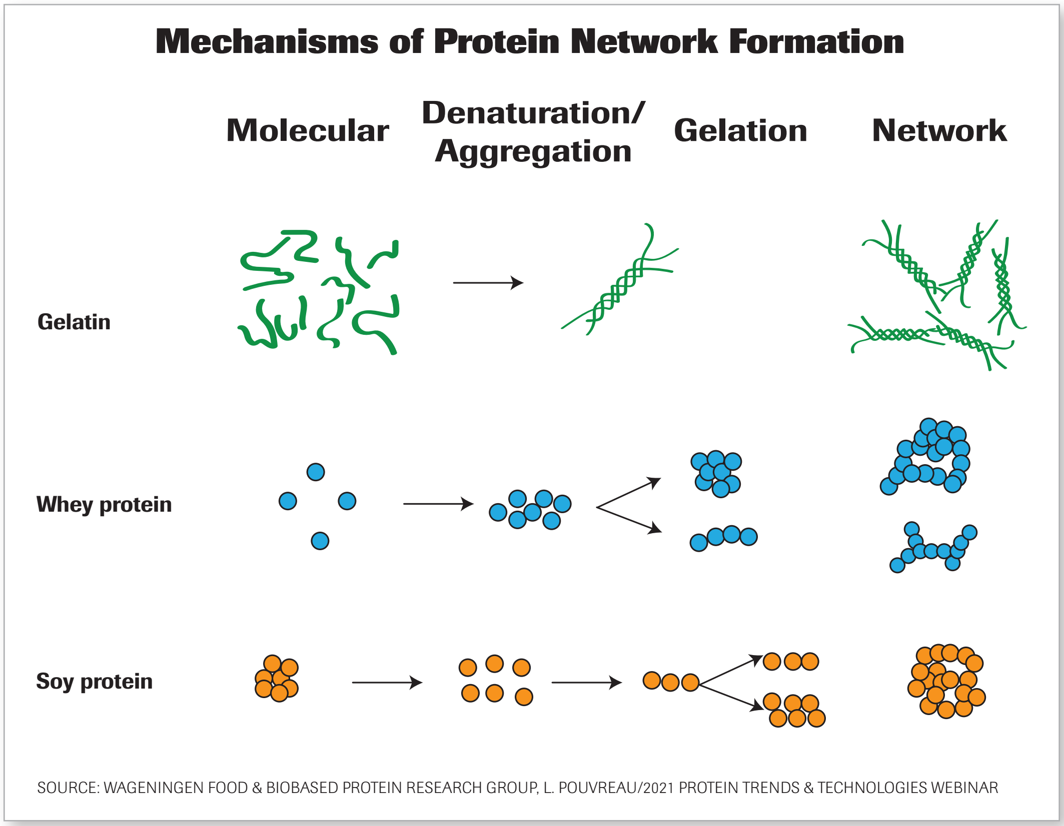
"Emerging Protein Trends & Interesting News Bites," Claudia Dziuk O'Donnell, MSc, MBA, Co-Owner, Global Food Forums

Blending Proteins to Increase Functional Performance

NUMEROUS DRIVERS HAVE FUELED the transition to using more plant proteins. Food production contributes some 25% of global greenhouse gases. World population is growing, and the demand for protein is expected to increase up to 50% by 2050. Public health could be improved by switching to a more plant-based diet. An important contribution toward this goal is blending proteins to increase functional performance.

A balanced diet between plant and animal protein is necessary to face these problems. "Protein blending can improve nutrition and also reduce the cost of protein in formulation," said Laurice Pouvreau, Ph.D., Research Scientist and Expertise Leader Plant Protein Technology, Wageningen University and Research. The presentation, "Beyond Nutrition: Protein Blending for Improved Structure and Texture," was given by Pouvreau at Global Food Forums' 2021 Premium Protein Webinar series.

In nutritional quality, for example, legumes are generally deficient in cysteine and methionine, while cereal proteins are



Every protein is unique in how it gels. High-temperature, heat-induced gels include those from whey and soy protein. Lower temperatures can induce gelatin-based gels. Examples of cold-set gels are those from acidification (yogurt); enzyme induced gels (rennet in cheese making); and through salt addition (release of calcium ions for tofu production).

deficient in lysine. Protein blends can improve amino acid profile and digestibility. Blending protein can also improve technical functionality by creating new and improved textures.

Established sources of protein include animal-derived proteins, cereals and grains. Emerging proteins include legumes and co-products, such as potato protein. Future proteins will include fermentative biomass and novel protein sources, such as duckweed.

It's important to choose the protein with the right functional properties for each specific application, noted Pouvreau. When working to develop a cheese- or yogurt-identical product, source proteins that are soluble and that provide aggregation and gelation are needed. For a bakery application, select a protein with good visco-elastic property, said Pouvreau.

For some applications, solubility is key. Solubility is impacted by many factors. For example, soy protein isolate exhibits different solubility profiles as a function of pH. There is less variation in less pure fractions, such as soy protein concentrate or soy flour, because they have been exposed to fewer processing steps than soy isolate. Examples of processing steps that impact solubility and functionality, and thus increase inconsistencies, include defatting, toasting, heating and (spray-)drying.

Plant proteins, in general, have lower gelling properties than animal proteins. Blending plant and animal proteins may lead to a synergistic affect, yielding higher gelling properties than for plant proteins alone. In some cases, the gelling properties of plant protein concentrates are higher than for isolates, due to the presence of other types of proteins or other compounds.

Many of the same consumers who seek plant-based proteins are also looking for clean label products. Most plant alternatives have a much longer list of ingredients, including those that many consumers don't understand or expect on their labels. One example is methylcellulose. By blending proteins, food companies can create the desired functionality and texture—while eliminating or minimizing added functional ingredients.

Some acidified gels partially replace sodium caseinate with soy protein. This replacement will affect the microstructure of the gel and may have significant effects on the mechanical properties of the gel. The right pre-treatment conditions of the soy proteins and sodium caseinate together can allow fine-tuning to achieve higher gelling properties in the finished product, Pouvreau advised.

Combining whey protein with soy protein can create a synergistic gel. Adding more soy protein increases the water-binding properties of the mixture, which might add juiciness to a finished product.

Another example of blending proteins to increase functional performance is the combination of soy protein and wheat gluten in textured vegetable protein and in high-moisture meat analogs. The addition of wheat gluten creates a more fibrous texture in both applications. Gluten is a unique protein with viscoelastic properties that provides elasticity and stretchiness to protein gels.

The take-home message for the food industry is that it needs to transition to more plant-based protein sources for sustainability and to feed the world. Every protein has its place, and a balanced approached to protein sourcing will be required to feed more people in the future.

Food scientists need to understand the effect of processing on the functionality of proteins, as well as on the amino acid pro-

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file and digestibility of protein. Blending can address both nutritional and textural issues and can create cleaner taste profiles. With the right protein blending, less added flavor is required. The vision of Wageningen University & Research is to find ways to create and use sufficient nutritious, delicious, sustainable proteins for the growing global population, concluded Pouvreau.

"Beyond Nutrition: Protein Blending for Improved Structure and Texture," Laurice Pouvreau, Ph.D., Research Scientist and Expertise Leader Plant Protein Technology, Wageningen University and Research

PDCAAS Values Directly Impact Label Claims

PROTEIN SOURCE	PDCAAS	CORRECTED PROTEIN CONTENT IN RACC (G)	PERMITTED PROTEIN CLAIM
Almonds	.39	2.5	No claim
Sunflower seeds	.66	4.1	No claim
Peanut butter	.45	3.2	No claim
Navy beans	.47	5.7	Good Source
Whole green lentils	.43	5.8	Good Source
Split red lentils	.54	5.6	Good Source
Split yellow peas	.64	5.7	Good Source
Chickpeas	.74	5.9	Good Source
Tofu	.56	8.22	Good Source

PDCAAS: GOOD SOURCE = 5 – 9.9G PROTEIN/RACC
DIAAS: GOOD SOURCE = 5 – 9.9G PROTEIN/RACC IF DIAAS >75

SOURCE: MODIFIED FROM MARINANGELI, CPF AND HOUSE, JD (2017) NUTR. REV. 75
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PDCAAS values of raw plant materials vary greatly. Blending of protein ingredients, whether plant and/or animal sources, permits higher values in finished products for stronger protein content claims.

Increasing PDCAAS Values with Ingredient Blending

"PROTEIN QUALITY IS GENERALLY ASSESSED as a function of its ability to meet the biological needs of the consumer. Different methods exist to determine the quality of dietary proteins, each with their own advantages and disadvantages." So began Dr. Matthew Nosworthy, Ph.D., Food Science and Nutrition Research Scientist, Guelph Research and Development Centre, Agriculture and Agri-food Canada, when introducing his talk, "Increasing Plant-Based Products' Protein Quality through Ingredient Blending." In turn, protein quality, as quantified by PDCAAS values, directly impacts label claims.

Aspects of protein quality include sustainability, functionality, color, taste, its health profile, its cost and the focus of this talk—nutritional quality. The evidence necessary to support protein claims on food labels includes assessing quality, i.e., how well does the amino acid (AA) pattern of the food match human AA needs. The second parameter is protein digestibility, defined by how easily the protein is broken down after consumption.

"Regulatory frameworks for protein content claims in Canada and U.S. are underpinned by the protein efficiency ratio (PER) and protein digestibility-corrected amino acid score (PDCAAS), respectively," explained Nosworthy. Presented in 2013 by the Food and Agriculture Organization of the WHO, the digestible indispensable amino acid score (DIAAS) is a novel approach to measuring protein quality but is not currently used for regulation of protein content claims. One important difference is that PDCAAS uses protein digestibility, whereas DIAAS is based on amino acid digestibility.

In 2020, Health Canada accepted the calculation of conversion from PDCAAS to PER (PDCAAS X 2.5 = PER) in an attempt to better harmonize regulations, said Nosworthy. (*Health Canada*. 2020/ https://bit.ly/3rVY8sX)

The PDCAAS is determined from the product of the amino acid score, AAS (calculated by dividing the food AA by the AA in the reference pattern, with the lowest ratio being the AAS), and true fecal protein digestibility. Fecal protein digestibility is determined by comparing fecal nitrogen output to the dietary nitrogen input, correcting for endogenous losses.

Protein content claims for foods are based on the product of the PDCAAS and the protein content of the Reference Amount Customarily Consumed (RACC).

Nosworthy explained that "the advantages of the PDCAAS are that it's simple; there are robust AA datasets; and values are additive to permit calculations of PDCAAS values for blends. But, as with the PER, the PDCAAS is determined using a rodent bioassay. Also, fecal protein digestibility is impacted by gut microbiota—which can lead to an overestimation of protein digestibility."

Additionally, PDCAAS values are truncated at 1.00, so proteins of higher quality are not easily identified and the quality of the blend may be inaccurate. PDCAAS assesses nitrogen digestibility

rather than amino acid digestibility—the two of which are not necessarily equivalent.

A PDCAAS value of 0.20 or more is considered a "significant source of protein" for all non-infant foods. Foods with PDCAAS corrected protein values of 5 to 10 grams per Reference Amount Customarily Consumed (RACC) can make a claim of being a "Good Source of Protein." And, "Those with PDCAAS corrected values of 10 grams or more can claim to be an 'Excellent Source of Protein,'" said Nosworthy. Several plant-based examples are noted in the table "PDCAAS Values Directly Impacts Label Claims."

Improving protein quality by blending is important in dietary planning/interventions and for ingredient selection related to quality for package labelling. Among the limitations of plant proteins in general include allergenicity (pea and others) and secure supply chains (rice, pumpkin seed, microalgae). Compositional challenges include low protein content (cereals) and anti-nutritional factors in pulses that reduce digestibility, such as protease inhibitors, tannins, phenolics and phytate. Limiting AA in cereals include lysine, leucine in ancient grains, and methionine, cysteine and tryptophan in pulses.

There is an increasing desire to reduce animal experimentation to determine protein quality (i.e., rats and ileal-cannulated pigs for PDCAAS and DIAAS, respectively). "PDCAAS is mathematically friendly and can be used to determine optimal blends," noted Nosworthy.

He described research that found a correlation between both digestibility and PDCAAS values generated from *in vitro* and *in vivo* methods. The blending of 50:50 of extruded buckwheat/pinto resulted in higher protein efficiency ratio values, increased digestibility and greater PDCAAS (Nosworthy et al. *J. Agric. Food Chem.* 2017/ https://bit.ly/2RgcSGF). Nosworthy suggested that "the use of *in vitro* digestibility analysis could be a potential replacement for current rodent assay for determining nutrient content claims with much less cost and effort." (Nosworthy, MG, House JD. *Cereal Chem.* 2017/ http://bit.ly/316GlTc.)

In one experiment (J.D. House, unpublished data), blending wheat flour with increasing amounts of pulse flour increased PDCAAS values from 0.39 to upwards of 0.80. This supports how blends can be designed to maximize quality.

During follow-up questions, Dr. Nosworthy indicated that achieving a PDCAAS of 1.0 or even greater [*Note: before truncation to one*] is possible, and ingredient companies are blending multiple sources for both nutrition and functionality. "It is important though that the processing method doesn't reduce levels of the limiting AA," he concluded.

"Increasing Plant-Based Products' Protein Quality through Ingredient Blending," Matthew G. Nosworthy, Ph.D., Food Science and Nutrition Research Scientist, Guelph Research and Development Centre, Agriculture and Agri-food Canada

Proteins and Flavors: A Challenging Mix

FOOD SCIENTISTS AND CONSUMERS increasingly utilize more plant proteins. "The whole area of flavoring proteins has been a long-term problem. It's even more interesting today, because people are seeking more protein from all sources in their diets," said Gary Reineccius, Ph.D., Professor Emeritus, Department of Food Science & Nutrition, University of Minnesota. In his presentation, "Flavoring Protein Foods and Beverages," Reineccius went on to discuss how proteins and flavors are a challenging mix for researchers and R&D staffs.

Protein is particularly good at binding volatile flavor compounds, and this is problematic in several ways. First, the challenge of working with novel proteins is that, as ingredients, they bring unique inherent flavors to the finished food. For example, plant metabolites that make peas taste like peas will get trapped in the protein and remain with the protein throughout the isolation process, impacting the final product, noted Reineccius. Unfortunately, this pea flavor will not be desirable in most product applications. Research is looking into how these proteins can be processed to remove undesirable flavors.

Secondary issues include how to initially flavor protein products. The challenge in initially flavoring a protein product is that added flavorings can be bound to protein side chains weakly via ionic, hydrophobic or hydrophilic bonds—or more strongly by covalent bonds, explained Reineccius. Hydrophobic amino acid

While cherry and lemon flavors are highly reactive, several fruit flavors, such as apple, banana, peach and grape, are fairly stable.

Q&A Provides Practical Advice

After the event, Reineccius followed up with responses to several questions regarding flavoring protein foods, beverages and supplements. Here is a sample. The complete published Q&A session can be found at the end of his posted PowerPoint presentation.

QUESTION: What are some flavors that are more stable currently on the markets?

ANSWER: Some of the fruit flavors are quite stable. For example, Concorde grape is exceedingly stable, while apple, peach, banana and other grapes are fairly stable. Some, such as cherry, lemon, coffee and almond, are terribly unstable.

QUESTION: How do we increase protein concentration without the plant protein off-flavor?

ANSWER: The off-notes that you are referring to are inherent in the protein that you are using. If you use more protein, you are going to bring in more off-flavor.

QUESTION: What are some tactics that could be taken to mitigate protein taste issues in a formula?

ANSWER: The industry has found ways to manage the off-flavors that accompany most protein sources. If the protein carries very mild or less noticeable off-notes, one might just put a flavoring that is more intense or defined. When the protein is carrying more pronounced undesirable flavor, the flavor company can design/compound a flavor that's more in line with the characteristic inherent flavor. The company would just reduce in their formula notes that would be provided by the protein source. It is a fairly successful approach but does limit one's choice of flavorings. Practical research is needed to address protein flavor interactions.

chains contribute to folding of the protein, creating hydrophobic pockets where flavor molecules can bind. The result is a part or all of a flavoring disappears within hours of flavor addition.

While these weak bonds are problematic, these protein/flavor interactions come to equilibrium over a short time and can be managed through flavor reformulation.

In contrast, carbonyls, thiols and sulfides create sites for covalent chemical bonding to occur. These strong covalent bonds form rapidly during heat processes, such as pasteurization or sterilization. These bonds also develop slowly over shelflife, resulting in flavor loss or off-flavor buildup. Covalent bonds do not come to equilibrium. They consume both the amino acids and the flavor. Weak bonds are manageable, but covalent bonds are not, said Reineccius.

Flavor research over the past 60 years has focused primarily on weak bonds. We have learned that the more hydrophobic a flavor molecule is, the more it will bond to a protein. If a functional group moves to a different part of molecule, it will change the bonding. The location of hydrophobic groups in molecules determines how strongly they will bond. A great deal has been learned about these reactions. To the contrary, we know very little about the factors that influence covalent bond formation.

Covalent bond formation may determine the shelflife of a food product. At the end of product shelflife, off-flavors may have increased; desired flavors decreased; or perhaps both have occurred. Traditional ways to extend shelflife, e.g., use of antioxidants, encapsulation or expensive gas packaging, maybe unnecessary, if we can protect the added flavoring from reaction with proteins.

Strategic Flavoring Tactics

When a protein carries very mild or less noticeable off-notes, industry might just add a flavoring that is more intense or defined. When the protein is carrying a more pronounced undesirable flavor, the flavor company can recommend or design a flavor includes the protein off-notes—build a flavoring “around” the inherent, characteristic off-flavor. Finally, the flavor company can create a protein-stable flavoring.

Since covalent bonding is the biggest problem and a flavoring issue researchers know relatively little about, Reineccius's research team was motivated to developed new methodologies to measure covalent bond formation. They have found aldehydes and some sulfur compounds to be very reactive. In fact, within 10 minutes of adding a flavor to a protein, one can detect flavor-protein bonding.

Proteins differ in affinity for flavor components. A recent article showed that in a 5% casein solution with added citral (lemon) flavor, casein consumed 70% of the citral flavor.

Many of the commonly used flavor compounds have high rates of covalent reaction. The base for butter flavor is diacetyl, which is very reactive with proteins. Other highly reactive flavor compounds include those for common cherry, cheese, lemon and meat flavors. Sulfur compounds, which are used to create grapefruit, mustard and garlic, are also very reactive. In contrast, some of the fruit flavors are quite stable.

Reineccius's group has studied factors that affect flavor reaction, including food water activity, temperature and pH. These interactions are not fully understood. Industry should know more about which proteins are most reactive and how to protect flavors in protein applications. One solution might be to block the protein by adding a sugar molecule.

Reineccius would like to challenge the flavor industry to develop novel, stable flavorings. Flavor chemists have traditionally created cherry flavor by using benzaldehyde. Could they create something

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red, sweet and tart, built around a different aroma chemical that is not as reactive with protein, and that would ultimately be more stable? He mentioned that letters from industry may help the university to secure additional funding for research into protein-flavor interactions; he asked anyone interested in offering a supporting letter to contact him.

"Flavoring Protein Foods and Beverages," Gary Reineccius, Ph.D., Professor Emeritus, Department of Food Science & Nutrition, University of Minnesota

The Big 9: Allergens and a Proactive Approach

THIS YEAR, SESAME became the ninth major food allergen for which labeling is required under the Food Allergen Labeling and Consumer Protection Act (FALCPA) in the U.S. With sesame's addition, the "Big 8" became the "Big 9."

As with most food allergens, proteins are central to triggering allergenic responses. The topic has been the subject of excellent presentations given by Steve Taylor, Ph.D., then Professor and Co-director, Food Allergy Research and Resource Program (FARRP), University of Nebraska (since retired), at past Global Food Forums' Protein Trends & Technologies Seminars.

Some 85 million Americans have food allergies and intolerances—of which 32 million have a potentially life-threatening condition, according to the Food Allergy Research & Education (FARE) website (foodallergy.org). A Google search using a phrase such as "increasing rates of food allergies" results in a multitude of surveys, published research and news items providing credence that the health issue is a growing global phenomenon. Although some studies have questioned the evidence (Messina, M. and Venter, C., 2020, *Nutrition Today*/ https://bit.ly/3gSw1cB), there is little doubt consumer allergy awareness has grown in the last few decades. On April 23, 2021, President Joe Biden signed the FASTER Act which "expands the definition of 'major food allergen' for purposes of certain food-labeling requirements to specifically include sesame," as well as requiring food allergy research to be given higher priority. The Act is scheduled to be implemented January 1, 2023.

With the topic of allergens fresh on the food industry's collective mind, we offer Steve Taylor's sage insights and advice here with only the most minor of updates. As he joked during his introduction at the first of two presentations: "It takes a lot of guts to talk about allergens at a protein conference." However, his take-away messages remain as important as ever.

Could Novel Food Sources of Protein Become Allergenic?

- Inevitable; reports of allergic reactions should not be a surprise
- But perhaps not commonly; prevalence will be predictable to some extent
- Not all adverse reasons will be allergic reactions (Quorn)
- Should not be a deterrent to development of new protein sources
- Clear labeling is the key

SOURCE: FOOD ALLERGY RESEARCH AND RESOURCE PROGRAM (FARRP), UNIVERSITY OF NEBRASKA

Allergens and the Importance of Communications

There are a lot of myths about food allergens, including the myth that certain proteins are non-allergenic. In fact, every protein has the potential to become an allergen.

The key to dealing with allergens is careful management within manufacturing facilities and clear communication to consumers on the food label. So advised FARRP's Taylor during his presentation, "Allergens—It's Really Just a Management and Communications Issue," at the 2015 Protein Trends & Technologies Seminar.

Food allergies are abnormal responses of the human immune system to substances in food. "When an individual is exposed to protein, that exposure can stimulate the creation of IgE antibodies that create sensitivity to that protein. Individuals don't have symptoms during the sensitization phase. The next time the individual is exposed to the protein, however, the body reacts and releases a host of physiologically active substances in tissues and the bloodstream," explained Taylor.

In the U.S., eight foods (cows' milk, egg, crustacean, fish, peanut, soybean, tree nuts and wheat) are the most common causes of food allergies. [*Editor's Note: To this list, sesame has now been added.*] These Big 9 are responsible for some 90% of all food allergies on a global basis. Common allergenic foods in other countries include buckwheat in Japan and lupine in the EU.

The most common allergenic foods tend to be consumed frequently and in relatively large quantities. With the exception of crustaceans, they are typically consumed in early life stages. Most are excellent sources of protein. Another factor that determines allergenic capability of a food is resistance to digestion in the stomach, which allows the proteins to enter the small intestine in an immunologically active form.

To predict the allergenic potential of a novel protein, one should first perform a thorough review of global allergenic literature. Explore if the protein ingredient is allergenic in other countries; if it contains a potentially cross-reactive protein; or if it is botanically

related to other allergens. Insects are invertebrates, as are crustacean shellfish. Taylor recommended putting a warning on insect ingredients, such as "Not suitable for individuals with shrimp allergies."

Food allergens are commonly classified into families by their shared amino acid sequences and conserved 3-D structures. Knowing if a novel food source contains any of these amino acid sequences could help predict if that food could one day become allergenic.

There are four main families of plant-based food allergens.

• **Prolamin superfamily**—this includes Ara h 2, which is present in peanuts. This family includes allergens in walnuts, peanuts, sesame, mustard and sunflower. It also includes gliadin, a component of gluten.
• **Cupin superfamily**—includes seed storage proteins, peanuts, soybeans and other legumes.
• **Bet v 1 family**—present in birch trees.
• **Profilins**—present in all species of animals and plants, but not a major concern, because they are heat-labile.

There are also three main families of animal-based food allergens.

• **Tropomyosins**—the major allergens of crustacean shellfish and, probably, insects.
• **EF hand proteins**—includes parvalbumin, the major allergen in fish.
• **Caseins**—the major allergens in milk.

Foods should not be marketed as non-allergenic. It would be more accurate to state that the product "contains no commonly known allergenic foods." Companies working with novel protein ingredients might consider seeking insights from the FDA as to how that organization will handle new information about potential allergens, advised Taylor. Companies should also be aware that regulations for novel food products in other countries may differ from U.S. regulations.

With clear labeling, consumers who develop allergic reactions will be able to avoid the offending food. Allergenic potential should not be a deterrent to marketing of novel food protein sources, said Taylor.

The Allergen Challenge of Emerging Proteins

Taylor again spoke at the 2018 Protein Trends & Technologies Seminar. In a richly illustrated presentation with global and historical examples on the topic of "Food Allergies: A Challenge for Current and Emerging Proteins," he discussed a key and growing concern that food manufacturers need to keep in mind when using novel proteins in food products: Can novel food sources of proteins be allergenic?

Beyond the buckwheat in Japan and lupine in the EU, other countries include additional foods, such as mustard, molluscan shellfish and lupine on their lists of allergens.

If a "new" protein source is biologically related to a known allergen, such as insects and crustacean shellfish, it is advisable to put a warning on insect ingredients like "Not suitable for individuals with shrimp allergies."

While the importance of the Big 9 allergens is not debatable, data on prevalence, potency and severity to support the inclusion of these other foods on allergen lists may be limited.

When new proteins are introduced as foods, allergenic reactions in some individuals will inevitably occur. As precedent, Taylor cited the emergence of soybeans as a novel food source in the U.S. in the 1930s. While soybeans had been consumed in Asia for thousands of years, it wasn't until the 1950s, when soy-based infant formula was developed for milk-allergic infants, that soy allergies were recognized in the U.S.

Reactions to multiple allergens are usually due to cross-reactivity to a similar antigen found within different foods. Sometimes, the allergenic potential of novel proteins can be predicted because of their similarity to other allergenic proteins. Lupine, a legume that has historically been used in cattle feed, is botanically similar to peanuts.

Lupine protein can trigger allergic reactions in some individuals with peanut allergies, which raises a labeling conundrum: Do you warn those with peanut allergies not to eat the food, when only about 20% of Europeans with peanut allergies also have lupine allergies?

Cross-reactivity can also make it difficult to pinpoint the precise allergen that triggers a reaction, especially when cross-reactive allergens with differing potencies may be present. Taylor described severe allergic reactions that occurred in peanut-allergic individuals who consumed a soy-containing, muscle-building supplement. Taylor's group demonstrated that there was no peanut (a highly potent allergen) present; instead, very high levels of soy protein in the product likely caused the reactions.

Soy protein has a low potency that appears to share cross-reactivity with the peanut allergen. Proteins that demonstrate allergenic cross-reactivity may also be challenging to distinguish

analytically. Pea protein is surging in popularity right now and may have allergenic cross-reactivity with peanuts. The analytical challenges in differentiating pea vs. peanut protein have elicited food recalls for potential peanut allergen presence—even though pea, not peanut, was present.

It's important to remember that not all adverse reactions associated with novel proteins are allergic reactions. Quorn is a fungal mold product that is high in both protein and fiber and is popular in the U.K. In some individuals, the high fiber content of Quorn can trigger gastrointestinal symptoms that may be confused with allergic reactions.

As consumer appetite for protein grows, novel proteins will continue to be developed. Determining the allergenicity of new proteins can be difficult and expensive. However, knowing whether the food product has already been shown to be allergenic (in other parts of the world where it is consumed), and whether the food is related to known allergenic foods (i.e., is it a legume?), may help predict whether a novel protein could have significant allergenic potential, concluded Taylor.

[*Of potential interest to insect protein enthusiasts, Australia and New Zealand require the labeling of the insect products bee pollen/propolis and royal jelly. Examples of other protein sources not included in the U.S.'s Big 9 but have regulatory requirements elsewhere include beef, chicken and pork in Korea. Japan recommends voluntary labeling for these three popular American proteins as well as for gelatin, matsutake mushrooms, squid and salmon roe. Only a very few countries, the U.S. being one of them, do not require the labeling of cereals with gluten.*]

The original PowerPoints from of Steve Taylor's 2015 and 2018 presentations are available for downloading.

- **Food Allergies - A Challenge for Current and Emerging Proteins** https://foodproteins.globalfoodforums.com/food-protein-rd-academy/protein-and-food-allergies-presentation
- **Allergens - It's Really Just a Management and Communication Issue** https://foodproteins.globalfoodforums.com/food-protein-articles/of-allergens-and-proteins/

As with many other companies and industries, the Covid-19 pandemic accelerated developments within Global Food Forums. A more robust expansion into webinars, as well as a redesigned website became top priorities when in-person conferences were put on hold.

Global Food Forums continues its focus on three themes: Food Proteins, Clean Labels and Sweetener Systems. Since its inception, the company has strongly relied on independent, expert sources of information on applied food science—such as professors, consultants and R&D at consumer food companies. The result is objective advice on the use of ingredients in formulated foods and beverages. Additional presentations on updated regulations, market trends, nutritional insights and emerging technologies help put ingredient choices within the broader industry context.

This section provides a brief overview on the types of content that are available, suggestions on how to navigate the websites and tips on where to access various categories of information. Although there continues to be fine-tuning at GlobalFoodForums.com, we believe that our content—generated by food scientists for food scientists—will prove invaluable.

• In-person Events – Back in 2022!

Despite the heightened push into digital products, Global Food Forums again will offer in-person events for which it is best known. Combined Clean Label and Sweetener System Conferences will be held May 24-25, 2022, in Itasca, Illinois, a suburb near Chicago. On October 25-26, 2022, the 9th Protein Trends & Technologies Seminar will be held at the same location. Events' details will be updated at:

- https://cleanlabel.globalfoodforums.com
- https://sweeteners.globalfoodforums.com
- https://foodproteins.globalfoodforums.com

• Webinars

Https://globalfoodforums.com/global-food-forums-webinars offers a running list of free webinars, both up-coming and from the past, that are on-demand. Webinars are designed by Global Food Forums' staff and others by outside companies.

Ingredient supplier webinars provide detailed information on benefits and use of their products. Written summaries of most, if not all, webinars will be available. As of June 2021, examples include the following.

One can navigate between Global Food Forums' four websites by clicking on the logos at the top of each page.

• https://bit.ly/34Rfgag - A webinar titled "Protein Ready-to-Drink Formulation Tips" by Idaho Milk Products focuses on the importance of achieving nutritional quality and functionality in enteral formulas. Recent developments in reduced-mineral milk protein concentrate (MPC) and reduced-mineral milk protein isolate (MPI) enable enteral product formulators to achieve product quality and functionality.

• https://bit.ly/3vvARQB - "New Clean Label Texturizing Solutions for Dairy & Plant-Based Alternatives" by AVEBE delves into the formulation of products with a desired sensory profile, cost and shelflife by use of Etenia™. This consumer-recognizable ingredient is traceable and sustainably sourced, explains the company.

• https://bit.ly/3cou0ld - "Plant-Based Protein Debate: What Consumers Want" is a webinar by US Soy and Quality. It provides information on the sustainability, nutrition, as well as protein quality and functionality of a variety of protein ingredients. Consumer insights from an April 2020 United Soybean Board survey are also included.

• https://bit.ly/3x1Liwy - Global Food Forums produced the "2021 Premium Protein Webinar—Overcoming Technical Issues: Formulating with Protein Ingredients" that consisted of four presentations. Three were food technology-based and designed to deliver practical insights into common product development challenges for product formulators. A session each was presented by Laurice Pouvreau, Ph.D., Wageningen Food & Biobased Protein Research Group (on protein ingredient functionality); Matthew G. Nosworthy, Ph.D., Agriculture and Agri-Food Canada (on protein quality, which impacts label claims); and Gary Reineccius, Ph.D., Dept. of Food Science & Nutrition, University of Minnesota (on flavorings and proteins). These presentations are the basis of this publication.

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Additional Resources (cont.)

• Magazines – Print & Online
The webpage https://globalfoodforums.com/magazines provides access to download publications with articles based on presentations given at Global Food Forums' conferences, seminars and its own webinars. Examples range from the 2013 Protein Trends & Technologies Seminar post-seminar publication summarizing presentations, such as "Protein Packing Products: The Nutritional Rational," by Christine Steele, Ph.D., then Director, Science, Innovation & Education, Abbott Nutrition; and "Using Protein-rich Components to Achieve Desired Labeling," by Scott Martling, MS, then Group Leader R&D, International Food Network. And the most recent: this publication!

• Websites
Four websites now comprise GlobalFoodForums.com. Besides the parent website, https://GlobalFoodForums.com, each of the company's three areas of focus—Food Proteins, Clean Labels and Sweetener Systems—has its own dedicated site. The sites are extensively interlinked, and one can easily navigate between them by clicking on the desired site's logo at the very top of each page.

Work continues adding and updating tags attached to posts articles. For example, if one clicks on the tag "plant proteins" found at the bottom of many articles, presentations and other posts on the food protein website, a dynamic list of relative pages appears. See https://foodproteins.globalfoodforums.com/tag/plant-proteins. Similarly, clicking on the tag "antimicrobial" found on pages on the Clean Label website brings up a number of relevant presentations (please see https://cleanlabel.globalfoodforums.com/tag/antimicrobial) or the tag "sweet taste perception" on the Sweetener Systems website brings up relevant information in that area (https://sweeteners.globalfoodforums.com/tag/sweet-taste-perception).

Global Food Forums, Inc. wishes a hearty thank you to all the speakers, sponsors and staff that have and continue to contribute to its efforts to provide helpful and interesting information to the industry.