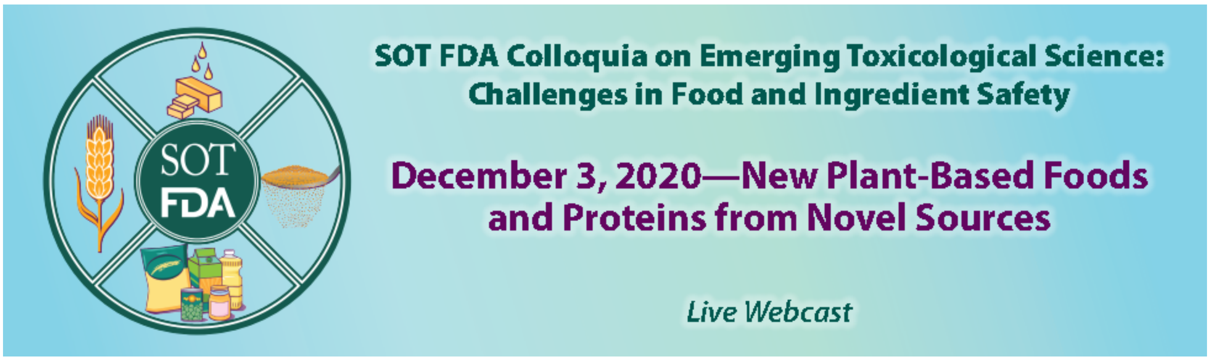

Real-Time Captioning

Note: This is not a transcript.

December 3, 2020

New Plant-Based Foods and Proteins from Novel Sources

Schedule

Schedule (All times are Eastern US, UTC -4)

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9:45 AM–10:20 AM	Functions and Appeal of Plant-Based Proteins and Novel Proteins Baraem (Pam) Ismail, University of Minnesota, Minneapolis, MN
10:20 AM–10:30 AM	Break
10:30 AM–11:05 AM	Future Developments in Plant-Based Foods Michael Leonard, Motif FoodWorks, Boston, MA
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11:40 AM–12:35 PM	Roundtable Discussion Moderator: Jason Dietz All speakers

Jason Aungst: Good morning. Welcome today's colloquium sponsored by the FDA Center of Food Safety and Applied Nutrition and Society of Toxicology. We have a new topic, New Plant-Based Foods and Proteins from Novel Sources. Before we get started, I would like to run through some house-keeping items. We'll have four talks and panel discussion. We'll take a break between the second and third talks, about 10:20 AM Eastern Standard Time. If you have questions either during the talks or the panel discussion, please type those into the chat box. If you can, you can send those to me, my name is Jason Aungst. We have more than 200 people registered for today, so it's possible we might not get to all the questions, but we'll do our best. I would like to turn it over to Dr. Dennis Keefe for some introductory remarks, and we'll hear from the moderator Jason Dietz. Good morning.

Welcome
Dennis Keefe, OFAS Office Director, US FDA, CFSAN, College Park, MD

Welcome to today's colloquium. I'm Dennis Keefe, I am a director of the Office of Food Additive Safety in FDA Center for Food Safety and Applied Nutrition. The role of our office is tone sure the safety of substances added to food. We do this to premarket review of submissions. Some of the substances are like high intensity sweeteners, like Stevia or sucralose. Also, we look at the materials use food handling and preparation such as polymers used in food packaging. We look at products of biotechnology, such as genetically engineered plants or protein carbohydrates or lipids produced in microbes. Some of which you'll hear about today.

In a typical year the office receives and reviews about 400 submissions and referrals from other agencies for new ingredients. The ofice itself is staffed with about 100 scientists with advanced degrees and experience ranging from chemistry to toxicology to biology. We have about 100 or so highly trained multidisciplinary scientists in one organization working together committed to ensuring safety of food ingredients, including food packaging. If I could have the next slide. Actually, it's the next slide.

One aspect that's key to our work is understanding new trends and technology. We see remarkable advances in technology, at work, at home, in our communities and even in the grocery store. Technologies such as artificial intelligence or AI, blockchain, big data, genome editing, nanotechnology are all examples impacting food protection and distribution in ways that consumers see and in some ways they don't see. For us at OFAS, it is important that we understand those technologies and their impact on food safety. You must keep up with the technological advances of new ingredients to inform safety assessments of new ingredients. This information is also important for other scientists beyond OFAS. That’s why we’ve teamed with SOT to host these colloquia as a forum to explore the advances in science and technology and their impacts on toxicology and food safety. We started this collaboration back in 2014. Over the years it’s had proven to be very successful, stimulating a dialogue among experts on future oriented science relevant to food. These colloquia are a forum to discuss the latest toxicological science in the context of food chemical but they are not a forum for regulatory advice or food ingredient regulatory issues. For example, we're not going to discuss the policies around food labeling today. The purpose of the series is to discuss scientific and technological advances as they relate to food toxicology and safety assessments. I think the next slide.

Finally, these colloquia are all open to the global scientific community and to the public via web cast at no charge. This is the second colloquium we've had this year. If you missed past colloquia, I'm pleased to let you know all of the colloquia are available, all the past colloquia are available on the Society of Toxicology website which is www.toxicology.org. Now moving. If I could have the next slide.

I want to recognize the FDA SOT [inaudible]. This is a group composed of FDA scientists as well as scientists around the world. They have done a lot of work in the background. While they may not be presenting today, the series would not be possible without their work. We're are excited to bring you a colloquium on plant-based foods and proteins from novel sources. We have lined up an excellent slate of speakers from government, academia and industry and have had over 200 already from around the world. We look forward to another informative discussion today. So, thank you, and I hope you enjoy the colloquium. Jason, I will turn this back over to you.

Overview and Speaker Introductions
Jason Dietz, Colloquium Chair, US FDA CFSAN, College Park, MD

Thank you. Can I have the next slide please. Wonderful. Thank you, Dennis. Very much appreciate those welcoming remarks. My name is Jason Dietz, the biotechnology coordinator at the FDA Center for Food Safety and Applied Nutrition, and in that role, I coordinate biotechnology issues across our center. I will be the moderator and chairperson for today's discussion.

I think you'll find it to be a very stimulating discussion as Dennis mentioned. This is one of the hottest trends. In fact, if you've been to the grocery store recently, you've probably noticed there's an increase in foods described as plant based. According to the International Food Information Council's 2020 Food and Health Survey 28% of consumers say they're eating more protein from plant sources. 24% are eating more plan- based dairy. 17% are eating plant-based meat alternatives. These foods take the form of burgers, milk, jerkies and even eggs. The ingredients that often make these foods work or function as we anticipate similar products to function are plant proteins from novel or what we might think of as nontraditional sources of protein and this list of sources can be quite interesting including, peas, cashews, green banana, chickpea, sunflower, and I've seen hemp, just to name a few.

When we say plant-based proteins, we may mean a collection of proteins or individual protein used to do a specific job. For instance, to optimize flavor or provide color to a product. I've also seen instances in which one plant protein maybe substituted for another with food functionality. We'll hear about this as part of our series. Next slide.

Some during the colloquium, Jeremiah Fasano of FDA will talk about where we might find proteins from novel proteins in our food supply and some examples of these proteins. Pam Ismail from the university of Minnesota will focus on how these proteins perform in foods and why consumers and food manufacturers are interested in foods with the proteins. Michael Leonard of Motif FoodWorks will give us a glimpse of what the future might bring from an industry perspective and how some of the modern technologies are being applied to this field. Finally, we'll hear from Supratim Choudhuri of FDA to discuss how toxicologists have assessed the food safety of new proteins. Then we'll have a panel discussion with all our speakers where you will have the opportunity to ask questions. I want to stress our focus is on a scientific discussion around the foods. So, we will not be getting into regulatory and policy issues. To reiterate what Jason Aungst has said, if you have a question, please direct it to him in the chat and we will do our very best to try and address them as best we can, recognizing the limits of time. What I envisioned is after each talk, if there's a question of clarification or two, I think we'll have time to entertain them there. For broader questions, they would be useful to save for the broader panel discussion.

So, let's move to our first speaker today. Jeremiah Fasano a senior policy adviser in the Office of Food Additive Safety’s Regulatory Review Branch. He's worked on a variety of issues during his time at CFSAN, including premarket evaluation of new food ingredients, the assessment of genetically engineered new plant varieties, the development of a safety assessment framework for new food technology and functionalities. So, I think we're in good hands with Jeremiah here. Jeremiah, take it away.

Proteins in Our Diet from Novel Sources
Jeremiah Fasano, US FDA CFSAN, College Park, MD

Thanks, Jason. Good morning, all. As Jason said, my name is Jeremiah Fasano. I work in the Office of Food Additive Safety here at CFSAN. Next slide please. I have no conflicts of interest to declare. Next slide.

So, my goal today is just to provide an overview to sort of kick this topic off. We'll hear from a number of other speakers who will dive into various aspects in more detail. I’m going to provide some context. Next slide.

So, it used to be when people thought about plant proteins in the context of food, they were thinking about things like soybeans, legumes, consumed in relatively unprocessed state, but as Jason already alluded to, that's not where we are anymore. The landscape has gotten more complex. Next slide.

There's interest in this complex landscape on all sides as evidenced by this colloquium today, but you can easily see it from a scan of the trade pres or the popular press. There’s interest from consumers about the kinds of foods that are becoming available to them and what's actually in those foods. There's interest from the food industry, folks looking to develop new sources of plant proteins or taking advantage of these protein sources, and as always, there's interest from the regulators whose job it is to make sure that the food supply is safe and to understand new substances being added to the food supply. Next slide.

So, for all these groups and perhaps for some of you today there may be some value in trying to sort through the developments and how they're reflected in the foods that are now or soon will be available. When I was asked to give this talk, given all the complex developments, what's the useful way to organize them and think about what's going on in a way that's analytically useful? Next slide.

So, one way to think about these novel sources of proteins and where they're coming from is to focus on the drivers of novelty. Three of those you can think about would be the expansion of economically and technically feasible sources of plant proteins, use of new processing techniques, and the identification and refinement of specific functionality for plant proteins. Of course, there are interconnections between the drivers. One may feed into another, but this, you know, is a potentially useful place to start. For each of my three drivers, I'll start with some familiar examples and move into less familiar and more innovative aspects. Next slide.

Let's consider first, new sources of plant proteins, sort of an expansion of sources. These could be conventional crops. They could derive from innovations in cultivation or application of biotechnology. Next slide. The most obvious source is something I already mentioned, pulses, protein-rich legumes commonly used as a common source of protein. There's not a lot of novelty in soybean, pea or fava bean. We'll hear more detail later. We're seeing novel uses of these proteins even from traditional sources. In addition, we don't necessarily think of grains, things like oats, rice or wheat as protein source. We think of them as carbohydrates, fiber, but they, too, can represent sources of proteins with the right processing techniques. This is a conventional crop that could be considered as a protein source. Then, finally things like oil seeds, tubers. They can increasingly represent sources of proteins in the diet. Next slide.

Moving on from conventional agriculture, we can consider other systems of cultivation. This can involve nontraditional agriculture. Perhaps growing things like duckweed or algae in a pond-type setup. There are some challenges with growing them in an open setting. You can get contamination. You can also grow algae in a bioreactor. So, that's a nontraditional culture that can represent a significant source of protein. You could grow other things in that bioreactor, such as filamentous fungi, cyanobacteria, or bacterial that can feed on hydrogen. These can represent significant sources of protein. The culture of microbial or fungal or algal sources of protein, called single cell protein, is something that was pursued in the second half of the 20th century, but it did prove that this was difficult to scale economically for a number of reasons. However, there are a number of firms that are exploring these options today. Next slide.

Finally, applications of biotechnology can also drive new sources of protein or increase the use cases for current ones. One significant and interesting one involves cotton. Cottonseeds could be an excellent protein source. Cotton is widely grown with it made into fiber, clothing. The problem with cottonseed is it contains [inaudible] which is toxic and has an effect on the liver, heart and reproductive system. There is still some use of cottonseed for certain ruminants. Because the problem was identified, there were a number of attempts to breed [inaudible] cotton, but the problem there is that removes the key defense the plant has against insect pests. When you grow them in the field, they don't do very well. Recent efforts have resulted in a cotton plant that produces [inaudible] everywhere but the seeds and thus is protected against insects but also represents a new protein source where you don't have the concerns about [inaudible] toxicity.

People have also used biotechnology to alter nutritional content of existing protein sources, including rice, corn, mung bean. Finally, biotechnology can be used to express individual plant proteins in a recombinant platform. Yeast. This is available to a wide number of proteins. Because of the expression of the single protein, can greatly simplify potential issues of isolation at scale, thereby opening up a number of new applications that would not be economically feasible through ordinary isolation extraction. Next slide.

So, another driver of novelty is processing, starting from a baseline of very minimal processing moving on to concentrates and isolates and moving to modifications. In the realm of minimal processing, where the original source is simply milled or ground, you need to start with a relatively high protein source, like legumes or seeds. That can enable the use of new applications in the use of the plant proteins in foods where you wouldn't ordinarily find them. This is a fairly well-established source of new availability of plant protein in the diet. Next slide, please.

An even more important one and significant driver of new protein sources is valorization, development of ways to use byproduct from an existing process and repurpose them so they become high value on their own. This could be pressed cake from oil seed production. It could be rice grain left over from milling, even residuals algal mass from biodiesel production. So, all of these, sort of, by-products or way streams can be repurposed, and many of the things I mentioned earlier such as potatoes and oats are being driven by this, the ability to reuse this sort of secondary streams. Perhaps they would have been used for animal feed or something like that, but people are finding that with new isolation techniques, those products can be directly repurposed as human food ingredients. However, it's not always that simple to recover these proteins, many they may be associated to cell walls or other structures and may require strategies to concentrate the proteins and make them available for reuse as food ingredients. Another consideration because plants are complex is that many other things may come along with the extracted proteins, other substances that could cause problems for functionality or consumer acceptance. There are colors that would complicate use in various kinds of foods. The extraction technology, you have to take this into account and it can be challenging.

One final aspect to consider includes the modification or fractionation of isolated proteins. This could open a lot of new use cases. Hydrolysis can be used to facilitate protein extraction from plant sources, but this same sort of efficacy could modify the properties of the proteins, perhaps rendering them suitable for new applications. On a similar note, it's possible to isolate certain proteins, for things like legumes or seeds. If you're looking at something like a single cell protein source, you're likely to have a much more heterogeneous distribution of proteins of various metabolic functions, but with something like a seed or a legume, the bulk the protein you extract will be a relatively small number of proteins that are high in abundance. You open up the possibility of isolating fractions. This is something I'll talk on briefly and others will talk more about in detail either. That is a driver for novelty is the ability to isolate these fractions and turn them into particular applications in food.

One last sort of process is fermentation. This is something with a long history in food processing. It's being explored both as a way of hydrolyzing plant proteins which can alter or improve the functionality, but it can also potentially remove some of the secondary metabolites, removing potential off-notes or undesired flavors. Next slide.

The last driver of novelty is functionality. Next slide. So, one starting point is basic nutrition. Particularly for certain kinds of plant proteins that can be tightly bound other cellular structures. There may be some direct issues with access to the protein from a digestive context, particularly for things like algal protein sources where you may have robust cell walls that interfere with digestion. Things like mechanical cell disruptive, thermal processing. Another aspect and something I touched on is alterations in amino acid profiles. It is possible to alter the amino acid profile for some plant protein sources, and this may open up new applications. Finally, something else I mentioned was this issue of other constituents of the plants could have consequences like protease inhibitors and lectins. Focusing on processing that removes these from the plant protein could be important for enhancements and open up new applications. Next slide.

A very interesting area now and significant driver of novelty, however, relates to the texture and taste of plant proteins. Plant proteins tend to be globular rather than fibrous. This could have an impact on the texture of foods made with them. Developments in extrusion technologies have had a big impact in being able to generate fibrous textures using a combination of high temperature, high pressure, moisture and sort of sort of compression to generate these fibrous textures. Another aspect of plant proteins, of ones tending to be globular, is that they're not very soluble, and this has a downstream impact on the ability to serve as emulsifiers or stabilizers foams, form gels, a lot of things that are important in food technology applications, again, something we'll hear a lot about later. The greater ability to isolate or modify for specific plant protein fractions for applications has really enabled a large number of new uses, new applications. Similarly, things like water holding capacity, properties of proteins, having increasing ability to serve select specific protein fractions or sub fractions and make use of. That, again, is sort of a big driver for increased uses for plant proteins. Next slide.

Then, finally and to briefly touch on some targeted functional properties. One mentioned is soy leghemoglobin. There are things like proteins that can impart a sweet taste, there are things like plant proteins and peptides that can impart umami flavor. These are another sort of targeted application from plant proteins. There's some exploration of antioxidant activity, things like soy and oat and canola. There's interesting work going on in sort of complexes of proteins and carbohydrates like carbohydrate polymers associated with proteins of different structures that can lead to potential new applications and include functionality. The next slide.

So, that closes out my sort of brief overview here. I talked about three drivers of novelty as a way of organizing some of the developments we're seeing, expansion of new sources, new processing and production techniques, and new capabilities in identifying and isolating proteins. They interact to produce this interesting and complicated landscape that we find ourselves in. So, I hope that helps set the stage to some extent and I look forward now, as I'm sure all of you do, to the next talks. Thanks very much.

Thank you, Jeremiah. That was an excellent summary of the number of ways developers are using technology to bring to our grocery stores, a diversity of protein sources and a number of challenges that developers have to consider and overcome. It was interesting to hear the wide range of technology that are being applied to this issue, everything from genetic engineering to food processing techniques. We have a little bit of time if there are questions for clarification, I think we can take those. Again, folks can address them to Jason Aungst, who will be curating the questions. I don't see any. Jason, do you see any? Not yet. I will give folk as moment or so. If we have no questions, then we can move on to our next speaker. Okay. Thank you, Jeremiah. I'm not seeing questions.

Let's move on to Pam Ismail. She is a professor from the Department of Food Science and Nutrition from the University of Minnesota. She is the founder and director of the Plant Protein Innovation Center. Dr. Ismail has over 20 years of experience in food chemical research, focused on analytical chemistry, protein chemistry, chemical and fate of bioactive food constituents. Her research focuses on chemical characterization and enhancement of functionality, safety, bioavailability, bioactivity of food proteins following novel processing and analytical approaches. She will talk about the functions and appeal of plant-based proteins and novel proteins. She is clearly an expert in this area. Dr. Ismail, take it away.

Functions and Appeal of Plant-Based Proteins and Novel Proteins
Baraem (Pam) Ismail, University of Minnesota, Minneapolis, MN

Thank you, Jason. It is my pleasure to be here today. Thank you for the opportunity to present. Also, I would like to thank Jeremiah for a nice introduction in covering a lot of the background on plant proteins and some of the functionality and potential uses. That will set the stage for what I will talking about. Next slide, please.

So, it's no surprise to anybody that the demand for plant protein is increasing tremendously and that has to be related to the population of the 7.8 billion. With that, the demand exceeds 200 million tons. For protein ingredients specifically, the proteins that are used in formulation including plant and animal sources, they are expected to reach about 7 million tons by 2025 and a market revenue of about $70 billion by the same year and the rate of growth is significantly high, about 8% annually. Of this market plant protein ingredients account for 40%. Next slide, please.

So, there's no surprise about the population growth as a factor for increase in demand for protein, also changes in the socioeconomic status, so increasing income or household income, increased urbanization, and also, we see an increase in the aging population and therefore protein is associated with healthy aging. So, the aging population is encouraged to increase their protein intake. Consumers in general are recognizing the beneficial role of protein in their diets. If we look at developing countries, we see an increase in the demand for animal protein whereas in developed countries there's an increase in the demand for alternative protein. Next slide, please.

So, reasons behind increased interest in plant protein, I look at it from a consumer perspective first. Consumers right now are more aware of the environment and they have interest in sustainable and environment-friendly sources accordingly and they're also interested in animal welfare. So, we see a lot of increase in vegetarians and flexitarians, so they are more oriented towards plant-based foods for animal welfare reasons and sustainable environment concerns. The recognition of the plant-based diet being healthy and understanding that aspect. So, when you say flexitarians, these are folks that consume meat, but they have preference for plant based. So, this part of the population is increasing. We also see an increase in allergenicity to certain proteins, such as wheat, dairy, as well as soy. So, also looking for protein sources of low allergenicity. Now, if we look from a producer's perspective. Next, please.

So, obviously, the producers are going to address consumer demands. So, the consumers are demanding more plant based. So, the producers are seeking those alternative proteins to add to their formulations. However, they're also seeking low cost compared to traditional ones. So, trying to formulate plant based to place these traditional proteins to reduce the cost. Also finding a unique and competitive place in the market. Everybody in this market, producers are looking for a new source of protein that they can hold an advantage. We heard a little bit about valorization. Definitely valorization of by-products is a big deal where you get oil out from the oil seeds, you end up with this protein rich meal, and valorizing that and enhancing the functionality of that. Not only the by-products from oil seeds but from production of proteins. So, you get pea flower only 20% protein. You and up with starch and fiber, so we have to valorize by-products. And the clean label is of interest to the consumer, they want to understand the ingredients that are listed and see less number of ingredients. So, what producers are trying to do is formulate with functional ingredients such as proteins to eliminate some of the chemical ingredients. Driving the clean label. Next, please.

And, of course, not to forget the population growth where it's estimated to reach 10 billion by 2050. So, we need to increase the protein supply somehow. So, I'm not saying or we're not saying there won't be any animal protein consumption in the future, but we still need to have additional protein sources for the growing population. Next slide, please.

So, if we step back a little bit and look at the role of proteins in food, it was touched upon earlier in the earlier presentation, but nutrition is a big component of consuming protein or high protein diet. So, when we look at the different sources of protein, we think about essential amino acids. Those are the building blocks that our body can't produce. And we also look at digestibility of the protein. So, different proteins, depending on their structure, will have different ease of digestion. That means the enzymes or digestive enzymes will be able to digest them at different rates. So, we have to take into account the amino acid composition as well as the digestibility. And this brings us to the PDCAAS value some of you are familiar with. But it stands for protein digestibility corrected amino acid score. Plus, the score combines the essential amino acid limiting plus the score multiplied by the score of digestibility or percent digestibility. So, if you have a score rough one, that means it's 100% digestible. If we look at the graph, we look at the animal sources. They're all about one. If we look at plant sources, we see soy right up there. That's why soybeans get a lot of traction as being a nutritious source of protein. If we look at different plant protein sources, we see them trailing behind in that aspect in PDCAAS. [Inaudible] but also digestibility and how we process them, how we extract this protein, and then we formulate with it those different processing that can lead to further reduction in digestibility.

Also, proteins have other functions than just nutrition, and We have the physiological functionality and muscle growth or regeneration. Athletes and body builders have to have a high protein diet for building those muscles or regenerating the muscles after having exercised. We go back to proteins that are very important for regenerating or growing muscles. Next, please.

So other benefits, other physiological benefits other than muscle deterioration or reduction in muscle deterioration in aging population, also associated with weight loss and metabolic health. Also, the aspect of protection of bioactive peptides. So, upon digestion of these proteins whether digestion in our gut or hydrolysis of the protein outside, we are generating sequences or peptides. They could have some physiological activity such as reduction of hypertension, acting as antidepressants, promote satiety, or even act as antioxidants or anti-inflammatory as well. Next, slide, please.

Now if you look at the role in foods, in particular, their physical property or physical functionality, we look at color. So, proteins can contribute to color, either native. Next, please. So, we can see here the casein micelle, so when you form the cheese curd before adding any color it is white and that is the casein scattering light. Next, we have the chromaphores in myoglobin, for example, in meat betalins in red beets, and then you have the browning that happens during the processing. For example, the non-enzymatic browning by [inaudible] you see this nice brown color of the bread loaf and we have enzymatic browning. We see sometimes it's detrimental. It's not appreciative such as in fruit or produce in general. There's this browning that this polyphenol oxidates cause. However, if you breed or genetically modify applies like Granny Smith, for example, to reduce these polyphenol oxidate enzymes, then you won't get that browning or the browning is reduced. However, sometimes the browning is desired such as in tea. So, it is desired to get that brown color. Next, please.

They contribute to flavor. For example, if you have a protein that has a high percentage of hydrophobic amino acids or if you break down the protein into peptides that have hydrophobic amino acids then you get the taste of bitterness. Sourness, if you also hydrolyze and you end up with free amino acids, then these are acids, right. So, they would have the sour taste or a protein with a high percentage of acidic amino acids, you would get the sour test. If you have cysteine such as in eggs you got that sulfur taste. Next, please. Glutamate gives you that umami flavor and proteolysis that can happen during the ripening of cheese, this causes proteins to break up and give you the smaller peptides and they would contribute to specific and unique flavor in cheeses. Finally, the sweetness. The peptide here—next slide, please. So, Aspartame, for example, that gives you that sweetness.

Next, we're going to look at functionality in terms of building the texture. So, in this one the protein has so many the structure allows them to work in different ways to build structure. The ability to bind water and hold water and form a gel also gives us the unique texture of jelly or tofu or yogurt, for example. That's another form of forming a gel and holding water. Solubility is a big deal for beverages, and different proteins have different solubilities. The golden standard for beverages with high protein, it’s [inaudible] and allows the beverage to remain clear, which is really attractive in a clear bottle to see your beverage and not to see sedimentation at the bottom of that bottle. Emulsification and foam are another important functionality. For example, look at ice cream, it is an emulsion, and it is a foam at the same time. So, having your fat emulsified. You have the air stabilized by the protein. Then, of course, not to forget viscosity for building for sauces, for example, or gravy, and then you have the elastic properties that are essential for bread that is unique for wheat protein, the gluten-forming protein.

So, what are some of the trendy applications right now, and it’s not a surprise to anybody? Next, please. We have the meat analogues, which are growing tremendously in the market. It is estimated that the plant-based meat protein sector will reach about 5.2 billion by 2020 or this year and could potentially make up one third of the market by 2050. So, it might even grow bigger than that. It's growing at a steep rate. Next, please. Next, please.

Protein beverages is also a growing market, and we see that the trend is going to be an increase in the market up to about $2 billion in market value. Next, please. Dairy analogs are also growing at a fast pace as well. So, alternatives to milk, to dairy cheese, dairy yogurt, as well as ice cream. This is another area where folks or producers are seeking proteins that can be functional in a way that can help in producing the texture that is common for daily products. Protein bars is another area. You want to incorporate protein that does not lead to hardening of the bar over time. You want to make sure it does not cause hardening so the bars can have a decent shelf life before failure. Extruded products and snacks is also a big area.

However, producers are seeking alternative to soy protein and gluten. Due to modification in soy and gluten sensitivity and allergenicity to both proteins, consumers are seeking alternatives and therefore producers are exactly doing so. Next, please.

So, we just heard from Jason, the different types of proteins out there. In terms of the plant proteins, we can see that peas is no longer really an alternative protein, it is growing so much in the market and gaining traction, as well as other pulses including chickpeas, lentils, beans. And we have a slew of oil seeds, canola, sun flower, a lot of research in Europe in the sun flower area. Camelina. There are oil seeds as well. Then we have other plant protein sources including oats, hemp, potato, corn, almonds and other sources of nuts, leaves and the list goes on and on. We have single cell proteins, lab-grown proteins, insect proteins. So, the list is infinite at this point. Can we go to the next one, please?

Let's talk about peas and why it's a trendy source and growing so much in the market right now as an alternative to soy specifically. Peas are easy to grow. The crop has a short season or growing season. [Inaudible] It improves nutrient levels in the soil, especially if used in crop rotation. It's currently non-GMO. I always say this with caution, it has low occurrence of allergenicity. But any protein, people can form or become allergic to that with more exposure. 50 or 60 years ago, soy was not among the big 8, but now it is, and that's due to increased exposure to it in Europe and North America, where it wasn't part of the diet. So, I always say that with caution but for now, it's desired because it's not one of the big allergens or does in the contribute to a significant number of allergic reactions. Next, please.

So, here's a look at protein product launches. So, we see over the years and I know this is a little bit older, but the trend is still the same. Soy is going down in terms of new product launches, whereas pea, we can an increase over time. However, there's still a gap and this gap is due to some disadvantages with pea proteins. Next, please.

So, some of these disadvantages have to do with the nutritional quality of the pea protein. Again, we go back to the PDCAAS. It's not that bad. It has a PDCAAS of 0.8 to 0.85 depending on extraction processes and formulations, but that is less than soy that has almost 1, between 0.9 and 1 PDCAAS, depending on processing, so that makes it lagging behind soy and other animal-based proteins. It does have inferior functionality. We do have flavor issues. It doesn't have the years of that soy has, so there is research still ongoing to reduce some of that flavor and oxidation of fats during processing. Some of the residual fat gets oxidized during processing. We do have that starch and fiber byproducts. We have to figure out a way to utilize them and valorize these byproducts. Next.

If we look at why pea is behind soy in functionality, both are legumes and they have very common globular proteins, so the globular proteins are the functional proteins, and since soy, glycinin and beta-conglycinin are the globular proteins. So glycinin belongs to the legumin family and beta-conglycinin belongs to the vicilin and convicilin family that belongs also in pea, but if we look here closely at the distribution and the difference in percentages of these globular proteins and also the globulins, if we look at the profile of these protein component, although there is high homology in amino acid sequences, they are different in percentage. If we look at glycinin in soy, it is 38% to 51% of protein in soy. However, the legumin, which is a close relative. It's has as low as a 6% and could be up to 25% depending on variety or environmental conditions. So, that causes a lot of differences in functionality. Next, please.

So, if we want to compare how the structure impacts function, I will give you a little bit, not dive a lot into the chemistry but give you a hint the on why some proteins behave differently. I get a lot of requests, as we have our research centers and laboratories. We get requests to, can you make this plant protein behave as whey protein? It's not possible, they are genetically different so you can't make pea protein behave as whey protein simply because of the makeup of the protein and the structure of the protein. So, looking at the simple function, which is solubility, and if you look at the whey protein, which as we said earlier is the golden standard for beverages, so it maintains 100% solubility at a wide range of pH. However, if you heat it, then it does have a lower solubility around the pH where it loses its charge. However, if we look at soy protein, for example, it has low solubility at a wide range of pH, it ranges from a little bit of acidic pH around 3 to around 5 or even 6. And it's more soluble at very low acidic conditions but higher in the alkaline condition over pH 7. Next slide, please.

I just want to show you the difference in solubility at pH 3.4. that's the pH of an acidic beverage. We see whey protein maintaining high solubility whereas soy has less, about 20%. It really depends on the source, depending on where we get soy protein from. It's lower than whey. However, pea protein has very low solubility, especially where you can make a protein claim, and this is about 5% protein. Next, please.

So, in the way of pea getting ahead, again, if we look at the functional properties that are essential in other than beverage applications, emulsification, gelation, texturization, pea protein is behind soy in these categories. And again, this is mostly attributed to the differences in their globular protein composition. So, we see that soy protein remains dominant over pea protein in these functionalities. Texturization, pea protein can be texturized; however, it doesn't have the same high molecular weight composition that soy protein has to form fibrous structure that gives you the dense, and the more put-together structure, I would say. It doesn't get as well structured fibers as soy proteins and gluten to perform upon extrusion. Next, please.

So, if we want to compare daily protein and emulsification, we look at casein. It's a natural emulsifier. It has the ability to interact with the fat and the aqueous phase more readily because of this open structure that has the hydrophobic component and hydrophilic component on the surface. It can act as a natural emulsifier. However, the globular plant protein. So, the globular, you can see here this is a soy protein structure. It is globular. It has to unfold a little bit and be able to orient its different components to the different phases, aqueous versus lipid. So, we can form an emulsion with sodium caseinate, for example, we can form a really nice thick emulsion. We call the emulsification capacity is basically the ability to form an emulsion and determining how much oil can be emulsified by 1 gram of protein. So, the emulsification capacity as .5% is 1800 grams of oil per grams of protein. If we look at soy, we need at least 1% protein to be able to form an emulsion that is considerably think. It's less. If we look at pea, we can't form an emulsion at .5 or 1%. It has to be 2% at least. The emulsion is not very thick and can only emulsify 400 grams. Next, please.

Again, I bring back the difference in soy and pea in terms of the legumin, which is glycinin in soy. That I bring it back and I show you here the profile surrounding a gel, and this is a different component within soy versus the different component in pea. The higher percentage of glycinin that can enhance the formation of cross linking, then you can form a stronger gel. So, for example, soy protein forms a gel at 10% protein; however, pea protein does not form a gel at 10% and forms a very weak gel at 15%. If we want a stronger gel, it has to be at least 20%, and even at 20%, the gel is not as strong as soy. Next, please.

So, with that, I'm going to introduce camelina and pennycress, which are two oil seeds that are short season, winter cover crops they call them, and they belong to the *Brassicaceae* family. They are short season. Because they are short season, they can grow between different crops when the land doesn't have any cover on them. They act as preservers to the land or to the soil. So, basically, their roots will hold on to the soil and prevent soil and water erosion and help with reduction of nitrate leaching and help with increased carbon sequestration. They lead to reduced inputs of energy and pesticide. So, basically, if we can entice the farmers to plant them as cover crops, then they will protect the land and help with sustainable agricultural systems. However, the farmers really would like to see a market for them so they can cover the cost of production or planting. So, we're looking at them as potential sources since they're oil seeds, so potential sources of oil, as well as a protein rich meal that we can utilize for protein extraction and production of ingredients. So, they're both rich in lipids. That's why they are a good source of oil. They have 25% to 30 % protein. So, after pressing the seeds, you end up with, again a rich cake, a protein and fiber rich cake. Next.

So, again, the advantages here we highlight low occurrence of allergenicity. That is still there as an advantage. Also, the environmental benefits I just mentioned and looking at PDCAAS, it's not great as soy. It's not terrible, it's .72, which is higher than gluten, which is .5 or lower depending on processing. Next.

So, disadvantages. We do have protein extraction challenges. After you press the oil and extract every bit of oil you can, you end up with a complex matrix where the protein is entangled with dense fibrous matrix, so to get the protein out is a challenge. They are mostly soluble at 10, 11 or 12, which is high alkalinity that causes the protein to denature, to oxidize. Another potential problem is glucosinolates. There are breeding programs right now at the University of Minnesota to look at selective breeding to produce lines that have low levels of glucosinolates. So, decrease that sharp flavor. Glucosinolates at high levels have a negative impact. Next, please.

Hemp is another source of protein now gaining a lot of attention. Of course, hemp belongs to the *Cannabis sativa*, which includes marijuana, however, it does have .3% of THC, so it doesn't have psychoactive effects when ingested. It is high in fat and high in protein and grown for many years. Next, please. So, again, the advantages is non-GMO, can be used as a rotation crop, can enrich the soil and prevent erosion. Pesticides are not required, thus making the cost of production low. It's nonallergenic, and it's highly digestible, the seed with about 30% protein. Next, please. It does have lower PDCAAS compared to soy protein. And that is due to lysine deficiency. It does have potentially fewer functionality, but we don't know yet much about it. There's not a lot of research on it. So, this does need a little bit more looking into. Next, please.

So, here, I'm wrapping up a little bit with areas to be addressed. I presented some of the plant proteins as an example of some of the plant proteins out there with limitations in terms of functionality due to their structural differences. We need to do is understand how these novel proteins can lead or can replace or be combined with traditional proteins and we always hope to deliver three main components. I call them the three pillars: nutrition, functionality and flavor. Also to determine viable and cost effective ways to extract these proteins and preserve their properties without damaging their structure or characteristics. Looking at characteristics we can't make them behave like whey but let's figure out formulations. What can we combine them with to bring out some of their functionality? Looking at crop diversity and breeding. We breed for protein quality. We need to secure abundant and sustainable supply.

Here's a snapshot of what we do at the Plant Protein Innovation Center in terms of research to enhance plant protein viability. Extraction methodology to maintain structure integrity, functionality, nutritional quality, and flavor. Looking at varietal differences and initiating breeding programs. Also make sure that we always look at nutritional quality, whether we are looking at different extraction procedures, different varieties as well as under functionalization where we try to functionalize these plant proteins to enhance their characteristics. We work on different forms of functionalization, whether it's enzyme modification to enhance polymerization or reduce polymerization in terms of the final application. A combination of polysaccharides and nonthermal treatments such as cols plasma. So, this is an example of what we do to functionalize the protein. Next, please.

So, ultimately, we hope to produce or develop new quality driven protein isolate protocols that are functionally enhanced and provide feedback to the breeders in terms of what do they need to breed for to get a protein with good functional and nutritional quality and entice the farmers with economic revenue and prosperity and provide the food industry with what they need to get successful ingredients and formulations that are accepted by the consumers.

With that, next, please, I would like to just thank my group. Never mind the animation. Just the last slide is basically thanking the group and also the center, Plant Protein Innovation Center and my department.

Dietz: Thank you, Pam. That was wonderful. I think that helps us understand some of the consumer and producer drivers behind the use of some of these ingredients and very clear to see the role that proteins play, not just in nutrition but in making some of our favorite foods by improving their taste, texture and function. I found it interesting why some proteins are better suited for certain functionalities and there is a cross protein and why one protein would be better for a particular product than others. I have one question. You mentioned when you talked about hemp being nongenetically engineered and the advantages. The way I interpreted that is that would be in terms of consumer interest and not necessarily because of safety concern. Is that the correct interpretation on my part?

Ismail: Yes. That is absolutely correct. The consumer is looking more into nongenetic modification, sustainable agriculture, and also some sources of proteins that are currently nonallergenic. It doesn't necessarily—GMO is kind of a controversial issue. It's hard to dive into it. I don't think consumers understand a lot about GMO. So, the stereotyping more than anything else, we want something natural, organic.

Dietz: Thank you for clarifying that. Jason Aungst, do we have questions from the audience?

Aungst: Yes, I think we have time for two of these here. One is to increase the umami flavor and increase the glutamate content, does this increase consumer concerns due to potential MSG sensitivity or MSG allergy? While there's still a debate as to the prevalence of MSG sensitivity, there are data showing that some consumers do have a physiological response to MSG or products with high glutamate content. Is there a way to increase umami other than glutamate?

Ismail: So, again, it has to do with how much consumption is happening with glutamate to induce the umami flavor. Yeah, there is a concern of sensitivity and, yes, there are some consumers that have that sensitivity to MSG. In terms of how much it is, I'm not quite certain. In terms of other flavors that might cause umami, I'm sure there are, but since I'm not the specialist, nothing is coming to mind right now.

Aungst: Thank you. Another question was, any safety certain concerns about the presence of cannabinoids in hemp seeds?

Ismail: No, the hemp seed is basically bred to have less than .3% of THC, so there is no psychoactive components that would elicit any of the reactions that you would get with marijuana, for example. Also, when you produce the protein, a lot of different components are eliminated from the extract, so there's no concern there, not at all.

Aungst: Okay. Thank you. I think we're going into our break here. Jason, I will turn it back to you.

Dietz: Wonderful. Thank you, Pam, and thank you to everyone who has posed questions. I think that will help develop a rich discussion later today. I have 10:23 on my computer. I would like to give everyone a ten-minute break or so. So let's reconvene at 10:35. That will give everybody to stretch and we'll start off with Michael Leonard at 10:35. Thank you, everyone.

Break

Dietz: Okay. I hope everyone has enjoyed their break. It is 10:35. Our next speaker is Michael Leonard. Dr. Leonard is the chief technology officer for Motif FoodWorks where he leads the technical strategy in building out its research and development capabilities and joined September 2019 and has over 17 years of technology and commercial leadership experience in the food ingredient and consumer products industries. I think he has some very interesting material to share with us, so Michael, please take it away.

Future Developments in Plant-Based Foods
Michael Leonard, Motif FoodWorks, Boston, MA

Jason, thanks very much for the introduction. It's a pleasure to be here. I'd like to cover a few topics on the plant-based food industry specifically as it relates to ingredients and tackling some of the longstanding challenges in making these foods more delicious and nutritious. So, I will go over some of the concepts that Pam mentioned and building on some of the themes Jeremiah talked about. Hopefully you can derive an industry context of this and give you a sense of the hurdles that are facing us as product developers and approaches to try to manage that. So, we'll start with where we see consumers going in this industry, some of the themes that we as. Ingredient companies and large CPGs are dealing with and some of the challenges we're taking on, and some approaches to advance our knowledge and to create even better ingredients in products in this space. Just a little bit about my company Motif FoodWorks. Motif is a food ingredient startup here in Boston. We are focused on improving the quality and nutrition of plant-based foods through ingredient innovation. It's in the space of developing new tools to improve nutrition, flavor and texture of these products through new science and technology. We believe very much in the plant-based movement and we’re one of many companies trying to make a difference here and we know to truly realize the sustainability benefits, we need to make these products taste better. So, our ambition is to unleash the promise of plant-based foods, and we have some big opportunities. All right. Next slide, please.

I think we can all agree that the world is hungry for better plant-based foods. I know a good portion of the people in this meeting are plant-based enthusiasts, flexitarians, vegans, vegetarians, plant curious or fully bought in, but there's no denying this is not a trend. It's a secular shift in the way people are viewing food. It makes the world very exciting for people in our community both on the industrial side, academic side as well as the regulatory area. This will be a huge area of growth for us over the coming years. Next slide, please.

And I think Pam covered some of this in her talk, but I wanted to revisit some of the drivers of why consumers and people like us adopt a plant-based lifestyle. There are really three things that we think are driving this movement. Number one is the belief that plant-based foods and a plant-based lifestyle are healthier for you and I say belief because we're not really delivering as an industry on nutrition and health the way we should be. I think looking at one of those three pillars that Pam talked about, nutrition, that's a huge area. If you look at the nutritionals on an Impossible burger versus an animal-based burger, the nutritionals aren't all that different. So, if you’re eating a plant-based food with the perception that it’s better for you, you need to check the data because it's not always the case. So, I think we need to do better delivering on that as an industry. This is the reason why people adopt this lifestyle. Second is sustainability. It's a really fascinating and I think powerful concept that I might not be able to park a Tesla in my garage or put solar panels on my house, but I can make choices about the foods I purchase and eat that positively impact the planet, the society and make a statement about what I believe. This gives people an agency that very few other movements. Animal welfare is third on the list. It is subordinate to sustainability and health. These are the drivers that make people feel good about adopting a plant-based lifestyle. Next slide, please.

There's no denying that these are getting to be big markets globally. We estimate that the plant-based meat and dairy globally is around $30 billion. That was last year. This year it's even bigger. Most of it is driven by the dairy alternatives, the growth of almond milks and alternative milks. You've seen the growth in that segment, but the meat-alt segment is growing, too. Next slide, please.

From a growth standpoint you have plant-based meat and dairy dramatically outpacing their animal-based counterparts. Part of it is moderate increases in small baselines to start with. For me 15% growth is off a smaller base than the 7% base you see in the animal-based products, the red bar, but any time you see a growth rate of 15%, 12%, double digits in the food business, it really gets your attention. Food and CPG tends to be a slow growth industry, but the plant-based movement is a significant exception to that. All right. Next slide, please.

But despite this, the size of the market, the belief, the energy behind the market, plant-based foods aren't living up to expectations. It's the basis for why companies like Motif and others come to the table to take on the challenge. We know there are significant gaps in plant-based foods versus their animal counterparts and there are big opportunities there. All right. The next slide, please.

And the reason is pretty clear. It's taste. Going back to one of the three pillars we just talked about, flavor. Turns out taste is more complicated than just flavor, but this is what consumers will tell you. This is what consumers say. There are studies, Yale Climate Change Report, Health Focus International, GFI. It's pretty clear. 44% of consumers don't like the way plant-based foods taste. 67% of consumers said they would eat plant-based products over animal products if they tasted better. If you can't satisfy the basic need for food to taste good, people will not come back for more. Next slide, please.

We all vote with our wallets. We can give our opinions about what we think about foods but if we're not coming back to buy more, that's a sign of the opportunity we're dealing with here. This chart shows repeat purchase rates of meat alternatives versus the fresh meat analogs and milk alternatives versus cow's milk. Repeat is about half in both cases. So, if this is true, if people aren't coming back for more, taste is a big barrier, how do we expect to get the adoption to realize the benefits of sustainability, health and animal welfare that we all bought into when we got into this space as consumers and professionals in the industry. That's the big question we're all trying to deal with and find ways to solve so we can realize those benefits. Unless people come back for more, we're going to be stuck.

We did our own research last year just as we were getting off the ground as a company to create some more detailed insights, what do consumers mean when they say it doesn't taste good to target a portfolio that delivers specific benefits around what consumers want. Most companies are doing the same thing. We all come up with similar insights. We looked at plant-based milk, yogurt, cheese, ice cream and plant-based meat. Some of the hottest emerging categories in this space. I'd like to create some suspense here. You wouldn't be surprised when you see the outcome.

Taste is the biggest issue. We've broken this down into a few different components. When consumers say taste, it's not just flavor. Taste is very much in the mouth of the beholder. In some cases, it means straight-up flavor. Others a combination of texture and flavor, mouth feel, color, smell, the overall opinion you develop of a food. So, what we did, we took the data, broke them down into specific functional attributes and started to develop a portfolio of solutions. Throughout the presentation I'll talk about the approach we're taking. It's really something that's general. The reason I wanted to talk to you about this, you'll see some of the same themes from other companies. We're all working as a community to try to move the needle here so the trends that I'm showing are general and useful for you to understand as we start to advance this industry. So next slide, please.

We have to back up for a second and maybe reprise a little bit of what Jeremiah and Pam talked about in terms of the protein sources that comprise the bulk of a lot of these plant-based formulas. A tremendous amount of progress has been made in improving the processing and quality of plant-based proteins that can be used for animal product analogs. Really, the protein sources in most cases are the root cause of why these products are so hard to formulate and why people are coming back half as often to buy the plant-based foods. Protein sources present a lot of challenges from a formulation standpoint as well as a nutritional standpoint. From a functional perspective, the main sort of sources of proteins you'll see are presented here, so soybeans, we're all familiar with soy and the challenges. Gluten we've talked about before. Pea protein. One of the fastest growing scale crops that's now being used in plant-based foods, has nutritional challenges. Mycoprotein. You've seen advances in mycoprotein as well as companies making whole slabs of steak analogs with novel mycoprotein processing techniques. That's exciting. It's still emerging, boutique. A tough texture to work with. If you've bitten into one of those products you know what I mean. Then nuts, using nut textures, almonds, cashews, to provide scaffolding and textural benefits in products like cheeses and yogurts. There are issues with nut sources, too. Ultimately it comes down to the function issue that we talked about already today. The difference between globular proteins and fibrous proteins. Globular proteins serve a very different purpose than fibrous proteins. Over the years we developed techniques to narrow the gap. We're still not there. How do we get globular storage proteins in those cases and protein sources that were evolved for a different purpose than their fibrous animal protein counterparts?

We as developers in this space have been dealing with this for many, many years. So, some of that new thinking you're starting to see emerge through our company and others in this space. As a community we're seeing much more investment in science and technology to help understand what are the root causes of functional gaps, not just in protein structure but the whole formulation? How does it need to work together to perform better? This is as much about the drivers of novelty that Jeremiah mentioned earlier as it is about developing a basic understanding of how food needs to be designed. I think it's clear that we can no longer rely on a cook and look approach if we really want to make a quantum leap. The challenges are just too big. Okay. Next slide, please.

So, we'll be successful if we can create these great tasting, more nutritious, plant-based foods that people actually crave. It's all about adoption. The bar has to be much, much higher. Without creating cravable foods, that repeat purchase rate will continue to stay low. Next slide, please.

To give you some sense of target applications, so moving beyond the protein sources, what are the types of food forms that we as an industry are really investing in and promoting? You've seen some of these products in the stores. You bought some of them. I'm a heavy consumer of these types of products myself. These are the areas where we'll see continued see continued growth and where the focus is going to be, we think, more generally. So, ground meat applications have had the limelight now for a few years thanks to Impossible Foods and Beyond Meats and others, the new science and technologies. Snacks and jerky application. Vegan jerkies are one of the fastest growing categories within the meat space. And within the dairy space, you’ve seen the explosive growth in alternative milk, yogurt, cheese. We haven't come close to getting cheese right. Have you ever tried a vegetarian or vegan cheese, you've experienced the oily starch paste. Ice cream, some great work.

Also, moving outside of animal analogs, if we talk about plant-based nutrition, products already in a plant-based space but are suffering from nutritional gaps in terms of amino acids, and then functional gaps, like mouth feel, graininess, stability issues, there are opportunity there for new ingredients to play a role to make those products more mainstream acceptable and more nutritious. These are the spaces we think we'll see more and more growth, some of the spaces we're investing in as a company as well. Next slide, please.

To get back to this concept of what I said on this cook and look approach won't work, I want to talk about that here. Over the past decades we all as product developers have been brought up with a set of tools to help design products in the plant-based space. I think of them as a modify and mask context. We're not getting to the root cause of why globular interact with starch and lipid matrices differently than animal proteins do. We developed some good tools to, number one, describe what the gaps are through sensory testing and how do we in some cases solve a root cause and in others cover it up where a consumer will say, yeah, I will buy that product. Using flavor strategies, formulation strategies with gums and stabilizers, words with a lot of vowels in them and process strategies. I know Jeremiah mentioned extrusion has come a long way. How do you brute force your way to a fibrous product? If more basic understanding is required, of food design rules, how does food need to be put together to perform optimally in a way that consumers really appreciate and love the product? We have to bring some existing scientists to the table. So, bringing some science that's been around for a while but applying some new lenses to the food industry.

We're doing that in a few areas. Number one, a deeper understanding of oral processing physics, how you consume food, how you experience it in your mouth and bringing in cognitive metrics. How does the brain actually interpret liking versus what it doesn't? Then synthetic biology is something we'll talk about a little bit later today. That's a very significant engine for us and others to develop a portfolio of animal-free ingredients or animal proteins without using animals. We're using microbes. How do you get a formulation to work? How do you optimize interactions? That's where soft matter physics and materials science come into play. So, we believe that applying a more rigorous scientific lens to root causes of these gaps is critical. And then we have to develop technology to address that. This is a theme you'll see. If you look at food science in general, historically, there's been a high level of underinvestment, I would say, compared to the other life sciences. There hasn't been a lot of investment in how food works. Next slide, please.

Here's some of the attributes. If we look at some of the categories, what are the specific targets that we're going after. As an industry, you talk to any company that will see similar themes. So, with meat it's taste, appearance, texture and nutrition. Check out nutritionals between a plant-based burger and a burger an animal burger that you buy. They're not that different. In the dairy space, cheese is a big target for all of us. Getting the right stretch, gooey and melty, that's really important. Then within the plant protein space, how do you raise the bar on improvement of appeal of those products as well? So, these are some specific targets that we're factoring in. Next slide, please.

So, as I mentioned, the level of underinvestment in food science has really been shocking over the past decades in this area, and the trend is to look into science and technology as a tool. I'm showing some examples here of what we've done at Motif. You can look at everything through a general lens and start to anticipate and contextualize. We're relatively small company. Right now, we're little more than 20 people and rely heavily on partnerships to advance the agenda. A few of those are highlighted here. We'll talk about our partnerships in a minute. We're working with U Mass Amherst to develop microscale food functionality that can interface with a high-throughput screening environment. That's critical. As soon as you get expression data from a microbial host, you'd like to know, how do those proteins function? From a food standpoint? Within texture, we're working with University of Queensland and Jason Stokes's lab to understand oral processing of plant-based meat. The University of Illinois system and Texas Tech on microscale and macroscale nonlinear analysis of foods, both in process and during consumption, getting back to, how do we think of food as a material? We need to think of food as a material. And as a complex fluid. As a soft matter system to be able to design better foods and to understand interactions. And then getting fat right is critical. And you are seeing this if you follow the plant-based industry news. You've seen fat mentioned quite a bit. We are taking a crack about the partnerships at the University of Guelph and Coasun.

This idea of leveraging partnerships to advance the field is a general theme you will see in the industry. Most big companies and even startups like us are looking to accelerate the pace of innovation. In getting new thinking to the table by leveraging partnerships. You will see less and less of in-house development and focusing on internal capability over the next few years. More focus on how to accelerate and leverage expertise and work together in more of an open innovation paradigm. Our company is kind of a microcosm of that. We think that approach is really important. Next slide please.

A couple of examples of how we are doing this. I mentioned oral processing. We have a couple detailed slides on that. As I mentioned using nonlinear rheological techniques, we are developing up in those with the University of Illinois. Moving away from rheological techniques that are easy to do things that are easy to measure to more realistic but kind of scary spaces in rheology and come where the physics are little bit more complicated. You have to be more brave in terms of how you approach the experiments. I mentioned neurobiology. Let's talk about oral processing. Next slide, please.

Oral processing is a very dynamic process. It is something that has opened my eyes. I have not been a student of this until I came to Motif and I realized the potential benefit and understanding oral processing can really deliver. If you think about eating food. And the way we talk about food performance and the measurements we do in the lab. We do a lot of measurements on first bite impression. Maybe even second and third bite to texture profile analysis. You imagine the result. There is a lot more to consuming food than understanding first bite or some basic force measurements. It is a very complex physical process will you have the structure of a food. When developing a project, you have to structure it correctly, you've got to formulate it correctly so when you buy it, you look at it on the shelf it looks right, it feels right. You pick it up and take that first bite, then, it has to break down the way you expect too.

Not only do you have to engineer the construction, but you have to engineer the destruction as well. That is really hard. That is where science can help us. That structure is related to the properties of the proteins, the carbohydrates, and the lipids that constitutes the matrix. You transform that whole thing in your mouth through chewing, mixed with saliva, interaction with your mucous membranes, then you can relate that to a sensory output. This is some of the work we are doing with the University of Queensland. It is a very dynamic process. Next slide, please.

This is an example of how we are doing that. The top line shows the stages of oral processing. First bite is important, second and third bite are important as you granulate the product in your mouth. Then it goes from this bulk mechanical experience, and bulk mechanics that dominate very quickly to surface events and try biology. Now you have the coating of services with saliva and bolus that you form when chewing a product. What your brain is giving you signals about, do I like this or not, is not necessary based on what you experience in that first bite. Our goal is to understand the route this whole process, what are the physics? How can we use that knowledge to design foods better so that the physics are more amenable to consumer liking? And we monitor consumer impression and sensory impression through this process called temporal dominance profiling, so whatever step in this process, we’ve got a way to understand the sensory impact and performance. And then how do we measure this in the bulk? We have new techniques that we are developing that will be adopted by the industry, eventually. To understand the bulk affects all the way down to the surface and [inaudible] effects. Very exciting stuff. We hope to be publishing a lot of this work and talking about it more broadly, but we feel that oral processing, understanding this deeply is key to unlocking some insights and design better. Next slide, please.

I have talked a lot about diagnosing the gaps and an approach towards understanding the problem and analyzing and setting up the right physical understanding to design a portfolio. What I would like to spend the last part of this talk on is how we actually start building some of that portfolio. That is where we discussed synthetic biology and the partnership we had with Gingko Bioworks to develop animal free proteins through synthetic biology. Ginkgo if you're not familiar with them they're based in Boston. They are still in startup mode, they're not yet a company, but they’re one of the world leaders in biotechnology and synthetic biology and metabolic engineering. We are very fortunate to be co-located with them. And have access to their facilities and expertise. Next slide, please.

Using synthetic biology as an engine to produce ingredients is a really fascinating thing. It is not a new idea. A lot of companies have been doing this. A lot of companies are more on the down the road than we are. We think we are at a real crossroads in that industry now. We believe that biology is fundamentally programmable. This is where Ginkgo has helped to advance the industry through the scale, and level automation that they have been able to achieve in terms of screening, sequencing and synthesizing DNA. And creating new organisms to produce ingredients across a variety of industries. The hypothesis is that you can program these microbes like a computer. Next slide, please.

You do it this way. Schematically, this is an example of how that process works. There are fundamentally three steps. There is sequencing which has already been done. The genome most animals have been fully sequenced. We already know which genes correspond to which functional proteins, which algorithms have, which proteins and other materials in the animal are produced. Those databases exist. Ginkgo is great at searching those. And there are a lot of companies that have good algorithms to pull out specific targeted genes you're looking for to make a certain protein. The next step is programming an organism. First you have to assemble the gene. So, DNA synthesis happens much the same way as solid-state synthesis works. So, this is works like an inkjet printer. You've got your four base pairs. You put them all together in a synthesizer and make solid-state DNA. And to give you a sense of the scale we are operating at with Ginkgo. They're responsible for over half of the world since the synthesized DNA. It is synthesized by Ginkgo or on their behalf. That is a kind of scale we are leveraging.

Print fragments of that DNA, put them into a vector that the microorganism can adopt. An engineer an organism. Transforming the DNA and eventually being able to produce a product. And animal protein through fermentation. You synthesize the DNA, transform the organism, engineer metabolic pathways, and you ferment this the same way you ferment beer or other products. That's a very simple view of how the process works. Everyone who talks about making animal proteins through fermentation, this is what they're doing. Next slide, please.

We talk about programmability and high-throughput screening and the ability to really program biology, that is possible because of the dramatic advances that have been made in scale and automation in this space. This chart shows the cost of DNA sequencing and synthesis. This chart is current up to 2018. The point here is that the cost of sequencing which is reading DNA and synthesis that writing of new DNA, has dramatically reduced. The cost has dropped much faster than Moor's law would predict. If you look at what you usually see in the technology space with semiconductors and chip manufacturers. The pace of innovation there and the reduction of costs and the reduction of size keeps pace with Moor's law. Costs have dropped much more dramatically than you have expect. That is why it is possible for us to look like at this microorganism, high-throughput engineering approach as viable, because it no longer costs an arm and a leg to get this done. Next slide, please.

The cost per straining to test is much lower. If you look at what Ginkgo can do. We're talking roughly $90 per strain to test today versus what industry standard is almost $500 per strain. These costs will get lower and lower over time. This means we can experiment, we can access a very ride range of genetic diversity to look for differences in functionality in different types of proteins. We are all about using this engine create new types of food functionality and to provide animal free ingredient options for the industry. Next slide, please.

This is a little cartoon on how we do that. Let's say we are interested in an egg protein. We are not just interested in chicken eggs. We are interested in all the different type of egg proteins that we can access in nature. We work with Ginkgo to screen those libraries and find the genes that code for the specific proteins across all the genetic diversity we can access. Doesn't matter how many birds exist in nature. As long as we have the genes, we can access the protein. We design an organism; we build the organism. And figure out which organism can actually express those proteins. It's not a fully flushed out science to predict how exactly expressions can go. You have to use experiments. That is why it is great that the cost is relatively low so we can integrate a lot. And then we test and see, do we actually get those functional proteins expressed, and through our microscale assays, do we get the right data? Then we go and ferment those at a larger scale and you can actually produce animal free egg proteins to innovate in the food space. That is hypothetical. To indicate food space. That is a quick example of how we do that. Next slide please.

Some quick examples on some other targets that we have looked at. So transglutaminase, alpha-lactalbumin, these are PCA plots showing some expression screening results from our collaboration with Ginkgo across a variety of different genetic sources, different animal sources. You can see what we've got the ability to work in a wide range of different species and the functionality differences are significant. Next slide please.

In closing, we really believe that the approach where we understand in much more detail how food actually works, what are the design rules that need to be refined to make these foods better as well as ingredient portfolio developed from new technologies. If we believe that approach works, there should be space for an entirely new categories of plant-based foods. We need to get away from this mimicry. I'm going to copy a burger or copy cheese. Ultimately if we unlock new sources of functionality, we should be as an industry be able to create new foods. That can really bring plant-based foods to their own and create some iconic food forms that aren't just mimics. Next slide please.

This is an industry-wide desire. We are taking our own crack at it. We are part of a larger community that is helping to push the thinking here. We are looking way beyond mimics of burgers and cheese to new food forms that are vegetable forward but still offer some of the functionality of animal proteins through synthetic biology and other technologies that we are bringing to bear into plant-based products that can become iconic in their own right. This is our big vision of how we can inspire growth in the industry. This is a very exciting thing for us. As food developers and designers, how we look to create and inspire, and with the tools that are now available, we can really accelerate our progress to this endpoint. Next slide please.

We can't do it alone. As I mentioned, the intent of today's top is give you a sense of some of the industry trends, as we see them through our lens as a company. We feel these are general themes that in your roles that you play in this industry and this ecosystem that you will encounter these trends and will hopefully give you some good context and background for understanding and developing your own view and point of view on how we can evolve the space together. With that I will conclude. I really appreciate the invitation.

Dietz: Can a conference coordinator confirm that our audio is coming through?

Conference Coordinator: The audio is working. He had got muted but he is un-muted now.

Leonard: I just wrapped up. I was just thanking everyone for their attention. I'm not sure where I got muted. That is the end of the talk, thanks again for the invitation. Look forward to the Q&A.

Dietz: Thank you, Michael, for a very visionary talk about what is a currying in the industry and some of the common challenges that the industry faces. I find it very interesting to see the taste changes that you have outlined. Some of the progress that has occurred to date. The understanding that what kind of progress might be desirable in the future and some of the new thinking that you are applying to the development process. As you mentioned, it is not the cook and look process anymore. You are taking a different kind of approach. I found it particularly interesting when you make a point that part of the product develop process is engineering not just the construction of the food but the destruction of the food as well as we eat it as consumers. I think we have some time for a few questions. I would like to turn to Jason Aungst. Jason, other a few questions we have?

Aungst: We have two here that we can get to. So, how much of the innovation in food technology is driven by trying to emulate animal meat taste? I think you addressed that somewhat. Maybe just summarize again.

Leonard: Can everyone hear me? Okay?

Conference Coordinator: You are good to go.

Leonard: Yeah, right now, [inaudible] is plant-based meat technology. And getting that flavor right is a significant priority for us as a company. And for the industry in general. I would say, the bulk of the plant-based food-based are focused on meat and dairy. I don't know how to split between those two, but meat, ground meat, replicating the flavor of meat. If you talk to any of the flavor houses, they will be happy to show you extensive portfolios a meet flavor solutions they have developed. There is a significant demand for those products now. A lot of time and effort has been spent on trying to mimic that flavor. There is no substitute for the actual meat proteins themselves. That is where using synthetic biology is a tool to do that. And to provide an animal free option. To provide proteins and favor components that they can impact plant-based products is important. Right now, we are focused on how to get the highest level of fidelity between animal product and the plant-based product. That is important. Eventually, I see us as an industry working on new spaces, new categories, new food forms. Moving beyond mimicry to really create more growth.

Aungst: Thank you. The next question fits well with this. If you make an animal free egg using proteins that are major allergens in animal products, will consumption by the allergic individual differ?

Leonard: Will allergenicity be any different? In the animal free version?

Aungst: Yes.

Leonard: The answer is no. The proteins are the same. So, allergenicity would continue, you would have to deal with that. And we need to do at that as developers and food companies. The providence is less important than the actual composition in the protein and the protein composition. The answer is no.

Dietz: Thank you, Jason and Michael. I do want to be cognizant of our time. I also do recognize there are some other questions that had been submitted. Hopefully we have time to get to them during our panel discussion today. I would like to turn now to our final talk this morning to talk about safety considerations for proteins from novel sources. That will be from Dr. Supratim Choudhuri, who is a senior toxicology reviewer in FDA's Office of Food Additive Safety. Dr. Choudhuri has reviewed GRAS notices, biotechnology notices, food additive petitions, and has played a pivotal role in issues pertaining to the use of bioinformatics in the review process. Before coming to FDA, Dr. Choudhuri was a research faculty member at the Department of Pharmacology and Toxicology at the University of Kansas Medical Center. Dr. Choudhuri, take it away.

Safety Assessment Considerations for Proteins from Novel Sources
Supratim Choudhuri, US FDA CFSAN, College Park, MD

Supratim Choudhuri: Hello.

Dietz: Yep, we can hear you.

Choudhuri: Okay, great. So far you have had quite a few very nice talks. Now we can get to Safety Assessment Considerations for Proteins from Novel Sources. Next slide please. I have no conflicts to declare. Next slide please.

The objective of this talk is to discuss general biological aspects of proteins in food. Known and potential adverse effects of proteins in food. And discuss in more detail the principles of safety assessment of new proteins in food. Next slide please.

Proteins provide us with structural and functional components. Structural components such as actin and keratin. And functional components just that it intra- and extracellular transporters. Ferritin and transferrin are involved in ion transfer and storage. Uptake and efflux membrane transporters. As well as the nuclear transporters. Antibodies, enzymes, messengers such as growth hormone, and various offense-defense effectors, such as plant and animal toxins. Next slide please.

Dietary proteins are also macronutrients. There is increasing consumer preferences for plant-based proteins, which have been triggered by increasing health consciousness and environmental awareness. The plant-based proteins are less costly than beef, lamb and goat. And plant-based proteins facilitate in reducing the greenhouse gas emission.

FDA's experience in terms of submissions for proteins from novel sources are of two types broadly. One are submissions of proteins with high proposed daily intake such as from plant sources which is the increasing trend, from fungal sources *Fusarium* species, and new proteins and also proteins with loaded intake. This includes bacterial or endogenous proteins genetically engineered into plants. And that is used in cheese making and many enzymes. Proteins with low daily intake usually have in micrograms. This group of proteins will not be the focus of this talk. We will focus on those proteins with high proposed daily intake. Next slide please.

Adverse effects of various food proteins could be mediated by excess protein intake, mis-folded proteins, and allergenicity and toxicity. So, it could be a problem for people with a sedentary lifestyle if they consume excessive protein. More than 2 to 3 grams per day for a long period of time, it creates a metabolic burden on various organs. Including your kidney and bone. These proteins we know prions cause neurodegenerative diseases. And proteins of course responsible for some allergenicity and toxicity which will be the focus of this talk. Next slide please.

The big eight is a group of proteins that cause allergic reactions in some people. Source labeling is a primary means of protecting consumers. But allergenicity can be caused by other foods. Their most foods are fruits and vegetables examples are kiwi, celery, sesame, mustard and lentil. The incidence of food allergy is about 6 to 8 percent in children and 1% and 2% in adults. And they often develop during infancy and early childhood. Many of them outgrow the allergies. About 80% of all food allergies in children are due to peanuts, milk and eggs. Fortunately, childhood food allergies are usually outgrown. Unfortunately, allergies to peanuts, trees, and fish are rarely resolved in adulthood. Next slide please.

Some propose characteristics of allergens. This is not universal. High levels in the source. For example, peanut allergy. They are present in very high levels in the source. About 15% or more than that. Many allergenic proteins tend to be heat stable. They have disulfide bonds, and they are stable in SGF which is essentially pepsin. The disulfide bonds confer heat stability as well as stability in SGF, so if the protein has many disulfide bonds, that could be a problem. As a food protein. Glycosylation was thought to be an important contributor to allergenicity but now it’s thought that glycosylation all by itself may not be that important. Many proteins have allergenic and toxic structural motifs that have been identified. A [inaudible] motif, which is [inaudible] stabilized by three disulfide bonds. Toxic motif which is the ration between glutamine and [inaudible]. Most of the toxic motifs are found in proteins that are in the [inaudible]. Next slide please.

Allergenic and toxic plant proteins and food are restricted to a small number protein families or superfamilies, such as cupins, prolamins, plant defense system proteins, pathogenesis-related proteins. And seeds contain various naturally occurring compounds toxic to humans and animals, such as trypsin inhibitors, phytic acid, lectins. Fortunately, many foods we eat have those nutrients but at a very low level. For safety assessment, [inaudible] is that if a protein is from an allergenic or toxic source the protein is assumed to be allergenic or toxic unless demonstrated otherwise. This is a presumption that we go by. Next slide please.

There are two issues with protein, one is toxicity and allergenicity. Allergenicity being the primary group. There are two factors important to remember that most food allergens are proteins. But most food proteins are not allergenic or toxic to the majority of the population. Any source of protein could be allergenic to some individuals in the population. This is very important to remember. It is probably no source of protein that can be guaranteed not to be an allergen to some people somewhere in the world. So, the protein source and its history of use in food are important considerations in the safety assessment of both total proteins and individual proteins. Next slide please.

For individual proteins we file a weight of evidence approach for both toxicity and allergenicity. It was recommended by FOA, WHO, and CODEX. Some of the elements of the weight of evidence approach include the source of the protein and dietary intake levels, biological function, history of safe use, and sequence comparison with known allergens and toxins, in vitro digestible in simulated gastric fluid, oral toxicity studies. And additional studies, as needed. There is room for a case-by-case approach, depending on the protein itself, as well as the source. And the history of use of the source. Next slide please.

I will discuss four examples here. Pea protein and protein from *Fusarium* as examples of total protein, beta-lactoglobulin soy and soy leghemoglobin as examples of individual proteins. These submissions came part of a GRAS notice. All GRAS submissions are publicly available so you can check them out on the web.

For the pea protein is a new use but it has been historically widely consumed. It doesn't consume forever. The history of use is a very serious element of pea protein, total protein from pea. It is up to 50 grams of pea protein, we have received many submissions on pea protein and the exposure, the intake, varies from about 10 to 15 grams other way to about 45, 50 grams. Safety data includes ADME studies in humans, which was conducted with pea proteins labeled with [inaudible]. There was subchronic toxicity studies in rats. And mutagenicity and genetox studies were provided. And there were a number of studies on humans, investigating intolerance, satiation, and benefit. And all submissions had a discussion on the relatively low allergenicity of pea and discuss labeling to inform consumers. This is the overall safety paradigm for pea protein. And of course, there were no toxicology that were evident. From animal studies or even human studies. Next slide please.

Safety assessment of *Fusarium venenatum*. Protein from *Fusarium venenatum* is also known as mycoprotein. By now it has 40 to 50 years history of use. It was approved in the UK in 1985 for use in food and FDA the GRAS in 2001. So, intake was up to about 46 grams for the general population and the safety data impacted quite extensively included compliance with several specifications. That is an important element because it was chemistry and toxicology. For example, the final protein the final product has to have less than 2% content but because too much RNA because of metabolism from uric acid it is not good for people with gout or kidney stones. Several long-term published studies were submitted. They were in rats, dogs, baboons. That included from 90-day subchronic study all the way to two-year carcinogenicity study, reproductive and developmental toxicology study. The studies were also submitted. Adverse GI symptoms were noted. Approximately one per 140 thousand. They concluded that about 5 percent of the reported adverse effects were true allergies whereas the rest were food intolerances. The product is labeled. Next slide please.

Moving on to the example of a single protein. Beta-lactoglobulin expressed in *Trichoderma reesei*. High levels of proposed intake up to 48 grams and for general population it is lower and safety assessment is quite simple, straightforward, rather, a discussion on the safety of *Trichoderma reesei* at the host of expressed protein. It is non-toxigenic, nonallergenic, nonpathogenic, and widely used organism for expressing proteins and enzymes used in food. And a history of exposure to Beta-lactoglobulin through milk and whey protein is also wide, and source labeling for people allergic to milk. The safety assessment of Beta-lactoglobulin relatively straightforward.

Soy leghemoglobin is an individual protein, it's a new and novel protein. It was never, to our knowledge, it was not used or consumed before. It is obtained from a novel tissue source. It was expressed in *Pichta pastoris* and soybean is one of the big eight. Next slide please.

The facts to remember for Soy leghemoglobin is that soybean contains several naturally occurring compounds toxic to humans and animals, such as trypsin inhibitors, and phytic acid and soybean toxin. Which is a protein containing two chains of 127 amino acid. 117 amino acid connected by a disulfide bond. All these are found in soybean feed. None in root nodules. The source is root nodule, which doesn't contain all of these toxic elements. Toxic compounds. Next slide please.

So, the toxicology package had 28 day feeding study in rats. No evidence of systematic adverse effects. The feeding study was preceded by a range-finding study for 14 days. The doses used in the range finding were increased in the final 28 day feeding study. There were Ames assay and chromosomal aberration assay, which shows that it is non-mutagenic and nonclastogenic. And the Toxin Database search and BLASTp did not return any positive hits with low toxins or allergens. We had a good discussion on *Pichta pastoris* and the nontoxigenic, nonallergenic nonpathogenic host for protein expression. Which is widely used for expressing proteins and enzymes used in food. Next slide please.

So, moving on to allergenicity. This soybean itself is one of the big eight known allergens. Son we searched whether we could find Soy leghemoglobin as an entry in the allergenicity databases. This is the WHO-IUIS database of every allergen enlisted in this component. No hits. Next slide please.

This is an Immune Epitope Database and Analysis Resource. This is maintained by NIAID. No hit on leghemoglobin. All the non-toxins from soybean are listed. But no hit on leghemoglobin. Next slide please. This is the database of SDAP. All the known allergens of soybean they are returned, but no hit on leghemoglobin. Next slide please. This is the COMPARE database. Again Glycine max. 43 entries. No hit on soy leghemoglobin. Next slide please. This is allergen online. 43 entries. Search term was glycine max. No hit was returned on leghemoglobin or soy leghemoglobin. Next slide please.

Searching and multiple databases they do not reveal any report of allergenicity of soy leghemoglobin. An absence of evidence. That is not the same as evidence of absence. We had to analyze the other elements of the weight of evidence approach. Next slide please.

The dietary intake of leghemoglobin was 6.7 milligrams per kilogram per day which is less than 1% of IOM daily protein recommendation. The biological function, mode of action, was oxygen binding like hemoglobin and myoglobin. And similar to human cell structure, it was predicted, and it did overlap. History of safe use, we do not have the data. Digestibility and bioinformatics analysis, I will discuss them separately. Next slide please.

So, digestibility. Next slide please. Digestibility is studied one element of the weight of evidence approach. Allergenic proteins may show a greater resistance to STF *in vitro*. Specially under lower pepsin ratio such as one pepsin to one microgram of protein. A one unit of pepsin to 10 micrograms of protein. And different views exist in the literature. In real life, when we eat protein the amount of pepsin in the stomach that digests the protein is far less than the amount of protein. Also, the idea that allergenic proteins are more register for digestion than the non-allergen proteins were provided in many publications in the beginning in 1996 all the way to early 2000s. There are many allergenic proteins not registered to digestibility. Nonetheless, digestibility remains an important element of the weight of evidence approach.

The notifier performed the digestibility using the usual 10 to 1 ratio means 10 units of pepsin in one microgram of protein. We have a good information base on the kinetics of protein digestibility using the 10 to 1 ration because almost all protein that are engineered in the plants have been subjected to digestibility using this ration. We have quite extensive information based on that. One information base using the one-to-one ratio. Next slide please.

Here is the in vitro digestibility. On the left is 10 units of pepsin. On the right is a gel with one unit of pepsin. As you can see, the soy leghemoglobin is hardly visible. So, with 10, the soy leghemoglobin it is totally digested by two minutes, three minutes, or four minutes. But it is so. Next slide please.

This is an *in silico* digestibility prediction of soy leghemoglobin. On the top is the leghemoglobin protein, on the right we have prediction output. You can see it is pepsin. There are 33 cleavage sites. You see the numbers. It means that the pepsin starts, the target of the pepsin is located on the right side. Terminal that you can see. So, you can focus on number 19 through 29 which means that pepsin will digest the bond that links amino acid number 19 and 20. Therefore the fragment that will be created will contain a system through 29. This information has been tabulated on the left. As you can see, the numbers. Fragment span and fragment length. There are about 6 fragments that resisted pepsin digestion. When we do this fragment and plastic. They have to be in a database. There were overwhelming hits. Overwhelming. I mean more than 90%. There were some similarities with various enzymes. Such as oxidoreductases, transferases, and sigma fator, selenium binder, but no hits with nontoxins. And when we subjected these fragments to an additional in silico digestibility prediction using trypsin, many of these fragments are further broken down. So, the *in silico* digestibility prediction of soy leghemoglobin either complemented the experimental data and most likely the protein is broken down into very small fragments. So, one thing to emphasize here is that most proteins develop, no nonallergen proteins are digested down to a single amino acid. There are some fragments that are created, different transporters, various amino acid transporters as well as oglio transporter peptides.

Moving on to bioinformatics. Bioinformatic analysis is another element of the weight of evidence approach, again recommended by the FAO/WHO CODEX. The paradigm has two elements. One is searching for 8 amino acid identity for potential cross-reactive epitopes, and greater than 35 personal identity, over a sliding window of 80 or more amino acids. So, the sliding window is a window that works from one amino acid at a time and looks for greater than 35 percent personal identity. And according to Aalberse, there is one more element that should be greater than 50 percent amino acid identity to known allergens. So, these predictions are all based on sequence alignment-based approach. Now, these are the original approach sequence approach; in the meantime, other allergenicity prediction algorithms have been developed; for example, SVM prediction methods. Next slide, please.

Sequence alignment-based analysis predicted soy leghemoglobin will be a non-allergen. [Inaudible] SVM-based analysis, there were about four or five. All of them except one predicted it to be a non-allergen. It is still available on the web. They predicted soy leghemoglobin to be a potential allergen. Had to do some extra work. As I mentioned the protein function the body that function is an important element of the weight of evidence approach. They ran hemoglobin and myoglobin which also have the same function like oxygen binding. This program predicted allergen. As hemoglobin as a potential allergen. So, there may be something in the amino acid composition of the oxygen binding proteins that is [inaudible] prediction. It turns out that this particular human base program is a high false positive prediction rate. Which is okay because for allergenic protein would rather have a false positive, which you can pursue, rather than a false negative. All of this information was distilled, to conclude that soy leghemoglobin is not an allergen. There is reasonable certainty that it is not an allergen. Next slide please.

The question is why use non-sequence alignment-based methods? For a novel protein, the potential allergenic and toxicology of epitopes may be unique. And in alignment which is a sequence alignment-based analysis does. There is a signature sequence by introducing gap opening and gap extension to provide with the highest score. The non-sequence alignment-based methods should also be used and also a GRAS notice. For this conclusion. It is expected that they will harness whatever information is available to provide it to FDA. Next slide please.

I will move on to a different example to underscore the utility of bioinformatics. And an example of what I call a case-by-case approach. This example is HPPD. Next slide please. So, HPPD W336 is the same protein with one amino acid substitution. That is the amino acid number 336 which is substituted from glycine to tryptophan. This was used in GE soybean. MST-FG072-2. They predicted that HPPD protein could be a potential Mollison. But HPPD is digested in vitro. This is a using HPPD. On the left is an SDS-PAGE and on the right is a Western Blot. As you can see HPPD protein is gone in 30 seconds. In the Western blot you could not look at HPPD. Next slide please.

When we ran HPPD alignment with that of other food, other bacteria here. You see that the percent identity and percent similarity are very high. Percent identity is almost like 55 to 72%. Remember that if the proteins have greater than 50% identity, then it should be looked at more carefully. Next slide please.

This was a multiple alignment of all of the other five proteins. Using Clustal W 2.1. And on the right side you see the *in silico* digestibility prediction of HPPD. As you can see they are the ingredients of identity, two regions have been highlighted here, one is in gray and one is in blue. The gray sequences highlighted here it includes identical amino acids as well as other substitutions. Those substitutions are not expected to change the functionality of the protein. Involving those amino acids. The gray highlighted totally digested as in silico digestibility. But the blue highlighted regions, they survived through digestion. You can see it involves, on the right side amino acid 234 through 244 and amino acid number 342 through 355. Again, there is that question. Are these regions somehow involved in [inaudible] a true [inaudible? This analysis gives us a question which leads to the designing of experiments. Next slide.

HPPD was incubated with whole blood at one, 10, and 100 microgram of concentrations. The highest concentration was 100 microgram per mL was chosen based off theoretical prediction of his 10 milligrams per kilograms is injected intravenously, then the black concentration of that protein would be 100 micro per mL. That was the highest dose. There was no hemolysis. Positive control saponin caused maximum hemolysis. They designed additional studies. With 2000 milligrams of HPPD per kilo body weight did not show any hemolysis or any clinical signs. There is another one with 1000 milligrams of HPPD. No hemolysis, no clinical signs. So, the actual experiments with animals both *in vitro* and *in vivo* showed that HPPD is not a hemolytic protein even though the bioinformatic analysis indicated that it could be. This is an example of how bioinformatics can be used to direct further studies. Next slide please. Here is a publication in *Regularly Toxicology and Pharmacology*. Next slide please.

Some take-home points. In terms of food safety. The involvement of the consumer, the food industry, and the FDA. The allergenicity of the proteins is not widespread but could be a concern for some sensitive consumers. Avoidance is the key. Source labeling source labeling and packaged foods is useful tool to warn susceptible consumers. The [inaudible], for example a responsible practice would not introduce a known allergenic protein in corn, which is a food that cannot be labeled to warn the consumers. Continuous engagement of all stakeholders with FDA important in the process. To summarize, next slide please.

Allergenicity of food proteins is not widespread but could be a concern for some sensitive consumers. The WOE approach has served us well for the last 20 years. Depending on the protein, further targeted studies may be needed. Hence, a case-by-case approach of safety assessment is important. Further improvements in non-sequence alignment-based prediction methods and allergenic epitope mapping of food proteins should increase the prediction accuracy. And confidence in the outcome of analysis. Allergen source labeling and packaged food is an important regulatory tool to protect consumers. Responsible innovation practices by the food industry can ensure safe food. Next slide please.

I would like to thank SOT for organizing the symposium on this topic. I would like to thank OFAS management and OFAS colleagues for support and encouragement. Thank you very much.

Dietz: Thank you, Supratim, for a wonderful summary of the framework for assessing the safety of proteins. The ingredients that we eat every day. And the vast majority of which we do not have safety concerns about as individuals. For some proteins and some individuals there most certainly are. I thought your examples were very enlightening about how that framework is put into practice this on actual ingredients that come before us. Thank you very much. Jason Aungst, do we have a question or so that would be specific for Supratim?

Aungst: Yeah, we have one here. Two common proteins, that is, whey protein and casein protein, are cross-linked. Assuming bound by [inaudible], is there any safety concern?

Choudhuri: Whey protein has a number of proteins [inaudible]. It's whey protein and the other one is?

Aungst: Casein protein.

Choudhuri: OK. First of all, these are coming from milk, which is a known one of the big eight. This will be labeled to alert the consumers. In terms if they are linked by a bond, when it could be a problem. Theoretically, it could be. We consume proteins that have disulfide bonds. Usually, they don't have 3, 4, 5 disulfide bonds. If these two proteins are linked by just one disulfide bond. It's hard to see if it would be a problem. It would be a problem for certain people. That's difficult to predict.

Dietz: Thank you, Supratim. Jason, other any other questions specific for Supratim, or can we move into the panel discussion?

Aungst: Nope, that was it.

Roundtable Discussion
Moderator: Jason Dietz
All speakers

Jason Dietz: OK, thank you for folks who had speaker-specific questions. Let's move into the panel session. I would invite folks to share their questions for the panel in the chats. And Jason Aungst will curate them as we go along. I would like to get things started with a very broad question. I think it will give all of our speakers an opportunity for some input. This question would be for the entire group. What you see as the greatest impact of the application of new technologies to food production on our food system; is it food safety impact the greatest, food security, nutritional quality, food supply chain, or any just general food system impact? I'm thinking for this let's go in the order of our speakers from Jeremiah to Pam to Michael and Supratim. Jeremiah, do you have particular thoughts on this?

Jeremiah Fasano: Sure. It really strikes me, especially after listening to some of the subsequent discussions, it seems that the biggest impact is likely to be not in safety or anything like that. But more just about the range of possible tools that are kind of available to us. And the number of options folks will have in developing particular kinds of foods and functionalities. I was particularly interested in Michael's comment about developing sort of new, sort of iconic foods. Foods that might stand on their own rather than being mimics; that is certainly an interesting direction. I really enjoyed all these talks. the range possibilities opening up is a very interesting one.

Dietz: How about Pam, do you have thoughts on this?

Baraem (Pam) Ismail: Yeah, I think the impacts, I agree with Jeremiah. I also think the impact could really impact be or include food security as well as the food supply chains. Really all of them including nutritional quality as well. With more technologies for food production using alternative sources, giving a variety of options for consumers. That are seeking really high nutrition. They are seeking sustainable sources. And they are obviously seeking food safety or safe sources of food. I think with these new technologies that we can provide alternatives to these consumers based on their ask. I do agree that the impact would be on all of these altogether.

Dietz: Thank you. Michael?

Michael Leonard: I think the environment we have all witnessed around Covid and continue to witness with Covid spread over the past nine plus months now has provided the need for diversity and the ag supply chain and we have seen increased adoption of plant-based foods for variety of reasons during this period. Food safety is a significant element of this. You can produce proteins that have historically only been able to be produced in an ag supply chain. In a much more controlled environment. We actually have a roof on the factory versus an ag situation. Food safety considerations could be really brought to the front with alternative proteins especially those from fermentation in a positive way. I think also it de-risks the ag supply chain in general as we grow the plant-based food supply. You have now got a more healthy coexistence of alternative proteins and animal-based proteins. I don't see the alternative protein space overtaking or completely replacing the animal ag supply chain. I do not think that is realistic. I think will always be looking at coexistence. Finally, I think as a benefit to consumers materialize. As we deliver on nutrition, taste, performance, I think that provides, it helps cultivate the environment for more acceptance of technology and food. When consumers can really see the benefit and it is tangible to them, especially in food, which is a very intimate thing for everybody. It opens it up for discussion around technology that has not been possible previously.

Dietz: Thank you. Supratim? Supratim, you may be on mute. Can our AV staff help us here?

Conference Coordinator: He is unmuted.

Supratim Choudhuri: I am sorry, I was somehow disconnected. I just joined again.

Dietz: Okay, Supratim, why don't we catch up with you on the next question then, okay?

Choudhuri: Okay.

Dietz: Actually, I have a question I think really would be relevant to Michael and Pam, what you had talked about. Pam, you talked about the need for protein as our population grows. Are there specific advantages of plant proteins in terms of their ability to be transported, for instance without refrigeration or things like that, that would enable these proteins to have places in the food system that historically animal proteins may not have been able to have because of transport requirements and or storage requirements, and shelf life?

Ismail: It depends on the crop itself. And the composition of the crop, and in what form it is being transported. If it is being transported as a grain or the seed or is it being transported as an ingredient already after production. Many factors can play a role, depending on the composition. If we are talking about a grain of seed with low moisture content, transportation would be easier. If we are talking about the ingredient as a powder, it would be this same, applies, kind of similar to a powder form of an animal source. Again, composition plays a big part of it. If it is high in fats, if it is, again, low moisture food but it is being transported under certain humidity and temperature that might cause caking. That can cause having an adverse effect on the formulated product. It is very dependent on the composition and the state, what are we transporting? In some cases, it is better than animal, and in some cases, not. I know I didn't give you a very clear answer. It is really dependent on the situation.

Dietz: That was perfectly clear. That the answer is really depends on the situation. That's very good. Could we go to Jason; are there questions from the audience we could go to?

Jason Aungst: Yes. In general, consumers have historically been left accepting that anything artificial is not natural. What is the approach to drive consumer acceptance of these synthetically made proteins?

Leonard: I can take a crack at that one to start with; that is the key question. It goes back to this point around benefits that I talked about. The ongoing concern around genetic modifications, synthetic biology comes from a lack of ability to connect benefits with technology and a lack of understanding of what this stuff really is. As an industry we have done a bad job of communicating that. I don’t think we have to reprise the how we got here discussion. It creates a mandate for us as developers at these from an industrial standpoint to raise the bar on the consumer experience. The best way to open that conversation is to show people foods can actually be better, more nutritious, more tasty, and cravable. It requires a concerted effort from all of us to align on the message and to talk about the technology in a way that is not confusing. So far, that is a work in progress. A lot of players in the industry are talking about it. We're trying to figure out the best way to cultivate that environment for acceptance. We are still very much building a concerted view on how that works. For me it starts with benefits. People have to see the benefit outweighing the uncertainty and inviting them in to learn more.

Ismail: Yeah, I might add here. I agree with Mike completely. How are we going to educate the consumer? It is very important. Making sure they have places they can go to and listen and read and understand about these technologies. The fact that they might not be as concerning from a safety perspective as they might have perceived. Education is very important in terms of having them accept these new technologies and these proteins.

Dietz: Thank you. Jason, do you have a second question?

Aungst: Yeah. Are there concerns about adding too much food additives and flavors to make plant-based meat more like animal meat?

Ismail: I can speak to that briefly too. Yes, there are concerns. That's what we hear also from formulators and food producers. Asking about how can we deliver good flavors with less food additives. Because consumers are looking more and more into lesser number of ingredients on the label. The work that has to be done really is looking at ways we produce our protein ingredients or novel protein ingredients, how can we eliminate the production of off flavor or how can we eliminate flavor that are innate to the original crop? Through extraction without impact on the protein characteristics. This is an open area of research. Needed right now, much needed. So that the protein formulation for meat based—or I mean plant-based meat or any other plant-based project, is really to get rid of flavors that might come from the production of the ingredient itself. That is one step. The other step is protein flavor interaction is a big deal. You have a protein, no matter what its source is. You are adding some flavors that mimic the natural cherry flavor for example. Not necessarily a meat alternative in this case. But protein can interact very strongly with natural and synthetic flavors. Sometimes in covalent bonding and then render a flavor absent. So, you no longer can perceive that flavor. Given the interaction with the protein. That is another area that research is focusing on now. We have several research going on with flavor houses to understand the flavor interaction with protein. You have the off flavor bit and you have the flavor interaction where the protein hangs onto that flavor and does not allow us to perceive it.

Leonard: To quickly build on that. In addition to everything Pam said. If we are good at actually producing the target protein that we want, so we can produce an animal free version of a muscle protein, it alleviates the need to support an analog with a whole bunch of other things. If we get the right functionality out of our ingredient development work. In this case as an example just fermenting the actual protein that is in the animal product using microorganisms. That serves a variety of functions that no longer require us to add certain ingredients that might complicate the label. It's an ingredient development task, the interaction part is absolutely key. Regardless of what approach you take to develop the ingredient, if we are good at producing those proteins that are faithful to the animal version, then that can typify the formula quite a bit. Formulators can take things out, instead of put things in.

Dietz: Thank you, very interesting. Jason, do we have more questions?

Aungst: One question that came during Pam's talk. The main petitions of PDCAAS does not take into account anti-nutrient factors, which limit the absorption of protein among other nutrients. FAO has recommended a new DIAAS, digestible indispensable amino acid score to supersede PDCAAS. [Inaudible] measuring amino acid digestion through the ilium, which alternative do you envision as feasible for the food industry?
Ismail: Yes, this is a give very good question. Again, a question that has been seeing a lot of attention recently. Especially from industry. So, the DIAAS, yes, it is a newer method that takes into account factors that is not considered with PDCAAS. Currently though, some food industries are also requiring that we use a method that

does not involve animals. That is another issue. Again, with animal welfare and the use of animals in studies. Even though DIAAS might serve a better, it might be a better indicator, it is also utilizing an animal source. Basically, there have been a lot of research looking at the *in vitro* method for a DIAAS as well as PDCAAS so that we can simulate some sort of prediction of the PDCAAS or DIAAS. It is not yet official method of analysis. I know there are several labs trying to get the approval for such methods, *in vitro* methods. There are a couple of research studies out there comparing the results from *in vivo* to *in vitro*. Plotting and determining the correlation and looking at square, if you get good correlation between *in vivo* and *in vitro*. That proves the *in vitro* method can replace. Research is still ongoing in that area. I think it is going to go in that direction of *in vitro*. There are always going to be limitations in terms of how well that prediction is, and is it really taking into account all of the different factors, including the [inaudible]. Say you have enzyme inhibitors. Or any component that might interact with the protein at prevent its digestibility. It's an open area for research.

Dietz: Thank you. I have a question. Thinking about the presentations today, I recognize that one of the major factors here is to be able to do things in a laboratory. And get results, that you find useful. The challenge is to then scale it up into a factory level to make a product. That is successful, performs as you hope. Can you all talk about some of the challenges associated with scale up? With proteins from novel sources or plant proteins?

Leonard: I can kick that one off. We can talk about this from a couple different perspective is. We can start from a fermentation standpoint. This is one of the critical challenges facing companies that hope to use synthetic biology and fermentation techniques to produce proteins or other ingredients. Fundamentally you're limited by tank volumes and how much tank capacity you can install. You've got a bit of a different set of economics governing the scale up process than you do with more traditional approaches. Cost becomes a huge driver of how decisions get made. In food, we're not talking about pharmaceuticals here. We're not talking about very large margins and profits, but the margins are a bit thinner so cost sensitivity is much higher than other industries where fermentation might be a no-brainer as a mainstay technology. Grappling with that scale curve from a cost standpoint for fermentation is something we are all trying to figure out. Producing things, producing animal proteins in stainless steel tanks we have to keep installing is a very different composition. Cost is a significant thing there. With respect to other proteins from ag sources, process development work to scale from bench to commercial is highly nontrivial. Despite the fact we made a lot of investments in soy protein processing, pea protein processing, there is still quite a bit of fundamental process element knowledge that is being built now. And has to be built. This gets back to my comment earlier about investment in science and technology. It applies not only to the discovery end but also to the processing and scale end. This is an area that is still emerging. We are still learning how to optimize scale level.

Fasano: This is Jeremiah, I want to chime in. No, go ahead.
Ismail: I'm sorry. I will quickly add to what Mike said in terms of processing and scaling up from a plant protein perspective from novel sources such as those sources, some I mentioned earlier in my talk. They're not just entangled with fiber they are also, they don't dissolve in neutral pH. Which makes it really hard to try to

get them out to this matrix and yet have them remain functional. The research going on to look at other ways to extract them in a way that can potentially be scaled up. Use of enzymes for example to break down some of that solvable material. Or using technologies other than alkaline extraction which is very common in the protein world or plant protein world. There definitely need to be process developed to help with the ease of extraction first. And scalability second. It's not just a challenge with scaling up; it’s also would challenge with all these new novel sources of proteins. With the different characteristics. Definitely poses a challenge in terms of extracting the proteins feasible at a cost-effective manner. At the same time, preserve the structure of the protein. Otherwise, your extracting protein, yes, you’re getting yield, but if the structure is destroyed. You can't do much with it. Definitely an area for more research. Sorry, go ahead Jeremiah.

Fasano: That's okay you said very eloquently exact the point I was going to make. Better than I could've. I will just say, it is easy to assume, once you identified a potential source that all the rest is just process. But process can be really involved. The margins are often thin. Pam as you were saying, now we have more concerns than ever about maintaining functionality, that’s another consideration. Techniques that were originally developed for doing bench top level research where you could spend a lot of time and effort with extra steps all becomes much more difficult to scale. So, basically to agree with what both Michael and Pam said.

Dietz: Thank you, I think that makes a really good clear that just the discovery is not the end of the research. Supratim, I would like to turn to you for a question. You talked about the weight of evidence and the case-by-case approach to assessing the safety of proteins. Recognizing that we could have proteins from new sources in the future, do you think we have a framework that is amenable to addressing any safety concerns from them? Supratim, you might be on mute.

Choudhuri: Hello?

Dietz: Supratim, we able to hear a question?

Choudhuri: I did hear your question, could you hear? I started talking.

Dietz: We can hear you now.

Choudhuri: Okay. Safety assessment is actually it is not straightforward; it’s not like a small molecule safety assessment. The weight of evidence approach has purpose as well. I think we will have to rely on the weight of evidence approach. The only thing I would expect the scientific community to do going forward, is, and I talked about this in the summary, more epitope mapping and improve the bioinformatics capability. The computational capability is increasing by leaps and bounds every year. Other than that, I cannot envision any change in the weight of evidence approach at this moment.

Dietz: Okay, thank you appreciate that input. One of the questions I had for the group. You talked a little bit about valorization of products. The ability to use products we might have thought of as waste products in the past. We are now finding new uses for them. Are there very specific examples you could share with us, or some compelling stories around that, that you could share?

Leonard: I'm not an expert in this area, but one thing that kind of struck me as I was preparing for this talk was this example of a biodiesel refinery. Where you are primarily using photosynthetic algae to produce biofuels, essentially, but the point was that, in order to make the economics of that work. It might be actually critical to be able to use all of those waste streams. It wasn't just an example of picking opportunity for additional process but actually looking at an entire facility with multiple streams coming out of it as an integrated whole from an economic perspective that we need to take advantage of those synergies to make that work. That struck me as an interesting perspective that I had not been thinking about before. In many cases, you're looking at valorization as basically additional usage for things that were just animal feed before. In some cases, it actually may be critical to the overall success of server facilities products.

Ismail: I might add from a plant-based perspective. Same story was soybean. It was originally used for oil production. The meal was discovered to have a high amount of protein that is nutritious. Same thing right now is other oilseeds are gaining the same kind of attention. Such as canola for example. You have the oil, and you have the meal. What can you do with the meal to valorize it for high-value protein ingredients? And sunflower is the same thing and other oilseeds. Again, the problem is we need to understand that when you produce the oil you want to keep in mind, you want to keep the structure again and the function of the protein and the meal. There is this dilemma of, are we going to take, extract every drop of oil no matter what? Or are we going to extract whatever we can of oil and then treat the meal more gently to get that protein out? That is where you had to have a balance between you want to get the oil out and also you want to get protein out that is still functional. Still some work in that space. Similar to what happened with the soy protein in the soy business. This has to also kind of repeat itself with other oilseeds. That is an example that I can think of in terms of valorizing a meal after production of oil. We do get, for example at the center, we do get requests for so many different byproducts, valorizing byproducts. Such as spent grain for example. Can we get that protein out and would it still have value to be leveraged? There are a lot of different examples to think about it definitely one has to think about how we are processing the initial ingredient. OK, we are taking starch out from corn; what are we doing to the protein that is left off? We are taking the oil, what are we doing to the protein that is left off? We have to have that mentality that we want to valorize the byproduct. What can we change in the upstream processing to protect that resource?

Dietz: Thank you. As we are wrapping up today. I want to ask our panel, having considered the talks that you've delivered and heard and the discussion here in the panel session, I want to give you an opportunity to make any points you think are important to escape today's discussion but may not have come out so far in the talks and the panel discussion. I open the floor to the panel for that. I think, then, it sounds like we had done a good job capturing the relevant points that we intended to set out to capture today and discuss. I am also looking at the time. I don't think we have more questions from the audience at this point.

I would like to thank our panel. And to say how impressed I have been personally with the messages that you had and the clarity and in which you presented them. And the very great interest in this topic. It has been a bit very interesting topic that is relevant to us every time we go to the grocery store. I appreciate the expertise that you brought for us today. Jason Aungst, I would like to turn it back to you for our company words for today. I very much appreciate the work of our panel. It has been an absolute delight working with you throughout this process. So, thank you.

Aungst: Thanks, Jason. On behalf of the Society of Toxicology and the food and Drug Administration, I would like to thank all our speakers for this great educational experience and for the engaging participation from our audience. It's great to see so many people participated in this colloquium. As a reminder, please keep an eye on the SOT FDA Colloquium website. We are working on developing the next colloquium for April 2021. The topic will be "The Toxicology of Nanoparticles." I hope to see you there. Thank you.

Betty Eidemiller (Conference Coordinator): This is a reminder to everyone you will be receiving a survey link. We hope that you will fill that out. Also look to the SOT website for the recordings of this and previous colloquia.

Dietz: Thank you, Betty. Thank you, everyone. Have a wonderful day.