Begin at the end when isolating plant proteins

Katie Cottingham

From body builders to vegans to those just wanting to lose a little weight, it seems that everyone is trying to get more protein in their diets. In specialty nutrition stores and the health food aisle of the grocery store, protein-packed products dominate. High-protein bars and shakes are popular, as is pure protein powder.

- **Consumers are increasingly desiring plant protein in their foods for health, cultural, ethical, and environmental reasons.**
- **Extraction methods can affect how proteins behave in products—sometimes with beneficial effects, but often with negative consequences.**
- **How a protein will be used in an end product is an important factor in deciding which isolation or concentration approaches to include.**

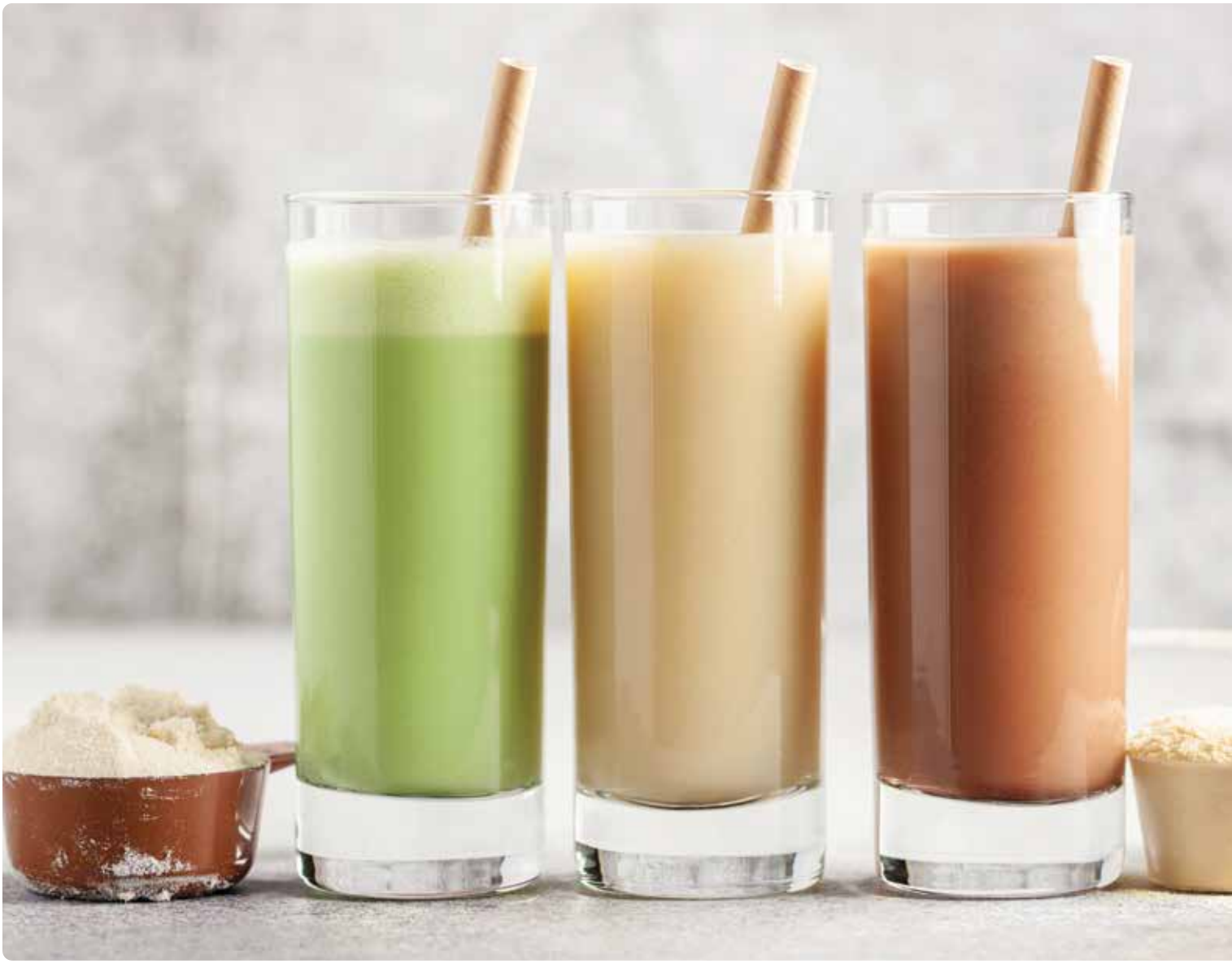
But whereas animal meat is naturally high in protein, plants also contain a high percentage of oils, fats, carbohydrates, and fiber. To concentrate or isolate protein requires a complex series of techniques that can change the structure and properties of these compounds along the way. Understanding the properties and composition of the end product is crucial for processors.

"It is really about tailoring the process to fit the final functionality in the food matrix," explains Jennifer Kimmel, head of R&D Americas at Roquette America, Inc in Geneva, Illinois. Roquette specializes in providing plant-based ingredients to other companies that make consumer products. "The properties that you would want for, say, a ready-to-drink beverage are going to be different from something you might want to put into a baked product."

WHY PLANT PROTEIN?

In the body, proteins do a lot of the heavy lifting, carrying out enzymatic processes and building muscle and bone. But not all protein is the same. Animal proteins from meat and dairy products, for example, are "complete," meaning they include all of the nine essential amino acids in the required ratios that humans cannot make on their own (https://tinyurl.com/3r56k8az). Although some plant proteins are complete, many are not, which means that a plant-based diet often lacks some essential nutrients. Vegetarians and vegans must be mindful of their protein consumption and combine the right plants, seeds, and nuts to have a balanced diet.

The reasons for eating a plant-based diet are varied. For some, it is a matter of geography or culture. Animal, or meat, protein has long been the go-to for complete nutrition in the Americas and Europe. But that is not the case elsewhere. "If you look on the global scale, plant protein is a large part of a human diet, especially in China and India, and the people in these countries have been consuming lentils, peas, and soybeans as a regular part of their diet," says Bishnu Karki, assistant professor at South Dakota



State University in Brookings. It is common practice for people in those locations to combine different plants in their meals. "My grandparents used to mix rice with lentils and beans without really knowing the science behind it," says Pam Ismail, professor and Plant Protein Innovation Center director at the University of Minnesota in St. Paul. "But they are nutritious—the protein from these sources complement each other."

Other people turn to plants because they take issue with raising animals just to eat their meat. Environmental and sustainability concerns, such as fecal run-off into waterways and the production of greenhouse gases, are other reasons that people cite for removing animals from their diets. And some consumers have allergies to dairy or meat products, so they have to seek out other protein sources.

Supplementing a diet with protein-packed bars or beverages, or sprinkling concentrated protein powder on foods are increasingly common ways to incorporate more of these nutrients. Although a large portion of these products contain animal-based whey obtained as a byproduct of cheesemaking, alternative proteins from plants are gaining traction in the market.

"The plant protein market continues to increase, based on 2023 reports," says Ismail. "The demand for plant protein is increasing, so we are seeing a surge in the global protein market." Aside from plants, alternative proteins can come from microbes, cultured animal cells, and even insects. In fact, Tyson Foods announced in October 2023 that it is investing in an insect-ingredient company with an eye toward using this type of protein in animal feed (https://tinyurl.com/d9n2wbte). But by far, the largest segment of this class is protein derived from plants.

Soy is considered to be a complete protein (https://tinyurl.com/4h7bwbrf) and is undoubtedly the major player in the market, as seen in the myriad of tofu products and alternative soy dairy drinks, but other plant sources are increasing in popularity. For example, pea protein is becoming popular, and is an ingredient in Beyond Meat® products and some protein bars. According to Ismail, pea is an easy

crop to grow and it has not been genetically modified. "Also, right now, pea is not allergenic, though things might change with more exposure to it," says Ismail. There is some debate as to whether pea is a complete protein—it has all of the essential amino acids, but some are not present in high quantities. Other plant sources being explored include chickpea, canola, and potato, Ismail adds.

THE COMPLEXITY OF THE IDEAL PROTEIN

The ideal properties of an isolated or concentrated protein depend a great deal on what the ultimate product will be, say experts. "There is a number of factors that are important, but you prioritize those depending on what the end application is," says Lolly Occhino, senior food scientist at the Agricultural Utilization Research Institute in St. Paul, Minnesota.

Perhaps the first question to ask is whether the product will be for animal or human consumption. Karki develops fish feed and says that purity is not as much of a concern for animal applications compared to when proteins are used in human food. She is concerned with removing glucosinolates, which are plant metabolites that have anti-inflammatory and antioxidant properties in humans, but can be toxic to animals.

However, Mila Hojilla-Evangelista, research chemist at the US Department of Agriculture-Agricultural Research Service in Peoria, Illinois, explains that even in human diets, certain glucosinolates, such as sinigrin, can be unwelcome, producing a pungent, sinus-clearing sensation. Proteins isolated for human food also would have to undergo regulatory screening processes for safety, she says.

If the protein is meant to go into a consumer product, taste is always a high priority, but then another important consideration is the form that the product will take. For example, will the protein go into a drink, a baked good, or something else, like yogurt? Occhino points out that a protein that foams could be a problem for companies that make beverages because it might not flow well through some types of equipment. However, foaming is generally desirable for a good crumb texture in a muffin.

Solubility is important for many drinks and liquid systems. "If we are targeting a beverage application, we look for solubility—the ability to stay dispersed in the beverage and not sediment or gel over time," explains Ismail. She also says that plant proteins are not that soluble, so they can be tricky to incorporate into liquids, but adding stabilizers can help.

Protein properties to consider for human foods:

- Nutritional value
- Digestibility
- Taste
- Solubility
- Thermostability
- Texture
- Color
- Gelling
- Foaming
- Emulsification
- Water retention

Preparing peas for protein separation. Source: Roquette

Proteins in meat substitutes or used as binders in animal meats have another set of ideal characteristics. Gelling and forming a fibrous 3D structure are important to provide a texture and mouthfeel comparable to that of animal meat. The protein should also retain water to make the product juicy.

If a product is made of components in two phases, such as an oil and an aqueous phase, then a protein that emulsifies is desirable. For example, muffins, yogurts, and cream cheeses require proteins that can help blend different phases. "If I am an ice cream producer, I want to replace the milk proteins with an alternative protein that has functional and nutritional properties, similar to the milk protein—something that would work well with the fats in the product," says Karki. "And from the vegan consumer's perspective, if I am able to replace the milk protein with the plant protein, how wonderful would that be?"

Another consideration is thermostability. "If you are trying to use the protein as an alternative dairy creamer, will it be able to withstand the hot temperature of a beverage and not precipitate out of it?" asks Hojilla-Evangelista. This is also a concern with products that a formulator or consumer would heat in an oven or microwave.

POSSIBLE PROBLEMS WITH PROCESSING

"Certainly, processes will change the protein structure, which will have a direct impact on the protein's functionality—sometimes in the way you want it to go, sometimes not," says Kimmel. But also, chemists with a deep understanding of how methods affect compounds can tweak methods to obtain proteins with ideal properties for their applications.

Soy protein isolation is generally considered the conventional approach that researchers start with when developing

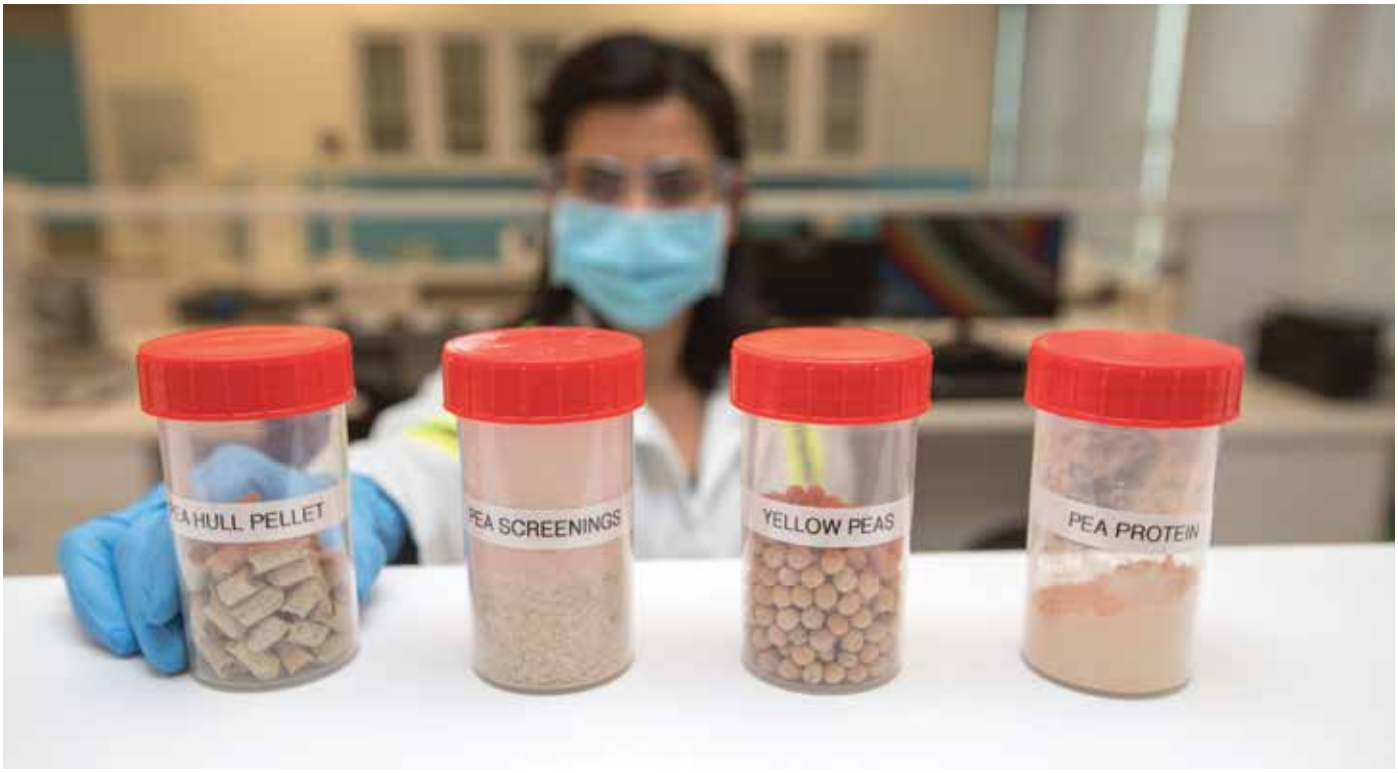
Replacing hexane

Hexane defatting is problematic for many reasons—hexane can be neurotoxic, is highly flammable, and is derived from petroleum—so researchers have been trying to replace it (https://doi.org/10.3390/foods11213412). Hojilla-Evangelista used ethanol instead and found that although it reduced the yield, the properties of the proteins were not affected negatively. To get around hexane, Karki has added enzymes sequentially—one enzyme releases the fats, another removes proteins. Because the conditions are less extreme than the conventional approach, enzyme-assisted extraction has potential, she says, though it is costly. Some researchers use a cold-pressing method instead, and other researchers have proposed deep eutectic solvents (https://doi.org/10.1021/acssuschemeng.1c01848, *INFORM*, February 2024 cover story).

new strategies. First, the protective hard shell, the fibrous hull, is removed. Defatting removes the oils and fats and usually involves an extraction with hexane, along with high temperatures and pressures. Then, the resulting meal undergoes alkali solution-acid precipitation in which the plant proteins dissolve at a high pH and then precipitate at low pH. The solid can be washed, and then it is typically spray-dried.

Some processing steps can have negative effects. Ismail explains that defatting can denature the protein. “Exposure to high temperatures during processing can result in protein denaturation and polymerization,” she says. “When the protein polymerizes, it forms large aggregates, and they cannot function as well.”

Because plants can have different compositions and characteristics, what works for soy may not work for other sources. When working with smaller seeds, even the first step is challenging. Karki says that seeds such as canola or camelina are so small that the hulls cannot be removed with existing techniques, so the whole seed is processed. In this case, the protein becomes contaminated with large amounts of fiber from the hull, forming a complex structure that is difficult to work with. The characteristics of the proteins in a particular plant also can make processing difficult. Whereas most soy protein can be extracted in alkaline conditions, Karki says that proteins in rapeseed meals require additional extraction steps using different solvents.



Stages of separation for pea proteins. Source: Roquette

The presence of phenolic compounds or residual lipids can lead to oxidation and off-flavors, as well as a darker color, says Ismail, and some compounds called anti-nutrients can interfere with digestion or soak up nutrients, preventing them from being bioavailable. But Hojilla-Evangelista, who works with an oilseed called pennycress, notes that some types of residues can be beneficial for certain applications. For example, residual oil in her extracts improved the emulsifying properties of some of the proteins in her samples.

In addition to testing different varieties that might respond better to processing, Ismail and Hojilla-Evangelista are developing new methods, altering and optimizing conditions for their plant of interest. Ismail closely controls the steps, uses mild pH conditions, and includes multiple washes and a membrane filtration step. Hojilla-Evangelista uses a functionality dependent approach. Pennycress proteins extracted with the conventional method had excellent foaming properties and were heat-stable. The saline-extracted proteins, made up of mainly water-soluble albumins and saline-soluble globulins, were good emulsifiers that were highly soluble across a wide pH range. For fish feed, Karki is exploring fermentation—having microbes do the work, producing concentrated protein from camelina meal.

SCALING CONCERNS

Developing methods at the bench or at pilot-scale is one thing, but the approaches do not always work well at a large-scale commercial level. "That is the biggest hurdle," says Karki. "That is where a lot of things need to be tackled." She notes that companies that isolate proteins want a technology that will fit easily with their existing equipment and workflows. For example, an overnight step or constant stirring might not be possible in an industrial setting, where continuous workflows and short residence times reign.

"We will run the plant for several days, so things like protein building up on pieces of equipment need to be taken into consideration," says Kimmel. As protein builds up on heat exchangers, the heat transfer properties can change, and build-up on membrane filters can alter what substances can flow through. In addition, moving gallons of solution per minute through industrial pumps will have a different effect than stirring a solution gently on a hot plate at the bench.

"I think when you are at an industrial level, there are always trade-offs," says Kimmel. Industry-scale processes can be energy intensive, and food safety is always an important consideration. Ismail notes that companies can sometimes make high protein yield a top priority, but doing so with high pH conditions, for example, can result in denaturation. "There is a balance between saving costs, increasing yield, and protecting the protein structure," she says. "Something has got to give."

GETTING A PRODUCT TO THE CONSUMER

Whether an ingredient supplier thinks they have the perfect protein does not matter if it is incompatible with the final consumer product. "We will manufacture an ingredient through one set of processes, and then our customers will take that and put it through another set of processes when they are making their final food application, combining it with different ingredients," says Kimmel.

Open lines of communication are key, says Occhino. She suggests that formulators let processors know what the final product will be, since that dictates what properties the proteins should have. Being aware of costs and possible supply chain issues are important, as is whether a food manufacturer wants to avoid the use of solvents like hexane. But often, communication is a one-way street because product and process information can be considered proprietary. "Frequently, bigger companies do not want to share details, but that will result in more work for them because they will have to then screen through all the different options that a supplier would have to send in," she says.

The final determinant is really the consumer, who could end up using the product in various ways, like heating it in a microwave or putting it in a blender. "It is important to know what you want the experience to be for the consumer, and then relating that to the science behind how proteins function, how they interact with other ingredients, and how they respond to processing changes," says Kimmel.

In the end, Kimmel says that putting all of the factors together requires a holistic approach, not just having a focus on high yield or low cost. "It is really knowing that the isolation process is only one step, and then food manufacturing is another step, and then the consumer experience as well," she notes.

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