

Breeding pulse crops for enhanced plant-based protein

Perspectives, challenges, and recent research efforts

By Alison Jennings

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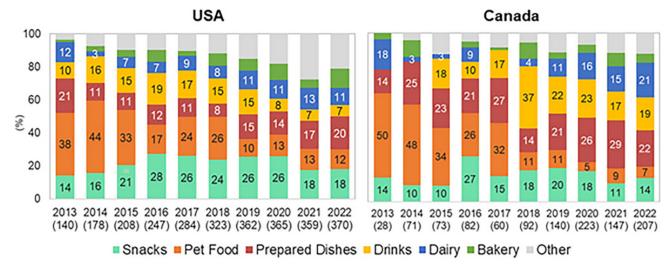
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 Pulse crops are a healthy, environmentally friendly alternative source of protein for consumers. With a rising demand for more plant-based food and drinks, more research needs to be done to unlock the true potential of beans, peas, and other pulse crops.

A review paper and two research studies, recently published in *Crop* Science, highlight how and why plant breeders are trying to enhance protein content and palatability of pulse products.

It doesn't matter if you're strictly vegan or more of a "flexitarian": if you aim to add more vegetables to your diet, you're part of an ongoing consumer trend that's seeking out plant-based meat and dairy alternatives.

Consumers have been steadily switching out their chicken for chickpeas and beef for beans. While animal sources of protein are still dominating the West, the rise of plant-based diets has nevertheless increased demand for more, higher quality, plant-based sources of protein in the market. There are good number of reasons for this, too. Plant-based proteins are cited to be low in saturated fats and high in fiber with cropping systems that are more environmentally sustainable than traditional animal protein production.



The demand for plant-based food and drinks has increased steadily in the U.S. and Canada over the past decade. Figure 2 from "Breeding for Plant-Based Proteins in Pulse and Legume Crops: Perspectives, Challenges and Opportunities." Courtesy of Valerio Hoyos-Villegas.

This demand has opened the door for an often-overlooked food group to step into the spotlight: Pulse crops—the dry, edible seed of legume plants, like beans, peas, and chickpeas—have gained attention as a viable source of protein. Recent market insight from Towards Food and Beverages predicts the legume market to expand from \$15 billion to \$24 billion in 2034, largely due to increased consumer interest in plant-based meals.

The ideal party food

Beans, lentils, and other pulse crops are more than just a dietary fad. In many countries, they're staples—the star of a Sunday night dinner. Valerio Hoyos-Villegas, assistant professor of plant breeding and genetics at Michigan State University, ultimately centered his career around pulses because of his childhood family meals.



Valerio Hoyos-Villegas found his love of agriculture growing up on a farm in his home country, Colombia. "I've been in contact with agriculture all my life, and I've always loved it," remarks Hoyos-Villegas. "It was so obvious to me that that's what I wanted to do." Photo courtesy of Valerio Hoyos-Villegas.

"What drew me to bean (*Phaseolus vulgaris*) breeding," explains Hoyos-Villegas, "Was the fact that [beans] were my primary source of protein growing up in Colombia. ... Food also serves as a bonding mechanism, and in Colombia, when there are family meals or social events—they frequently involve beans. So, beans have always felt personal to me."

Hoyos-Villegas runs a dry bean breeding program at Michigan State. "I did my Ph.D. at Michigan State, in the lab that I lead now. It's a great honor," adds Hoyos-Villegas. "I had a unique opportunity to lead a breeding program that is over 100 years old now."

As a crop breeder, Hoyos-Villegas' job is to produce new bean varieties that are superior to older lines. They are meant to be healthier, resistant to diseases, and sustainable. In a

new *Crop Science* review that Hoyos-Villegas coauthors, he advocates for the importance of agriculture in this field—it produces all the raw materials for these plant-based products, after all.

Adding amino acids, eliminating antinutrients

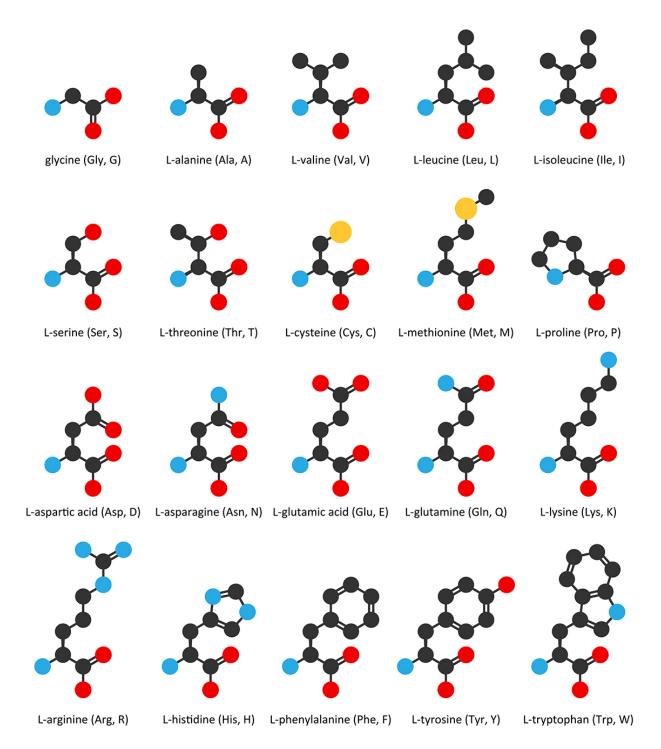
Beans, like many pulses, are already valued by consumers for their nutritional value—they provide energy, fiber, micronutrients, vitamins, and protein. So, what's there to improve?

"We call [beans] a near perfect food," says Hoyos-Villegas. "They're near perfect because they are exactly two amino acids shy of perfection."

Hoyos-Villegas explains that beans, while rich in other compounds, are lacking in two special, sulfur-containing amino acids: methionine and cysteine.

"If we could increase that profile, we would have a pulse species with a full array of amino acids," he says.

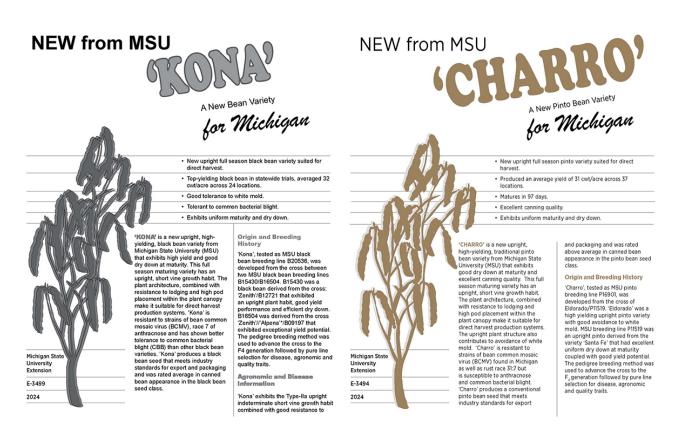
"We call [beans] a near perfect food. They're near perfect because they are exactly two amino acids shy of perfection."



Amino acids are the building blocks of protein. Beans and peas, like many other pulses, lack a substantial amount of methionine and cysteine, amino acids that contain sulfur (second row from top: yellow dots). This lowers the plant's protein digestibility corrected amino acid score (PDCAAS), or how well the human body can digest the available protein. Certain beans also lack a sufficient amount of tryptophan (bottom right), which, while not

a sulfur amino acid, is an essential amino acid for human diets. Photo courtesy of Adobe Stock.

But what is so special about these two, tiny molecules? Reduction in sulfur amino acids actually reduces protein digestibility, reducing the crop's nutritional value. Not having sufficient amounts of amino acids (especially methionine, which the human body cannot produce on its own) also makes this pulse crop an incomplete protein.



Breeders at Michigan State University (MSU) have been improving their bean crops for nearly a century. Not only are breeders attempting to increase protein concentration, but they are also aiming to increase micronutrients, yield, and disease resistance, in addition to making them grow upright instead of as a bush—which allows for easier harvest and less opportunity for mold. Above are factsheets for two 2024 variety releases: Kona black bean and Charro pinto bean, developed by MSU. Photo courtesy of Valerio Hoyos-Villegas and

There are other challenges bean and pulse crop breeders face. Many pulse crops contain "anti-nutrients," compounds that can have negative effects if eaten raw and in sufficient quantities. Think about how raw, dry beans need to be correctly prepared in order to eat safely.



Beans need to soak and be boiled on high heat in order to denature lectins. Soaking the beans (left) will remove some of the lectins as they are water soluble. Then, heating up lectins denatures them (right): The high heat causes the proteins to lose their shape, unravelling and effectively destroying them. This cooking combo makes beans safe to eat. Photos courtesy of Adobe Stock.

"So, beans do have to be cooked," explains Hoyos-Villegas. "They cannot be eaten raw.

They have heat-labile proteins (e.g., lectins) that we cannot digest readily. And so those

are what we call anti-nutritional factors. When cooked they are denatured, and that's what makes them safe to eat. ... But if we could reduce most of this anti-nutrient activity, then the potential of beans really changes. Using beans and pulses in industrial processes as whole seeds or ingredients in plant-based products will require innovations from crop breeders and industry processors in order to minimize the presence of anti-nutrients."

Improving amount and quality of protein in pulses, in addition to increasing micronutrient content, decreasing anti-nutrients, and increasing yield, are all ways to increase the value of pulse crops and combat malnutrition in areas where pulses are the predominate source of protein.

Using RNA-seq to identify genes related to pea protein

Just how do breeders attempt to tackle these goals? There are many methods agriculturalists can use to increase protein content in their crops. Researchers at the University of Saskatchewan recently published a *Crop Science* study about how they're doing this in pea (*Pisum sativum*) plants by analyzing RNA sequences (RNA-Seq) with supercomputers.

Unique challenges for different legumes and pulse crops

Crop	Main Challenges	Potential approaches and solutions	Traits of interest	Available Resources	Reported trait or QTL	References
Common bean (Phaseolus vulgaris L.)	Low sulfur-containing amino acids (Met - Cys). Trypsin inhibitors and Lectins. Low throughput phenotyping.	Breeding for: Increased protein hydrolysis and digestibility. Increased phaseolin and bioavailable Met. Higher SPC. Improved protein fractions. Flour characteristics.	Protein quality. Protein hydrolysis and digestibility. Biological Nitrogen Fixation (BNF) and N metabolism.	Available QTL for biological nitrogen fixation. BNF phenotyping QTL for seed protein content (SPC). Recombinant populations. Genetic markers. Lectin-deficient germplasm (phytohemagglutinins - PHA)	Protein Ndrolysis and digestibility. Sathe (2002); Luna-Viral et al. (2015); BBN: Olacad et al. (2020); Kamfwa et al. (2015); Protein quality: Viscarra-Torrico et al. (2021); De Lumen et al. (1999); Kochlier et al. (1997); Mecha et al. (2021); Voelker et al. (1986).	Portion Quality SNP:: Marquice et al. (2024) Protein cuality Germplasm De Ron et al. (2015). Jha et al. (2022). Marquice et al. (2021). Macha et al. (2022). Marquice et al. (2021). Marquice et al. (2021). Lectin Gene Editing: da Costa Moura et al. (2021). Lectin Gene Editing: da Costa Moura et al. (2024). Lectin Germplasm Surveys: Taylor et al. (2008); Yang et al. (2021). Sparvol et al. (2016). BNP Phenomics: Jafart et al. (2024).
Pea (Pisum sativum L.)	Low genetic diversity. High GxE. Negative seed protein. content and yield correlation. Traits with low heritability.	Use of QTL with major and minor effects on SPC. Genomic selection and gene editing. Breeding for: Amino acid composition. Protein digestibility	Increased Seed Protein Content (SPC).	Elite and wild germplasm surveys. QTLs for SPC and composition. Improved phenotyping methods. GXE assessment. Mapping populations and genetic markers. Genomic selection (GS) Gene Editing (GE)	SPC: Karjalainen and Kortet (1987) Tar'an et al. (2004). Krajewski et al. (2012). Ferrari et al. (2016). Tools and Morris (2012). Franck et al. (2024). SPC and N content: Burstin et al. (2007).	SPC Elite and wild Germplasm Surveys Undice et al. (2024). Winther et al. (2023) Twinther et al. (2023) Tarkins et al. (2006), has et al. (2013), Phenomics Taglivael and Smith (2007). Inglivael et al. (2022) markers: Galler al. (2024) Germanics: Galler al. (2024). See jake et al. (2019). Undice et al. (2024). See jake et al. (2019). Windice et al. (2024). See jake et al. (2019). Yang et al. (2020). See jake et al. (2022). Saludares et al. (2022); Saludares et al. (2022).
Chickpea (Cicer arietinum L.)	Traits with low heritability, complex to breed for.	Exploitation of wild relatives. Breeding for: Increased SPC. Higher S-containing amino acids.	Increased Seed Protein Content (SPC).	Variation in SPC among materials. Evaluation of wild relatives. Multi-year studies. QTL for protein concentration. Genetic markers and diverse collections.	SPC and amino acid composition: Paredes Lopez et al. (1991): Clemente et al. (1999): Sanchez- Vioque et al. (1999): Wang et al. (2010): Zhang et al. (2023).	GWAS and SNP markers: Varshney et al. (2021); Upadhyaya et al. (2016); Jadav et al. (2015); Samineni et al. (2022); Karaca et al. (2019); Mugabe et al. (2023); Sari et al. (2024)
Lentil (Lens culinaris L.)	High GxE Traits with low heritability	Exploitation of wild relatives for shifted balance among storage proteins. Breeding fo increased SPC. Despite GxE in SPC, there is potential for improvement	Increased Seed Protein Content (SPC).	Variation in SPC among materials. Draft genomes of cultivated and wild accessions. Diverse collections, introgression populations. Genetic markers, exome capture array.	SPC : Erskine et al. (1985); Salaria et al. (2022); Jha et al. (2022).	Wild Germplasm Surveys and introgression: Erskine et al. (1985): Kumar et al. (2016): Gautem et al. (2016): Ggucem et al. (2017): Sal
Soybean (Glycine max L.)	High GxE Traits with low heritability. Sub-optimal levels of Met and Cys. Limited studies about amino acids composition.	Rebalance amino acid composition. Gene editing. New cultivars with hypocholesterolemic effects. Removal of allergens and antinutrients.	Increased Seed Protein Content (SPC). Increased S-containing amino acid contents	Studies on domestication process and wild relatives. Extensive genomic resources: Mapping populations, QTL studies, molecular markers. Improved phenotyping and genotyping.	SCP and protein accumulation: Panthee et al. (2005); Vaughn et al. (2014); Zhang et al. (2017; Karikari et al. (2019); Fliege et al. (2022). Amino acid composition: Medic et al. (2014)	Genetic diversity and wild gemplasm: hyter et al. (2006) general et al. (2006) deline; when et al. (2002) Lakhsaassi et al. (2024); Yang et al. (2024) Allergens and antinutrients: Sugano et al. (2020); Song et al. (2022); Kim et al. (2024).
Faba bean (Vicia faba L.) and other pulses	Low yield potential despite high SPC. Low sulfur-containing amino acids (Met - Cys), and Tryp Antinutritional compounds (Vicine and convicine)	Increased use as protein isolate for food industry. Breeding for protein quality. Gene editing for low vicine and convicine. Use of protein from cowpea leaves. Mungbean as an emerging plant protein product.	Increased Seed Protein Content (SPC). Protein isolate for food industry. Protein Quality. Protein functionality. Antinutritional compounds (Vicine and convicine).	Studies on faba bean fractioning for food industry. Identified genes for low antinutritional compounds. Other pulses (mung bean-cowpea) with genomes and variation in SPC.	SPC: Naik et al. (2000): Lisiinwaka et al. (2007): Crepon et al. (2010): Fernandes Santos et al. (2012): Boukar et al. (2015): Weng et al. (2019): Hoet et al. (2015): Dhull et al. (2022). Protein isolate: Shi and Nickerson (2022): Protein fraction and amino acid content: Jayathilake et al. (2018). Artifuctivitional compounds: Khazael et al. (2019).	Genomic prediction: Chen et al. (2023). Antinutrients and gene editing: Khazael et al. (2019): Badjona et al. (2023). Genomic resources: Guerra- Garcia et al. (2023): Somta et al. (2022). Genomic resources: Guerra- Garcia et al. (2023): Somtone et al. (2022). Food industry: Krause et al. (2022). Food industry: Krause et al. (2023): Yang et al. (2023): Sone et al. (2023): Yang et al. (2023): Sone et al. (2024): Feng et al. (2024): Sone et al. (2024): Feng et al. (2024):

Breeders of pulse crops and soybeans (legumes, but technically not pulses because of their oil content) are interested in increasing seed protein content and quality. However, certain crops possess special challenges. For example, lentil protein content is highly dependent on how its genes interact with the environment (GxE), not just the genes themselves. Figure 3 from "Breeding for Plant-Based Proteins in Pulse and Legume Crops: Perspectives, Challenges and Opportunities." Courtesy of Valerio Hoyos-Villegas.

While all breeders of pulse crops and legumes are trying to increase digestible seed protein, not all crops are made the same. Breeders who specialize in chickpeas will encounter different challenges compared with pea breeders. Hoyos-Villegas summarizes key challenges for different plant-based sources of protein as thus:

 Beans: Reducing compounds that reduce protein digestion (trypsin inhibitors, lectins).

- Peas: Decoupling the negative correlation between yield and protein. Typically, due
 to the way pea plants metabolize nitrogen, increasing yield decreases protein
 concentration.
- Lentils: Understanding how genes of interest interact with the environment,
 making lentil crops more reliable.
- **Soybeans** (which are *not* pulse crops, but they *are* legumes): Decoupling the correlation between amino acid and oil production.
- Minor pulses (faba beans, mung beans, etc.): Increasing research and knowledge about minor the minor pulses—as a whole, these plants are unfortunately understudied.

And other, legume-specific issues, are detailed in the table above.

Junsheng Zhou, postdoctoral fellow and lead author of the study wanted to identify genes that are potentially associated with total seed protein and sulfur amino acid concentration. By comparing three lines of pea—one with high total seed protein, one with high amounts of sulfur amino acids, and one with low amounts of both—he could analyze which lines express which genes.

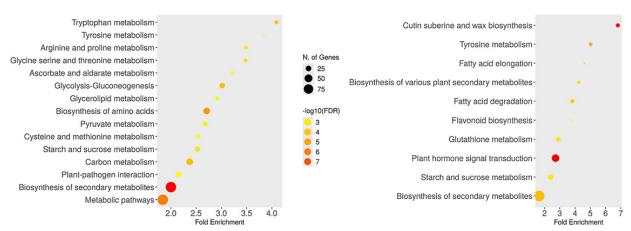
Zhou harvested developing pea pods, extracted RNA sequences from them, and compared the sequences to determine which lines increased (upregulated) or decreased (downregulated) certain genes at different time points during plant growth.



Field pea and soybean research site. Saskatchewan, Canada. Photo courtesy of Junsheng Zhou.

"We compared the gene list of the high-protein line to the gene list of the low-protein line. What's overlapping?" asks Zhou. "There might be some genes that are fundamental for the overall developmental process. And those that are uniquely expressed in high-protein lines—those might be the genes that associate with high protein."

To confirm that these genes are associated with protein development, the authors compared these genes with a public database to determine each gene's function.



High-protein pea plants downregulated (*left*) and upregulated (*right*) certain genes compared with the low-protein pea line. Larger circles indicate the number of genes

discovered while darker/redder colors indicate stronger overrepresentation of genes with specific functions. The high-protein pea plants decreased expression of genes related to metabolism and synthesis of specific amino acids and increased expression of genes related to reproduction, defense, and preserving nutrients. Figures 11 and 12 from "RNA-Seq-Based Gene Expression Analysis Of Seed Protein And Sulfur Amino Acid Accumulation In Developing Pea Seeds." Courtesy of Junsheng Zhou.

"We used this list of uniquely expressed genes to do gene ontology and enrichment analyses where we got to know what the genes are involved in structurally (for the development of the protein) and which pathways these genes are involved in."

Zhou's study is a continuation of his previous work. He previously used quantitative trait loci (QTL) analysis to look for broad chromosome regions associated with protein concentration in his pea lines. This study builds off of those findings, using a method that locates specific genes controlling protein content.

"[RNA sequencing] provides higher resolutions," Zhou explains. "But I think the QTL mapping and RNA sequencings can be partnered together to increase [accuracy]."

Zhou's findings required a lot of processing in order to narrow down the specific candidate genes. "From our starting point, we found more than 20,000 differentially expressed genes, but we used different methods to filter and narrow them down to around 500 genes that potentially are related to protein or sulfur amino acid concentrations. From those 500 genes, we might have the potential to develop genebased markers that will help breeders know which [new] lines will have higher amounts of protein."

Zhou hopes to next measure metabolites (small molecules made during metabolism) in his lines in order to better "understand the biological mechanisms" behind high-

protein plants, establishing the indirect links he has found so far between the genes and traits as more concrete relationships.

No rest for the crop breeder

Clearly, developing new pulse crop lines is a process that takes years, despite any tools that can speed up a plant breeder's program.

"Protein is genetically complex," Hoyos-Villegas says. "Instead of being controlled by a few genes of large effect, it's controlled by hundreds and thousands of genes of very small effects that interact together in different ways. And so that makes breeding for protein ... really slow."

"All the successful breeders that I've talked to have multiple projects going on. And they 'saw the future' a long time ago."

But Dhyan Palanichamy, plant breeder and data scientist at Ingredion, says that consumers rapidly change their minds on what they want to buy. "The market doesn't wait for a breeder to release his or her variety."

So, how do pulse crop breeders cope with such volatile consumer markets, when plant breeding takes so long?

"All the successful breeders that I've talked to have multiple projects going on. And they 'saw the future' a long time ago," says Palanichamy, coauthor of the recent *Crop Science* review. For example, if his colleagues were to develop a fava bean protein powder for the market, that sort of product would have been in development for at least 20 years prior to sales—even if the timing seems "perfect" from an outside perspective. That's why it's crucial to juggle multiple different projects at the same time.

This product development involves several different teams working on several different avenues. "Those 20 years include foundational research (collecting plant genetic material and breeding), extension work (commercial seed production, building grower networks, and refining agronomic practices), and commercialization hurdles (finding markets, securing farm insurance, and getting inputs approved)," Palanichamy explains. "The market window is incredibly narrow and unpredictable, which is exactly why it's so important to have several different projects running in parallel—one of them will be ready when the opportunity finally opens."



Left: Drone picture showing Ingredion's commercial research plots of plant-based protein crops in 2022. Saskatchewan, Canada. **Right:** Ingredion South Sioux City plant. "With any

plant breeding program, we are standing on the shoulders of giants," Palanichamy explains. "We actually take over a lot of good ideas from other scientists and need to be good stewards of those ideas to take them to the end for commercialization and business success. While I have to plan for the future, I might not see the end of some of my projects. But future plant breeders will see them." Photos courtesy of Dhyan Palanichamy.

To demonstrate how quickly the market shifts, Palanichamy explains that when he started collaborating with Hoyos-Villegas on their paper, there was a high demand for meat substitutes. Since then, the market has shifted to producing more cold-pressed plant-based products, such as shakes, cereals, and protein bars.

He adds that consumers will likely shift back to wanting meat substitutes—the market perpetually shifts back and forth.

"It's cyclical," Palanichamy says. "And when [the market eventually] comes back [to meat substitutes], we better be ready with great varieties that are sustainable, have the best flavor, and don't require much processing. ... When the second wave happens, it'll be huge I think."



Faba bean varieties showing variation in seed coat color, hilum color, and seed size. Photo courtesy of Dhyan Palanichamy.

In the meantime, the industry has gotten creative with its plant-based products, too: Pea and other pulse crop by-products can be used as material in plant-based protein bars, shakes, and egg replacements, which reduces food waste and valorizes by-products.

Putting plant-based products to the (taste) test

But for any of these new, plant-based products to be successful, consumers have to like and purchase them. Another *Crop Science* study explored this by evaluating consumer perception of yogurt made with common bean.

Eric Owusu Mensah, food scientist at the Kwame Nkrumah University of Science and Technology (KNUST) in Ghana, explains that exploring a "locally available, affordable, and nutrient-rich" ingredient as a dairy alternative is "an opportunity to heighten food security, product diversification, and reduce over-reliance on dairy products."

His research team compared physiochemical and sensory properties of yogurt made with one of two varieties of common bean. The yogurts were made with either 45, 55, or 75% milled bean and compared with a yogurt made completely from milk powder. They analyzed properties such as pH, texture, and color in addition to conducting a sensory panel with 50 untrained panelists to determine acceptability of the yogurts. The researchers found that yogurt made with 55% Adoye bean was the second-most liked yogurt product after the control, as it was thicker and had a more suitable flavor compared with other bean-based yogurts.





Left: Researchers measured the color of each yogurt sample using a portable chromameter. Right: Two traits that might seem similar, but have different effects on consumer preferences, are pH and titratable acidity. Both are concepts in food science that have to do with acidity; however, titratable acidity can predict flavor better than pH, and pH can better predict how microorganisms can grow in food products. Mensah says that "higher acidity gives a sharper, tangier taste," optimal "pH promotes protein coagulation, improves firmness and gel stability," and higher pH "inhibits spoilage microorganisms." However, both are "key indicators of fermentation indices." Photos courtesy of Eric Owusu Mensah.

Unfortunately, one of the product's greatest challenges is what makes it so novel: The researchers needed to combat an excessive "beany" flavor while making the yogurt.

Mensah clarifies that despite the potentially off-putting flavor, common bean is a valuable source of protein that is worth the challenge.



By soaking the beans in 0.5% sodium carbonate, researchers were able to reduce off flavoring in the yogurt. Photo courtesy of Eric Owusu Mensah.

"Although the 'beany' flavor is a known challenge, common beans are nutritionally powerful—rich in protein, fiber, minerals, and antioxidants," he says. "Working with a challenging yet abundant resource allows opportunities for innovation in processing techniques (soaking, blanching, germination, fermentation, enzymatic treatment, etc.) to reduce the off flavor[MN1], making the product more acceptable." One of those pretreatment methods—soaking the beans for 12 hours in 0.5% Na₂CO₃—was successful in reducing some of the off flavoring.

They concluded that a yogurt product made with 55% common bean has potential in this industry. Mensah says he would like to further explore "flavor-masking techniques, optimized fermentation conditions, and additional stabilizers to improve texture" in addition to testing a wider demographic and developing flavored yogurts.

"I think it's all about taste," adds Palanichamy, further emphasizing that the success of plant-based protein depends on consumer perception. "And you know, if we can make plants taste really good, it will be good for everyone."

Dig deeper

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