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Optimizing Soybeans for Alternative Proteins

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I. Context

Price, texture, and flavor still limit the adoption of plant-based alternatives to animal products today. The high level of processing requirements not only increases costs but imposes a negative view on consumer perception as well. Lengthening the ingredient list also doesn't necessarily guarantee that target functionalities are met. Genetic modification of crops can potentially solve some of these issues by optimizing the ingredients from the source, thereby efficiently alleviating these second-order consequences.

II. Regulatory implications

Since genetic modification is already extensively used in food crops in the US, this market is ripe for such a solution. [1] Biotech firms continue to develop new GM seeds and farmers continue to adopt them at a robust rate, with seeds stacking multiple improved traits. [2] Moreover, the laws in the US don't regulate crops modified with site-specific methods as GMO crops, as these methods are considered "exempt". [3,4]

With molecular farming, we've seen significant resistance to introducing exogenous molecules, particularly those which are considered allergens, into commonly used food crops due to the fear of cross-contamination. [5] However, here a different approach is explored. We outline various strategies for organoleptic improvement, which modify the organisms's existing proteins and lipids thereby introducing no exogenous molecules. The crops carrying these changes could still be used for their current applications in a safe and effective manner - analogous to how some naturally existing varieties are better for making French fries and others are better for potato chips, although all varieties technically can be used to make French fries. [6,7]

[1] "Global Regulation of Genetically Modified Crops Amid the Gene ...." 2 Feb. 2021, https://www.frontiersin.org/articles/10.3389/fpls.2021.630396. Accessed 8 Dec. 2023.
[2] "Genetically Engineered Crops in the United States." 1 Oct. 2014, https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2503388. Accessed 8 Dec. 2023.
[3] "The evolving landscape around genome editing in agriculture." 19 May. 2020, https://www.embopress.org/doi/10.15252/embr.202050680. Accessed 1 Dec. 2023.
[4] "Deregulation of Soybean Developed Using Genetic Engineering." 14 Mar. 2022, https://www.aphis.usda.gov/aphis/newsroom/stakeholder-info/sa_by_date/sa-2022/basf-soybean-deregulation. Accessed 8 Dec. 2023.
[5] "FDA Issues Letter to Industry on the Food Safety Risks of ...." 13 Apr. 2023, https://www.fda.gov/food/cfsan-constituent-updates/fda-issues-letter-industry-food-safety-risks-transferring-genes-proteins-are-food-allergens-new?utm_medium=email&utm_source=govdelivery. Accessed 8 Dec. 2023.
[6] "Global Markets for Processed Potato Products - ScienceDirect.com." https://www.sciencedirect.com/science/article/abs/pii/B9780444510181500440. Accessed 8 Dec. 2023.
[7] "NDSU develops potato variety now approved for McDonald's World ...." 17 June. 2022, https://www.ndsu.edu/news/view/detail/68927. Accessed 8 Dec. 2023.

III. Removing off-flavors

The cause for off-flavors

Soybean is known to have an unpleasant "beany", "green", or "grassy" off-flavor which limits consumer acceptability and purchase of soy-based products. This off-flavor has been primarily attributed to the degradation of polyunsaturated fatty acid (PUFA) derivatives by endogenous enzymes to produce profiles of various volatile organic compounds (VOCs) which constitute the beany/grassy off-flavor. [8,9] There are about 30 main VOCs which contribute to the off-flavor of soy[10] Lipoxygenase enzyme (LOX) is thought to be the main cause of undesirable PUFA degradation, with more than 60 different isozymes (forms) found in various species of fruits and vegetables. [11]

Prospective GM methods for removing off-flavor

One method for removing the off-flavors can be to engineer the crop to produce less PUFA. Soybean generally contains 20-23% lipids, of which >60% is PUFA. [12] By shifting the lipid production to predominantly non-PUFA oils, these off-flavors can presumably be reduced (improved VOC profile). Indeed, natural soybean varieties with lower PUFA ratios have been shown to exhibit less off-flavor associated volatiles in laboratory measurements. [13,14] Genetically engineering soybean with low PUFA levels have been demonstrated before, although the effect on off-flavors in soy-based products from these varieties have not been characterized. [15]

Another method could be to remove the presence of these major degradation enzymes. In Soy, it is generally known that different naturally existing varieties have higher levels of LOX amounts/activities than others. [16] Removing LOX has been attempted in the 1980s and 1990s. [17,18] However there is some evidence to suggest that LOX plays an important role in the functionality of soy protein in the baking industry and generally contributes to food quality. [19,20] Therefore, removing LOX entirely might not be compatible with a wide range of applications.

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[8] "Flavor Aspects of Pulse Ingredients - Roland - Wiley Online Library." 29 Nov. 2016, https://onlinelibrary.wiley.com/doi/full/10.1094/CCHEM-06-16-0161-FI. Accessed 5 Dec. 2023.
[9] "Off-flavor precursors in soy protein isolate and novel strategies for ...." https://pubmed.ncbi.nlm.nih.gov/23297776/. Accessed 5 Dec. 2023.
[10] "Insights into formation, detection and removal of the beany flavor in ...." https://www.sciencedirect.com/science/article/abs/pii/S0924224421002764. Accessed 5 Dec. 2023.
[11] "Lipoxygenase in fruits and vegetables: A review - ScienceDirect.com." 5 Mar. 2007, https://www.sciencedirect.com/science/article/pii/S0141022906005904. Accessed 5 Dec. 2023.
[12] "Off-flavor precursors in soy protein isolate and novel strategies for ...." https://pubmed.ncbi.nlm.nih.gov/23297776/. Accessed 5 Dec. 2023.
[13] "In vitro kinetics of soybean lipoxygenase with combinatorial fatty ...." 1 Mar. 2014, https://www.sciencedirect.com/science/article/abs/pii/S0308814613012028. Accessed 3 Jan. 2024.
[14] "Oxidative and flavor stabilities of soybean oils with low-and ultra ...." 1 Feb. 2003, https://aocs.onlinelibrary.wiley.com/doi/abs/10.1007/s11746-003-0672-6. Accessed 3 Jan. 2024.
[15] "Breeding for Modified Fatty Acid Composition in Soybean - ACSESS." 1 Dec. 2007, https://acsess.onlinelibrary.wiley.com/doi/abs/10.2135/cropsci2007.04.0004IPBS. Accessed 3 Jan. 2024.
[16] "Lipoxygenase activity in different species of sweet lupin (Lupinus L ...." 1 May. 2015, https://www.sciencedirect.com/science/article/pii/S0308814614017580. Accessed 5 Dec. 2023.
[17] "Flavor improvement of soybean preparations by genetic removal of ...." 1 Oct. 1987, https://aocs.onlinelibrary.wiley.com/doi/abs/10.1007/bf02636994. Accessed 3 Jan. 2024.
[18] "Oxidative and flavor stability of oil from lipoxygenase ... - SpringerLink." https://link.springer.com/article/10.1007/s11746-998-0123-7. Accessed 3 Jan. 2024.
[19] "Biochemistry of lipoxygenase in relation to food quality." 29 Sep. 2009, https://www.tandfonline.com/doi/abs/10.1080/10408397709527229. Accessed 5 Dec. 2023.
[20] "Legume lipoxygenase: Strategies for application in food industry - Shi." 8 Jun. 2020, https://onlinelibrary.wiley.com/doi/full/10.1002/leg3.44. Accessed 5 Dec. 2023.

Competition with downstream solutions

For isolated soy protein powder, there are methods to reduce off-flavors, although they can degrade the quality of the protein and can be expensive. One such method is heating in order to denature and reduce the activity of LOX (a culprit which was described in a previous section). Heating is the most common post-processing treatment used to reduce the off-flavor of soy due to its simplicity. However, this method also reduces the functionality of the non-LOX protein by partially denaturing them as well. [21]

Additionally, another method is to supplement with phospholipase enzymes which cleave the lipids between the fatty acid and triacylglyceride backbone. Polysaccharides are then added prior to acid-mediated precipitation of the protein from the rest of the biomass. This method demonstrated the removal of >92% of the off-flavor precursor molecules, however it severely degrades the quality of the oil and was also found to cause significant structural changes to the protein. [22]

Additionally, it was found that not adding sugar to the powder mix prior to extrusion reduces the progression of the Maillard reaction with the amino acids from the denatured protein in the barrel and reducing off-flavor products. However, this still doesn't stop PUFA lipid hydrolysis and oxidation that occurs in the heat of the extruder, making for an imperfect solution. [23]

Lastly, it is worth mentioning that these methods could generally be competing with other alternative protein technologies looking to improve flavor. Notable methods include 1) AI-driven novel formulations of non-obvious plant-derived or fermentation derived ingredients 2) precision fermentation derived ingredients and 3) cultivated cells included at various ratios. These methods gained significant traction within the alternative protein ecosystem, and technologies are becoming more mature. Their effect on improving flavor is now being explored with some companies claiming major improvements in the organoleptic experiences introduced by these technologies. Cost and scale are also to be determined.

IV. Improving the texture for meat analogues and other alternatives

The cause for the texture parity

Plant proteins are fundamentally different from animal proteins in structure/function because they evolved to serve very different biological functions. This difference affects the way these proteins interact, resulting in a food matrix with significantly different organoleptic properties than that of animal-based meat matrixes.

[21] "Effect of soybean roasting on soymilk sensory properties." 9 Oct. 2018, https://www.emerald.com/insight/content/doi/10.1108/BFJ-11-2017-0646/full/html. Accessed 6 Dec. 2023.
[22] "Removal of off-flavour-causing precursors in soy protein ... - PubMed." 30 Oct. 2018, https://pubmed.ncbi.nlm.nih.gov/29853382/. Accessed 6 Dec. 2023.
[23] "Control of Beany Flavor from Soybean Protein Raw Material in Plant ...." https://www.mdpi.com/2304-8158/12/5/923. Accessed 6 Dec. 2023.

The texture of meat is primarily driven by a complex interaction between muscle, connective tissue, and fat [24]:

1. Muscle fibers bundled together in a hierarchical structure create the fibrous microtexture mouthfeel and contribute structurally to the macrotexture. [25]
2. Extracellular matrix proteins (collagen, elastin, etc.) forming networks with each other, cells, and fat (connective tissue) contribute to the chewiness and cohesiveness of meat. [26]
3. Intramuscular fat deposits and overall fat content level contribute to the tenderness and juiciness of meat. [27]

Therefore, for meat texture, protein structure/function, fat composition and protein/fat ratios are critical.

Improving soybeans for improved texture in meat substitutes

Without introducing exogenous protein into the soybean (e.x. Moolec's "Piggy Sooy") to change protein structure, the organoleptic effects of animal-based protein networks can only be mimicked using compensating alternatives. Until now, these approaches have manifested in the form of chemical/thermomechanical post processing techniques (e.g. transglutaminase, methyl cellulose, and extrusion). However, non-exogenous forms of genetic modification can still potentially change existing macronutrient ratios, protein ratios, and oil compositions to achieve a better starting ingredient platform for downstream formulation and adaptation. Benson Hill has perhaps set the most advanced commercial precedence by improving water holding capacity. [28]

Glycinin (11S globulins) and conglycinin (7S globulins) are the two most abundant proteins found in soybean (most plentiful storage proteins). Natural variations in soybean breeds and cultivars contain varying compositions and ratios of these proteins and it is generally recognized that the ratio of glycinin to conglycinin and the variation in subunit compositions are the largest contributors to soy protein functionality. [29,30] Higher gelation is associated with 7S rich ratios and higher solubility is associated with group I subunits and conglycinin. [31,32] . Feedback between this protein functionality research and breeding efforts has been attempted in order to create isolates with increased functional properties, however the bulk of the functionality research has been limited to isolated protein fractions in lab conditions. [33] Therefore, research

[24] "How Muscle Structure and Composition Influence Meat and Flesh ...." https://www.hindawi.com/journals/tswj/2016/3182746/. Accessed 2 Jan. 2024.
[25] "Comparison of Meat Quality and Muscle Fiber Characteristics ... - NCBI." 1 Sep. 2022, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9478981/. Accessed 2 Jan. 2024.
[26] "The role of intramuscular connective tissue in meat texture." 28 Jan. 2010, https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1740-0929.2009.00696.x. Accessed 2 Jan. 2024.
[27] "Effects of intramuscular fat on meat quality and its regulation ...." 8 Jul. 2022, https://www.frontiersin.org/articles/10.3389/fnut.2022.908355. Accessed 2 Jan. 2024.
[28] "The Secret to Tastier Fake Meat? Breeding Better Beans - WIRED." 7 Jun. 2022, https://www.wired.com/story/meat-substitutes-breeding-better-beans/. Accessed 13 Dec. 2023.
[29] "Soy Protein: Molecular Structure Revisited and Recent Advances in ...." https://www.annualreviews.org/doi/10.1146/annurev-food-062220-104405. Accessed 2 Jan. 2024.
[30] "Soy Protein Functionality: Emulsion and Gels" Oct. 2011. https://www.sciencedirect.com/science/article/abs/pii/B978008088504900324X?via%3Dihub. Accessed 29 Dec. 2023.
[31] "Protein Subunit Composition Effects on the Thermal Denaturation at ...." 6 May. 2008, https://aocs.onlinelibrary.wiley.com/doi/abs/10.1007/s11746-008-1238-6. Accessed 29 Dec. 2023.
[32] "Biochemical Characterization of Soybean Protein Consisting of ...." 17 Mar. 1997, https://pubs.acs.org/doi/10.1021/jf9604394. Accessed 29 Dec. 2023.
[33] "Soy Protein Functionality: Emulsion and Gels" Oct. 2011. https://www.sciencedirect.com/science/article/abs/pii/B978008088504900324X?via%3Dihub. Accessed 29 Dec. 2023.

using food-grade isolations/formulations at the pilot scale are needed to evaluate the effect of large-scale food-grade processing techniques on the functionality of these protein groups.

It is widely recognized that protein functionality is affected by heat, pH, harsh chemicals, causing proteins to unfold and lose shape and motif binding/valency. Soy protein isolate is typically prepared using isoelectric precipitation of an alkaline extract - which involves pH and harsh chemicals (solvents). Protein gelation requires heat. The glycinin fraction is more heat stable (with a thermal denaturation between 85-95℃) than conglycinin (between 65-75℃), caused by the increased amounts of disulfide bonds in its structure. [34] Genetically modifying the glycinin to conglycinin ratio can therefore impact the thermal denaturation profile of SPI. A different denaturation profile then alters the ratios of proteins which interact during gelation and also how they interact. For example, glycinin shows changes in the secondary structure and increase in surface hydrophobicity with heating while conglycinin shows a dissociation of the protein subunits. [35] Heating of soy protein isolates containing both glycinin and conglycinin causes the formation of soluble aggregates between the beta subunit of beta-conglycinin and the basic polypeptide chain of glycinin, creating a gel. [36,37] However, these soluble aggregates are also known to decrease emulsification because they rearrange less efficiently at oil-water interfaces. [38] Therefore, GM efforts of soybean which focus on modifying the heat stability and/or binding/valency of these proteins could potentially improve gelation and solubility/emulsifying functionality.

Solubility, emulsification, water-binding, and oil-binding capacity are affected by hydrophobic/hydrophilic moieties as well as the particular conformation that proteins assume at interfaces to minimize their free energy. The layer of assumed soy proteins at the interface was found to be thicker for glycinin than for beta-conglycinin isolated fractions since beta-conglycinin can pack/arrange itself at the interface more efficiently - as measured by thinner interface boundaries in laboratory tests. [39] As mentioned previously, heat reduces emulsification properties of these fractions and since these fractions have different thermal tolerances. Therefore, modifying these fractions for different thermal tolerances can impact the *effective* glycinin/conglycinin ratio at the interface thereby changing emulsification. Alternatively, GM methods could potentially explore sparse amino acid substitutions which could affect the conformational efficiency of these fractions, potentially boosting the emulsifying properties of glycinin, thereby boosting the overall emulsification potential for SPI. In plant-based meat analogues, hydrophobicity/hydrophilicity and emulsification can play important roles in meat fat mimicry and overall perceived juiciness or tenderness (oil holding capacity, water holding capacity).

As mentioned, SPI gelation is an interaction between both glycinin and conglycinin in the presence of heat. Therefore, different ratios of glycinin to conglycinin will affect gelation characteristics. Glycinin rich ratios were found to be associated with firmer turbid gels while conglycinin rich ratios were associated with softer more transparent gels. [40,41] It was also demonstrated that the A_3 subunit of glycinin is particularly important

[34] "The effect of pH on heat denaturation and gel forming properties of ...." https://pubmed.ncbi.nlm.nih.gov/10867183/. Accessed 2 Jan. 2024.
[35] "The effect of pH on heat denaturation and gel forming properties of ...." https://pubmed.ncbi.nlm.nih.gov/10867183/. Accessed 2 Jan. 2024.
[36] "The effect of pH on heat denaturation and gel forming properties of ...." https://pubmed.ncbi.nlm.nih.gov/10867183/. Accessed 2 Jan. 2024.
[37] "Changes in Characters of Soybean Glycinin Groups I, IIa, and IIb ...." 25 Feb. 2004, https://pubs.acs.org/doi/10.1021/jf030353s. Accessed 2 Jan. 2024.
[38] "Heat-Induced Changes Occurring in Oil/Water Emulsions Stabilized ...." 27 Jul. 2010, https://pubs.acs.org/doi/10.1021/jf101425j. Accessed 2 Jan. 2024.
[39] "Dynamic properties of soy globulin adsorbed films at the air-water ...." https://pubmed.ncbi.nlm.nih.gov/14611771/. Accessed 2 Jan. 2024.
[40] "Heat-Induced Gel Formation by Soy Proteins at Neutral pH." 16 Feb. 2002, https://pubs.acs.org/doi/10.1021/jf010763l. Accessed 2 Jan. 2024.
[41] "The effect of pH on heat denaturation and gel forming properties of ...." https://pubmed.ncbi.nlm.nih.gov/10867183/. Accessed 2 Jan. 2024.

in modifying the gelling properties of SPI. [42,43] Therefore, GM efforts to modify glycinin/conglycinin ratios as well as the relative proportions of the A_3 glycinin subunit can potentially create SPIs with improved gelation. Firmer and more opaque gels may potentially be associated with meat muscle mimicry whereas softer more transparent gels may be associated with meat connective tissue mimicry.

Extrusion trial data for plant-based meat production is limited within the public domain, perhaps because knowledge of various parameter effects is retained as trade secrets. The resurgence of interest in meat alternatives has spurred some mechanistic research studies in the extrusion process. [44,45,46] While these studies are focused on understanding the physical mechanism of structure formation, it was discovered that phase separation of the protein and water within the extrusion barrel is responsible for structure formation orthogonal to the temperature gradient in the barrel. These results suggest that GM modification of soybeans for varying solubility/hydrophilicity can affect structure formation. By modifying the soybean for increased water holding capacity, phase separation and structure formation could be amplified. More experimentally oriented extrusion trials with varying soy protein composition/ratios are more limited. In one particular extrusion trial, a more moderate ratio of 1.5-2.0 11S/7S yielded more favorable plant-based meat analogues although the effect size was not very large. [47] An additional extrusion trial testing different characteristic blends of plant-based proteins found that a higher ratio of “cold-swelling” proteins (characterized by a high-water absorption index, higher gelling concentration, higher viscosities, and higher solubility), improved the consumer liking of plant-based meat analogues by reducing the density and hardness and increasing tenderness. [48]

Competition with downstream processing methods

Transglutaminase and Methylcellulose are the most common additives used to increase gelation and binding. Transglutaminase modifies proteins moieties, enabling them to cross link and form networks which they couldn’t have naturally formed. These networks exhibit properties which more closely resemble those of connective tissue networks and contribute positively to macroscale meat texture mimicry in plant-based meat analogues. [49,50] More specifically, transglutaminase at optimal inclusions <1% (m/m) has been shown to improve hardness, gumminess, chewiness, and springiness. [51,52] Methylcellulose at ~3% (m/m) optimal

[42] "Protein Subunit Composition Effects on the Thermal Denaturation at ...." 6 May. 2008, https://aocs.onlinelibrary.wiley.com/doi/abs/10.1007/s11746-008-1238-6. Accessed 2 Jan. 2024.
[43] "Stability of soybean seed composition and its effect on soymilk and ...." https://www.sciencedirect.com/science/article/pii/S0963996901001259. Accessed 2 Jan. 2024.
[44] "High Moisture Extrusion of Soy Protein: Investigations on the ... - MDPI." 6 Jan. 2021, https://www.mdpi.com/2304-8158/10/1/102. Accessed 2 Jan. 2024.
[45] "Towards understanding the mechanism of fibrous texture formation ...." https://www.sciencedirect.com/science/article/pii/S0260877418303406. Accessed 2 Jan. 2024.
[46] "Protein physical state in meat analogue processing - ScienceDirect." https://www.sciencedirect.com/science/article/abs/pii/S2214799322000248. Accessed 2 Jan. 2024.
[47] "The Protein Composition Changed the Quality Characteristics of ...." https://www.mdpi.com/2304-8158/11/8/1112. Accessed 29 Dec. 2023.
[48] "Understanding Protein Functionality and Its Impact on Quality ... - MDPI." https://www.mdpi.com/2304-8158/12/17/3232. Accessed 2 Jan. 2024.
[49] "Effect of transglutaminase-catalyzed crosslinking behavior on the ...." https://www.sciencedirect.com/science/article/abs/pii/S0308814623013729. Accessed 28 Dec. 2023.
[50] "Texturization of plant protein-based meat alternatives." https://www.sciencedirect.com/science/article/pii/S2666833523000345. Accessed 28 Dec. 2023.
[51] "Effects of Glucono-δ-Lactone and Transglutaminase on the ... - MDPI." https://www.mdpi.com/2304-8158/11/21/3337. Accessed 28 Dec. 2023.
[52] "Mechanical and rheological effects of transglutaminase treatment on ...." https://www.sciencedirect.com/science/article/pii/S0268005X22007810. Accessed 28 Dec. 2023.

inclusion also increases harness, gumminess, and chewiness while decreasing moisture content. [53,54]. Other hydrocolloid-based systems including κ-carrageenan and xanthan gum can also be used with similar effects at <1% (m/m) inclusion. [55] In comparison to the aforementioned GM approaches, these additives do increase production cost and lengthen the nutrition labels for consumers.

Isolations of protein fractions have been recognized as potential strategies for improvement of texture. [56] The most prominent method for protein fractionation is the Nagano method which involves pH adjustment and ultrafiltration. [57] This method has been replicated at the pilot scale process and protein fractions have demonstrated distinct functionality differences with the glycinin fraction exhibiting higher solubility at lower pH. [58] Implementing this process at large scale for plant-based meat production will certainly add significant cost and create waste streams of unused fractions, likely rendering the strategy impractical.

Hydrolysis uses enzymes and/or chemicals to break proteins into smaller fragments. Soy proteins have also been selectively hydrolyzed targeting each of the two main protein fractions. [59] This resulted in similar levels of solubility, lower emulsifying activity and increased whippability. However, this process also adds cost (cost of the enzymes) and the benefits in plant-based meat analogue production are unclear with most extrusion/meat analogue studies focusing on the anti-oxidant and nutritional benefits of hydrolysis. [60,61] Overall, more research is needed to understand the functional effects of hydrolyzed soy protein in extruded plant-based meat analogue production.

Improved protein isolation methods for better preserving functionality may obviate the harsh pH and chemicals associated with traditional isoelectric precipitation methods. Enzyme-assisted isolation has been shown to increase protein extraction yields by ~20% at pilot scale with no significant change to foaming capacity and solubility, although emulsifying ability was decreased, and foaming stability increased.[62] Overall more research and novel methods are needed in order to develop a protein isolation process which preserves more protein functionality while being practical/cost-effective at scale.

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[53] "A Novel Approach for Tuning the Physicochemical, Textural, and ...." 8 Mar. 2021, https://www.mdpi.com/2304-8158/10/3/560. Accessed 2 Jan. 2024.
[54] "Effect of transglutaminase-catalyzed crosslinking behavior on the ...." https://www.sciencedirect.com/science/article/abs/pii/S0308814623013729. Accessed 2 Jan. 2024.
[55] "Quality improvement of plant-based patty using methylcellulose, κ ...." 12 Feb. 2023, https://ph04.tci-thaijo.org/index.php/JCST/article/view/298. Accessed 2 Jan. 2024.
[56] "Functionality of Ingredients and Additives in Plant-Based Meat ...." https://www.mdpi.com/2304-8158/10/3/600. Accessed 2 Jan. 2024.
[57] "Simplified process for soybean glycinin and beta-conglycinin ...." https://pubmed.ncbi.nlm.nih.gov/10898608/. Accessed 2 Jan. 2024.
[58] "Functional properties of soy protein fractions produced using a pilot ...." https://link.springer.com/article/10.1007/s11746-003-0735-8. Accessed 2 Jan. 2024.
[59] "Functional properties of soy protein hydrolysates obtained by ...." https://www.sciencedirect.com/science/article/abs/pii/S0023643804001574. Accessed 2 Jan. 2024.
[60] "Enzymatic hydrolysis of soy protein to high moisture textured meat ...." https://www.sciencedirect.com/science/article/abs/pii/S1878818123001019. Accessed 2 Jan. 2024.
[61] "Applications of soy protein hydrolysates in the emerging functional ...." 17 Sep. 2019, https://ifst.onlinelibrary.wiley.com/doi/abs/10.1111/ijfs.14380. Accessed 2 Jan. 2024.
[62] "Functionality of soy protein produced by enzyme-assisted extraction." https://link.springer.com/article/10.1007/s11746-006-1178-y. Accessed 2 Jan. 2024.

V. Commercial and Operational Factors to Consider

Beyond the regulatory and IP challenges, there are a number of potential factors that could slow or prevent the commercialization of these technologies, and therefore should be considered by startups looking into this area.

Size and value of the target ingredient market

- One key barrier is the operational viability through the supply chain if the traits a company focuses on are targeting a relatively small end addressable market.
- Targeting improved characteristics for plant-based meat for example, will be too small to warrant being the primary end market for genetic changes, particularly for crops grown at large scale. For context, assuming all PB meat in the US at present used soybeans for all its protein requirements, this would represent just 0.03% of US soybean production. Getting farmer and processing buy-in will therefore be very difficult.
- Targeting the removal of off-taste generally in soy could help broaden the potential end market, but the fact that this has been explored and not gained traction suggests there may not have been enough buy-in from end users even at this larger market sizing.

Attaining farmer uptake

- Getting farmer uptake if you are not one of the major seed companies, particularly in soy, is incredibly difficult unless you have a very strong value proposition and large end market.
- Other companies with theoretically strong value propositions such as improved protein content in soy have struggled to get farmer uptake, even if offering farmers notable premiums. Having partnerships with a major seed company will be very beneficial for startups in getting trial traction, or finding ways to give comfort around the ability of the farmer to sell the crop (this could be expensive!).

Attaining processor uptake

- Another hurdle is finding a location to process the crop. Particularly for large volume crops such as soy where economies of scale are fundamental and have driven the development of very large processing facilities. Given daily throughput of some plants can surpass 3,000 tons per day, it places more pressure on the seed companies as more widespread farmer traction is needed to generate sufficient product quantities to make it worthwhile for a mill to process. Moving to small mills is an option, but as has been seen by some companies, the economics did not stack up.
- Finding novel approaches and solutions for alternative processing or end uses for the crops will be fundamental to get farmer buy-in.

Crop selection and scale

- The plant species and scale of production at farm and processing have a notable impact. Selecting crops within which considerable economic gains can be derived is one interesting approach. Alternatively, targeting less extensively cultivated crops with smaller processing scale could improve potential uptake.
- NuCicer, a chickpea genetics company improving protein content by >80%, is an interesting example here. The new seeds provide a sizable economic value proposition here for farmers and

processors that will help the company generate traction in the market. It also helps that chickpea operations are notably smaller in scale than soy, and therefore the challenge of running smaller batches is much lower. Additionally, economics are a key barrier to the broader uptake of chickpea and its constituent parts in the market – solving this notably expands the addressable market. These positive factors across key areas therefore improve the viability of this crop genetics solution.

Some of the most successful GM crops have all fundamentally driven advantages in economics through the value chain, including for the farmer, at scale, and in general have not limited the crop to one use case. Insect resistant, bacteria resistant, herbicide tolerant, drought tolerant etc., these all provided considerable upside through the value chain.

VI. Conclusion

The strategies for GM of soy outlined here holds promise for reducing off-flavor and improving functionality for alternative protein applications. Much of the functionality benefits could also prove useful in other alternative protein categories such as plant-based dairy or egg products. Improving the functionality of the commodity crop from the source could significantly decrease the need for additives and extra processing steps, thereby reducing production cost and cleaning up the label for alternative protein products.

However, the effect size of these functional modifications is unclear and is perhaps the main contributor to risk. Further research and scaled proof of concepts are required to explore the potential of these modifications. Additionally, the effect sizes should be compared to those of existing additives and processes in order to evaluate the practicality of this approach.

To improve the quality of plant-based meat and dairy, there may be other more compelling methodologies that will achieve a similar or greater effect size, including cultivated and fermentation derived ingredients, and different processing technologies. This competitive angle represents another challenge to seed engineering to tackle this issue. GM traits that improve characteristics beneficial to large end markets, and by happenstance make plant-based meat and dairy better, could however represent interesting areas of exploration. Therefore, market timing, economics, scalability, and competing technologies are all important considerations for the implementation of this strategy moving forward. Perhaps in the future, when a large fraction of traditional animal agriculture is replaced with alternatives, a substantial proportion of soybean and potentially other crops will be used for the production of meat alternatives and other processed foods, thereby opening up the market for more targeted optimization of crop genetics.