

# Cultivating alternative proteins from commodity crop sidestreams



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# **About The Good Food Institute**

<u>The Good Food Institute</u> is an international nonprofit building a sustainable, healthy, and truly global food system. With unique insight across the scientific, policy, industry, and investment landscapes, we are using the power of food innovation and markets to accelerate the transition of the world's food system toward alternative proteins. GFI is powered by philanthropy, and all of the resources we produce and analyses that we conduct or commission are intended to be shared in an open-access manner to focus efforts on the highest-impact opportunities.

### **Executive summary**

This report identifies high-value crop "leftovers" or sidestreams with tremendous potential for maximizing food production via alternative proteins while cutting the costs and environmental impacts of agricultural wastes.

Ingredient manufacturers and agricultural product companies, equipped with access and expertise, can tap into these promising ingredients, turning costly waste into commercially viable food products. Further, to promote a thriving bioeconomy and diversified supply chain, governments and private industry should invest in sidestreams as valuable and sustainable sources of protein concentrates, nitrogen, and fermentable sugars. Lastly, by upcycling sidestreams, alternative protein production can enable circular bioeconomies that produce food more efficiently, affordably, and sustainably.

### Scope

This report analyzes the major agricultural sidestreams from the top crops produced in North America (Canada, Mexico, and the United States), as projected for 2030, that could be valorized for (1) protein concentrates for plant-based food ingredients, (2) protein hydrolysates for fermentation or cultivated meat media, and (3) lignocellulosic sugars for fermentation media.

Protein concentrates provide plant-based foods with desired sensory and nutritional properties, while protein hydrolysates supply nitrogen and amino acids necessary for efficient microbial and cellular growth. Lignocellulosic-derived sugars reduce reliance on traditional carbon-based feedstocks required for fermentation.

### **Top sidestream candidates**

The following sidestreams demonstrated high potential for economic return, low environmental impact, and basic functionality for alternative protein production:

**Protein concentrates:** soy meal, canola meal, wheat bran, wheat gluten, tomato pomace, corn distillers dried grain with solubles (DDGS), corn gluten meal, and brewer's spent grain

**Protein hydrolysate:** soy meal, corn DDGS, canola meal, brewer's spent grain, and corn gluten meal

**Lignocellulosic sugars:** corn stover, soy straw, sugarcane trash/bagasse, and barley straw/husks

# Challenges

Since most of these sidestreams are not currently optimized for food production, research and development are required to understand and improve sidestream functionality and conversion into alternative protein inputs. Additionally, most sidestreams lack established infrastructure and supply chains to ensure adequate stabilization and preservation to maintain food safety and to transport materials from regions of production to processing facilities.

This analysis highlights specific geographic opportunity areas and production densities for high-potential sidestreams that should be considered by interested stakeholders. Ultimately, public and private collaborations are needed to fine-tune processing technologies, improve infrastructure, and strengthen supply chains to convert sidestreams into mainstream assets and take full advantage of their economic and environmental potential.

From field to facility

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# **Key Recommendations**

We provide the following recommendations to food producers and governments motivated to valorize crops and create circular bioeconomies for alternative protein products:

# Recommendations for ingredient manufacturers and agricultural players:

- Assess the applicability of each sidestream-derived ingredient for end-product or production use based on functional attributes required for specific use cases.
- 2. Optimize sidestream processing conditions to maximize ingredient recovery, creating mature value chains to lower costs and increase recoveries.
- Improve sidestream drying methods, especially high-moisture sidestreams like brewer's spent grain and tomato pomace. Drying processes, typically involving rapid heating or spray drying, can be expensive, require large equipment with high energy use, and impact flavor, texture, and nutritional attributes.
- 4. Collaborate across the value chain to improve transportation and upcycling logistics.
- 5. Evaluate and prioritize food safety by ensuring sidestreams are dried, transported, treated, and stored with efficient, food-safe practices.
- 6. Collect data to perform life cycle and techno-economic analyses to quantify how valorization affects end products.

# Recommendations for researchers and alternative protein manufacturers:

- Assess the technological readiness of sidestream valorization processing methods for generating alternative protein inputs. Typically, academic researchers have better resources to explore the basic principles of a technology and validate proofs-of-concept, while industry can focus on upcycling sidestreams with advanced technology readiness levels, expediting alternative protein commercialization efforts.
- 2. Examine the valorization of alternative protein sidestreams to improve the environmental benefits and economics of production processes.

# Recommendations for policymakers:

- 1. Provide biomanufacturers with financial incentives to develop and scale up upcycling and circular operations.
- 2. Incentivize public–private and crop value chain collaborations to facilitate crop valorization, improve value chain efficiencies, and ensure feedstock availability.
- 3. Provide regulatory guidance for safety evaluations of upcycled food ingredients.
- 4. Prioritize investments in R&D and biomanufacturing infrastructure to enable sidestream valorization.

<sup>-</sup>rom field to facility

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# Introduction

# Motivations to use sidestreams in alternative protein production

By 2050, the human population is expected to reach approximately 10 billion people <sup>1</sup>. The World Resource Institute (WRI) has identified <u>three gaps</u> between business as usual in 2010 and sustainably feeding a population of 10 billion: (1) the need for 56% more crop calories, (2) an additional 593 million hectares of agricultural land, and (3) the mitigation of 11 gigatons of greenhouse gas (GHG) emissions from agricultural activity to keep global warming below a rise of two degrees Celsius <sup>2</sup>. To sustainably feed our rapidly growing population, food and agricultural systems must optimize natural resource utilization to form nutrient-dense foods for human consumption.

As climate change-driven drought could impact crop production and yield variability in the years to come, every fraction of crop production should be valorized efficiently to increase resources to feed a growing human population. Currently, significant amounts of waste are generated due to low-value utilization and disposal of agricultural residues, processing sidestreams, and food losses generated throughout the supply chain. In North America, only a small portion of crop biomass is used for human consumption or other industrial uses, leaving approximately 75% for low-value animal feed or even lower-value animal forage, fertilizer, landfilled mass, or incinerated mass (Figure 2) <sup>3,4</sup>.<sup>(a)</sup> These lower-value end uses are common because they are currently less expensive and time-consuming than investing resources into finding more sustainable, higher-value uses of these byproducts.

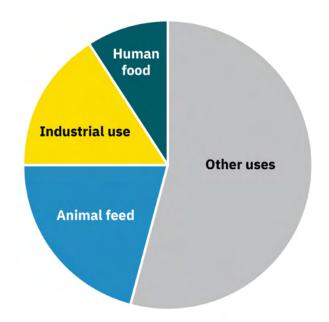


Figure 2: Estimated entire crop biomass allocation across food, feed, industrial, and other uses and losses for high-volume crops. Other uses refers to burned, landfill, animal forage, horticultural uses, or supply chain waste.

<sup>(a)</sup> Low-value end uses and supply chain wastes are not well-tracked in the agricultural industry and supply chain, so understanding their exact uses and environmental impacts is difficult.

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The valorization of agricultural sidestreams presents an opportunity to curb the environmental impact of agricultural waste products and access additional resources for feeding the human population. Agricultural and processing sidestreams are byproducts of crop use generated after producing a primary product or ingredient of high economic value. Many sidestreams retain a significant portion of macronutrients that could be made available for human dietary needs directly or through microbial or cultivated meat applications. Although a majority of agricultural sidestreams are not readily usable for direct human food, some processes offer the potential to elevate their value by extracting and converting biomass that would otherwise go to waste. These sidestreams can yield valuable inputs for the food sector, especially alternative proteins.

These innovative approaches align with the principles of a <u>circular bioeconomy</u>, wherein resources traditionally treated as waste products are instead reclaimed, converted, and valorized into new materials <sup>5</sup>. This paradigm shift offers the potential to reduce financial costs and environmental impacts of food production. Furthermore, <u>recent</u> <u>advances</u> made in alternative protein production science and technology enhance the capacity to leverage agricultural sidestreams as resources and ingredients tailored for enjoyable consumption <sup>6</sup>.

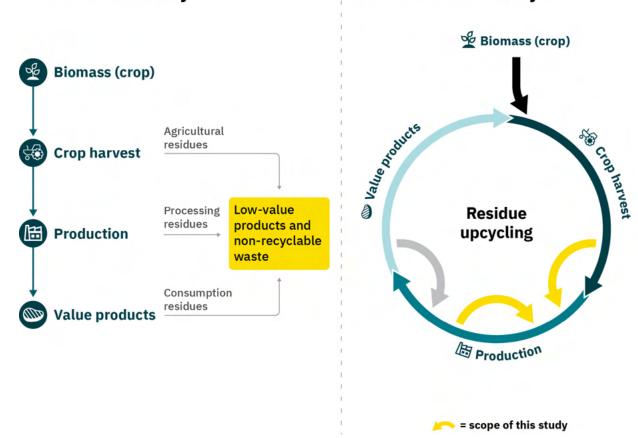


Figure 3. Linear versus circular bioeconomies and the scope of this study within circular bioeconomies.



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Recommendations & conclusions

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Recommendations conclusions

Approach & scope

Crop & sidestream landscape

Top sidestream candidates

From field to facility

Using sidestreams as ingredients in production will improve the sustainability of alternative protein manufacturing as we move closer to a sustainable, circular bioeconomy. As such, alternative proteins are well-positioned to valorize sidestreams, yet the marketplace for upcycled protein ingredients is underdeveloped, so there remain technological gaps and economic bottlenecks to accessing these resources.

The goal of this analysis was to identify highvolume agricultural sidestreams in North America with high potential for use in alternative protein production based on economic, environmental, and functional criteria. Additionally, we identified opportunities where investment into research and development may sustainably yield new resources for alternative protein manufacturing across plantbased, fermentation-derived, and cultivated food products. These results should point stakeholders toward the sidestreams with the greatest potential for alternative protein commercialization and marketplace adoption in North America.

# **Analysis overview**

This report analyzes the major agricultural sidestreams from the top crops produced in North America (Canada, Mexico, and the United States), as projected for 2030. Much of the burgeoning alternative protein industry relies on sources of bulk plant-based protein, as well as amino acids and glucose processed for food use for fermentation and cultivated meat production. Several agricultural sidestreams are readily available at high volumes and could serve as inputs for alternative protein production. Specifically, our study focused on using high-volume crop sidestreams in North America that could be valorized for the following:

- Plant protein concentrates as food ingredients.
- Protein hydrolysates for fermentation and cultivated meat media.
- Lignocellulosic sugar sources for fermentation.

Goal of study: Guide stakeholders interested in circular bioeconomics to crop sidestreams with the greatest potential for applications in alternative protein production in North America.

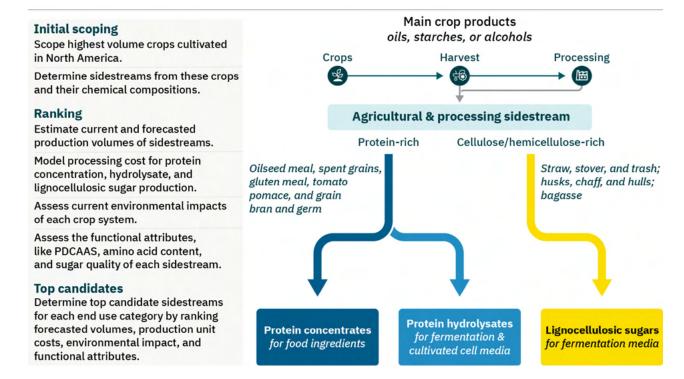


Figure 4: Approach and scope of analysis for potential sidestream uses in alternative protein production.



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# Analysis approach and scope

### Crop and sidestream scope

Our study focused on the valorization of sidestreams from high-volume crop production to investigate the early stages of food production, where new technologies could best be leveraged. We assessed the highest volume crops produced by Canada, Mexico, and the United States and identified the top eight: corn, soy, sugarcane, wheat, barley, canola, tomatoes, and rice. From these, we identified over 20 sidestreams for potential use as alternative protein ingredients. These sidestreams were assessed by their production volumes and chemical compositions to determine applicability to possible use cases in alternative protein production.

# **Ingredient categories**

Based on characteristics of interest for each use case, each crop sidestream was assessed for its potential as a plant-based protein concentrate ingredient, protein hydrolysate for fermentation and cultivated cell media, or a lignocellulosic sugar source for fermentation (Table 1).<sup>(b)</sup> The objective of this study was to identify sidestreams that could be converted into these ingredient categories for application to alternative protein production.

#### Table 1. Ingredient categories and use cases

Ingredient category	Use case	Objective
Protein concentrates	Plant-based protein ingredient	Identify high-protein content sidestreams that could be valorized for protein concentrate ingredients for plant-based foods.
Protein hydrolysates (e.g., peptones)	Nitrogen source for fermentation and cultivated meat	Identify high-protein sidestreams that could be valorized into protein hydrolysates that supply essential and non-essential amino acids for fermentation and cultivated meat.
Lignocellulosic sugars	Carbon source for fermentation	Identify lignocellulosic biomass that could be economically valorized as a sustainable source of reducing sugars for heterotrophic fermentation.

<sup>(b)</sup> The sidestreams were not assessed for valorization through solid-state fermentation, which has been extensively studied and offers a low processing means of upcycling many crop residues through fermentation.

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# Sidestream ranking and criteria

After evaluating the sidestreams for their potential to be converted into alternative protein inputs, we ranked them to determine which sidestreams have the highest potential for valorization using four primary criteria: forecasted sidestream volume, production unit cost, environmental impact, and functional attribute. Through our conversations with relevant stakeholders, we chose these four criteria as the top factors that companies and organizations consider when determining the suitability of a sidestream for commercial use. Production volume and unit cost are typically the most important indicators of a value chain's commercial feasibility. Consequently, we highlight forecasted sidestream volume and production cost by evaluating them together as an *economic ranking* (Figure 5). To feature crop sidestreams with the greatest potential benefit for alternative proteins and those that may benefit from production cost optimization, we provide an *integrated ranking*, which combines the economic ranking with environmental impact and functional attributes.



Figure 5: Ranking criteria for sidestream comparison.

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#### Forecasted sidestream volume

Crop volumes were forecasted in million metric tons (MMT) to assess possible annual output in 2030 by using the compound annual growth rate (CAGR) from 1961 to 2021 for each crop. Sidestream volumes were calculated by determining the percentage of sidestream material produced from each crop and estimating the total metric tons produced as a fraction of the crop output. Volume is a critical factor in determining the suitability of a sidestream for valorization because higher volumes of crops and their associated sidestreams will benefit from accessibility and economies of scale, thus reducing the overall cost.

#### **Production unit cost**

Production unit cost is the modeled unit cost to produce the ingredient categories of interest from the bulk "raw" sidestream. This economic consideration is, essentially, the cost of goods sold that determines the economic feasibility of any ingredient. The processing costs of an end product will vary, primarily because the compositions and bulk unit costs of the sidestreams vary. Unit costs were determined by first scoping the unit cost of the bulk sidestream and then modeling the production unit cost of processing each sidestream into (1) protein concentrate as USD per kg-protein, (2) protein hydrolysate as USD per kg-nitrogen, and (3) lignocellulosic sugars as USD per metric-ton sugar. See the Assumptions and Methods sections for more information on the underlying cost models.

#### **Environmental impact**

Environmental impact was used to assess the sidestreams, whereby crops with lower impact had a more favorable ranking. This environmental criterion is important for stakeholders to determine how to support sustainable crop cultivation and how the valorization of each sidestream could improve crop environmental impact. This study evaluated the environmental impact of each sidestream using the total crop cumulative environmental pressure calculated by Halpern <u>et</u> <u>al. 2022</u>. Environmental impacts are compared using average environmental pressures per tonne of crop grown in Canada, Mexico, and the United States, calculated using four dominant pressures: land disturbance; blue freshwater consumption; excess nutrients; and greenhouse gas emissions.

#### **Functional attributes**

A fourth criterion related to functional attributes of the end ingredient was applied for each category as follows. See <u>Assumptions</u> and <u>Methods</u> sections for more information.

**Protein Quality:** For protein concentrate ingredients only, protein quality was used as a criterion and represents the acceptability of a sidestream byproduct as a food ingredient to meet human nutritional requirements. Protein Digestibility Corrected Amino Acid Scores (PDCAAS), a method to evaluate the quality of proteins based on the amino acid composition and the ability of humans to digest them, was used to rank the quality of the protein concentrate ingredients. Higher values indicate better digestibility, are ranked higher based on this criterion, and suggest a protein is better suited for human nutrition.

**Culture Media Amino Acid Quality:** For protein hydrolysate ingredients only, an essential amino acid coverage score was used as a criterion. In cellular agriculture applications like cultivated meat, the cultivation media must provide all the essential amino acids needed for growth since the animal cells cannot generate these amino acids on their own. A coverage score of essential amino acids was calculated for comparison. A higher score represents better applicability to cell culture and is ranked higher based on this criterion.

From field to facility

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**Fermentation Sugar Quality:** For lignocellulosic sugars only, the ratio of cellulose to total cellulose and hemicellulose was used as a criterion and represents the theoretical quality of the total fermentable sugars extracted from the lignocellulosic material for fermentation. Cellulose is made up of mostly glucose monomers (hexose sugars), which is a preferred carbon source over pentose sugars like xylose. Assuming glucose is the preferred sugar source, a higher score represents a better feedstock and is ranked higher based on this criterion.

### Assumptions and caveats

Several assumptions were applied to reduce the complexity associated with this analysis and to focus on essential findings. Table 2 summarizes the key assumptions used in this analysis and the associated rationale. We recognize these assumptions may oversimplify aspects of the systems we are modeling, but the results are meant to uncover opportunity areas rather than serve as a quantitative predictive model.

#### Table 2. Key assumptions in the analysis

Assumption	Rationale
Sidestream volume forecasting model A simple economic model using CAGR is sufficient to identify valorization opportunities. Sidestream volume estimates are an accurate estimate of possible supply.	A simple economic model using CAGR was applied to project future growth in crop volume by using historical data from 1961 to 2021. This data was pulled from national databases, such as the <u>USDA ERS</u> , <u>StatCan</u> , and <u>FAO Stat</u> . We anticipate that demand for staple crops as major sources of edible protein will continue to increase steadily between 2022 and 2030, trending with future world population growth. Sidestream volumes were estimated by market-tracked volumes when available, such as corn DDGS and soy meal, or by crop composi- tion estimates, which serve as an estimate of possible volume supply. Otherwise, sidestream volumes were estimated by crop composition data.
<i>Sidestream unit cost</i> Raw material costs are accurately represented by literature, national databases, and commercial marketplaces.	Unit costs of raw materials have a strong impact on the modeled processing costs of protein concentrates, protein hydrolysates, or lignocellulosic sugars. The market costs of these sidestreams were pulled from research literature or national databases, such as the <u>USDA ERS</u> and <u>FAO Stat</u> . If data was not available from the databases, costs were pulled from commercial marketplaces (e.g., Alibaba) to inform the model cost of ingredient processing <sup>8</sup> . However, sidestream costs can vary by volume demand and partnerships with producers.

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<b>Protein processing steps</b> Basic processing steps currently used to produce plant-based proteins can be applied to extract and purify other proteins from sidestreams.	We used simplified models for protein processing to evaluate the costs associated with these steps, based on the most common technology used to extract proteins from crop matrices, such as acid or base extraction followed by precipitation, filtering, spray-drying, and air-drying. Corn protein ingredients were assumed to have an ethanol solvent extraction. There are a variety of technical strategies to improve the efficiency of protein extraction, such as using enzymes, microwaves, ultrasound, superheated water, and pressure. However, this study did not include them because of the limited techno-eco- nomic data linked to these technologies.
<ul> <li>Protein quality (rank factor)</li> <li>PDCAAS is a sufficient measure of protein quality for ranking.</li> <li>Protein quality for tomato pomace protein can be extrapolated from animal studies.</li> </ul>	<ul> <li>PDCAAS is used to evaluate the quality of proteins for human consumption. Values are based on the content of essential or indispensable amino acid requirements of humans and their ability to digest it from specific dietary proteins.</li> <li>Digestible indispensable amino acid score (DIAAS) is another value commonly applied to evaluate the nutritional quality of proteins. While we don't examine DIAAS here, the calculation and interpretation of these scores are similar to PDCAAS.</li> <li>PDCAAS values were not available for tomato pomace. A protein quality score was estimated by using digestibility scores derived from feeding poultry tomato seed protein and used in lieu of a verified PDCAAS value. Since chicken metabolism is different from humans, this estimate does not reflect the true quality of protein sourced from tomato pomace, which could be lower or higher than the estimated value.</li> </ul>
<b>Protein hydrolysis</b> <b>processing steps</b> Enzymatic processing steps currently used to produce protein hydrolysates can be applied across all protein- rich sidestreams.	The protein hydrolysis processing cost model used in this study was adapted from pre-existing commercial enzymatic methods to convert bulk protein into amino acids and peptides for media. However, we assume no additional processing steps are required, which may not reflect the physicochemical properties of the actual sidestream material. The degree of enzymatic hydrolysis also impacts the amount of free amino acids and other peptides. Pretreatment efficiency will depend on the feedstock composition and the input material loading concentration. Additionally, protease enzymes used to hydrolyze proteins will have varying efficiencies across materials and may be inhibited by other components present in each of the protein-rich sidestreams. There could also be compo- nents that must be removed before processing, such as oils. All of this would require changes in pretreatment, solids loading, and enzyme loading concentrations, which impact the cost of the process.

From field to facility

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Culture media amino acid quality (rank factor) Amino acids present in the raw sidestream will be present in the final hydrolysate with no loss since the loss would vary in each developed process. This serves as a best-case assessment.	The degree of hydrolysis and amino acid profile of each sidestream will impact its suitability for certain applications, such as cultivated meat growth media. Animal cell cultures in cultivated meat require organic nitrogen sources, which provide the essential amino acids needed for growth since the cells cannot generate these amino acids themselves. However, amino acid profiles will not impact fermen- tation as much since most microbes can convert the amino acids needed for growth. We assessed the sidestream amino acid profiles for suitability in cultivated meat media by assessing amino acid coverage relative to cellular protein demand for mammalian cell culture <sup>9</sup> . See the <u>Methods section</u> for the amino acid profiles of each candidate side- stream (Figure 16).
Lignocellulosic sugar processing steps The basic processing steps currently used to extract fermentable sugars from lignocellulosic biomass can be applied across sidestreams. We have not included an assessment of the potential concentration of inhibitors in the sidestreams and have chosen a more simplistic model to evaluate the cost of processing.	A general set of processing steps was modeled for converting ligno- cellulosic biomass into fermentable sugars. To extract fermentable sugars from lignocellulosic biomass, saccharification requires a pretreatment step to break the lignin and improve access to the cellulose, followed by enzymatic conversion of the cellulose and hemicellulose. Each lignocellulosic sidestream may have its own challenges. Pretreatment efficiency will depend on the feedstock and the material loading concentration. Cellulase enzymes used to degrade cellulose will also have varying efficiencies across materials and may be inhibited by secondary metabolites and byproducts in the sidestream material. Furthermore, each sidestream lignocellulosic saccharifica- tion could produce compounds, such as phenolics and furfural, that could be inhibitory to cell culture.
Fermentation sugar quality (rank factor) Glucose is the preferred sugar source over xylose in microbial fermentation.	The hydrolysis of both cellulose and hemicellulose results in a mixture of fermentable hexose and pentose sugars. Glucose will be produced from the cellulose, while hemicellulose hydrolysis will result in a mixture of xylose, arabinose, mannose, and galactose, depending on the feedstock. For many microbes, the utilization of xylose is inhibited by the pres- ence of glucose until the glucose has been consumed <sup>10</sup> . Thus, the co-fermentation of pentose and hexose sugars by microorganisms during fermentation is very desirable for lignocellulose sugar valori- zation. If a microorganism does not readily use pentose sugars, such as xylose, that carbon is essentially wasted feedstock cost <sup>11</sup> . When looking at the composition of crops, a higher cellulose content to hemicellulose content would therefore be desirable.

Recommendations & conclusions

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#### Environmental impact (rank factor)

The normalized environmental pressure is an accurate representation of the crop impact in Canada, Mexico, and the United States.

The impacts from "other oilseeds" and "other fruits" are representative of canola and tomato production, respectively. Environmental impact was calculated by averaging country-level average environmental pressure per tonne for crops grown in Canada, Mexico, and the United States <sup>7</sup>. The average environmental pressure per tonne of rice and sugarcane grown in Canada was not included, because both crops are seldom grown in Canada.

Additionally, the environmental pressure data for "other oil crops" from <u>Halpern et al., 2022</u> was used to estimate canola's environmental criteria but represents environmental pressure data for cotton, olives, rapeseed (canola), sesame, sunflower, and other oil crops. Similarly, their data for "fruits" was used to estimate the environmental criteria for tomatoes but represents environmental pressure data for apples, grapes, oranges, and other fruit. Both estimates should be relatively accurate environmental impact indicators for canola and tomato cultivation.

The greenhouse gas (GHG) emissions data from crop cultivation includes impacts from crop residue burning following IPCC guidelines which estimate "developed" countries burned 10% of their crop residues and "underdeveloped" burned 25%, as described in <u>Halpern</u> <u>et al., 2022</u>. It was not specified how Canada, Mexico, and the United States were classified in this method. It is likely that upcycling these crop residues, rather than burning them, has a strong potential to improve the environmental impacts of crop cultivation.

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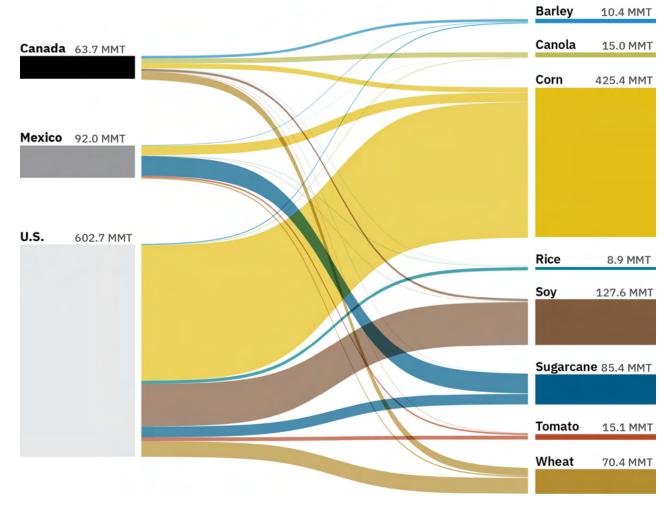
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# Crop and sidestream landscape

## Crop landscape

The top eight high-volume crops with sidestreams in Canada, Mexico, and the United States are corn, soy, sugarcane, wheat, barley, canola, tomatoes, and rice. These crops accounted for over 750 million metric tons (MMT) of production in 2021, with 425.4 MMT from corn and 127.6 MMT from soy. The United States is the predominant producer of corn and soy but also has significant wheat, sugarcane, and tomato production. The United States is also the largest producer of rice in North America at ~8.7 MMT, while 0.25 MMT was produced in Mexico (Figure 6). Crop production in Canada is predominantly canola, corn, barley, and wheat, given the optimal growing conditions for those crops in the country. Canada is the largest canola producer in North America at 13.7 MMT annually in 2021. Corn production was a sizable 13.9 MMT in Canada and 27.5 MMT in Mexico. However, this seemingly high production is dwarfed by corn production in the United States. Of the crops assessed, Mexico's crop production is predominantly sugarcane, corn, wheat, and tomatoes (Figure 6).



#### Figure 6: Total crop production by country in 2021.<sup>(c)</sup>

<sup>(c)</sup> North American production values extracted from national agricultural datasets were generated by summing the total amount of production for each crop or relevant sidestream across production for Canada, United States, and Mexico. The production year used was 2021 as it is the most accurate recent full-year data set for all crops assessed.

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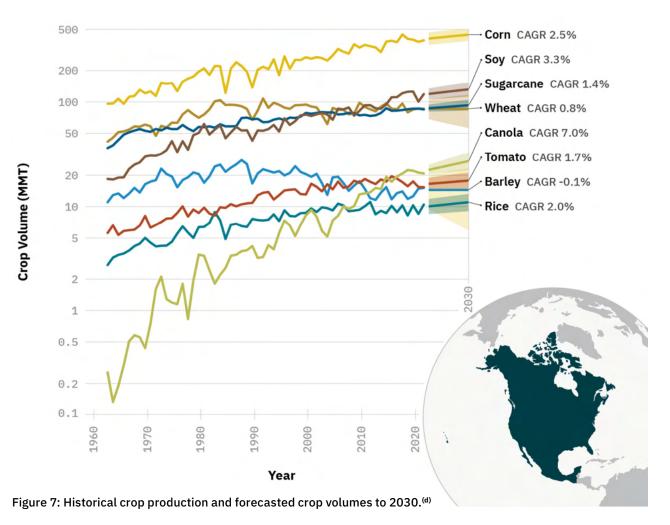
Recommendations & conclusions

Fop sidestream candidates

From field to facility

# Forecasted crop volume in 2030

We forecasted the volume of crop cultivation in 2030 to provide a roadmap for future production and to anticipate potential future bottlenecks or opportunities (Figure 7). Historical CAGR for each crop ranged from -0.1 to 7.0%, with a median and average annual crop increase of 1.8% and 2.3%, respectively. Canola had the highest production increase with 7% annual growth, with Canada as the primary producer. Forecasted volumes for canola were based on the 2021 harvest year, which had a significant decline from 2019 and 2020 due to extreme drought and heat. While the 2022 crop yield rebounded, climate change could impact canola production and the forecasted volumes. Annual growth of corn production in the United States has remained fairly steady over time with an average CAGR of 2.5% despite major price shifts from 2000 to 2010 when corn ethanol production increased. Barley was the only crop to see negative annual crop production over this time period (-0.1% CAGR). Shifting market demands and economics have affected barley production due to geographic competition from corn and soy, as well as a decline in feed barley production. Overall, these historical trends are a baseline for forecasting future crop volumes, as shifting market demands, economic factors, global events (such as the Russian invasion of Ukraine), climate change (such as drought), and land use changes can affect crop production regionally.



<sup>(d)</sup> Production data in metric tons from 1961 to 2021 for the top 8 crops in North America were collected from FAO-Stat and Our World in Data interactive webpage. CAGR was calculated from initial values in 1961 to final values in 2021.

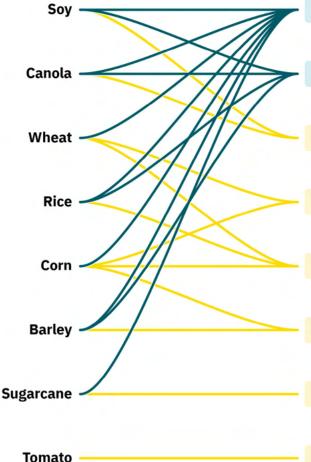
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### Sidestream landscape

From the top eight high-volume crops, we identified 24 sidestreams that could be further valorized for alternative protein ingredient production. These sidestreams can be categorized as agricultural residues and processing residues (Figure 8). From these sidestreams, we compiled the chemical composition, forecasted volumes, and raw material costs to identify high-potential candidates for plant protein concentrate, protein hydrolysate, or lignocellulosic sugar extraction (Figure 9, Figure 10).

# **Agricultural residues**

Agricultural residues are the leftover crop components after harvesting, dehusking, or dehulling and include crop fiber residues, such as corn stover, straws, and sugarcane trash, that are high in fiber content associated with the stems, leaves, and stalks of the plant material <sup>12–15</sup>. These straws, stovers, and trash residues are composed of lignocellulose, which is the predominant component of plant dry matter composed of cellulose encapsulated by hemicellulose and lignin (Figure 10). Harvested crop values are reported as



#### Straw, stover, and trash

Soybean straw, canola straw, wheat straw, rice straw, barley straw, corn stover, sugarcane trash

#### Husks, chaff, and hulls

Soybean hulls, canola hulls, canola chaff, rice hulls, barley husks

#### **Oilseed meal**

Soybean meal, canola meal

#### **Gluten meal**

Wheat gluten, corn gluten

#### Bran and germ

Wheat bran, wheat germ, rice bran, corn bran

#### **Alcohol spent grains**

Brewer's spent grain (barley), distillers dried grain (corn)

#### Bagasse

Sugarcane bagasse

Pomace Tomato pomace

# Agricultural residue Processing residue

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Figure 8: Major crops assessed and their associated sidestreams.

the edible food crop volumes, which does not account for many of the agricultural residues left in the field like corn stover, straws, and sugarcane trash. Thus, these are the highest volume sidestreams identified in this study (Figure 9).

Husk and hulls, such as soy hulls, barley husks, canola chaff, canola hulls, (e) and rice hulls, are another agricultural residue produced from harvesting grain or seed crops <sup>16,17</sup> (Figure 8). These are the outer protective coverings that enclose the grain or seed. The hulls, husks, and chaff sidestreams are also high in lignin, cellulose, and hemicellulose (Figure 10). However, their volumes are not as high as straws since they are a fraction of the harvested food crop that is removed prior to processing. Their volumes range from 5–20% of the harvested crop (Figure 9).

The market price of these fibrous sidestreams rich in lignin and cellulose ranges between \$11 to \$165 per metric ton (Figure 10). The price range is dominated by straws, stover, and bagasse, which are primarily left in the field, burned, or used for horticulture as the variable cost of fuel and transportation makes it uneconomical for sidestream producers to sell. These are sometimes used as backup animal forage but do not have high nutritional value. Transportation is usually embedded in the unit cost of the agricultural residue, which, in fact, makes up the majority of unit costs for these lower value sidestreams<sup>18</sup>. The higher-priced agricultural residues have a higher nutritional value for animal feed applications, such as soy hulls and wheat straw at ~\$165 and \$85 per metric ton, respectively (Figure 10).

## **Processing residues**

Processing residues are sidestreams generated after further processing of the harvested crop material and include oilseed meals, gluten meals, bran, brewer's spent grains (BSG), sugarcane bagasse, and tomato pomace.

Oilseed meals, such as soy and canola meal, are typically byproducts of oil extraction processing<sup>19,20</sup> (Figure 8). These meals are high in protein content and have a history of coproduct valorization for either human food or animal feed applications. Soy meal and canola meal are both high-volume sidestreams that contain high protein content (35–50%) but also contain high levels of starch, hemicellulose, and residual fat after extraction of the oil (Figure 9, Figure 10). Soy meal and canola meal command higher raw unit prices, \$525 and \$487 per metric ton, respectively, due to their market demand for use in feed and food production <sup>21</sup>.

Gluten meals, such as corn gluten meal and wheat gluten, are byproducts of wet milling of corn starch and wheat starch, respectively <sup>22,23</sup> (Figure 8). This results in a protein-rich sidestream (60-80%) with residual starch (~15%) leftover from the starch extraction (Figure 10). These gluten meals are a small fraction (<1%) of the processed starch crop, thus their estimated volumes are comparatively low at 0.1 MMT for wheat gluten and 2 MMT for corn gluten (Figure 9). These gluten meals command some of the highest raw unit costs, \$600 to \$1200 per metric ton, due to the high protein content and market demand (Figure 10).

<sup>(e)</sup> Canola chaff and canola hulls often refer to the same component. Here, we assess chaff as a mixture of canola straw, leaf material, and whole or cracked kernels. Canola hulls refers just to the outer covering of the canola seed pod.

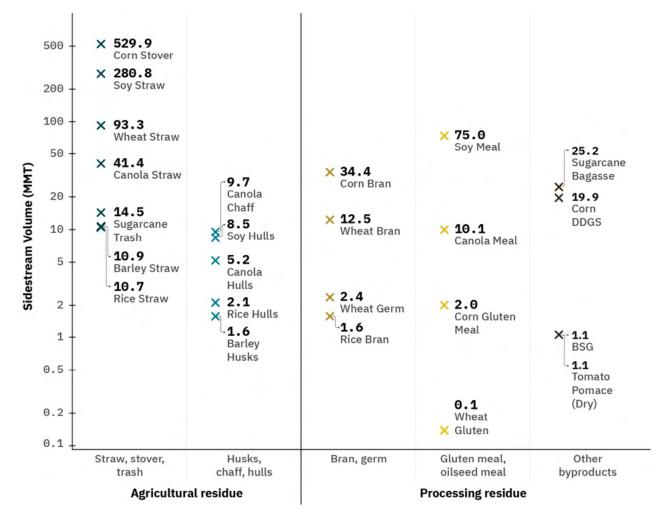
& conclusions

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Corn distillers dried grains with solubles (DDGS) and brewer's spent grains (BSG) are remnant grain material after sugar extraction for alcohol production (Figure 8). Corn DDGS is a dried sidestream of the corn ethanol (fuel or distillers) production process. BSG, made predominantly from barley grain, is a high-moisture byproduct generated from the beer brewing industry <sup>24</sup>.<sup>(f)</sup> Corn DDGS and barley BSG sidestreams have volumes of 19.9 and 1.1 MMT, respectively, with a high composition of protein (20–30%), cellulose (15-20%), and hemicellulose (12-20%).

Corn DDGS also contains residual starch (8%) and fat (10%). BSG has a high moisture content (20% solids) as it is generated from the wet mash process. Corn DDGS also goes through a wet extraction process, but it has a low moisture content since it is dried on-site by producers for feed valorization (Figure 9, Figure 10).

Bran refers to the outer layer or protective skin surrounding the endosperm of cereal grains like wheat, rice, and corn <sup>25-27</sup>. Bran sidestreams are produced during the grain and legume milling process (Figure 8).



#### Figure 9: Sidestream landscape and volumes with 2030 forecasted crop production.<sup>(g)</sup>

<sup>(f)</sup> In this analysis, we consider brewer's spent grain made only from barley grain.

<sup>(g)</sup> Sidestreams are not additive to crop volumes since crop volumes are the harvested edible crop volumes and processing residues have added moisture volume. The current and forecasted 2030 volume of sidestream production was calculated as a percentage of the total crop production. Components of sidestreams or dried versions of high-moisture sidestreams were calculated based on the fraction of dry matter available in each sidestream.

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Recommendations conclusions

Introduction

Bran sidestreams represent about 5–15% of the harvested crop volume and have a varied composition of protein, carbohydrates, fat, and lignocellulose depending on the crop (Figure 9). Rice bran has high levels of lignin (>40%) since it's the outer protective layer of the rice grain, but also contains significant protein (15%) and fat (18%) <sup>28</sup>. Wheat and corn bran have high concentrations of starch (20%), cellulose (10–20%), hemicellulose (18–33%), and protein (10–15%) <sup>29–31</sup>. Brans command a higher market value, \$220–250 per metric ton, due to their higher and balanced nutritive value for feed and food applications (Figure 10).

In sugarcane processing, bagasse is the fibrous remnants of the cane stalks after the sugar extraction process. Sugarcane bagasse is mostly comprised of cellulose (46%), hemicellulose (26%), and lignin (23%)<sup>32,33</sup> (Figure 10). It accounts for ~26% of the sugarcane crop, which creates a significant volume of waste biomass (Figure 9).

Finally, in the tomato industry, tomato pomace is the residual seeds and peels left over from the tomato processing for juice, sauce, and ketchup production <sup>34</sup> (Figure 8). It is a high-volume, high-moisture (24% solids) **sidestream** with a broad composition of protein (17%), fat (9%), cellulose (8%), hemicellulose (15%), and lignin (22%). The protein is predominantly from the seeds, while the lignocellulose is from the peels (Figure 9). The wet tomato pomace accounts for 25% of the tomato crop volume (Figure 9).

Processing sidestreams that contain a significant amount of moisture, such as tomato pomace and BSG, suffer from short microbiological and physicochemical stability, which requires on-site drying or limits their utility and value on the market <sup>35</sup>. Therefore, their raw cost, ~\$45 per metric ton, is estimated to be equal to the cost of transportation, which is dependent on the geographical location of production and processing facilities. Conversely, corn DDGS is a high-moisture coproduct that is dried on-site by ethanol producers and offers a long shelf life. These drying costs are associated with the final market price, ~\$250 per metric ton, which is welltracked on the market <sup>36</sup>.

Market demand across industries (food, feed, industrial) is the main cost factor for higher value agricultural and processing residues. Transportation and drying costs are another major cost driver of these raw materials. Other variables include crop seasonality, crop quality, producer storage, producer capacity, and material contamination with mycotoxins, pesticides, or heavy metal contamination <sup>37</sup>.

# Sidestream ranking summarized results

Based on the crop sidestream compositions, we evaluated each sidestream by its composition followed by modeled processing cost to either protein concentrates, protein hydrolysates, or lignocellulosic sugar extraction. Based on composition, there was a clear distinction between protein-rich crop residues that could be upcycled for protein extraction or protein hydrolysis and lignocellulosic-rich sidestreams that could be valorized for sugar extraction (saccharification). After scoping these sidestreams, we modeled the forecasted volume and production costs of the ingredients and ranked the resulting valorization opportunities. The scoped sidestreams and summarized results can be found in Figure 11.

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		Protein	Fat	Starch	Sugars	Cellulose	Hemicellulose	Lignin	Ash content	Dry weight (%)	Raw unit costs (USD/tonne)
Barley	Barley BSG	0	۰	٥	٠	0			•	20	\$49
	Barley husks	•	•	•	٠		ightarrow		•	88	\$40
	Barley straw	٠	٠	٥	٠				•	80	\$57
Canola	Canola meal		0	0	0	0	$\bigcirc$	0	0	89	\$487
	Canola hulls	$\bigcirc$	0	٥	0	$\bigcirc$	0	$\bigcirc$	0	88	\$40
	Canola straw	0	o	o	0		0	$\bigcirc$	0	83	\$62
	Canola chaff	•	٠	0	•		$\bigcirc$	0	$\circ$	80	\$40
Corn	Corn DDGS		0	0	٥	0	0	0	0	94	\$245
	Corn gluten meal		0	0	0	0	0	0	0	91	\$603
	Corn bran	0	0		0	0		0	0	92	\$255
	Corn stover	0		•				0	0	32	\$67
Rice	Rice bran	0	0	o	٥	0	0		0	96	\$221
	Broken rice	•	0						0	87	\$1,050
	Rice hulls	•		0				0		95	\$11
	Rice straw	•	•	•	٠			•	0	84	\$58
Soy	Soy meal		0	0	0	0	0	0	0	88	\$525
	Soy hulls	0	0	•			•	0	•	89	\$165
	Soy straw	0	۰	0	•	Ŏ			•	93	\$55
Sugarcane	Sugarcane molasses	•	0	0			0		•	73	\$200
	Sugarcane trash			•					•	70	\$37
	Sugarcane bagasse	•	•	0	•				•	50	\$50
Tomatoes	Tomato pomace		•	•		•			•	24	\$44
Wheat	Wheat gluten		0	0	0	0	0	•	o	94	\$1,200
	Wheat germ		0	0	۰	0	0		0	87	\$2,645
	Wheat bran	0	0	$\bigcirc$	•	$\bigcirc$	$\bigcirc$	0	0	87	\$221
	Wheat straw	0	0					$\bigcirc$	0	94	\$87

#### Figure 10: Crop sidestream composition and raw material unit costs.<sup>(h)</sup>

<sup>(h)</sup> Sugarcane molasses and broken rice were initially scoped as possible sidestreams but were not evaluated further within the end-use categories due to their composition, cost, and/or use in other industries.

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Recommendations & conclusions

From field to facility

Composition: Protein vs. Ce		a transmission of the transmission
<ul> <li>Protein concentrates</li> <li>Barley BSG</li> <li>Canola meal</li> <li>Corn bran</li> <li>Corn DDGS</li> <li>Corn gluten meal</li> <li>Rice bran</li> <li>Soy meal</li> <li>Tomato pomace</li> <li>Wheat bran</li> <li>Wheat germ</li> <li>Wheat gluten</li> </ul>	<ul> <li>Protein hydrolysates</li> <li>Barley BSG</li> <li>Canola meal</li> <li>Canola meal PC*</li> <li>Corn gluten meal</li> <li>Corn gluten meal PC</li> <li>Corn DDGS</li> <li>Rice bran</li> <li>Soy meal</li> <li>Soy meal PC</li> <li>Tomato pomace</li> <li>Tomato pomace PC</li> <li>Wheat germ</li> <li>Wheat gluten</li> </ul>	Lignocellulosic sugar Barley straw Barley hulls Canola straw Canola hulls Canola chaff Corn stover Rice straw Rice hulls Soy straw Soy hulls Sugarcane trash Sugarcane bagasse Wheat straw
deling	_	
Modeling	Ranking	
<ul> <li>Production unit cost models</li> <li>Forecasted production volumes</li> </ul>	<ul><li>(Halpern et al., 2022)</li><li>Functional attribute: prot</li></ul>	dels ive environmental pressure rein quality (protein d quality (protein hydrolysates)
o candidates —		
Protein concentrates	Protein hydrolysates	Lignocellulosic sugar
Economic	Economic	Economic
<ol> <li>Soy meal</li> <li>Canola meal</li> <li>Wheat bran</li> </ol>	<ol> <li>Soy meal</li> <li>Soy meal PC</li> <li>Corn DDGS</li> </ol>	<ol> <li>Corn stover</li> <li>Soy straw</li> <li>Rice hulls</li> </ol>
o. Wheat bran	0. 00	of filled filente

- 4. Canola meal
- 5. Barley BSG
- 6. Corn gluten meal
- 7. Rice bran

#### Integrated

- 1. Soy meal
- 2. Soy meal PC
- 3. Corn gluten meal
- 4. Corn DDGS
- 5. Tomato pomace
- 6. Tomato pomace PC
- 7. Barley BSG

#### Figure 11: Sidestream scoping and summarized results from modeling and ranking.

\*PC = protein concentrate; For hydrolysates, this is a hydrolysate of the protein concentrate. \*\*Integrated rank refers to equal weighted ranking for sidestream volume, modeled unit cost, environmental, and functional attribute.

4. Tomato pomace

2. Tomato pomace

5. Corn gluten meal

3. Canola meal

4. Corn DDGS

6. Wheat bran

7. Barley BSG

5. Wheat gluten

6. Rice bran

7. Barley BSG

Integrated\*\*

1. Soy meal

4. Sugarcane trash

6. Sugarcane bagasse

3. Sugarcane bagasse

4. Sugarcane trash

5. Barley husks

7. Canola straw

6. Soy hulls

5. Barley straw

7. Rice straw

1. Corn stover

2. Soy straw

Integrated

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# 4 Top sidestream candidates

Protein concentrates	
Protein hydrolysates	
Lignocellulosic sugars	

# **Top sidestream candidates**

### **Protein concentrates**

#### Definition

Protein concentrates are ingredients with moderate protein concentration, typically ranging from ~50 to 90% protein content as calculated on a dry basis (<u>International Pulse Ingredient</u> <u>Consortium; ADM Soy Protein Solutions</u>). Plant protein concentrates are commonly used in plantbased foods due to their excellent macronutrient content and functionalities, such as gelation, binding, and emulsifying characteristics.

#### Objective and rationale

Achieving high protein content in these ingredients requires beginning with protein-rich sidestreams. As a result, sidestream candidates were chosen based on their protein content. In addition to protein content, other properties, such as protein digestibility (PDCAAS), cost and ease of processing, protein functionality, and presence of antinutrients, off-flavors, and other undesirable compounds for food formulation, affect the usefulness of a sidestream to produce protein concentrate ingredients. While we do not quantify protein functionality and ingredient impurities here, we do include PDCAAS and cost of production as ranking criteria and suggest more open-access research to optimize these sidestream protein concentrates for human consumption (see <u>Recommendations and conclusions</u>).

#### Scoped sidestreams

Eleven sidestreams demonstrated high potential for input into alternative protein production as a protein concentrate based on their initial protein concentration by dry weight, ranging from 13.5 to 80%: wheat bran, rice bran, tomato pomace, corn bran, barley BSG, corn-based DDGS, wheat germ, canola meal, soy meal, corn gluten meal, and wheat gluten (Figure 10, Figure 11).

# Top sidestream candidates for plant protein concentrates

From the sidestreams assessed for protein concentrates, the top economic candidates were soy meal, canola meal, wheat bran, tomato pomace, wheat gluten, rice bran, and barley BSG. When considering economic, environmental, and protein digestibility factors, corn DDGS and corn gluten meal emerged as other promising sidestreams to enrich into protein concentrate ingredients (Figure 11, Figure 12).



Introduction

<sup>-</sup>rom field to facility

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	Volume	Protein unit cost	PDCAAS	Environmental impact	Economic rank	Integrated rank
Soy meal		$\bigcirc$			1	1
Canola meal					2	3
Wheat bran		$\bigcirc$	•	•	3	6
Tomato pomace					4	2
Wheat gluten		$\bigcirc$	•	•	5	8
Rice bran		$\bigcirc$	•		6	10
Barley BSG		$\bigcirc$			7	7
Corn DDGS		$\bigcirc$			8	4
Corn gluten meal	•	0			9	5
Corn bran					10	9
Wheat germ	•	•	•	•	11	11
Legend	Legend Volume, 2030 MMT/yr		cost /kg-protein	PDCAAS		mental impact /tonne crop
Excellent	>15.0	15.0 <\$2.50		>0.8	<0.000	1
Good	5.0-15.0	\$2.5	50-\$4.00	0.6-0.8	0.0001	-0.0003
Okay	2.0-5.0	\$4.0	00-\$6.00	0.4-0.6	0.0003	-0.0005
Low	0.5-2.0	\$6.0	00-\$10.00	0.2-0.4	0.0005	-0.0008
Poor	<0.5	>\$10	0.00	<0.2	>0.0008	

Figure 12. Plant protein concentrates results summary with volume, modeled unit cost, PDCAAS, and environmental impact data.

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#### Soy meal

#1 economic; #1 integrated

While soy meal, a byproduct of soy oil extraction, is mostly used for animal feed with minimal processing,<sup>(i)</sup> upcycling into protein concentrates for human consumption has an established value chain for plant-based meat products as well. In the United States, of the top 75 plant-based products in 2020, 48% of all burgers, meatballs, and grounds, 36% of sausage links and patties, and 40% of nuggets, tenders, and cutlets contained soy protein concentrate <sup>38</sup>. Soy protein concentrate is a popular ingredient due to the high annual production volume of soy and soy meal and the low cost of production. The low cost of soy protein concentrate production is a result of decades of research and commercial production of soy protein. Thus, it is unsurprising to see this sidestream rank highly when evaluating economic factors. Moreover, soy protein has an excellent PDCAAS score of 0.84 and a "good" environmental footprint. Overall, soy's ranking aligns with its popularity as an upcycled ingredient for plant-based foods.



#### Canola meal

#2 economic; #3 integrated

Canola is cultivated at high volumes in Canada and has an established <sup>39</sup>, low-cost protein enrichment process similar to other oilseeds like soy. Canola protein has excellent digestibility, with the highest PDCAAS value of proteins we evaluated at 0.93, so it demonstrates promise as an alternative protein for human consumption. Focused efforts to improve canola cultivation's "poor" environmental footprint would help optimize its use as a protein ingredient. It should also be noted that canola protein ingredients can be rich in phenolics, giving them a dark color and astringent taste, but there are strategies to remove phenolics from protein ingredients <sup>40</sup>. DSM-Firmenich, an ingredient company, recognized the high quality of canola protein and has developed CanolaPRO, a canola protein isolate that can be used for meat alternatives, dairy alternatives, and other grocery store staples.



#### Wheat bran & wheat gluten

#3 economic; #6 overall & #5 economic; #8 integrated

Wheat bran and wheat gluten both have low production costs, and wheat bran has a high forecasted production volume. Because of its low processing cost and unique dough-forming abilities, wheat gluten is a popular ingredient in plant-based meat products to provide elasticity and cohesiveness to end products. Of the top 75 U.S. plant-based products in 2020, 48% of all burgers, meatballs, and grounds, 72% of sausage links and patties, and 76% of nuggets, tenders, and cutlets contained wheat gluten <sup>38</sup>. Still, there are many areas to optimize protein-rich wheat sidestream utilization, including improving wheat protein's digestibility.

<sup>(i)</sup> 97% of all U.S. soybean meal is consumed by the global animal agriculture industry. <u>https://www.unitedsoybean.org/hopper/us-soybean-meal-a-dependable-feed-ingredient/</u>

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#### Tomato pomace

#4 economic; #2 integrated

Tomato pomace is a high moisture-content sidestream with a relatively low protein content that could be economically upcycled for protein concentrates or hydrolysates. From a technical point of view, the high moisture content could pose additional challenges from a drying cost and spoilage perspective. Additionally, tomato pomace protein concentrate has a low technological readiness level as there are no commercial efforts and sparse literature available for its production. The mixed seed and peel composition also poses a challenge to unlocking the high protein content of the tomato seeds <sup>41</sup>. While an enzymatic process for tomato pomace protein concentrate production has been developed, thus reducing its unit cost per kg protein 41,42, this method, or others, must prove scalable for tomato pomace to be a feasible protein concentrate input. Tomato pomace protein digestibility was the only PDCAAS value of the proteins evaluated here that did not have a literature value available. Therefore, we estimated PDCAAS using digestibility scores derived from feeding chickens tomato seed protein <sup>43</sup>. Finally, of all the proteins evaluated here, there is the least available information about the functionality of tomato pomace protein. Digestibility scores derived from human consumption and the functionality of tomato pomace proteins must be evaluated and optimized before fully understanding its value as a food ingredient. Still, tomatoes are one of the most widely cultivated crops globally, and tomato pomace has great potential for further valorization <sup>44</sup>. Tomato pomace sidestream demonstrated strong potential due to its low estimated cost of protein production, "good" estimated protein digestibility, and "good" environmental impact.



#### **Rice bran**

#6 economic; #10 integrated

The forecasted volumes of rice bran in 2030 ranked low compared to the other crops examined, but it still ranked high economically because of its low estimated cost per unit of protein. Rice bran protein content is low (14.5%), but an enzymatic process has been developed to create an enriched protein ingredient <sup>45</sup>, which lowered its estimated cost of production in this report. Rice bran ranked lower when comparing environmental and digestibility factors due to rice cultivation's poor environmental metrics as well as its mediocre PDCAAS (0.50). Rice cultivation is known to emit large quantities of methane and requires high water use, but there are some strategies to reduce these environmental impacts <sup>46</sup>. If the environmental footprint of rice cultivation is improved, rice bran could be a promising plant protein concentrate candidate due to its low estimated processing costs.



# Barley brewer's spent grain

#7 economic; #7 integrated

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BSG ranked well as a protein concentrate candidate because of the "good" digestibility of barley protein with a PDCAAS of 0.61 and "good" estimated production costs. BSG did not rank in the top five economic targets because it has a low forecasted production volume. Still, BSG has a high technological readiness level for its conversion into a protein concentrate, due to existing commercial-scale enzymatic processing <sup>47</sup>. AB InBev has recognized the value of brewer's spent grain as a protein ingredient and opened an ingredient company, EverGrain, in 2022 to upcycle barley protein from its brewing processes into nutritious ingredients. Terra Bioindustries is also focused on upcycling BSG into a barley protein concentrate and valorizes the sidestream further by using removed spent grain fiber to create fermentable sugars. Similar to tomato pomace, brewer's spent grain is a high-moisture sidestream, so drying before storage and transport could add significant costs to valorization and is an opportunity for drying and processing innovations. It should also be noted that for BSG and tomato pomace, drying the raw material before processing was not considered in production costs as the processes we modeled either directly wet milled (BSG) or used raw material (tomato pomace).



#### Corn distillers dried grains with solubles & corn gluten meal

#8 economic; #4 integrated & #9 economic; #5 integrated

Corn gluten meal (CGM) is a high-protein sidestream with high production volumes that has not been valorized much beyond <u>animal feed</u> and as a <u>natural herbicide</u>. Corn sidestreams ranked low for their use as protein concentrates when evaluating economic factors. This is mostly a result of modeled high extraction cost, which, due to corn protein's solubility, requires the use of large quantities of ethanol to extract. However, the process could be improved by effectively recycling or eliminating ethanol in corn protein ingredient production. When combining economic factors with environmental and protein digestibility criteria, both corn DDGS and CGM demonstrate much more promise. This is despite corn protein's low PDCAAS of 0.37 and is a result of its favorable environmental footprint, especially in the United States (the largest producer of corn) where corn breeding has been optimized. Despite this low digestibility, corn protein does present promise as a functional protein for plant-based food production due to its fiber-forming qualities <sup>48,49</sup>.

#### Conclusion

Overall, protein-rich sidestreams from commercial processes can be upcycled into high-value protein ingredients for human consumption. Most plant proteins have not yet been extensively researched and commercialized, with the exception of soy protein and wheat gluten-although these proteins would still benefit from more research focused on optimizing their use as food as opposed to animal feed. Besides protein concentrates, protein-rich sidestreams can be upcycled into other high-value protein ingredients, like protein isolates and protein hydrolysates-the latter of which we evaluate next. Depending on the sidestream source, it may be economically advantageous to upcycle it into one protein ingredient over another or to apply it for multiple alternative protein applications.

From field to facility

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## **Protein hydrolysates**

#### Definition

Microbial fermentation and cultivated meat production rely on nitrogen to support cell growth and produce food ingredients or other bioproducts. Protein hydrolysates, a source of free amino acids or small peptides, are frequently used in fermentation and cultivated meat media to supply essential and nonessential amino acids. These protein hydrolysates (e.g., peptones) have been historically sourced from various bacterial, plant, and animal biomass products, such as yeast extract, soy peptone, and beef peptone, respectively.

#### **Objective**

This analysis sought to identify high-protein, high-volume agricultural sidestreams that could provide cost-competitive protein hydrolysates for use in microbial fermentation and cultivated meat production media.

#### Scoped sidestreams

Based on chemical composition data, we identified nine sidestreams with high protein content (Figure 10): barley-based BSG, canola meal, corn-based DDGS, corn gluten meal, rice bran, soy meal, tomato pomace, wheat germ, and wheat gluten (Figure 10, Figure 11). Additionally, we modeled the cost of enzymatic hydrolysis of a few of the protein concentrates (PC) assessed in the protein concentrates section. These hydrolysates included canola meal PC, soy meal PC, corn gluten meal PC, and tomato pomace PC. Hydrolysates with more impurities, anti-nutrient, or inhibitory peptides could affect cell growth and productivity in cultivated meat applications. Thus, the more purified protein hydrolysates produced from PCs can be of higher quality for animal cell culture.

# Top sidestream candidates for protein hydrolysates

From the sidestreams assessed for protein hydrolysates, the top economic candidates were soy meal, corn DDGS, canola meal, barley BSG, and corn gluten meal. When combining economic and environmental factors, the top candidates were soy meal, corn gluten meal, corn DDGS, and tomato pomace (Figure 11, Figure 13).



From field to facility

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	Volume forecast	Unit cost (per kg-N)	Unit cost (per kg)	Essential amino acid	Env. impact	Economic rank	Integrated rank
Soy meal						1	1
Soy meal PC			$\bigcirc$			2	2
Corn DDGS		$\bigcirc$	$\bigcirc$			3	4
Canola meal	$\bigcirc$		$\bigcirc$			4	8
Barley BSG	$\bigcirc$			•	0	5	7
Corn gluten meal	0		$\bigcirc$	•		6	3
Rice bran	$\bigcirc$	•				7	12
Tomato pomace	$\bigcirc$	•		•		8	5
Canola meal PC	$\bigcirc$	•				9	11
Tomato pomace PC	$\bigcirc$	•	•	•		10	6
Wheat gluten		•	•		$\bigcirc$	11	13
Wheat germ	•			•	$\bigcirc$	12	9
Corn gluten meal PC	•					13	10
Legend Volume, 2030 MMT/yr		Unit cost USD/kg-nitro	Unit ogen USD/		itial amino overage	Environme pressure/t	ental impact onne crop

Legenu	MMT/yr	USD/kg-nitrogen	USD/kg	acid coverage ratio	pressure/tonne crop
Excellent	>15.0	<\$30	<\$2	>0.890	<0.0001
Good	5.0-15.0	\$30-\$40	\$2-\$4	0.80-0.90	0.0001-0.0003
😑 Okay	2.0-5.0	\$40-\$60	\$4-\$6	0.75-0.80	0.0003-0.0005
Low	0.5-2.0	\$60-\$100	\$6-\$10	0.60-0.75	0.0005-0.0008
Poor	<0.5	>\$100	>\$10	<0.60	>0.0008

Figure 13. Protein hydrolysate ranking results summary with volume, modeled unit cost, amino acid quality, and environmental impact data.<sup>(j)</sup>

<sup>(i)</sup> Modeled production unit cost per kg-hydrolysate was not used in ranking. Information only relative to the ranked unit cost per kg-nitrogen value.

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#### Soy meal/soy meal PC

#1/#2 economic; #1/#2 integrated

The media supplier industry already produces a range of soy peptone products from either soy meal or soy PC, which are used in many lab and commercial processes. When produced from the raw soy meal, other components such as sugars remain through the extraction process. Given the current and projected volume of soy production, there would be an opportunity for further production of this reliable protein hydrolysate. Soy meal ranked well based on its low modeled unit cost and high sidestream volume. Soy meal also has a great amino acid profile that can provide many essential amino acids for cultivated meat production (Figure 13, Figure 16). Soy peptone is the assumed hydrolysate source of amino acids in the Humbird 2021 cultivated meat techno-economic assessment with an assumed price of \$2–3/kg hydrolysate <sup>9</sup>. Our simple model estimated a \$3/ kg hydrolysate price from soy meal, but ~\$6/kg soy hydrolysate from soy PC. Though the production of soy peptones is a technologically mature and highly optimized process, production costs could increase with the rising demand and price of soy meal in the market.



#### **Corn DDGS**

#3 economic; #4 integrated

Corn DDGS is a high-protein sidestream of the corn ethanol industry that is dried on-site and is currently valued for its use as animal feed. Corn DDGS hydrolysate modeled cost ranked well ("good") on a cost per nitrogen basis at ~\$32 per kg-nitrogen (\$2 per kg hydrolysate). However, it has a higher fat content (~10%) that could impact the hydrolysis. A defatting step to remove excess oil content was not cost-modeled. Corn DDGS is a very high-volume, readily available sidestream that offers a long shelf life after drying, though its storage and handling would need to be foodgrade for application. It also ranked well overall with low-moderate environmental impacts of corn crop production relative to the other crops.



#### Corn gluten meal (CGM)

#6 economic; #3 integrated

CGM ranked high when examining economic factors due to the low modeled production costs and moderate sidestream volumes. The low forecasted volume of the corn gluten sidestream slightly lowered its ranking versus other highvolume sidestreams like soy meal and corn DDGS. The CGM hydrolysate modeled production cost was ~\$30 per kg-nitrogen content and \$3 per kg-hydrolysate. While corn crop volumes are very high, CGM is <1% of the crop production volume, but it has consistent production from corn wet-milling.

Both corn DDGS- and CGM-based hydrolysates are not readily utilized as peptone sources despite having a low modeled cost per nitrogen content. Additionally, the high starch content of both, upwards of 18%, makes them more desirable for use in microbial fermentation since the starch supplies carbon. Microbial evaluations of corn gluten hydrolysates have yielded favorable results <sup>50</sup>. In fact, CGM has been previously explored and patented by Cargill to supplement fungal fermentations <sup>51</sup>. For cultivated meat media applications, the corn DDGS amino acid profile is limited in glutamine, lysine, and tryptophan, while CGM is limited in glutamine and lysine (Figure 16). Additionally, some literature indicates that CGM peptide hydrolysates can negatively affect cell

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culture if not hydrolyzed sufficiently <sup>52</sup>. Elsewhere in food applications, CGM hydrolysate production has also been patented by Sempio Foods <sup>53</sup> and Cargill <sup>54</sup>. These patents are mostly intended for direct food applications. In fact, Cargill has a pending <u>GRAS application</u> for a CGM protein hydrolysate for commercial food applications.



#### Canola Meal

#### #4 economic; #8 integrated

Canola meal ranked well economically due to its very high sidestream volume and low hydrolysate cost of ~\$34 per kg-nitrogen (<\$3/kg hydrolysate). Canola meal has a very comparable composition profile to soy meal, which lends itself well for use as a hydrolysate for microbial fermentation. However, unlike soy meal, canola meal has a high lipid content from residual oil not extracted, which could impact the hydrolysis and end product. A defatting step to remove excess oil content was not cost-modeled, which could affect cost and efficiency. Its use in microbial fermentation has been demonstrated with positive results <sup>50,55</sup>. Canola meal's essential amino acid profile closely resembles soy protein and scored well for amino acid coverage (Figure 16). This amino acid composition could suit its use in cultivated meat cell culture applications, and it has been previously assessed with success 56.



#### **Brewer's Spent Grain**

#5 economic; #7 integrated

Brewer's spent grain (BSG) is a high moisture content processing residue with great potential for protein upcycling to a protein hydrolysate. BSG ranked as the top candidate for economic volume and unit cost. The modeled unit cost of protein hydrolysate was excellent and was based on a previous BSG hydrolysate techno-economic analysis <sup>47</sup>. The essential amino acid score for BSG was moderate and is mostly limited by glutamine and lysine (Figure 13, Figure 16). BSG has been evaluated for use in submerged and solid-substrate microbial fermentation processes where the material is directly processed by the microorganism without further purification due to high protein and cellulose content <sup>57,58</sup>. While BSG could be used directly in fermentation, the low shelf life due to moisture content warrants upcycling to a shelf-stable, dried hydrolysate ingredient that could be used for fermentation or food applications. Finally, BSG hydrolysates have been studied and commercialized (i.e., EverGrain) for food ingredient use, with many researchers evaluating improvements to the process 47,59,60.

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#### **Tomato Pomace**

#8 economic; #5 integrated

Tomato pomace is another high-moisture, high-protein sidestream. However, it did not rank well economically for protein hydrolysates due to its low volume and moderate production cost model (>\$40 per kg-nitrogen), which was due to lower protein content in the initial material and the final hydrolysate. The low environmental impact of tomato production boosted its integrated rank. The cost model employed here did not account for pretreatment drying, defatting, or seed separation from the peel since the seeds contain most of the protein. Thus, the costs could be higher than those modeled here. The amino acid profile of tomato pomace is rich in arginine and tyrosine, but limited in glutamine, lysine, and tryptophan, which could impact its application in cell culture media (Figure 16). Tomato pomace valorization might be better suited for direct solidstate fermentation due to the protein content, sugars, cellulose, and hemicellulose in the mixed peels and seed content. It would provide all key

nutrients in a single fermentation step from a current food sidestream and contains beneficial components like lycopene and beta-carotene <sup>61</sup>.

#### Conclusion

Overall, protein-rich sidestreams from commercial processes can be upcycled into protein hydrolysates for fermentation and cultivated meat media applications. While not a focus here, these protein hydrolysates can also be used directly as food ingredients. There are many plant-based peptones on the market for cell cultivation and food applications. Many major media component and food ingredient suppliers have the potential to valorize these sidestreams due to the technological maturity, infrastructure, and expertise in this field. There also needs to be a demand for these organic nitrogen sources, which can be difficult when inorganic ammonium sources can be obtained at lower prices despite their environmental impact. Nitrogen is a key media component of microbial fermentation, but there is a greater opportunity to reduce the environmental impact of fermentation processes through the use of lignocellulosic sugar carbon sources-which we evaluate next.

## Lignocellulosic sugars

#### Definition

Currently, most fermentation processes use first-generation carbon sources, such as sucrose derived from sugar crops and glucose from starch crops, for aerobic heterotrophic fermentation <sup>62</sup>. However, using these sources creates competition with other food sources and can raise sustainability concerns for the bioeconomy. An attractive alternative to sugar- and starch-based crops is the valorization of lignocellulosic biomass (LCB) for the extraction of fermentable sugars due to the cheap raw material and improved sustainability <sup>10</sup>. These sugars are extracted through a pretreatment step to break down lignin, followed by enzymatic hydrolysis of cellulose and hemicellulose. This process, known as saccharification, yields a mixture of fermentable hexose sugars (e.g., glucose) and pentose sugars (e.g., xylose) 62,63.

#### **Objective and rationale**

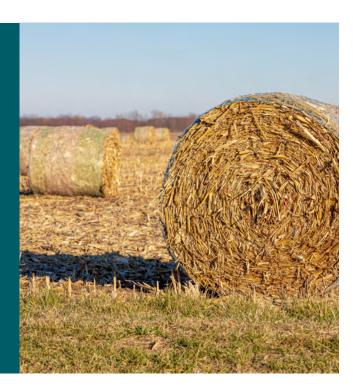
This analysis sought to identify lignocellulosic waste streams from high-volume agricultural crops that could provide cost-competitive fermentable sugars for use in fermentation-derived food ingredient production and an opportunity to upcycle crop sidestreams. This was accomplished by assessing the crop composition and developing a simple cost model for lignocellulosic sugar extraction to identify the most promising targets for further development.

#### Scoped sidestreams

Lignocellulosic biomass can come in various forms, including herbaceous crops, wood debris, and nonedible residues of food crops. We identified 13 sidestreams with high lignocellulosic content that could be valorized for lignocellulosic sugar extraction (Figure 10, Figure 11): barley straw, barley husks, canola straw, canola chaff, canola hulls, corn stover, rice straw, rice hulls, soy straw, soy hulls, sugarcane trash, sugarcane bagasse, and wheat straw. These sidestreams typically have a cellulose content of >35%, a hemicellulose content of >10%, and a lignin content of <25% (Figure 10).

#### Top sidestream candidates for lignocellulosic sugars

The sidestream volume forecasting and unit sugar cost modeling determined that corn stover, soy straw, and rice hulls were the top sidestreams with the most economical estimated conversion of cellulose-hemicellulose into fermentable sugars. Barley straw, barley husks, and sugarcane trash/ bagasse also ranked highly for integrated economic and environmental factors (Figure 11, Figure 14).



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The modeled total fermentable sugar costs for these top candidates ranged from \$320 to \$550 per metric ton of sugar, while the modeled glucoseonly sugar extraction costs ranged from ~\$550 to \$880 per metric ton of glucose (Figure 14). For reference, the commercial dextrose (corn glucose) market price has averaged \$840/metric ton over 10 years and \$1147/metric ton in 2022 alone (<u>USDA ERS Sugar</u>). Many commercial fermentation processes utilize lower-grade DE95 dextrose syrup. This liquid dextrose source has about 70% solids content with a ~95% dextrose equivalent content and had a bulk price in 2020 of ~\$560 per metric ton glucose (\$400/metric ton DE95 syrup)<sup>64</sup>. We considered these pricing benchmarks when ranking our sidestreams since there needs to be a strong economic and sustainability case to move to lignocellulosic sugar sources.

	Sidestream volume	Unit cost (sugars)	Unit cost (glucose)	Sugar quality	Env. impact	Economic rank	Integrated rank
Corn stover		0		0		1	1
Soy straw		•	•	•	$\bigcirc$	2	2
Rice hulls				$\bigcirc$		3	10
Sugarcane trash	•	$\bigcirc$		$\bigcirc$		4	4
Barley straw	•	$\bigcirc$	•		$\bigcirc$	5	12
Sugarcane bagasse	•		$\bigcirc$	•		6	3
Rice straw	•	0			•	7	13
Wheat straw		•	•		•	8	8
Canola chaff	0	•	•			9	9
Canola straw	•	•	•	$\bigcirc$	•	10	7
Barley husks	$\bigcirc$	•		$\bigcirc$		11	5
Canola hulls	0	$\bigcirc$		$\bigcirc$		12	11
Soy hulls	0				$\bigcirc$	13	6

	Legend	Volume, 2030 MMT/yr	Unit cost sugar USD/tonne	Unit cost glucose USD/tonne	Sugar quality cellulose/ carbohydrates	Environmental impact pressure/tonne crop		
	Excellent	>200	<\$350	<\$600	>0.8	<0.0001		
	Good	50-200	\$350-\$450	\$600-\$700	0.6-0.8	0.0001-0.0003		
•	Okay	5-50	\$450-\$600	\$700-\$800	0.5-0.6	0.0003-0.0005		
0	Low	1-5	\$600-\$800	\$800-\$1,000	0.4-0.5	0.0005-0.0008		
•	Poor	<1	>\$800	>\$1,000	<0.4	>0.0008		

Figure 14. Lignocellulosic sugar extraction ranking results summary with volume, modeled unit cost, sugar quality, and environmental impact data.<sup>(k)</sup>

<sup>(k)</sup> Modeled production unit cost glucose was not used in ranking and is provided for information only.

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#### Corn stover

#1 economic; #1 integrated

Over 300 million metric tons of corn stover are produced from corn each year, which is an astounding amount of carbon that could be valorized for microbial fermentation. Corn stover ranks well mainly due to its production volume, but does not model well for the unit price of glucose (>\$875/metric ton) or total sugars (>\$550/ metric ton). Corn stover has been extensively studied as a source of fermentable sugars, so the pretreatment and enzymatic extraction are robustly understood <sup>9,63</sup>. Corn stover has been developed for use as a feedstock for cellulosic ethanol production with a few commercial facilities in the United States. Thus, the supply chain, infrastructure, and technology are commercially mature. However, the production processes may not be directly transferable. Food-grade fermentation processes would likely require a more purified sugar stream than the simultaneous saccharification and fermentation process currently used in cellulosic ethanol production facilities.



#### Soy straw

#2 economic; #2 integrated

Soy straw ranked second for economics for its large volume sidestream availability. As with the economics of plant-based proteins, the large production volume for corn and soy crops leads to abundant sidestreams from these agricultural products. These crops have over 70% cellulose/ hemicellulose dry weight content, costing only \$55–65 per metric ton of feedstock. However, the modeled unit costs were high for both total sugar (~\$500/metric ton) and cost of glucose (>\$800/ metric ton). Soy straw has a lower hemicellulose content than corn stover, which would result in a lower total fermentable sugar extraction. Its moderate environmental impact kept soy straw among the top candidates for integrated economic and environmental rank. Soybean straw has also been extensively studied for lignocellulosic saccharification.



**Rice hulls** 

#3 economic; #10 integrated

Despite having a low sidestream volume, rice hulls rank extremely well economically for their low modeled cost of total fermentable sugar (<\$350/metric ton) and glucose only (<\$600/ metric ton). In fact, rice hulls had the lowest modeled unit costs of extracted sugar and glucose due to the high cellulose and hemicellulose content, as well as low feedstock cost (Figure 10). Rice hulls have a higher cellulose ratio, which would result in a predominantly glucose-rich sugar feedstock. However, rice hulls contain a high percentage of inorganic material in the form of silica. This can impact the saccharification process and require removal before processing, which could increase the pretreatment costs <sup>65</sup>. Rice straw did not model as well as rice hulls, but it does have a higher volume. It was extensively evaluated from a techno-economic and sustainability perspective for mycoprotein production <sup>66</sup>. Additionally, rice straw is one of the most commonly used solid substrates for mushroom farming, providing another revenue stream and alternative protein production opportunity 67. Valorizing these rice sidestreams would reduce the overall environmental impact of rice production through coproduct generation.

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#### Sugarcane Trash/ Bagasse

#4/4 economic; #6/3 integrated

Outside of the top three, sugarcane trash and bagasse ranked high economically for their high sidestream volume and low modeled sugar cost (\$350–450 per metric ton). They also model well for glucose-only production costs (~\$650–700 per metric ton). Sugarcane trash and bagasse also ranked highly with integrated ranking due to the low environmental impact. Both sugarcane trash and bagasse have been extensively evaluated for use as a lignocellulosic sugar source. Thus, they are technologically mature crop sidestreams for possible lignocellulosic valorization that may otherwise be incinerated or open-burned in the field.



#### Barley Straw/Husks

#5/12 economic; #11/5 integrated

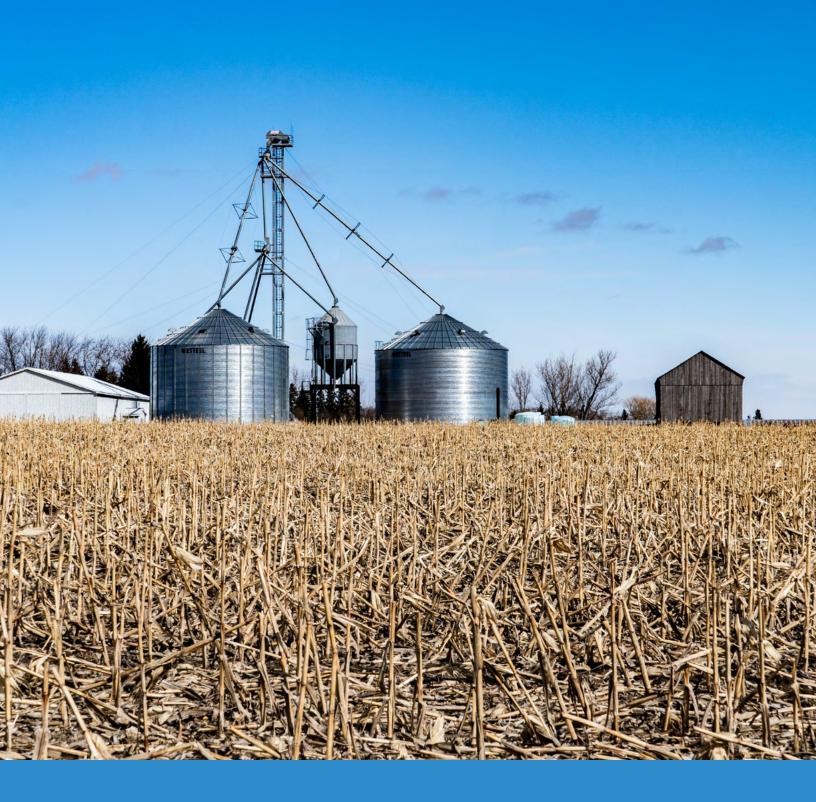
Barley straw ranks as a top lignocellulosic sidestream for economic ranking due to the low modeled total sugar unit cost (<\$400 per metric ton), but contains equivalent cellulose and hemicellulose content (Figure 14, Figure 10). Barley straw's lower production volumes impacted its economic ranking. For integrated ranking, it did not rank well due to its environmental impact and lower cellulose:hemicellulose ratio. Barley straw cost per total sugar content could be quite competitive, but the modeled glucose-only sugar cost would likely cost more than commonly used DE95. Finally, barley husks have fairly low volume and moderate sugar costs but ranked well in integrated due to the high cellulose content to total cellulose and hemicellulose that enables a "good" cost per ton glucose (Figure 14).

Cereal straws, like barley and wheat, have been extensively studied for lignocellulosic saccharification for commercialized bioethanol and other fermentation-derived products 68. Wheat straw did not rank well here, but it is a high-volume sidestream that has potential applications in alternative proteins. Mycoprotein production of the fungi Neurospora intermedia has been demonstrated using a saccharified wheat straw at a pilot scale with very high enzymatic efficiency (86%) <sup>69</sup>. Additionally, the economic feasibility of mycoprotein and precision fermentation production using saccharified wheat straw has been confirmed via modeling <sup>70</sup>. There is a clear opportunity for the commercial valorization of straws for fermentation-derived alternative protein ingredients.

#### Conclusion

Overall, lignocellulosic sugars offer an opportunity to reduce environmental impacts from unused sidestreams and provide a more sustainable carbon source for fermentation processes. These sugars could become cost-competitive through optimized extraction processes and incentives for their use over food crops. Additionally, the mixed sugar composition of these feedstocks should be utilized to its fullest potential by the microorganism. Access to and efficient use of lignocellulosic sugars could also be unlocked through novel strain selection and strain development that leads to efficient, simultaneous use of all reducing sugars (glucose and xylose) present in these feedstocks.

From field to facility



# 5 From field to facility: Geographic factors and storage considerations

# From field to facility: Geographic factors and storage considerations

The geographic location of sidestream production is a key factor in the economic and technical feasibility of sidestream valorization. Since these sidestreams are not yet high-value commodities, the cost of fuel, cold storage, distribution, and supply chain management are significant in the overall economic feasibility of using sidestreams as inputs, but the extent will vary by manufacturer location. Like other food materials, sidestreams have a limited shelf life depending on their storage conditions but most food manufacturing processes are not designed to make sidestreams suitable for human food applications. Food and agricultural processors rarely stabilize these byproducts due to the higher processing costs. Landfill disposal, incinerating, composting, or redirecting sidestreams to animal feed are usually more economical and less logistically complex.

We collected the largest regions of crop production and the major processing hubs linked to the analyzed sidestreams to provide a sense of geographic distribution (Figure 15). This analysis underscores the significance of geographical position, highlighting that the most efficient sidestream valorization would ideally correspond with specific regions in North America. This approach capitalizes on lowered costs associated with transportation, storage, and distribution. Manufacturers should consider constructing processing centers in close proximity to regions with high sidestream volumes or apply on-site technologies to pre-existing processing facilities to reduce the costs associated with transportation and logistics. The strategic commercialization of sidestream valorization processes in these rural agricultural regions could also foster economic development.

Many considerations and challenges are associated with storing and transporting each sidestream of North America's major crop production and processing regions. For instance, the production of brewer's spent grain (BSG) is widespread across brewery locations, yet its elevated moisture content mandates immediate drying or colocated processing facilities to ensure a consistent, stable supply of BSG. Furthermore, the output volumes would be influenced by brewery size.

Conversely, a substantial 95% of all tomatoes produced in the United States are grown in California, creating a centralized source of a highvolume sidestream. Thus, tomato pomace valorization could be colocated in California to minimize extraneous transportation costs <sup>71</sup> (Figure 15).

Other distinct crop concentrations, such as rice, sugarcane, canola, and barley, also shape regional sidestream prospects. Rice production occurs primarily along the Mississippi River delta and Texas, whereas sugarcane production is prevalent along the Gulf of Mexico, mostly in Florida, U.S.A., and Veracruz, Mexico. Similarly, canola production and canola crush facilities are highly clustered in southern Alberta and Saskatchewan, Canada, and in northern North Dakota, U.S.A. The barley production, malting, and milling sectors also converge in these regions, which coincides with barley husk availability (Figure 15). Consequently, respective sidestream processing centers would be best colocated in these regions, which exhibit lower production volumes than other crops (Figure 15).

Finally, there is a vast concentration of corn and soy production, complemented by extensive processing facilities and transportation hubs, characterizing the Midwest agricultural belt of the United States (Figure 15). There is a distinct opportunity for the further valorization of corn and soy sidestreams in this region to capitalize on the robust production, supply chain, processing facilities, and skilled workforce unique to this area.

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From field to facility

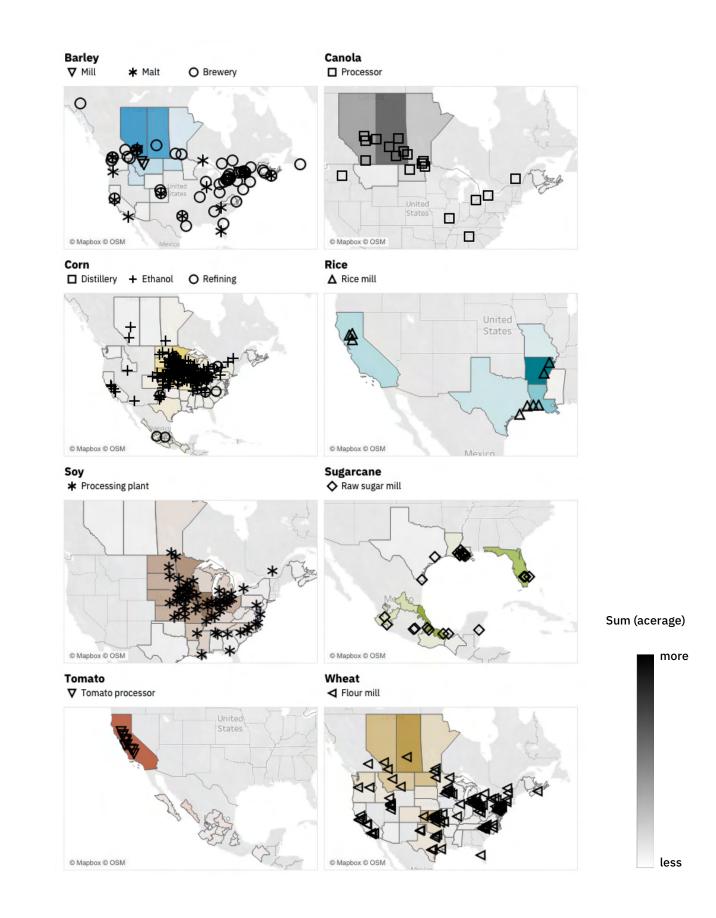


Figure 15. Regional triangulation maps for high-volume crops and major processing facilities. Color density represents harvested acreage for each crop. Processing facilities are the major facilities identified and are not comprehensive. For example, BSG facilities are major brewery sites.

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However, each sidestream type introduces a distinct set of challenges concerning moisture content, potential microbiological contamination, heavy metals, and pesticide residues of sidestreams. These concerns differentially impact food safety, material stability, and downstream applications. For instance, production regions with higher humidity are more likely to generate sidestreams contaminated with <u>mold mycotoxins</u>, which could limit their use in food applications. Longer storage and transportation duration increases the risk that sidestreams will arrive at their processing facilities unsuitable for conversion to desirable products.

Sidestream materials that contain water or oil are more challenging to process, store, and transport. BSG contains between 70 and 80% <u>moisture</u> <u>content</u> and requires substantial drying before storage and distribution to prevent spoilage. BSG producers could implement drying processes that the corn ethanol industry has already implemented to increase the storage shelf life of corn DDGS. Tomato pomace suffers from similar challenges as BSG due to its high water content. Likewise, sugarcane bagasse, the fibrous pulp left behind from processing sugarcane, contains at least 50% moisture content after processing and is prone to fungal contamination.

While meal and gluten sidestreams contain substantially less moisture than other sidestreams, they necessitate drying to ensure extended shelf life during transportation and storage. Rice and wheat bran contain substantial <u>oil and residual enzyme activity</u>, which can lead to rancidity issues. Therefore, heat treatment immediately after milling and before storage is vital to stabilize these sidestreams. Storage and transportation at cooler temperatures are recommended to prevent degradation of these bran sidestreams over time.

In contrast, non-edible agricultural residues like straw, stover, and sugarcane trash are relatively stable once dried. These <u>lignocellulosic materials</u> are dried to less than 25% moisture, baled, and transported via trucking. If stored in a dry, cool facility, these sidestreams are less susceptible to spoilage from mold or pests. Nonetheless, conversion economics often tread on thin margins, where excessive transportation can affect feedstock prices. The dispersed nature of barley production in the United States exemplifies this challenge, making it difficult to source substantial volumes of agricultural residues like barley straw.

Each sidestream poses a unique challenge to the issues involving storage and transportation. For sidestreams that can quickly spoil from rancidity or suffer contamination by microbial growth, food manufacturers will need to strongly consider the physical proximity of their processing facilities to sidestream producers and the distribution management of getting raw materials to these facilities. Additionally, manufacturers will need to account for the source of the sidestream and if any upstream processing is required to ensure the material will be fit for human consumption or use in fermentation processes.

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# 6 Recommendations and conclusions

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# **Recommendations and conclusions**

# Recommendations for stakeholders interested in using sidestreams for alternative protein inputs

While this analysis highlights several sidestreams with usefulness as alternative protein production inputs based on a preliminary evaluation of the economic, environmental, and functional factors, additional research must be conducted to fully assess the technological readiness levels of these ingredients, especially their functionality and commercial processing feasibility. Investing in these research areas will foster advancements in sidestream valorization.

# Recommendations for researchers and manufacturers

- 1. Fully assess the applicability of each sidestream-derived ingredient for end-product or production use: Researchers should identify optimized use cases for sidestreams by characterizing their relevant functional attributes-beyond those explored in this overview. Protein concentrates will have varying properties depending on the source, like emulsification, gelling, taste, and odor, that can affect end-product quality. Similarly, protein hydrolysates for fermentation and cultivated meat media need to be assessed for their application and suitability in those processes due to limiting amino acids (Figure 16) or properties that may alter cell productivity. These studies will allow farmers and manufacturers to better understand how they can valorize crops into the highest-value products and improve the cost and functionality of alternative protein products.
- 2. Fully assess the technological readiness levels of sidestreams as an alternative protein input: For each end product or production use, the commercial processing feasibility of sidestreams as alternative protein inputs must be evaluated. Technology readiness levels (TRL) demonstrate the maturity of technologies, ranging from TRL 1 to TRL 9 where 9 is indicative of competitive manufacturing. Strong public-private partnerships can drive innovation that enhances a technology's maturity. Typically, universities have better resources to explore the basic principles of a technology and validate proofs-of-concept (TRL 1 to 4), while industry researchers and manufacturers can focus on upcycling sidestreams into applications with high TRLs, expediting alternative protein commercialization efforts.
- 3. Optimize the processing conditions of sidestreams for maximized recovery of high-value ingredients: As many of these sidestreams do not have mature value chains, their use as alternative protein inputs has yet to be optimized, leading to high costs and low recoveries. Improving pretreatment processes, enzyme costs, and enzymatic efficiencies will help. Thus, economically sourcing enzymes, such as cellulases, and finding or engineering enzymes with superior activities, such as proteases, would improve ingredient techno-economics. Another need is improving protein extraction efficiencies and costs for many sidestreams. For instance, corn protein extraction is a costly process due to solvent-based extraction 72, which could be mitigated by ethanol recycling or alternative extraction methods or materials.

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- 4. Improve drying or stabilization of sidestreams: Drying processes, typically involving rapid heating or spray drying, can be expensive, require large equipment with high energy use, and impact the flavor, texture, and nutritional attributes of sidestream products. For high-moisture sidestreams like BSG and tomato pomace, more efficient drying or stabilization techniques are crucial to prevent spoilage and optimize downstream processing. Drying affects ingredient functionality, nutrition, quality, and food safety, so alternatives should be evaluated to ensure they provide high-value and safe end products.
- 5. Collaborate across the value chain to upcycle ingredients and improve transportation logistics: Through better collaborations between crop growers, ingredient producers, and end-product manufacturers, more high-value ingredients can be extracted for the alternative protein industry. Moreover, collaborations that ensure on-site drying and short transportation distances are crucial, especially for high-moisture sidestreams like BSG and tomato pomace, which are susceptible to contamination and degradation. Much of the success of corn DDGS as an animal feed results from its drying and storage by ethanol producers.

#### 6. Evaluate and prioritize food safety:

Challenges to food safety, especially from agricultural sidestreams or offtakes, could take the shape of microbial or biochemical toxins, such as furfural or aflatoxin contamination <sup>73,74</sup> that can arise during storage and transportation, especially from high-moisture sidestreams. Treatment or prevention strategies, such as monitoring programs and on-site drying, can help ensure food-safe alternative protein concentrates and feedstocks for fermentation or cultivated meat production.

- 7. Collect data to perform life cycle assessments and techno-economic analysis to quantify how valorization affects end product impact and cost: Comprehensive life cycle analyses (LCA) are necessary to evaluate the environmental and social impacts of utilizing agricultural sidestreams in alternative protein production and other bio-industrial processes. Research should quantify the potential environmental benefits, such as reduced GHG emissions, land use impacts, and water use, associated with sidestream valorization. Economic feasibility studies should also assess the cost-effectiveness and commercial viability of incorporating sidestream-derived ingredients in alternative protein products.
- 8. Examine the valorization of sidestreams from other agricultural crop systems or from alternative protein production systems: Other crops and their agricultural and processing residues in North America and other

processing residues in North America and other regions should be explored for their application as alternative protein inputs, as there are many region-specific crop sidestreams. Moreover, sidestreams from alternative protein production processes can be explored as inputs for other alternative proteins or for other industries. For example, in cultivated meat production, an ex ante LCA of commercialscale cultivated meat production suggests lactate, ammonia, and alanine waste streams could be valorized through other processes <sup>75</sup>. Specifically, they could be recycled for other fermentation purposes and are already being considered and evaluated for treatment valorization. Additionally, developing high-value applications for pulse starches, formed as a sidestream of pulse protein production, has been identified by Pulse Canada as a priority for the pulse industry. The valorization of alternative protein sidestreams would further improve the environmental benefits and potentially improve the economics of some alternative protein production processes.

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#### **Recommendations for policymakers**

- Provide biomanufacturers with financial incentives to develop and scale up upcycling and circular operations: Establishing supportive policy and regulatory frameworks is essential to incentivize and facilitate the utilization of agricultural sidestreams. Research should be conducted to evaluate existing regulations, identify barriers, and advocate policies that promote sustainable food systems, encourage research and innovation in sidestream valorization, and provide incentives for industry adoption of these practices.
- 2. Incentivize public-private and crop value chain collaborations to facilitate crop valorization, improve value chain efficiencies, and ensure feedstock availability: Academic and industry research collaborations are especially relevant in this context. Many upcycled alternative protein inputs still need robust functionality characterization and optimized processing conditions, ideal for academic research projects. At the same time, industry researchers can direct their focus on scaling processes and collaborating across crop value chains. Shortening transportation distances of sidestreams for high-value processing ensures better domestic economic contributions of the value chains while improving domestic crop utilization efficiency.
- 3. Provide regulatory guidance for safety evaluations of upcycled ingredients: For

upcycled ingredients that do not have a robust history of human consumption, it will be necessary to evaluate their allergenicity, toxicity, and other relevant safety metrics. Governments should help producers valorize sidestreams by providing guidelines for evaluating and communicating the safety of their upcycled food ingredients. 4. Prioritize investments in R&D and biomanufacturing infrastructure that will enable sidestream valorization: Many areas for development were outlined in the <u>U.S. Bold</u> <u>Goals</u> report for biomanufacturing that align with improving sidestream valorization (<u>Appendix 1</u>). Achieving a more circular economy will require investment in key R&D and infrastructure.

### Conclusions

The valorization of agricultural sidestreams presents an opportunity to move toward a more sustainable, circular bioeconomy by utilizing agricultural waste products as resources to feed the growing human population and reduce the environmental impacts of the food system. Many sidestreams retain a significant portion of macronutrients that could be made available for human dietary purposes as inputs to produce alternative proteins, such as plant-based, fermentation-derived, or cultivated meat, seafood, and dairy products. The food and agricultural industry has demonstrated that agricultural sidestreams can be widely utilized through the success of soy meal as a sidestream of soybean oil production. However, many economical sidestreams remain untapped, and it would benefit crop growers, alternative protein manufacturers, and the environment to use these valuable ingredients in production.

In this analysis, we evaluated the North American agricultural sidestreams with the highest potential to be converted into inputs for alternative proteins by 2030. We focused on three potential use cases: (1) protein concentrates for plant-based meats, (2) protein hydrolysates, and (3) lignocellulosic sugars for cell culture media used in fermentation and cultivated meat production. We identified that upcycling soy meal, canola meal, wheat bran, wheat gluten, tomato pomace, corn DDGS, corn gluten meal, and brewer's spent grain

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into protein concentrates had the highest potential for economic return, the lowest overall impact on the environment from a production standpoint, and the most suitable protein digestibility. For protein hydrolysates in fermentation or cell cultivation, the highest-ranked sidestreams included soy meal, corn DDGS, canola meal, brewer's spent grain, and corn gluten meal. Finally, for the production of fermentable lignocellulosic sugars, we found that corn stover, soy straw, sugarcane trash/bagasse, and barley straw/husks were the top-ranked sidestreams from economic, environmental, and fermentable sugar quality perspectives.

While many technologies already exist to transform these sidestreams into suitable inputs, R&D is still needed to reduce the cost of processing steps used in these conversions and improve the margins. We identified economic and technical opportunities to replicate the success of soy meal with other top sidestreams, though individual considerations might lead manufacturers to consider other candidates.

This analysis focused on high-potential sidestreams in North America but a protein transition will require global efforts. Sidestream coproduct analyses should be developed for lower-volume agricultural sidestreams and from other regions outside of the United States, Mexico, and Canada to identify opportunities for high-value ingredient valorization. Each sidestream has unique storage and transportation requirements. Thus, a deeper understanding of regional sidestreams and their processing requirements will help accelerate a circular bioeconomy and alternative proteins as a global solution. Successfully valorizing sidestreams across the agricultural sector is increasingly recognized as an urgent need. For example, this approach is outlined as one of many approaches to achieving the Sustainable Development Goals in the United Nations 2030 Agenda for Sustainable Development. In addition, in March 2023, the Bold Goals for U.S. Biotechnology and Biomanufacturing report released by the White House Office of Science and Technology Policy outlined the need to demonstrate viable pathways for circular food protein production and reduce biomass, GHG emissions, and food waste from our food systems within the next five years. Another pathway identified in the Bold Goals report is to improve feedstock availability, such as lignocellulosic sugars, for the growing bioeconomy. See Appendix 1 for Bold Goals aligned with the need for improving sidestream valorization.

Indeed, converting agricultural sidestreams into edible human food or ingredients for feedstock for alternative protein production is one of the most efficient ways to return these resources back to the food supply chain, improve the sustainability of our food systems, and narrow the loop on the circular economy. Given the outsized impact that food and agriculture production has on the environment, whether through the direct emission of greenhouse gases, ongoing conversion of rainforests into farmland, or the intensive use of limited resources such as water and fertilizer, how we generate, process, and dispose of food remains an economic and environmental priority if we are to navigate the coming decades successfully.

From field to facility

# Methods

# **Cost production analysis**

#### Volume forecast and compositions

The goal of the volume forecasting was to estimate the volume of crop and sidestream production in 2030 so that we could provide direction to stakeholders on which sidestream to pursue for investment into infrastructure and research. Crop production values from 1961 to 2021 were pulled from national agricultural agencies and research data.

The CAGR was calculated from previous data and used to extrapolate future production volumes by assuming constant annual growth from 2021 onward over 10 years, compounding yearly until 2030 <sup>76</sup>. Sidestream production volumes were then calculated from these 2030 crop production values by calculating the fraction of sidestreams generated in 2021 per unit of crop. Sidestream production volumes were then calculated from these 2030 crop production values by calculating the fraction of sidestreams generated in 2021 per unit of crop, and assuming this fraction remained constant through 2030. Composition data was pulled from the literature for each sidestream.

### Unit cost model: Plant-based protein

We estimated the unit cost to produce one kilogram of protein concentrate for plant-based meats. The unit cost per metric ton of each sidestream was extracted from existing databases and agricultural commodities markets. The percent protein content of each sidestream derived from literature values was used to calculate the amount of potential protein available for extraction from the bulk material. The cost of processing the sidestream into protein concentrate was calculated based on the material inputs. We added 14% of the cost of inputs for utilities, 12% for labor, and 3% for supplementary costs, averaged from foot plant operating costs based on the literature. <sup>77</sup>.

Processing methods used to extract plant-based protein concentrates for each sidestream were identified from the literature and used as a reference for the amount of reagents required to convert one metric ton of sidestream material into protein. The market cost of bulk reagents was used to determine their cost. Alternatively, if only benchtop methods were available, the cost of reagents used in processing the sidestream input was extrapolated and scaled up to one metric ton of sidestream. The yields from these protocols were used to calculate the amount of protein concentrate produced per metric ton of sidestream, and, further, to determine the total amount of sidestream required to produce one metric ton of protein concentrate. Sidestream unit cost, processing reagent costs, and utilities, labor, and supplementary costs were summed to estimate the total cost of producing one metric ton of protein concentrate from each sidestream.

Many of these methods are not yet optimized for producing protein concentrates for human consumption and commercialization, meaning this analysis is likely unrepresentative of the economic costs of protein concentrate production, but they provided an initial scope of how protein ingredients are currently made from these sidestreams. In this study, we modeled protein concentrate extractions as follows: corn sidestreams were extracted with ethanol and acid/base aqueous methods <sup>72,78</sup>; soybean meal, canola meal, and wheat germ were extracted via a traditional base/acid solubilization and precipitation method <sup>79,80</sup>; and wheat bran, wheat gluten, tomato pomace, barley brewer's spent grain, and rice bran sidestreams were extracted using enzymatic methods 47,81,82.

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Protein extraction yields were based on literature values or were assumed to have a 75% yield if not specified, except for BSG protein concentrate, which had a lower final protein content in the literature <sup>47</sup>. In reality, protein extraction yield is a nuanced variable, dependent on protein type and extraction process. In some cases, protein extraction yield can be much higher than 75% (soy protein isolate yield from wet extraction processes is typically higher than 90%) or lower than 75% (for less developed protein types and extraction processes). Higher protein concentrations also command higher market prices and can change the functionality of the ingredient (Food and Agriculture Organization). After consulting industry experts, a 75% yield was chosen as a good benchmark for a protein concentrate extraction to be commercializable.

In this study, the cost of utilities, labor, and other expenditures for the scaled production of plantbased protein was simplified by averaging values associated with various types of manufacturing plants in the food industry. Twenty types of foodbased production facilities, including corn starch production, dairy processing, and yeast production plants, were used as data sources. These costs were linearly scaled to the material costs associated with the production of proteins from each sidestream to reduce the mathematical complexity associated with techno-economic calculations to evaluate the cost of each unit operation of a production system for multiple hypothetical scales. In reality, these costs do not scale linearly and are partially a function of the plant's size and the operation units used in each processing approach. Additional costs not reflected here are the use of new technologies and patent royalty rates that would be essential for pioneering the production of new protein sources.

#### Unit cost model: Protein hydrolysates

Controlled enzymatic digestion is the key to the production of protein hydrolysate. Enzymatic digestion affects the degree of hydrolysis, which affects the free amino acids, peptide size, amino acid composition, and, thus the product quality. An enzymatic hydrolysis process resulting in free amino acids was modeled for each sidestream in this study. The production costs are most influenced by raw material cost, enzyme cost, and dry matter loading. Additionally, the degree of extraction, separation, cooling, and drying affect the product quality, including color appearance due to Maillard reactions.

In this analysis, the modeled protein hydrolysis processing cost and minimum selling price for each sidestream were based on techno-economic assumptions from He et al. 2021, which evaluated brewer's spent grain hydrolysate production of 16 kilotonnes annually. Specifically, the simple cost model employed in this analysis assumed a 9% dry-weight solids loading, 1% enzyme loading, and other processing costs estimated by He et al. 2021. The labor, utilities, overhead, and capital costs from their analysis were used in our hydrolysis processing model. The processing cost to hydrolyze each biomass type was scaled and adapted from this paper by applying a conversion factor for the solids-loading concentration to modify water and sodium hydroxide costs. The actual feedstock loading quantities and costs were adjusted by material dry weight and unit cost of the material to account for differences between BSG and other feedstocks. The enzyme costs were scaled from the original model, which had an enzyme load of ~0.5%, to be adjustable in the calculation. Finally, to estimate the protein concentration, a protein-volume concentration factor was calculated by subtracting out the insolubles (lignin, starch, cellulose, hemicellulose), adjusting for the soluble sugars that will come through to a final product, and then applying an approximate concentration factor seen in the reference paper for a protein conversion.

Unit modeled costs per kg material were then adjusted to a cost per kg-nitrogen equivalent to compare each hydrolysate based on their respective nitrogen content. Due to differences in

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protein content, the final nitrogen content varied in the final products. The nitrogen content was estimated by the amino acid profiles of each raw feedstock material before processing (Figure 16). While the degree of hydrolysis and recovered protein will vary in a commercial process, this provided a baseline estimate of nitrogen content and amino acid profile.

For cost comparisons, we assumed a 9% solids and 1% enzyme use, but recognize that this will be dependent on each sidestream. The production cost of any plant peptone hydrolysate is most influenced by raw material cost, enzyme cost, and dry matter loading. Thus, research would be needed to assess the maximum solids in the hydrolysis process. High-protein sidestreams with lower lignocellulosic content will have more efficient enzymatic hydrolysis, which could enable a higher loading concentration. Many plant-based hydrolysates, such as soy peptone, can be produced at higher solids loading rates of 15–30% due to their low lignocellulose content. BSG, however, has a high lignocellulose content, which requires lower solids loading. Thus, the lower solids loading rate used here provided a more conservative estimate as each material needs to be optimized.

### **Unit Cost Model: Lignocellulosics**

This analysis built a simple cost model based on previous techno-economic assumptions to assess the cost of producing one metric ton of glucose (a hexose sugar) or one metric ton of total reducing sugars, which include hexose and pentose sugars <sup>9,63,70</sup>. The main input variables included the cellulose/hemicellulose content of the feedstock, the feedstock price, cellulase enzyme loading, cellulase price, enzyme efficiency, the cost of food-grade ionic solvent, and the cost of electrical utilities. We determined the estimated cost of producing fermentable sugars from lignocellulosic sidestreams by first determining the amount of cellulose and hemicellulose available per metric ton of input material based on each of their chemical compositions.

The main cost drivers of the saccharification process are typically the feedstock cost, enzyme efficiency, and enzyme cost. Therefore, three enzyme cost and efficiency scenarios were modeled based on the assumptions from the literature. Percent yield efficiency of the enzymatic step converting celluloses and hemicelluloses to their respective sugars was applied to calculate the actual amount of sugars produced. Multiple scenarios based on variable enzyme efficiency, dry matter loading, and enzyme percent loading were applied to each lignocellulosic sidestream, yielding several production cost outcomes. These included enzyme efficiency ranges from 42.4% to 92.8% depending on whether the enzymes were food-grade, technical-grade, and/or produced in-house <sup>66,70</sup>. For ranking cost estimation, we used a conservative set of input values: 57% efficiency, 1% enzyme load, \$10/kg enzyme, and 20% dry matter load <sup>70</sup>.

We recognize that different materials will have their own processing needs and methods, which will affect the total cost of sugar production from these materials. Enzymatic hydrolysis models are very sensitive to enzyme cost and hydrolysis efficiency. Our base model assumed a 57% cellulase efficiency with a 1% enzyme loading and \$10/kg cellulase cost 70. Thus, any changes to the efficiency and enzyme costs can greatly affect the extraction costs. For instance, a 92% cellulase efficiency at \$6.2/kg enzyme would reduce the cost of glucose-only modeled costs to \$340-680/ metric ton glucose, depending on the feedstock, from our cost estimate of \$550-1100/metric ton glucose used for ranking in this analysis. Dry matter loading of 20% was also used to calculate the input costs of each feedstock and the output of sugars, as higher dry loading improves the sugar yield but can reduce enzyme efficiency.

Our model estimated the major raw material and utility costs in the process and used enzyme efficiency to determine how much hexose and/or

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pentose sugar monomers could theoretically be extracted from the cellulose and hemicellulose. The material and utilities cost of processing inputs, such as enzymes, solvents, and electricity, were calculated using market cost values from commercial sources or from literature data <sup>66,70</sup>. While capital expenditures and overhead costs (such as labor) were not quantified, we applied a 40% overage factor to the unit cost of sugar to account for a large portion of these unmodeled costs <sup>63</sup>.

For unit costs evaluated in the ranking, we considered total fermentable sugars to include both glucose and xylose generated from the enzymatic breakdown of the cellulose and hemicellulose, respectively. The unit cost of only glucose was also evaluated. This breakdown is important as not all microorganisms can readily use pentose sugars such as xylose, or may metabolize both glucose and xylose sugars. For many microbes, the utilization of xylose is inhibited by the presence of glucose (a hexose sugar) until the glucose has been consumed 10. Thus, microorganisms' cofermentation of pentose and hexose sugars during fermentation is desirable for lignocellulose sugar valorization. If a microorganism does not readily use pentose sugars, such as xylose, that carbon is essentially wasted feedstock cost <sup>11</sup>.

# Ranking sidestream candidates

# Rationale for two-step weighting and ranking

Criterion weighting is stakeholder-dependent. Industry stakeholders, especially those operating with smaller margins, cannot invest resources and time into a product without understanding its economic value. At the same time, policy stakeholders focused on improving climate and food security outcomes must understand environmental and nutritional impacts and other functional attributes of food and agriculture systems

before committing to new solutions. We therefore ranked sidestreams by either (1) economic factors or (2) integrated economic, environmental, and functional attributes. The functional attributes were broken into nutritional protein quality (PDCAAS) for protein concentrates, culture media amino acid quality for protein hydrolysates, and fermentation sugar quality for lignocellulosic sugars. Given the importance of economics for an ingredient to be commercially viable, we prioritized economic-influenced factors in our rankings. These factors include unit cost of production and forecasted sidestream volume in 2030. The environmental and functional attributes of alternative proteins and media ingredients are important market drivers and are necessary factors to consider in sustainable, secure food systems.

#### Economic ranking criteria

In this study, we prioritized two economic criteria: forecasted sidestream volume and modeled ingredient production unit cost. We used economic viability as the primary criterion to rank the sidestreams and determine the highest potential opportunity areas for valorizing these resources.

These values under each criterion were normalized into a [0,1] scale using <u>min-max normal-</u> <u>ization</u>. Volume values were normalized to the highest volume sidestream (max normalization), which was set to the value of 1 since higher volumes were a positive benefit to stakeholders and lower volumes ranked lower (Equation 1). Modeled production unit cost values were normalized to the lowest cost value sidestream (min normalization, Equation 2), because lower unit costs were a positive benefit to stakeholders and higher unit costs ranked lower.

$$x_{scaled} = \frac{x - x_{min}}{x_{max} - x_{min}}$$
(1)

$$x_{scaled} = 1 - \frac{x - x_{min}}{x_{max} - x_{min}}$$
(2)

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Forecasted sidestream volume values were weighted with a factor of 0.5, and unit cost was weighted with a factor of 0.5. The weights were multiplied across the normalized values, and the weighted normalized values for each sidestream were summed. The summed values were then ordinally ranked across the different sidestreams for each of the three possible alternative protein inputs of protein concentrates, lignocellulosic sugars, and protein hydrolysates generated from the sidestreams.

#### Integrated ranking criteria

The environmental and functional attributes criteria were our second priority in our ranking process. As in the method above, the values were normalized according to whether the highest or lowest values within a criterion were beneficial. For the functional attributes (PDCAAS, amino acid quality, and lignocellulosic sugars), higher values were beneficial, so these values were normalized using Equation 1. Since lower environmental impact scores are more beneficial, they were normalized using Equation 2. For the integrated rankings, the sidestream volume, ingredient production unit costs, environmental, and functional attributes criteria were all weighted equally with a 0.25 factor. The values under each sidestream were summed across all criteria and the sums were ordinally ranked.

# Environmental and functional attributes considerations

The environmental criteria were calculated by averaging the country-level average environmental pressure per tonne for crops grown in Canada, Mexico, and the United States <sup>7</sup> (Supplementary Data 3, Supplementary Methods). This data focuses on on-farm operations only, not including any processing that occurs after harvest, and was calculated using four dominant pressures: disturbance (km<sup>2</sup>eq); blue freshwater consumption (m<sup>3</sup> water); excess nutrients (tonnes NP); and GHG emissions (tonnes CO<sub>2</sub>eq). The final averages were normalized to the lowest value sidestream as 1 so lower environmental pressures ranked higher.

Functional attributes criteria were chosen for each ingredient category that was quantifiable and important to the specific category. Each functional attribute was normalized to the highest value sidestream, as better protein digestibility, media amino acid quality, and fermentable sugar quality are beneficial and ranked higher.

For protein concentrations, PDCAAS (e.g., protein digestibility) was used as the functional attribute criterion. PDCAAS values were extracted from available literature <sup>83,84</sup> and assumed to be the same for each crop sidestream. However, we were unable to find satisfactory PDCAAS data on tomato pomace protein in the literature. Instead, we estimated the digestibility of tomato pomace protein in humans by extrapolating digestibility data from chickens <sup>43</sup>. The adjusted digestibility of essential amino acids was calculated by multiplying the concentration of each essential amino acid found in tomato pomace by the True Amino Acid Digestibility Coefficient found in chickens. These adjusted amino acid values were divided by the ideal levels of each amino acid in a complete protein <sup>85</sup> to give the percentage of essential amino acids available per gram of protein. The concentration value of the amino acid in the tomato pomace protein at the lowest percentage compared to the ideal levels served as a proxy for the PDCAAS value.

For protein hydrolysates, the ranked functional attribute used was culture media amino acid quality. Sidestream amino acid compositions (mol-AA per mol-dry weight) were compared to theoretical cellular protein demand for mammalian cell culture <sup>9</sup> (Figure 16). The max ratio was set to 1.0 even though many plant proteins have excess amino acids. For instance, corn gluten meal has a 3.5x ratio of tryptophan to cellular protein demand needed for cell culture (0.039 mol vs 0.011 mol). For our ranking criteria, we

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averaged the normalized values for essential amino acids in mammalian cells (arginine, cysteine, glutamine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine).

For lignocellulosic sugars, fermentation sugar quality was applied for ranking. The ratio of cellulose content to total cellulose and hemicellulose combined content was taken for each crop sidestream to calculate fermentation sugar quality. The contents of cellulose and hemicellulose were estimated for each crop sidestream based on literature values and used to calculate each ratio. These ratios can be used as a proxy to understand how high the glucose content would be in the total fermentable sugars after hydrolysis. We did not include sugar and starch content, as most sidestreams had negligible compositions of these carbohydrates and the goal was to assess lignocellulosic wastes.

#### Essential amino acids (mol/mol-DW)

	Cellular protein	BSG	Canola meal	Corn bran	Corn DDGS	Corn gluten meal	Rice bran	Soy meal	Tomato pomace	Wheat bran	Wheat germ	Wheat gluten
Arginine	0.047	0.039	0.046	0.059	0.035	0.028	0.059	0.056	0.074	0.052	0.078	0.003
Cysteine	0.028	0.026	0.038	0.038	0.028	0.032	0.021	0.023	0.026	0.035	0.023	0.029
Glutamine	0.039	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Histidine	0.021	0.020	0.024	0.030	0.023	0.021	0.023	0.023	0.026	0.025	0.022	0.018
Isoleucine	0.046	0.040	0.043	0.032	0.037	0.048	0.053	0.046	0.040	0.035	0.038	0.036
Lysine	0.070	0.035	0.052	0.038	0.027	0.019	0.042	0.056	0.048	0.039	0.064	0.017
Methionine	0.017	0.019	0.024	0.019	0.021	0.032	0.025	0.016	0.022	0.018	0.022	0.016
Phenylalanine	0.035	0.039	0.033	0.026	0.039	0.057	0.038	0.040	0.042	0.035	0.032	0.042
Threonine	0.060	0.042	0.051	0.044	0.042	0.044	0.043	0.043	0.039	0.038	0.050	0.029
Tryptophan	0.011	0.008	0.008	0.004	0.006	0.039	0.011	0.009	0.007	0.009	0.006	0.005
Tyrosine	0.035	0.024	0.022	0.019	0.027	0.042	0.028	0.026	0.042	0.021	0.021	0.024
Valine	0.069	0.060	0.061	0.058	0.055	0.061	0.064	0.055	0.050	0.055	0.062	0.044

#### Non-essential amino acids (mol/mol-DW)

Alanine	0.090	0.078	0.068	0.101	0.105	0.055	0.094	0.065	0.067	0.071	0.091	0.042
Asparagine	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Aspartic acid	0.055	0.075	0.074	0.063	0.065	0.069	0.091	0.112	0.104	0.070	0.095	0.043
Glutamic acid	0.062	0.182	0.160	0.138	0.153	0.021	0.127	0.160	0.133	0.196	0.116	0.323
Glycine	0.075	0.074	0.092	0.092	0.071	0.055	0.094	0.075	0.094	0.092	0.110	0.062
Leucine	0.075	0.077	0.072	0.114	0.115	0.188	0.072	0.077	0.068	0.067	0.068	0.067
Proline	0.046	0.106	0.073	0.067	0.091	0.118	0.057	0.057	0.057	0.083	0.050	0.136
Serine	0.071	0.056	0.058	0.057	0.060	0.073	0.059	0.061	0.060	0.059	0.053	0.063

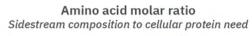




Figure 16. Sidestream amino acid compositions compared to theoretical cellular protein demand for mammalian cell culture. The cellular protein demands were extracted from the literature <sup>9</sup>.

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# Appendices

# Appendix 1. Key themes from The White House's Bold Goals for **U.S. Biotechnology and Biomanufacturing: Harnessing Research** and Development to Further Societal Goals report

U.S. Biotechnology and Biomanufacturing themes that align with improved agricultural sidestream valorization opportunities identified in this analysis include support from multiple U.S. federal departments and agencies.

#### **Department of Energy (DOE)**

**Theme 3: Climate-Focused Agricultural** Systems and Plants seeks "to develop restorative and resilient feedstock production systems, engineer better plants tailored as bioeconomy feedstocks, improve the usage of current feedstocks, and engineer more efficient protein production systems. These efforts will generate a variety of biomass feedstocks with increased resilience, yield, and nutrient use efficiency, laying the foundation for the expanding U.S. bioeconomy." Specifically,

"Goal 3.3: Engineer Circular Food Protein Production Systems - In 5 years, demonstrate viable pathways to produce protein for food consumption including from biomass, waste, and CO2 that achieve >50% lifecycle GHG emissions reduction and cost parity relative to current production methods."

calls for the following R&D needs:

 "Develop bioprocessing approaches that enable scale-up of biotechnology-based protein production while maintaining or improving quality, and thoughtfully matching large-scale waste feedstocks to efforts in synthetic biology and bioprocess engineering."

"Develop rigorous and transparent process analyses relative to existing food protein production pathways to inform development of sustainable bioprocesses."

Additionally, the U.S. DOE's "Industrial Decarbonization Roadmap" identifies the food and beverage industry "as a critical component of the U.S. economy, and one of the largest energy consuming and GHG emitting industries in the United States. To help achieve net-zero goals, the food and beverage sector can:

- Improve energy efficiency by advancing the electrification of process heating, evaporation, and pasteurization processes.
- Reduce food waste throughout the • supply chain through methods identified in life cycle assessments and collaboration between manufacturers.
- Pursue recycling and material efficiency through alternative packaging and package waste reduction."

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biomass and biogenic wastes are available as feedstocks for conversion to a range of products, and estimates indicate this could be expanded to more than 1 billion tons. There are, however, significant challenges to reliable and efficient biomass conversion to fuels and products stemming from the compositional variability of sources such as agricultural and forest residues, organic municipal waste streams, and dedicated herbaceous and woody crops."

calls for the following R&D needs:

- "Conduct research, development, and demonstration projects to reduce the carbon footprint of feedstock production, collection, transportation, and preprocessing."
- "Develop technologies capable of cost-effectively and sustainably pretreating heterogeneous waste streams and separating contaminants to increase the quantity and quality of available waste feedstocks."

### U.S. Department of Agriculture (USDA)

**Theme 1: Transportation and stationary** 

fuels "addresses the need to develop

more carbon-neutral transportation and

stationary fuels by expanding renewable

feedstock availability and producing more

fuels." While this theme focuses on fuels,

that align with sidestream valorization for

sustainable aviation and other strategic

there are common technological goals

fermentation application in alternative

"Goal 1.1: Expand Feedstock Availability. There is great potential for expanding the

use of renewable feedstocks in the United

unparalleled ability to grow, harvest, store,

States due to a unique national strength

in agriculture. The United States has an

and transport agricultural products on a massive scale. While this productivity is

most evident for grain and oilseed crops,

the potential exists to expand agricultural

roughly 368 million metric tons per year of

production of purpose-grown crops for

bioenergy and bioproducts. Currently,

proteins. Specifically,

Theme 1: Improving Sustainability and Resource Conservation While Increasing Agricultural Productivity describes "goals for increasing agricultural productivity, increasing climate-smart feedstock production and biofuel usage, reducing nitrogen and methane emissions, and reducing food waste. The future of U.S. agriculture depends on improvements in production capacity, efficiency, and environmental stewardship. To spur economic growth and meet the demands of a growing global population, agricultural lands need to be more productive and use both inputs and outputs efficiently." Specifically,

"Goal 1.1 Increase Agricultural Productivity – Over the next 10 years, increase agricultural total factor productivity growth to meet global food and nutrition security needs while improving natural resource use efficiency and conservation, toward the global goal of increasing agricultural productivity by 28% in the next decade."

- "Use accelerated breeding strategies and biotechnology to improve plants, animals, and microorganisms to enhance productivity and reduce environmental impacts of agriculture."
- "Bolster research in innovative approaches and technologies—including precision agriculture and circular and nature-based solutions—that improve sustainability; reduce inputs; and rebuild soil health, carbon, and organic matter."

"Goal 1.2: Increase Climate-Smart Feedstock Production and Biofuel Usage – By 2030, increase climate-smart production of conventional and alternative agricultural and forestry feedstocks for biomanufacturing, biobased products, and biofuels;..."

calls for the following R&D needs:

- "Develop tools that rapidly assess and track feedstock qualities to evolve markets that reward producers for product quality in addition to yield."
- "Develop biochemical and biomanufacturing processes, including enzymatic and microbial processes, for efficiently converting feedstocks into intermediates and products at scale."
- "Develop technologies to economically move, store, and process biomass prior to its use as feedstocks for biomanufacturing or conversion into biobased products."

 "Expand upon biorefinery technologies to efficiently break down biomass into its components (e.g., lignin, hemicellulose, and cellulose); to convert lignin and hemicellulose into plastics, adhesives, and low-energy building materials; and to convert cellulose fiber into nanomaterials and cellulose derivatives for fibers, coatings, renewable packaging, and other products."

"Goal 1.5: Reduce Food Loss and Waste – By 2030, reduce food loss and waste by 50%, including by developing and commercializing new technologies and encouraging the adoption of new and existing technologies."

calls for the following support and R&D needs:

- "Develop and expand strategies to increase food recovery or recycling programs at scale, including advanced biochemical and microbial systems for efficient conversion of food waste into feed, fertilizers, materials, bioproducts, and fuels."
- "Improve or develop strategies to measure food waste, including both edible food and non-edible waste such as banana peels, bones, and eggshells, anywhere along the food chain."

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Theme 2: Improving Food Nutrition, Quality, and Consumer Choice describes "goals for developing new food and feed sources, enhancing nutrient density in foods, and reducing foodborne illness. Innovations in food and feed can boost both dairy and cultivated protein companies, for example, sustainably expanding the range of available protein options. Improving nutritional quality and reducing foodborne illness are essential for increasing food security." Specifically, theme 2 calls out...

"Goal 2.1: Develop New Food and Feed Sources – Develop new food and feed sources, including production of novel or enhanced protein and fat sources at scale, to support the United Nations Sustainable Development Goal to eliminate global hunger by 2030."

and for the following R&D needs:

- "Identify and conduct feasibility studies for high-volume, low-cost protein and fat sources that could be used in food or feed, including products resulting from precision fermentation and coproducts or waste streams from other industries."
- "Expand research into food components that make novel foods more palatable, affordable, easier to prepare, and more easily incorporated into manufactured foods."

#### **Department of Commerce (DOC)**

Theme 2: Biomanufacturing Innovation to Enhance Supply Chain Resilience calls for advanced supply chain and ecosystems so that the United States and its partners can expand advanced biomanufacturing capabilities, including biomanufacturing that is regionally located close to feedstocks. Specifically related to sidestreams,

"Goal 2.4: Supply Chain Flexibility. Largescale production of new biotechnology products requires a robust global manufacturing ecosystem and infrastructure. The most functional and economical way to expand the biomass-to-chemicals industry is to locate biomass processing facilities close to feedstock production. Such colocalization can promote economic growth across the Nation, strengthen resilience to domestic

supply chains, and address the policy goal of revitalizing the economies of rural communities, as well as those facing hardships associated with the loss of traditional manufacturing jobs. To promote a robust biomanufacturing ecosystem, investments are needed to develop and implement fit-for-purpose manufacturing platforms, including modular and/or mobile platforms, for converting biomass, enzymes, metabolites, and other sources into viable products. Importantly, biomanufacturing technologies must keep pace with rapid biotechnology innovations to produce a diverse product portfolio for multiple sectors. Additionally, continued improvement in biomanufacturing technologies is necessary to make processes cheaper, more efficient, and more sustainable."

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#### calls for the following R&D needs:

- "Develop platform technologies and standards to accelerate the development, production, and interoperability of biomanufacturing equipment, components, and consumables and improve the characterization and testing of biomanufacturing processes and products."
- "Develop standard sets of microbial strains, cell free systems, key reagents, sequences of known function and

performance, and supply chain precursor molecules and compounds that can be rapidly produced, distributed, and scaled up on demand."

 "Develop standardized quality metrics for raw materials and reagents to enable interoperability from multiple vendors, and advanced algorithms to enable adaptive stockpiling capable of using alternative feedstocks or processes when supply chains are limited or disrupted."

#### National Science Foundation (NSF)

Theme 1: Leverage Biodiversity Across the Tree of Life to Power the Bioeconomy focuses on the discovery and understanding of the diversity of life and how it has adapted to harsh conditions and hard problems. Specifically,

"Goal 1.1: In 5 years, sequence the genomes of one million microbial species and understand the function of at least 80% of the newly discovered genes.

Goal 1.2: In 20 years, speed the discovery of new gene sequences, metabolisms, and functions by 100-fold over current practice across all types of organisms."

call for the following R&D Needs:

 "Develop a national strategy for selecting organisms to sequence so that comparative analyses are likely to reveal functional variation that can be used for biological design. Accelerate development of computational and experimental tools to enhance comparative discovery of sequence and functional elements that define genotype-to-phenotype relationships from evolutionarily diverse organisms and provide the basis for new biotechnology innovations.

- Put biodiversity to use in new biotechnology applications: Create new and improved technologies to move genes from one organism to another.
- Use outcomes of functional discovery to expand the number of organisms that can be used as hosts (chassis) in engineered biological systems."

Theme 2: Enhanced Predictive Modeling and Engineering Design of Biological Systems recognizes that the knowledge gained by tapping into Earth's biodiversity must be coupled with improved capabilities to predict the function and behavior of complex biological systems and to use that information for new bio-inspired design.

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# Theme 4: Advance Scale-Up and Control of Biological Systems

"Goal 4.1: In 5 years, advance bioprocess design, optimization, and control tools to enable predictable scale-up to commercial production of any bioprocess within 3 months with a 90% success rate.

Goal 4.2: In 20 years, advance integration of all aspects of feedstock use, organism design, process design, and end-of-use disposal with techno-economic analysis such that sustainability and commercial goals can be achieved for more than 85% of new bioprocesses within the first year of deployment." calls for the following R&D Needs:

- "Develop robust tools for techno-economic analysis and life cycle assessment that can be integrated within the design process.
- Integrate optimization parameters across all aspects of the bioprocess, including design, upstream and downstream processes, product end-of-life, and non-conventional bioprocess environments.
- Improve bioproduct supply chain resiliency by advancing process design methods to transition from (semi)batch to continuous and intensified processes, including through the use of modular, geographically distributed, and potentially reconfigurable processes or facilities."

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Appendix 2. Crop Snapshots



# Barley

Good protein production costs and high-moisture sidestreams with good protein and sugar contents.

# **Benefits**

- Good protein concentration and hydrolysate production costs.
- Good protein digestibility: PDCAAS = 0.63.

# **Opportunities**

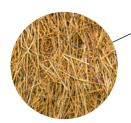
- Optimize use and drying of high-moisture brewer's spent grain.
- Lower environmental footprint through improved breeding and cultivation.





#### **BSG** (Brewer's Spent Grain)

- #5 Protein hydrolysate economic candidate
- #7 Protein concentrate candidate
- Affordable protein enrichment process
- Ideal amino acid profile for cell media
- Relatively low-volume sidestream
- High-moisture sidestream requires immediate use or drying



#### Straw

- #5 Lignocellulosic sugars economic candidate
- Favorable economics for total sugars extraction



#### Husks

- #5 Lignocellulosic sugars integrated candidate
- Good fermentable sugar quality
- Low-volume sidestream











**Canola** Oilseed crop with excellent protein content and digestibility with opportunities to improve property digestibility with opportunities to improve properties.

# **Benefits**

- · Established oil production produces proteinrich sidestream at high volume.
- Excellent protein digestibility: PDCAAS = 0.93.
- Protein enrichment is affordable, similar to other oilseed crops.

# **Opportunities**

- Remove phenolic compounds, which contribute to meal off-color and bitterness.
- · Reduce environmental impact through breeding efforts and resource optimization.

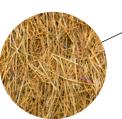


# **Sidestreams**



#### Meal

- #2 Protein concentrate economic candidate
- #4 Protein hydrolysate economic candidate
- Good protein content (36.3%)
- High-volume sidestream
- Protein enrichment is affordable with room to optimize ingredient color and taste



#### Straw

- #7 Lignocellulosic sugar integrated candidate
- Good sugar quality
- Moderate cost of total sugar production



### Hulls & chaff

- Not ideal candidates for alt. protein inputs
- Moderate-volume sidestreams





# Corn

High-volume sidestreams with opportunities to optimize byproduct enrichment.

# **Benefits**

- #1 crop volume, providing a steady stream of byproducts.
- Corn protein (zein) has unique fiber-forming properties.
- Good environmental footprint.

# **Opportunities**

- Reduce byproduct processing costs through recycling ethanol and other optimizations.
- Improve low protein digestibility: PDCAAS = 0.37.

# **Sidestreams**



#### Stover

- #1 Lignocellulosic sugar candidate
- Economical source for fermentation sugars
- High-volume sidestream
- Commercially mature supply chain as a fermentation feedstock in ethanol production



### DDGS

(distillers dried grains with solubles)

- #3 Protein hydrolysate economic candidate
- #4 Protein concentrate integrated candidate
- Good protein content (29.7%)
- Low-moisture; dried onsite in ethanol industry
- Efficiently processed via enzymatic hydrolysis



#### Bran

- High-volume sidestream
- Established industrial uses
- Not an ideal candidate for alt. protein inputs
- Low protein content (16.40%)
- Protein enrichment is costly due to low water solubility



#### Gluten

- #3 Protein hydrolysate integrated candidate
- #5 Protein concentrate integrated candidate
- High protein content (61.7%)
- Protein enrichment is costly due to low water solubility
- Moderate-volume sidestream
- Hydrolysates are currently being explored for food applications











**Rice** Large environmental footprint to cultivate, but affordable sidestream enrichment processes are available sidestream enrichment processes are available.

# **Benefits**

- Affordable sidestream enrichment processes.
- High cellulose/hemicellulose content sidestreams.
- · Established sidestream use in mushroom farming.

## **Opportunities**

- · Lower cultivation environmental footprint.
- Reduce inorganic silica content in hulls.
- Improve low protein digestibility: PDCAAS = 0.50.





#### Bran

- #6 Protein concentrate economic candidate
- #7 Protein hydrolysate economic candidate
- Low sidestream volume
- Affordable protein concentrate and hydrolysate processes
- Rice bran oil is also currently being explored for animal-free fat production (Paragon Pure)





- #7 Lignocellulosic sugar economic candidate
- Rice straw is one of the most commonly used solid substrates for mushroom farming

#### Hulls

- #3 Lignocellulosic sugar economic candidate
- Low sidestream volume
- Affordable extraction of sugars due to low feedstock costs and high cellulose/ hemicellulose content

# **Current efforts**



Imperial College London



Well-established supply chain and valorization **Sov** Well-established supply chain and valorization efforts can serve as a model for other crops.

# **Benefits**

- #2 crop volume, providing a steady stream of byproducts.
- Excellent protein digestibility: PDCAAS = 0.84.
- Established enrichment methods.

# **Opportunities**

- Optimize meal enrichment for food ingredient inputs.
- Improve cost of processing lignocellulosic sources.



# **Sidestreams**



#### Meal

- #1 Protein concentrate candidate
- #1 Protein hydrolysate candidate
- Excellent protein content (49.5%)
- High-volume sidestream
- Protein enrichment and hydrolysis are affordable and well-established



#### Straw

- #2 Lignocellulosic sugar candidate
- Okay fermentable sugar quality
- High-volume, readily available sidestream
- Extensively studied for lignocellulosic saccharification



#### Hulls

**BENSON** • HILL

- #6 Lignocellulosic sugar candidate
- Excellent fermentable sugar quality
- Processing costs need improvement





# **Sugarcane** Excellent cellulose and hemicellulose content in sidestreams for sugar sources.



# **Benefits**

- Regional distinct, concentrated feedstock access.
- Excellent environmental footprint.

# **Opportunities**

· Optimize fermentable sugar quality of lignocellulosic sidestreams.

# **Sidestreams**



### **Trash & Bagasse**

- Bagasse: #3 Lignocellulosic sugar integrated candidate
- Trash: #4 Lignocellulosic sugar candidate •
- Has been evaluated for use as a lignocellulosic source •



# Tomato

Good environmental footprint and high-moisture sidestream with moderate protein content.



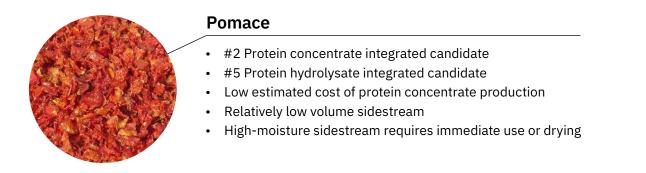
# Benefits

- Good amino acid profile and moderate protein content in pomace sidestream.
- Good environmental footprint.

# Opportunities

- Optimize use and drying of high-moisture tomato pomace.
- Evaluate tomato pomace protein digestibility in humans and ingredient functionality.

# Sidestreams







Wheat High-volume sidestream production with opportunities to optimize protein enrichment and digestibility.

# **Benefits**

- High-volume sidestreams.
- Wheat gluten has unique dough-forming properties and excellent protein content.

# **Opportunities**

· Improve essential amino acid profile and low protein digestibility: PDCAAS = 0.43.



# **Sidestreams**



#### Bran

- #3 Protein concentrate economic candidate
- High-volume sidestream
- Affordable protein enrichment •



#### Gluten

- #5 Protein concentrate economic candidate
- Excellent protein content (79.8%)
- Low-volume sidestream
- Applied in the majority of restructured plant-based meats in the U.S. due to its binding properties



#### Straw

- #7 Lignocellulosic sugar candidate
- Moderate-volume sidestream



#### Germ

- Not an ideal candidate for alt. protein inputs
- Moderate-volume sidestream











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Recommendations & conclusions

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