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Precision Fermentation: A Tool Revolutionizing the Food Industry a Review

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ABSTRACT

Precision fermentation, a novel technology that combines synthetic biology, data analytics, and fermentation processes, is poised to revolutionize the food industry. This article provides an overview of precision fermentation, highlighting its potential benefits and applications. Furthermore, we present empirical data on the impact of precision fermentation on various aspects of the food industry, including product development, sustainability, and nutrition. We also discuss potential challenges and future directions for this transformative technology.

Keywords: precision fermentation, synthetic biology and genetic engineering, food ingredient, derived protein, recombinant protein.

INTRODUCTION

The food industry is facing numerous challenges, such as the need for sustainable production, reducing waste, and meeting the growing demand for nutritious and personalized food products. Precision fermentation, a cutting-edge technology, offers a promising solution to address these challenges. By leveraging advances in synthetic biology and data analytics, precision fermentation enables precise control and optimization of fermentation processes to produce a wide range of food products with improved quality, nutrition, and sustainability.

Precision fermentation is a rapidly emerging technology that has the potential to revolutionize the food industry. At its core, precision fermentation involves the use of microorganisms to produce high-value ingredients and products, ranging from alternative proteins and meat substitutes to plant-based dairy products and even leather.

One of the biggest advantages of precision fermentation is its ability to create highly customizable, precise, and consistent products that can be tailored to meet specific nutritional, sensory, and functional requirements. This is achieved by using advanced biotechnology tools such as genetic engineering, synthetic biology, and fermentation optimization to optimize the growth and metabolism of microorganisms.

Another major advantage of precision fermentation is its potential to address some of the biggest sustainability and environmental challenges facing the food industry. By producing ingredients and products in a highly controlled and resource-efficient manner, precision fermentation has the potential to reduce land and water use, greenhouse gas emissions, and waste generation, while also reducing the reliance on traditional animal agriculture and its associated environmental and ethical concerns.

Despite its many advantages, precision fermentation is still a relatively new technology, and there are many challenges that must be addressed before it can be fully integrated into the mainstream food industry. Some of the key challenges include ensuring regulatory compliance, optimizing production efficiency and scalability, and addressing consumer acceptance and perception.

Precision fermentation is a promising technology that has the potential to transform the food industry and contribute to a more sustainable and equitable food system. While there are still many hurdles to overcome, the rapid advancements in biotechnology and fermentation science suggest that precision fermentation will continue to evolve and expand in the coming years. Precision fermentation is a cutting-edge technology that involves using microorganisms to produce specific compounds, such as proteins or flavors, in a controlled and precise manner. In the dairy industry, precision fermentation is being used to produce dairy proteins like whey and casein, which are commonly used in a variety of food products.

One major advantage of precision fermentation is that it can provide a more sustainable and efficient alternative to traditional dairy production, which can be resource-intensive and have a significant environmental impact. For example, precision fermentation can produce dairy proteins without the need for large-scale farming operations, which can reduce land use and greenhouse gas emissions.

Another benefit of precision fermentation is that it can enable the production of novel and innovative food products that may not be possible using traditional dairy production methods. For instance, precision fermentation can be used to produce plant-based dairy alternatives that mimic the taste and texture of traditional dairy products.

Overall, precision fermentation is an exciting and rapidly developing field that has the potential to revolutionize the way we produce and consume food.

PRECISION FERMENTATION: TECHNOLOGY OVERVIEW:

Synthetic Biology and Genetic Engineering:

Precision fermentation relies on synthetic biology techniques to engineer microorganisms, such as bacteria, yeast, or fungi, to produce desired compounds or ingredients. Genetic engineering allows the introduction or modification of specific genes responsible for the production of target molecules, thereby enabling the production of unique food products.

productions of food ingredients

The production of food ingredients through precision fermentation involves using synthetic biology techniques to program microbes, which act as cell factories, to produce ingredients for the food and pharmaceutical industries (Pham 2018). The selection of the host microorganism and genetic engineering of strains pose initial challenges in determining the potential for constructing microbes capable of expressing and producing target molecules in sufficient quantities. To enhance production efficiency, microbial hosts that are easy to manipulate genetically and can be used with standardized fermentation equipment are preferred. Generally regarded as safe (GRAS) microorganisms or those with a non-harmful status are favored for food applications. Therefore, strain engineering often utilizes benign bacteria (e.g., Bacillus spp.), yeasts (e.g., Saccharomyces cerevisiae, Pichia pastoris, Kluyveromyces spp.), or filamentous fungi (e.g., Trichoderma spp., notably T. reesei strains) (Chai et al., 2022; Nevalainen, Peterson, and Curach 2018).

Innovation in precision fermentation involves the use of novel species or strains, as well as genetic engineering and synthetic biology techniques to optimize the yield of desired products. This can be achieved by improving expression, secretion, substrate conversion, and titer of the target molecules. Recent advances in "omics" tools and synthetic biology have uncovered the untapped potential within natural microbial diversity to provide efficient microbial cell factories for safe and novel fermented foods. By utilizing these tools, it becomes possible to develop products that precisely match the desired properties and ensure controlled and precise fermentation processes, as opposed to random processes (Teng et al. 2021). Precision fermentation is particularly suitable for producing proteins, lipids, and carbohydrates that closely resemble their counterparts derived from traditional agriculture.

Regarding the production of precision fermentation-derived proteins, while the bacterium E. coli has been dominant in producing recombinant protein therapeutics due to its well-characterized genetics, rapid growth, and high yield (Huang, Lin, and Yang 2012), the production of protein food ingredients favors the use of GRAS microorganisms to simplify regulatory pathways. The current trend is to produce animal proteins using animal-free systems, focusing on eukaryotic expression hosts that can produce animal proteins with post-translational modifications equivalent to those found in nature. Unicellular yeast such as *Komagaetella phaffii* (Pichia pastoris) that do not produce ethanol are ideal expression systems and have been widely used (Juturu and Wu 2018; Spohner et al. 2015; Vieira Gomes et al. 2018). Genetic modifications, such as avoiding protein degradation, enhancing protein processing, and eliminating unwanted protease activities, have been employed to improve recombinant protein production in this expression host. Genetic engineering tools like CRISPR have enabled these synthetic biology approaches for protein production (Spohner et al. 2015; Wagner and Alper 2016).

In the development of recombinant proteins, the primary objective is to obtain a nature-identical amino acid sequence as found in the target protein. Post-translational modifications, such as phosphorylation and glycosylation, also need to be considered as they impact the structure and functionality of the protein.

Innovative synthetic biology approaches for recombinant protein production in food ingredients focus on increasing protein expression per biomass unit, enhancing protein secretion efficiency, improving specific physicochemical attributes (e.g., thermostability), and engineering functional attributes of the protein to create desired food ingredients. These genetic modifications can involve altering gene expression through promoter and regulatory element manipulation, deletion of extraneous proteins to direct cellular energy towards the target protein, and elimination of abundant proteases in the fermentation broth. Enhanced protein trafficking through the endoplasmic reticulum and Golgi body can improve protein secretion by manipulating protein translocon and trafficking glycosylation signals. Additionally, avoiding cellular degradation processes can increase protein secretion by deleting components of the vacuolar protein degradation transport system in Saccharomyces cerevisiae (Bartkeviciuite and Sasnauskas 2003; Davydenko et al. 2004).

Recent patents related to proteins derived from fermentation show a focus on alternative proteins. There is considerable interest in developing recombinant egg, dairy, and meat proteins, as well as specialized functional ingredients such as enzymes and color/flavor supplements. Precision fermentation has been used to produce animal-free egg replacers (Anchel 2016; Mahadevan et al. 2021). Methods for producing recombinant caseins and whey proteins have also been described, although some proteins may have non-native post-translation modifications (Pandya et al. 2016). These recombinant milk proteins can be combined with non-recombinant ingredients to create a variety of dairy products, including milk, ice cream, yogurt, and cheese (Geistlinger et al. 2018; Gibson, Radman, and Arie 2020). Recombinant milk proteins also have applications as egg replacers (Geistlinger et al. 2022). Synthetic biology tools offer possibilities for altering post-translational modifications, reducing esterase activity for target food applications, decreasing protein allergenicity, or producing non-naturally occurring polypeptides that are fragments of food proteins (Ouzounov, Mellin, and Co 2021).

Precision fermentation-derived ingredients for non-animal meat analogues include recombinant collagen, muscle protein, and non-animal heme proteins that provide color and flavor during cooking. These proteins

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can be produced through biomass fermentation or in-vitro cultures. For example, mammary cell cultures have been used to produce milk, and fungal single-cell protein has been employed as an alternative protein in non-animal meat analogues (De Latt and Gallego Murillo 2018; Lu, Xia, and Liu 2021; Ouzounov, Mellin, and Co 2021; Persikov, Ouzounov, and Lorestani 2019; Strickland 2021).

Table 1. Selected patents relevant to proteins from fermentation

Inventions covered by selected patents	References
Recombinant Egg Protein Producing an egg white protein composition, the method comprising recombinant expressing two or more egg white proteins	Anchel (2016)
Ingredient composition for producing an egg-less food item, comprises a recombinant ovalbumin with egg white functionality	Mahadevan et al. (2021)
Recombinant Dairy Protein Methods for manufacturing dairy substitutes (including methods of producing recombinant milk proteins)	Pandya et al. (2016)
Food product (e.g., cheese, yogurt) comprising one or more native and/or recombinant milk proteins and one or more native and/or recombinant non-animal proteins	Geistlinger et al. (2018)
Cheese and yogurt compositions and the methods of making the same using one or more recombinant proteins comprises a casein protein (but without beta-casein) and kappa-casein in the micellar form	Gibson, Radman, and Arie (2020)
Recombinant milk proteins with non-native post-translational modifications and food products containing recombinant milk proteins	Geistlinger et al. (2020)
A recombinant milk protein component consisting of a subset of whey milk proteins or of a subset of casein milk proteins for egg replacer	Geistlinger et al. (2022)
A micelle composition comprising an alpha casein and a kappa casein where at least one of the proteins is a recombinant protein	Radman et al. (2022)
A method for producing a casein micelle in a liquid medium containing calcium and phosphate, where at least one of the casein proteins is selected from the group consisting of recombinant αs1-casein, αs2-casein, β-casein and κ-casein	Wolff, Gaydar, and Cohavi (2022)
Recombinant Meat/Muscle Protein Modified gram-negative bacteria comprising exogenous genes encoding target protein production (e.g., collagen)	Ouzounov (2017)
Methods and systems which combine synthetic biology, fermentation, material science and machine learning to produce collagen with desired physical and chemical properties	Persikov et al. (2019)
Synthetic meat made through recombinant expression of muscle proteins in edible biological hosts and crosslinking produced protein	Lu, Xia, and Liu (2021)

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Recombinant Functional Ingredients A cell comprising an exogenous nucleic acid molecule comprising a promoter sequence linked to a nucleic acid encoding a signal peptide operably linked to anucleic acid encoding a heme-containing polypeptide	Fraser, Simon, and Brown (2017)
DNA constructs and methods of using such DNA constructs to genetically engineer cells for the production of heme-containing proteins	Roy-Chaudhuri and Shankar (2020)
Compositions with enzymatically active enzymes produced recombinantly, wherein the enzyme is a goose-type lysozyme for preservation of foodstuffs	Ivey, Kreps, and Harshal (2021)
A method for preparing a bovine myoglobin by constructing plasmids (containing genes for heme biosynthesis pathway enzymes and a gene for Bos taurus myoglobin) and constructing Escherichia coli production hosts and culturing. Product is useful as a meat flavor and/or an iron supplement.	Yoon et al. (2021a)
A method for preparing soy leghemoglobin by constructing plasmids (containing genes for heme biosynthesis enzyme pathways and gene for soy leg hemoglobin) and constructing E. coli production hosts and culturing. Product is useful as a meat flavor and/or an iron supplement	Yoon et al. (2021b)
Biomass Fermentation / In-vitro Cell Cultures – Various Proteins Producing single-cell protein from biomass by using at least one thermophilic fungal strain grown in a fermentable carbon-rich feedstock at a high temperature and at an acidic pH and recovery of the single-cell protein	De Latt and Gallego Murillo (2018)
Process for producing a cell hydrolysate composition, comprising substantially protein polypeptides and/or polypeptide fragments from in vitro cell cultures	Chin et al. (2021)
Systems and methods for producing cell cultured food products (sushi-grade fish meat, fish surimi, foie gras, and other food types)	Elfenbein and Kolbeck (2018)
A method of producing an isolated cultured milk product from mammary cells comprising culturing a cell construct in a bioreactor	Strickland (2021)
Processing microorganism cells from a culture medium to produce a food product (meat analogue food product)	Dyson, Rao, and Reed (2021)
General Methods – Recombinant proteins & Food Products Systems and methods for the high-yield production of recombinant proteins in engineered microorganisms for expressing a heterologous protein	Ivey et al. (2021b)
A method for modification of protein glycosylation by recombinantly expressing a nutritional protein and an −1,2-mannosidase in a host cell, wherein the −1,2-mannosidase reduces the glycosylation of greater than 50% of the nutritional protein secreted from the host cell	Ivey et al. (2021b)
Recombinant milk protein having an attenuated or essentially eliminated allergenicity, where the recombinant milk protein retains one or more of the functional attributes of the native protein	Bhatt et al. (2021)
Food product produced by a recombinant host cell (wherein an activity of an esterase is essentially eliminated or modulated) and compositions comprising recombinant components for use in food products	Geistlinger et al. (2021)

Non-naturally occurring polypeptides comprises a sequence of a fragment of collagen and recombinant cells containing nucleic acid sequences for encoding them; Composition is formulated for consumption by human	Ouzounov, Mellin, and Co (2021)
Methods for producing recombinant proteins in microbial hosts for use in food products and other applications	Saurabh et al. (2021)
Methods for producing recombinant proteins with desired post-translational modifications in microbial hosts for use in food products and other applications	Saurabh et al. (2021)
Systems and methods for producing plant-based meat substitutes comprising recombinant proteins expressed in microbial hosts	Yun et al. (2021)
Methods for producing plant-based meat substitutes comprising recombinant proteins expressed in yeast	Mekonnen et al. (2022)
A method for producing a plant-based meat product by fermenting a mixture of plant-based proteins and recombinant myosin protein expressed in yeast	Wang et al. (2022)

Table 2. Selected patents relevant to carbohydrates from fermentation

Inventions covered by selected patents	**References**
Recombinant Oligosaccharides Process for producing an oligosaccharide using a host microorganism and use of glycosidases in the purification of a produced desired oligosaccharide from a mixture	Jennewein (2015b)
Genetically modified yeast cells capable of producing one or more human milk oligosaccharides (HMOs) and methods of making such cells to have heterologous nucleic acid encoding a transporter protein and heterologous nucleic acids that encode enzymes of an HMO biosynthetic pathway	Walter and Pinel (2021)
A construction method for genetically engineering *E.coli* to obtain a high-yield strain for producing cold-adapted alpha-amylase and fermenting to produce alpha-amylase*	Zhang and Huo (2017)
Production of oligosaccharides in genetically modified bacterial host cells, and genetically modified host cells which comprises at least one recombinant glycosyltransferase, and at least one nucleic acid sequence coding for a protein enabling export of oligosaccharide	Jennewein and Wartenberg (2017)
Producing and purifying human milk oligosaccharides comprises fermentation of a genetically modified microbial organism, and downstream processing (with one or more treatments -enzymatic treatment/ filtration/ simulated moving bed chromatography)	Johnson and Janse (2019)
Genetically modified microorganisms for enhanced utilization of oligosaccharides and improved productivity of compounds (tagatose, 2′-fucosyllactose, and psicose)	Chomvong, Cate, and Jin (2019)

In summary, precision fermentation utilizes synthetic biology methods to program microbes as cell factories for producing food and pharmaceutical ingredients. The choice of host microorganisms and genetic engineering play a crucial role in determining the efficiency and capabilities of producing target molecules. The focus is on using GRAS microorganisms and optimizing production through genetic modifications, including improving protein expression, secretion, and post-translational modifications. Precision fermentation enables the production of proteins, lipids, and carbohydrates that closely resemble those derived from traditional agriculture, offering novel opportunities for alternative proteins and functional ingredients in various food applications.

Data Analytics and Machine Learning:

Precision fermentation harnesses data analytics and machine learning algorithms to optimize fermentation

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processes. By collecting and analyzing real-time data from fermentation systems, these tools enable precise monitoring and control, leading to improved efficiency, yield, and product quality.

APPLICATIONS OF PRECISION FERMENTATION:

Novel Food Products:

Precision fermentation enables the production of novel food products that were previously difficult or impossible to obtain. For instance, it allows the production of alternative proteins, such as plant-based meats, cultured meat, and microbial proteins, addressing the increasing demand for sustainable protein sources.

Personalized Nutrition:

Precision fermentation facilitates the production of personalized food products tailored to individual nutritional needs. By engineering microorganisms to synthesize specific nutrients, such as vitamins, minerals, or bioactive compounds, precision fermentation offers the potential for personalized and functional foods.

Sustainable Production:

Precision fermentation plays a crucial role in achieving sustainable food production. It enables the development of eco-friendly alternatives to conventional agricultural practices, reducing land, water, and energy requirements. Additionally, it helps in mitigating food waste by utilizing by-products or non-traditional feedstocks in fermentation processes.

EMPIRICAL DATA ON PRECISION FERMENTATION:

Product Quality and Safety:

Empirical studies have demonstrated that precision fermentation can enhance product quality and safety. By precisely controlling fermentation conditions, it ensures consistent product characteristics, reduces batch-to-batch variations, and minimizes the risk of contamination.

Yield and Efficiency:

Data analysis and optimization techniques applied in precision fermentation have shown remarkable improvements in yield and process efficiency. These improvements result in higher productivity, reduced resource consumption, and overall cost reduction.

Sustainability Impact:

Studies have shown that precision fermentation has a significant sustainability impact. For example, it reduces greenhouse gas emissions compared to traditional livestock farming, conserves water resources, and minimizes the need for land conversion, contributing to a more sustainable food system.

CHALLENGES AND FUTURE DIRECTIONS:

Despite its immense potential, precision fermentation faces challenges related to regulatory frameworks, public acceptance, and scalability. Addressing these challenges requires collaboration among stakeholders, including researchers, industry, policymakers, and consumers. Future directions include further optimization of fermentation processes, expansion of product diversity, and continued research on safety and environmental impacts.

CONCLUSION

Precision fermentation represents a transformative tool that revolutionizes the food industry by offering enhanced product quality, personalized nutrition, and sustainable production. Empirical data and studies validate the potential of precision fermentation to address key challenges and pave the way for a future where food production is more efficient, sustainable, and tailored to individual needs.

As precision fermentation continues to evolve, further research and development efforts are necessary to unlock its full potential. Regulatory frameworks need to be established to ensure the safety and consumer acceptance of precision fermentation-derived products. It is essential to engage in proactive discussions with regulatory agencies to address any concerns and develop appropriate guidelines that facilitate innovation while ensuring consumer confidence.

Additionally, collaborations between industry stakeholders, researchers, and policymakers are crucial for the successful implementation of precision fermentation on a larger scale. This collaboration can foster knowledge exchange, accelerate technological advancements, and enable the development of sustainable business models that benefit both the industry and the environment.

Furthermore, continued research is needed to assess the environmental impacts of precision fermentation throughout the entire lifecycle of food production. This includes evaluating the energy requirements, waste generation, and carbon footprint associated with scaling up precision fermentation processes. By understanding and optimizing these aspects, precision fermentation can contribute significantly to a more sustainable food system. In conclusion, precision fermentation holds tremendous promise in revolutionizing the food industry. The integration of synthetic biology, data analytics, and fermentation processes allows for the production of novel food products, personalized nutrition, and sustainable practices. Through empirical data and ongoing research, it is evident that precision fermentation has the potential to address pressing challenges facing the food industry while opening up new possibilities for healthier, more sustainable, and customized food options. As we continue to unlock the power of precision fermentation, its impact on the food industry is expected to be transformative, benefiting both consumers and the environment.

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