REVIEW

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Application of Omics Methods in the Study of Endophytic Microorganisms: A Scoping Review

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ABSTRACT

Introduction: Since 1994, the dynamic development of biotechnology and the widespread application of recombinant enzymes have led to new technological solutions in food production. Modern technologies enable the production of sugar, bread, beer, cheese, sausages, and other products using biotechnological processes and industrial food enzymes. The bioproduction of recombinant proteins has replaced natural enzymes, offering enzymes with enhanced catalytic functions, stability, and an extended range of operating conditions. These recombinant enzymes have proven to be economically more advantageous compared to natural and previously used recombinant enzymes.

Purpose: To delineate the scope of research on recombinant proteins and their role in modern food production from 1973 to 2024.

Materials and Methods: Sources were searched in the databases PubMed, RSCI, and Google Scholar. The review methodology adhered to the PRISMA-ScR protocol. The chronological scope of the review spans from 1973 to 2024.

Results: The initial search with keywords identified 121 sources: 101 from databases and 20 from other sources. After removing duplicates, 113 sources remained. A total of 111 full-text publications were assessed for eligibility, with two full publications excluded as ineligible. The main body of research indicates a trend towards the use of recombinant enzymes modified for improved physicochemical and catalytic properties. There is a noticeable trend towards the more widespread use of recombinant proteins produced by precision fermentation methods. General information on the application of recombinant proteins in the food industry is provided. The role of recombinant proteins in modern food production is highlighted.

Conclusion: The development of molecular biotechnology has led to the creation of new enzymes and proteins for the food industry, expanding their use in cheese making, confectionery, and baking. Challenges exist in developing new enzymes, expression systems for bioproduction, and bioprocesses with fundamentally new characteristics, leading to greater economic feasibility. The analysis revealed challenges related to the need for regulatory compliance with current capabilities and trends in the bioproduction of recombinant proteins for the food industry. The re-sults obtained can be used to improve the catalytic properties of recombinant enzymes and en-hance the stability of enzyme preparations. These findings are useful for the targeted develop-ment of recombinant protein and enzyme production systems, increasing their productivity through a better understanding of the main directions of the modern recombinant enzyme indus-try for food production.

Keywords: enzymes; recombinant proteins; producer strains; regulation of recombinant enzymes; genetically modified organisms; GM products; industrial protein synthesis biotechnology; protein bioengineering; genetically modified food sources

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Использование рекомбинантных белков в современной пищевой биотехнологии: обзор предметного поля

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АННОТАЦИЯ

Введение: С 1994 года динамичное развитие биотехнологии и широкое применение рекомбинантных ферментов привели к новым технологическим решениям в пищевом производстве. Современные технологии позволяют производить сахар, хлеб, пиво, сыр, колбасы и другие продукты с использованием биотехнологических процессов и промышленных пищевых ферментов. Биопродукция рекомбинантных белков заменила природные ферменты, предоставляя ферменты с улучшенными каталитическими функциями, стабильностью и расширенным диапазоном условий функционирования. Использование этих ферментов оказались экономически выгоднее по сравнению с природными и ранее использовавшимися рекомбинантными ферментами.

Цель: Выявить границы предметного поля по исследованию рекомбинантных белков и их роли в современном пищевом производстве за период с 1973 по 2024 гг.

Материалы и методы: Поиск источников осуществляли в базах данных PubMed, РИНЦ и Google Scholar. Методология обзора опиралась на протокол PRISMA-ScR. Хронологические рамки обзора: с 1973 по 2024 г.

Результаты: Первоначальный поиск по ключевым словам позволил выявить 121 источ-ник: 101 в базах данных и 20 из других источников. После удаления дубликатов осталось 113 источников. Оценено 111 полнотекстовых публикаций на приемлемость, в качестве неприемлемых исключены две публикации. Согласно основному массиву исследований, заметна тенденция к использованию модифицированных по физико-химическим и каталитическим свойствам рекомбинантных ферментов. Наблюдается тенденция к увеличению частотности использования рекомбинантных белков, продуцированных методами прецизионной ферментации. Приведены общие сведения о применении рекомбинантных белков в пищевой промышленности. Показана роль рекомбинантных белков в современной пищевой промышленности.

Выводы: Развитие молекулярной биотехнологии позволило создать новые ферменты и белки для нужд пищевой промышленности, расширив их использование в сыроделии, кондитерском производстве и хлебопечении. Существуют вызовы в разработке новых ферментов, экспрессионных систем для биопродукции и биопроцессов с принципиально новыми характеристиками, что приводит к большей экономической целесообразности. Анализ выявил вызовы, связанные с необходимостью соответствия нормативно-правовых актов текущим возможностям и тенденциям в области биопродукции рекомбинантных белков для пищевой промышленности. Полученные результаты могут быть использованы для улучшения каталитических особенностей рекомбинантных ферментов и повышения стабильности ферментных препаратов. Эти результаты полезны для направленной разработки систем продукции рекомбинантных белков и ферментов, увеличения их продуктивности за счет лучшего понимания основных направлений современной индустрии рекомбинантных ферментов для пищевого производства.

Ключевые слова: ферменты; рекомбинантные белки; штаммы-продуценты; регулирование рекомбинантных ферментов; генно-модифицированные организмы; ГМ-продукты; биотехнологии промышленного синтеза белков; биоинженерия белков; генетически-модифицированные источники пищи

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INTRODUCTION

The food industry is highly conservative in terms of product quality standards because people's health and safety depend on these standards. However, new food production technologies introduce new technological solutions. For instance, the rapid development of biotechnology that started back in the 1990s and continues to this day brought about such advanced solutions as recombinant enzymes produced by genetically modified organisms (Deckers, 2020), a rational design of highly efficient recombinant enzymes (Liu, 2024), precision fermentation that yields specific recombinant proteins (Ashok, 2023), etc. With the industry developing so fast, the flow of innovation is likely to increase (Siddiqui, 2023).

Proteins are natural high-molecular biopolymers that consist of amino acid residues linked together by peptide bonds. Most proteins contain twenty standard genetically encoded amino acid residues. Multiple combinations of amino acid residues make it possible to obtain different proteins that perform different functions (Tekaia, 2006). Recombinant proteins are proteins with an artificial DNA. They can be produced by bacteria (*E. coli*, *B. subtilis*), yeasts (*S. cerevisiae*, *P. pastoris*), or filamentous fungi (*A. niger*, *T. reesei*). This approach allows producers to standardize and scale up the processing and purifying (Crowell, 2021).

Since 2010, the bioproduction of recombinant proteins and enzymes has received a lot of public attention, which, in its turn, has increased scientific interest in such practical issues as genome editing of producer strains, metabolic engineering, and precision fermentation in the food industry (Deckers, 2020). Recombinant proteins can be divided into three categories (Augustin, 2023): enzymes used in food processing (Raveendran, 2018), taste-modifying proteins (Dufossé, 2019), and alternatives to natural proteins obtained by fermentation (Linder, 2023).

New methods of genetic engineering (Shankar, 2017), scale-up of fermentation processes, and isolation and purification of recombinant proteins have revolutionized the production of recombinant proteins (Augustin, 2023). These changes affect many areas of modern biotechnology, including food biotechnology. Traditional natural enzymes are becoming economically uncompetitive and give way to recombinant enzymes (Adrio, 2014).

The current review shows the main trends in the field of recombinant protein bioproduction and attempts to answer the following questions:

(1) What industries need amylases, proteases, and lipases? Why do recombinant enzymes replace natural ones?

(2) What technological platforms are used for the expression of recombinant enzymes?

(3) What are the new areas to employ recombinant proteins?

MATERIALS AND METHODS

Protocol and Transparency Statement

This review is a transparent, accurate, and honest report that relied on the PRISMA-ScR protocol.

Selection Criteria

The review included research articles and conference proceedings that met the selection criteria specified in Table 1. The sampling presupposed no linguistic or geographic limitations. The criteria were in line with the PCC principle, i.e., Population, Concept, Context (Table 1). Free access to full-text articles served as an additional selection criterion. In its absence, we sent a request to the authors; if the fill text was not provided, the work was excluded from the review.

Search Strategy

The review of articles registered in Scopus, PubMed, and ScienceDirect included the following search queries: "food enzyme", "food industry", "recombinant protein production", "enzyme", "amylase", "protease", and "lipase" with operators AND, OR. We filtered out such subject areas as "Health", "Social Sciences and Humanities", "Earth Sciences", "Pharmaceuticals", and "Toxicology and Pharmacology". The search query for Russian Research Citation Index was as follows: (enzymes OR recombinant protein) AND "food production" OR "regulation".

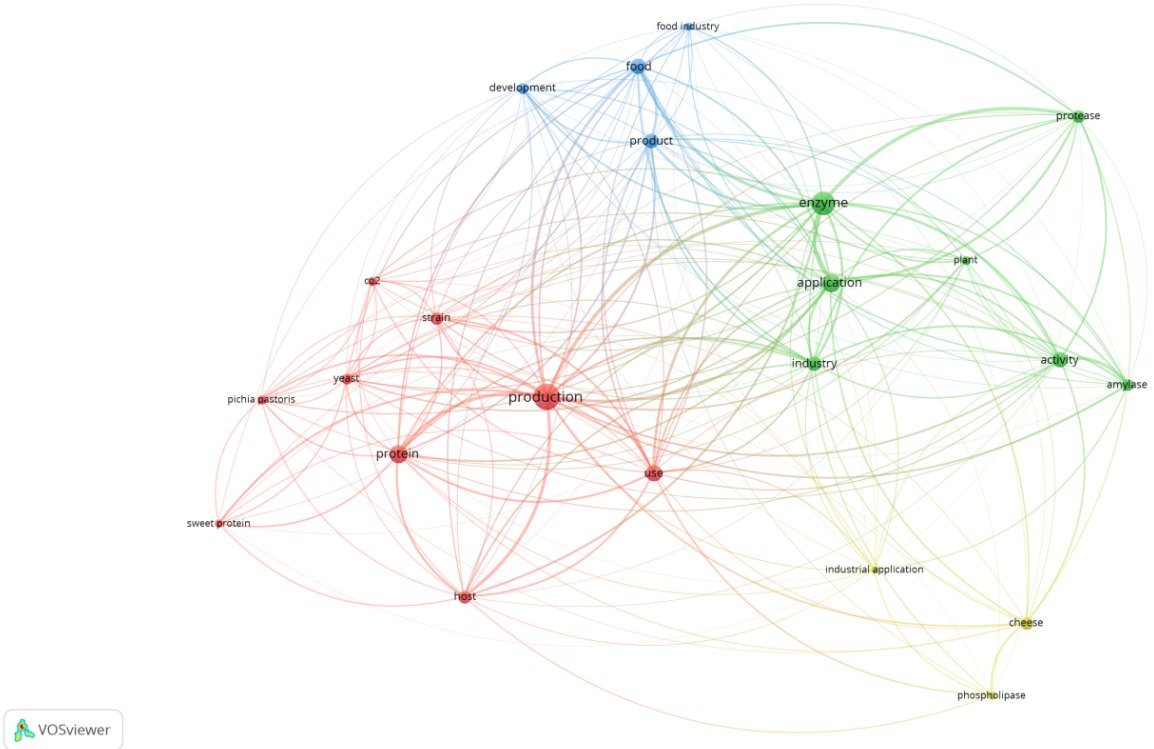
Table 1

Selection Criteria

Criterion	Included	Excluded	Grounds for exclusion
Population	New strains that produce recombinant proteins and enzymes in food technologies.	Transgenic plants / animals that produce recombinant proteins with predesigned properties; metabolic engineering of microorganisms with targeted properties to produce additional nutritional compounds; microorganisms with altered properties used in winemaking and lactic acid fermentation that yield a final product with targeted properties.	Microbial recombinant proteins and enzymes in the food industry.
Concept	Proteins and enzymes in food technologies, i.e., their classification, bioproducts, and food safety regulation in different countries.	Other biotechnological objects used in food production, e.g., lipids, vitamins, sugars, etc.	Recombinant proteins that facilitate the development of the food industry.
Context	Recombinant proteins in the food industry.	Other industries.	Papers that report the use of recombinant proteins in the food industry.
Language	No limitations	No limitations	Relevant sources published in any language.
Time	1973 — March 2024	Before 1973	No articles published before 1973 met the search requirements.
Origin	No limitations	No limitations	The issue of using recombinant proteins in the food industry has no geographic boundaries.

Figure 1

Keyword Frequency Analysis



Note. The larger the dot, the greater the frequency; the colored lines show the connections between the keywords.

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Selection Process

The search results were processed in line with the PRISMA-ScR protocol. First, the list of publications and abstracts were downloaded as *.ris* files and uploaded to the Zotero link manager. The full texts were downloaded as *.pdf*. Next, the Zotero list was checked for duplicates. After excluding duplicates, the articles were screened for compliance with the selection criteria in two stages, i.e., by title and abstract and by full text. The remaining publications entered the review.

Data Extraction and Analysis

The final sampling included authors' names, country of origin, study objective and design, conclusions, and the year of publication. All sources were processed in VOSViewer to visualize the frequency of keywords (Figures 1, 2, 3).

Figure 2

Keyword Frequency Analysis by Year

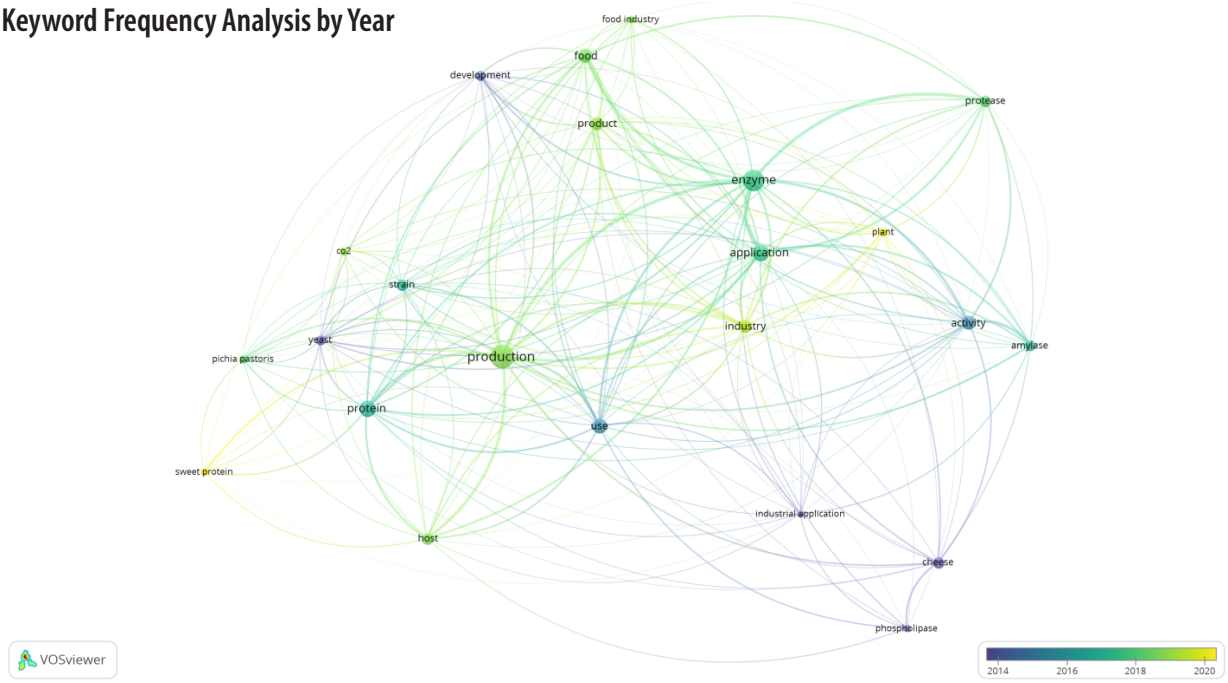
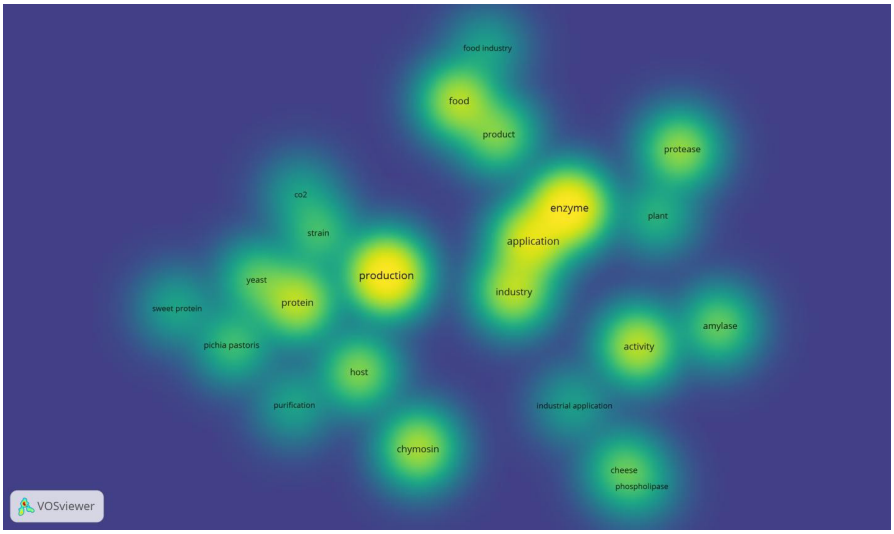


Figure 3

Keyword Density Analysis



Note. The larger and brighter the yellow circle, the greater the density.

RESULTS AND DISCUSSION

This review covered 109 sources: 22 from the United States, 31 from the European Union (including 11 from Germany), 17 from the Russian Federation, 24 from the Global South (including 10 from India and 5 authors from China). As for the language, 83.5 % were published in English and 16.5 % in Russian.

Chronologically, 79.2 % were published in 2014–2024, with 13.2 % published in 2021 or 2023.

The VOSViewer keyword network recorded clustering by industry. The cluster for the search-word "enzymes" could be divided into three parts, i.e., enzymes in cheese production, amylolytic enzymes, and proteases. A separate cluster related to targeted yeast fermentation included the expression of various target proteins in *Pichia pastoris* and sweetener proteins.

Enzymes in the Food Industry

General Characteristics

Many food products involve industrial food enzymes. Table 2 lists the most popular industrial enzymes.

Table 2

Industrial Enzymes

Code	Food enzyme
A.01	α-acetolactate decarboxylase
A.02	Aminopeptidase
A.1	Amylase
	(i) β-Amylase
A.2	Amylase (maltogenic)
	(i) α-Amylase (maltogenic)
A.3	Asparaginase
B.1	Bovine rennet
B.2	Bromelain
C.01	Carboxypeptidase D
	(i) Carboxypeptidase D
C.1	Catalase
C.2	Cellulase
C.3	Chymosin
C.4	Cyprosin (natural / recombinant)
F.1	Ficin
G.1	Glucoamylase
G.2	β-Glucanase
G.3	Glucoxidase
G.4	Glucoisomerase
G.5	Glutaminase
H.1	Hemicellulase
H.2	Hexose oxidase
I.01	Inulinase
I.1	Invertase
L.1	Lactase
L.2	Lipase

Continuation of theTable 2

Code	Food enzyme
L.3	Lipoxidase
L.4	Lysozyme
M.01	Mannanase
M.1	Milk-clotting enzyme
P.1	Pancreatin
P.2	Papain
P.3	Pectinase
	(i) Pectin lyase
	(ii) Pectinesterase
	(iii) Polygalacturonase
P.4	Pentosanase
P.5	Pepsin
P.5.1	Peroxidase
P.5.2	Phospholipase
	(i) Lysophospholipase
P.6	Protease
	(i) Acid prolyl endopeptidase
	(ii) Thermolysin
	(iii) Subtilisin
P.6.1	Protein glutaminase
P.7	Pullulanase
R.1	Rennet
T.01	Transglutaminase
T.1	Trypsin
U.1	Urease
X.1	Xylanase

Note. Table 2 relies on the List of Permitted Food Enzymes in Canada: SOR/2012–206 Government of Canada (2023)[1] as adapted by Dekkers *et al*. (Dekkers, 2021).

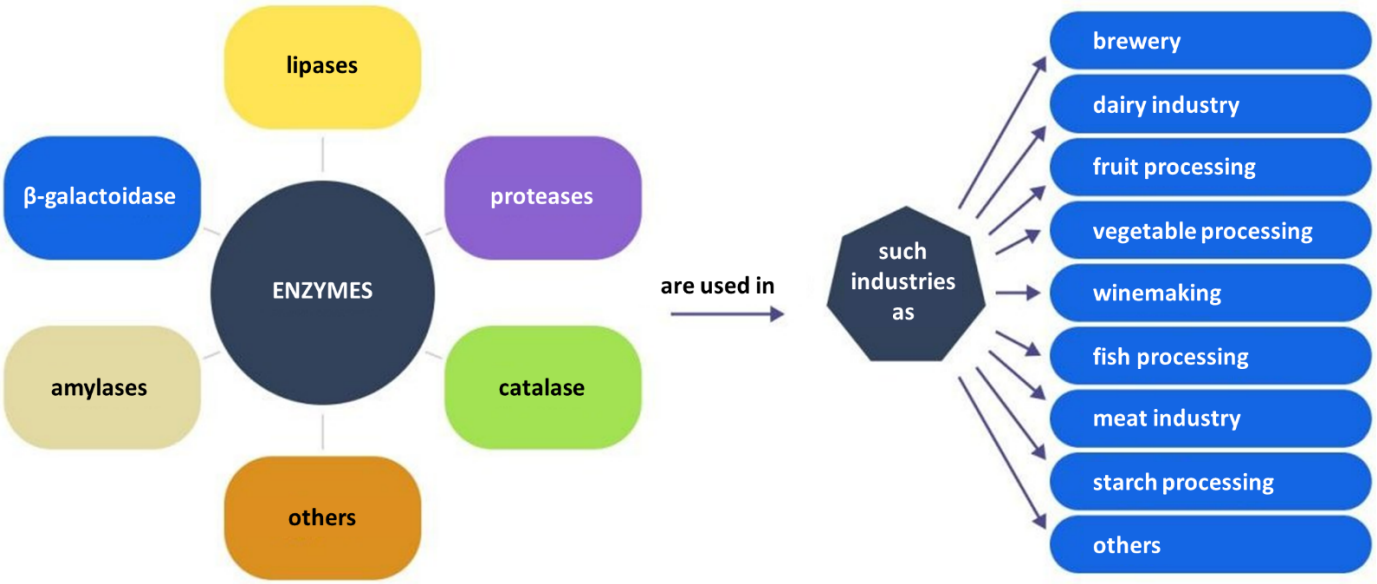
The food industry uses enzymes to process proteins, fats, and carbohydrates (Dahiya, 2020). Proteases break down individual proteins into monomers; amylases, glucoamylases, and other enzymes hydrolyze complex hydrocarbons; lipases and phospholipases break down lipids and emulsify fatty acids. Each enzyme requires optimal chemical sequence, expression system, producer strain, and cell culture environment, as well as an optimal method for isolation, purification, storage, and technological application (Figure 4).

Food enzymes can be classified by the reaction they catalyze into hydrolases, esterases, oxidases, etc., see Table 3 (Motta, 2023). Another classification takes into account technological processes and the enzymes involved. Table 4 visualizes the main milestones in the industrial use of enzymes and shows the prospects of the industry (Singh, 2016).

[1] Government of Canada (2023). List of Permitted Food Enzymes (Lists of Permitted Food Additives).

Figure 4

Industrial Enzymes and Their Applications



Note: Figure 4 was compiled based on Kaur & Gill (Kaur, 2019).

Table 3

Food Enzymes Classified by Reaction

Class	Functions	Subclasses of enzymes in foods
Oxidoreductase	catalyzes oxidation-reduction reactions	peroxidase, polyphenol oxidase, catalase, lipoxygenase, glucose oxidase
Transferase	transfers chemical groups from the substrate to acceptor molecules (except hydrogen and water)	transglutaminase
Hydrolase	catalyzes the reaction by adding water molecules, thus breaking several chemical bonds	amylase, invertase, lactase, lipase, pectinase, lysozyme, proteases
Lyase	catalyzes bonding and breaking, except for hydrolysis or oxidation-reduction reactions	pectin lyase, pectate lyase
Isomerase	catalyzes isomerization (isomers have the same molecular formula but different structural properties)	glucose isomerase

Note: Table 3 was compiled based on Motta *et al.* (Motta 2023).

Table 4

Enzymes by Food Industry Sector

Food industry sector	Enzyme	Use	Producer
Bakery	Amylase	increases softness	*Aspergillus sp., Bacillus sp.*
	α-Amylase	increases shelf-life	*Bacillus strearothermophilus*
	Xylanase	improves punching	*Aspergillus niger*
	Lipase	improves punching	*Aspergillus niger*
	Transglutaminase	facilitates dough-making	*Streptomyces sp.*
	Neutral protease	stabilizes pasta	*Aspergillus oryzae*
Dairy industry	Chymosin	facilitates cheese production	*Aspergillus sp., Kluyveromyces lactis*
	Lipases	enhance cheese taste and aroma, reduce maturation period	*Aspergillus oryzae*
	β-Galactosidase	yields lactose-free products	*E. coli, Kluyveromyces lactis*
	Aminopeptidase	facilitates cheese maturation	*Lactobacillus sp.*
	Catalase	facilitates cheese maturation	*Aspergillus niger*
	Transglutaminase	facilitates milk processing	*Streptomyces sp.*
Juices and soft drinks; wine and liquor industry; brewery	Glucose oxidase	removes oxygen to increase the shelf-life of beer	*Aspergillus niger, Pichia pastoris*
	Cellulase	facilitates fruit liquefaction	*Aspergillus niger*
	Neutral protease	facilitates wine clarification, brewing	*Aspergillus oryzae*
	β-Amylase	facilitates starch hydrolysis	*Bacillus, Streptomyces*
	β-Glucanase	facilitates clarification of beverages	*Bacillus subtilis, Aspergillus spp.*
	Protease	facilitates clarification of beverages	*Aspergillus niger*
	Pullulanase (amylo-α-1,6-glucosidase)	saccharifies starch	*Bacillus sp.*
	Naringanase	debitters citrus drinks	*Aspergillus niger*
	Limoninase	debitters citrus drinks	*Aspergillus niger, A.oryzae*
	Aminopeptidases	facilitate protein degradation	*Lactobacillus brevis*
	Pectinase	facilitates pectin removal	A.oryzae, Penicillium funiculosum

Note. Table 4 was compiled based on Singh *et al.* (Singh, 2016).

Food enzymes speed up technological processes, increase the yield and quality of finished products, save valuable raw materials, and reduce waste (Borrelli, 2015). Enzyme preparations can be natural, e.g., of plant or animal origin, and artificial, i.e., recombinant (Robinson, 2015). Enzyme preparations of animal origin come from organs and tissues of farm animals. For example, rennet, which is a mixof 70 % chymosin and 30 % pepsin, is used in cheese making (Kumar, 2010).

Natural plant enzymes are obtained by squeezing juice from various plant parts. For instance, the proteases papain and bromelain are obtained from the sap of *Cárica papáya* and the core of *Ananas comosus*, respectively (Fernández-Lucas, 2017). Ficin is another popular industrial enzyme. It is obtained from stems and leaves of *Ficus insipida* (Aider, 2021). Bromelain is a mix of four different proteases. Papain, ficin, and bromelain tenderize meat, etc. (Arshad, 2014).

Enzymes can be produced by special strains of microorganisms, bacteria, mycelial fungi, and yeasts (Cairns, 2018). Recombinant enzymes are grown in artificially created microorganisms; recombinant enzymes are no different from natural ones. Natural strains were used for enzyme production in the early XIX century. Initially obtained by selection, artificial microbial enzyme producers are now created by genetic engineering (Ashok, 2023).

Enzymes are of enormous benefit for the food industry. They facilitate technological processes, increase the yield of finished products, and improve their sensory properties, e.g., taste, smell, texture, color, appearance, etc. (Solanki, 2021). Biotechnology is especially effective when enzymes are needed in big quantities. For example, traditional rennet production presupposes chymosin extraction from calf abomasum. To obtain 10 kg of rennet, a calf has to be fed and cared after for several months. However, a fermenter of 1,000 L with the recombinant chymosin producer *Bacillus subtilis* yields 20 kg of enzyme in 12 h (Robinson, 2015). Apparently, recombinant microorganisms are both economically and ethically preferable.

Producer strains are created in line with the following a protocol: select the optimal expression model; select the vector for the expression of the target gene; select individual clones; choose the optimal purification scheme (Spohner, 2015). Yet, each project is unique because it depends on the physicochemical properties of the enzyme and its function (Robinson, 2015). Not only the yield of recombinant enzyme is important, but also the efficiency, scalability, and affordability of the purification method. The more enzyme is needed, the cheaper and easier the biotechnological process should be (Khootama, 2018). To meet these requirements, all elements of the process require constant improvements, not only the producer strain and the fermentation process. The recombinant protein is also genetically refined: for instance, its catalytic functions and specificity can be advanced by protein engineering (Liu, 2019). One and the same recombinant enzyme can be used in different food production sectors. For example, α-amylase is part of glucose-fructose syrup, bakery, and juices (Farooq, 2021).

Many of the abovementioned recombinant proteins are used as complex solutions. Enzyme mixes emulsify egg yolk fats, clarify wine and fruit juices, facilitate beer brewing, etc. Ready-made enzyme mixes are used in cheese production and bakery (De Maria, 2007). Ready-made stratified enzyme mixes make it possible to standardize formulations and monitor the quality of the final product.

Microorganisms that produce recombinant proteins are diverse (Deckers, 2020). Bacterial production systems are easy and cheap. However, they lack a complex eukaryotic folding system and can produce only the simplest proteins (Baeshen, 2015). Bacterial systems cannot produce proteins with post-translational modifications, such as glycoproteins. Moreover, they produce too much side-protein and require a more complex purification (Jia, 2016). *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus amyloliquefaciens* are the most popular bacterial systems that produce recombinant proteins. *E. coli* allow for more diverse molecular biological tools, but *Bacillus* strains give a higher yield of recombinant protein, especially α-amylase and various proteases (Vojnovic, 2024).

As for mycelial fungi, *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei* are often used as expression systems. They give high yields of recombinant proteins, especially if the original enzyme was also expressed in fungi (Meyer, 2015). Although mycelial fungi have a eukaryotic folding system, they are more effort-consuming and produce many by-products, which complicates purification. Fungi-based expression systems are popular producers of lipases, glucose oxidase, and many other enzymes (Meyer, 2008).

Yeasts have a good potential as a platform for recombinant enzyme production (Spohner, 2015). They have strong promoters, produce relatively pure recombinant protein with a high expression level, and possess a eukaryotic folding and glycosylation system. *P. pastoris* expression system demonstrates all these advantages. It produces such recombinant enzymes as inulase (Zhang, 2004), laccase (Püllmann, 2021), lactase (Bankefa, 2022; Sun, 2017), and phytase (Herrera-Estala, 2022).

The total global market for industrial enzymes was estimated at $7.4 billion in 2023 and is growing at an average rate of 6.6% per year[1]. At the end of 2022, the global whey protein market was $19.6 billion; by 2032, it will have reached $40.3 billion[2]. The demand for lactose-

[1] Formulation and Region - Global Forecast to 2028. (2023) Industrial enzymes markets by type (Carbohydrases, Proteases, Lipases, Polymerases, Nucleases). https://www.marketsandmarkets.com/Market-Reports/industrial-enzymes-market-237327836.html

[2] Shahbandeh M. (2023) Value of the whey protein market worldwide from 2022 to 2032. https://www.statista.com/statistics/728005/global-whey-protein-market-size/

free protein is also growing: 68 % of the global population is believed to be lactose intolerant (Bayless, 2017). The largest enzyme producers are Novozymes (Denmark), BASF (Germany), DSM (Netherlands), Epigen Labs (UAE), Du Pont (USA), Tex Biosciences (India), and Chr.Hansen (Denmark). The Russian market of food enzymes is dominated by such foreign companies as Barentz Group, Chr. Hansen, Kerry Group, Cargill Inc., and ADM. It was expected to grow by 5.3 % per year in 2020–2025[1].

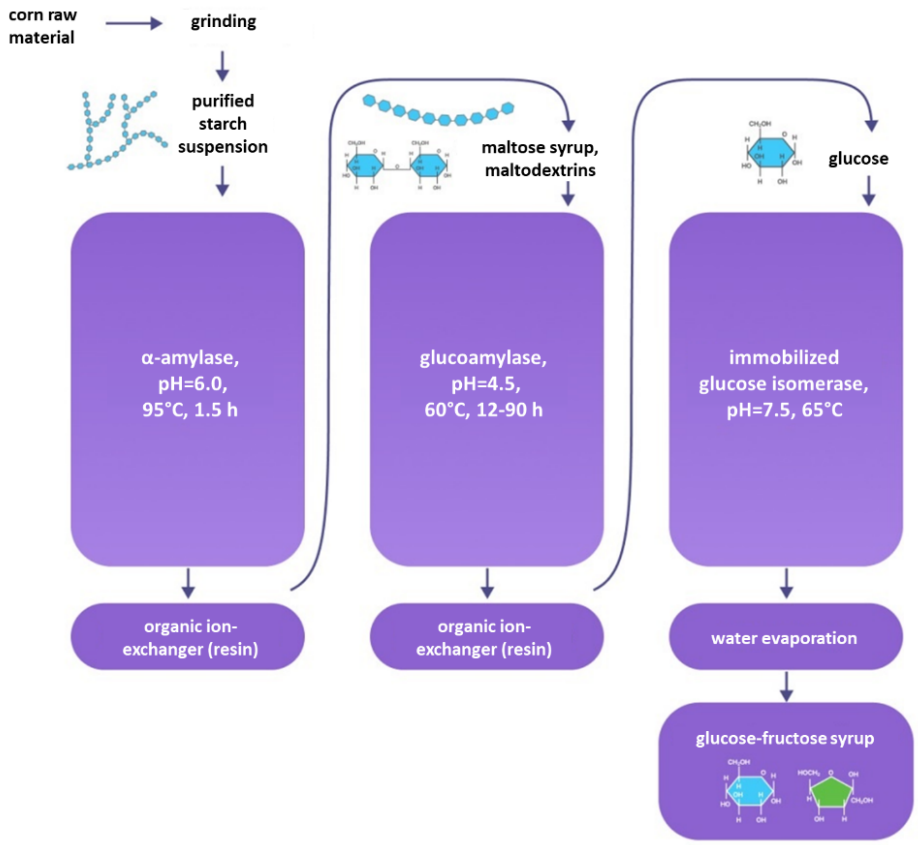
Production of Glucose-Fructose Syrup: Amylase and Glucoamylase

Sugar and such sugar-containing products as honey or fruits have always been part of the human diet. Sucrose remains the main measure of sweet taste. However, the industrial production of sucrose from sugar cane and beet faces a number of technical difficulties, e.g., purification and syrup refining.

Glucose-fructose syrup is a liquid sweetener used in many foods and drinks. Glucose-fructose syrup is the largest technological production in the food industry and an unrivaled triumph of biotechnology. The global production volume is 20 million tons per year[2]: indeed, modern technology is so advanced that it is capable of producing such fantastic volumes. Corn is the most popular raw material for glucose-fructose syrup, although wheat and potato starch are also used in Europe and the Russian Federation. The first enzymatic approach to glucose-fructose syrup dates back to the early 1960s in Japan, followed by industrial production in the late 1960s. Glucose-fructose syrup production has been growing for

Figure 5

Enzymatic Stages During Glucose-Fructose Syrup Production



Note: Figure 5 was compiled based on (Casey, 1976).

[1] Mordor intelligence. (2023) Analysis of the size and share of the food enzymes market in Russia: growth trends and forecasts (2023–2028). https://www.mordorintelligence.com/ru/industry-reports/russia-food-enzymes-market

[2] Bode J. W. (2018). Corn Refiners Association Industry Overview 2017. https://corn.org/wp-content/uploads/2018/04/CRA-Industry-Overview-2017.pdf

over 35 years and is currently one of the most successful projects (White, 2008).

Glucose-fructose syrup is an important alternative to sucrose. It is stable in liquid form and can be used in acidic products. Initially developed in Japan, its technology was actively popularized in the United States to stabilize sugar prices: corn as the main raw material made it possible to lower the price of sugar syrup, which made the United States independent from South-American sugar cane (Ballinger, 1978).

Glucose-fructose syrup is as safe as sucrose, glucose, or fructose; it has no negative impact on health, except teeth (Glinsmann, 1986). Obesity and microflora issues are a matter of wise consumption, which makes it the responsibility of consumers and nutritionists, not regulatory agencies (Bray, 2004).

Glucose-fructose syrup production involves at least three different enzymes, i.e., amylase, glucoamylase, and glucose isomerase (Figure 5).

First, corn kernels are cleaned of foreign impurities and soaked in warm water with SO_2. After softening, they are ground into a starch suspension, which is split into shorter oligosaccharides under the action of immobilized α-amylase. Under the action of glucoamylase, they split into monosaccharides and glucose. The resulting solution undergoes demineralization using an ion-exchange resin. Finally, it goes through immobilized glucose isomerase to obtain high fructose corn syrup (HFCS 42) with 50–52 % glucose and 42 % fructose (Bhosale, 1996) (Table 5).

This process involves three enzymes, i.e., α-amylase, glucoamylase, and glucose isomerase. They are recombinant proteins produced by microorganisms. α-Amylase accounts for 25–30 % of the total recombinant enzyme market (Singh, 2022). Amylase was discovered in St. Petersburg, Russia, in 1811 by K. S. Kirchhoff as a result of observing starch degradation. In 1833, E. Leutsch described a certain *ptyalin* involved in starch hydrolysis in saliva. In 1833, the French chemists A. Payen and J. Persaud isolated amylase and called it *diastase*. In 1894, J. Takamine patented the industrial production of amylase from *Aspergillus oryzae*, which became the first microbial enzyme obtained in the United States. The term was coined by α-amylase in 1925 by R. Kuhn (Singh, 2022).

The enzymatic technology for glucose-fructose syrup production has undergone numerous qualitative changes. The catalytic activities of amylase, glucoamylase, and glucose isomerase have improved; a new, enzyme-saving technology for immobilizing glucose isomerase has appeared. However, the main problem remains: all three enzymes require different conditions, temperatures, and pH. As a result, multi-ton production needs ion-exchangers in great amounts (Bessler, 2003). Bringing the catalytic conditions of the three enzymes to a single format remains an urgent biotechnological problem. If rational protein engineering solves it, the production will be even cheaper and more sustainable.

Lipases, Phospholipases, and Enzymes for Emulsification

Lipases and phospholipases are in high demand in the food industry (Fernandes, 2010; Filkin, 2020). Lipases are used in the production of edible oils, dairy products, bakery products, or emulsifiers (De Maria, 2007; Raveendran, 2018; Alekseenko, 2008). Phospholipase A2 secreted from porcine pancreas or snake venoms has long been used as an egg yolk emulsifier in mayonnaise, sauces, bakery, or refining of vegetable oils (De Maria, 2007).

Table 5

Carbohydrate Composition of Basic Sweeteners

Component	HFCS-42, %	HFCS-55, %	Corn syrup, %	Fructose, %	Sucrose, %	Invert sugar, %	Honey, %
Fructose	42	55	0	100	50	45	49
Glucose	53	42	100	0	50	45	43
Others	5	3	100	0	0	10	5
Moisture content	29	23	20	5	5	25	18

Note: Table 5 was compiled based on (White, 2008).

In 2024, commercial products with phospholipases A2 can be found under different trade names. Lecitase® 10 L (Novozyme A/S, Denmark) is a phospholipase of animal origin obtained from porcine pancreas and originally developed for degumming vegetable oils. Microbial phospholipases Rohalase® MPL (AB Enzymes, Germany) and Maxapal®A2 (DSM Food Specialties, Netherlands) are produced using *A. niger*; it improves the emulsifying properties of eggs and egg yolk. The microbial phospholipases in the enzyme mixes CakeZyme® and BakeZyme® (DMS Food Specialties, Netherlands) are used for baking purposes (Filkin, 2020).

Expression systems based on *Streptomyces sp*. and *A. niger* are the most common strains for large-scale industrial production. These strains account for 80 % of the total oil degumming production market (Borrelli, 2015). Enzymes can be reused or immobilized, which increases the yield and reduces operating costs. Therefore, the use of phospholipases in food oil seems an attractive alternative to more traditional degumming methods (Khamies, 2024). Phospholipases make it possible to reduce water consumption and CO_2 emissions by 12,000 tons per year during industrial degumming of vegetable oil (Borrelli, 2015).

Bakery

Bakery is another sector of the food industry that needs lipases. Various emulsification methods facilitate processing and help to utilize poor-quantity and low-quality raw materials. These methods include adding amylases, oxidases, hemicellulases, and proteases during dough preparation (Fraatz, 2014). Of all the enzymes used in the food industry, one third goes to satisfy bakery needs (Casado, 2012).

Dough contains a relatively small amount of lipids, e.g., 2–2.8 % solids in wheat flour. However, their ability to form lipid monolayers at the gas/liquid interface affects the stability of air bubbles. Unfortunately, this amount of lipids is not enough to maintain the quality of dough and bakery products. Exogenous lipids or emulsifiers improve the quality and increase the shelf-life of bakery products by stabilizing air bubbles, which increases bread volume, improves gluten qualities, strengthens the structure, and prolongs shelf-life (De Maria, 2007).

Dairy Products

In dairy products, lipases improve fat stability or increase the yield of cheese, butter, and ice cream. Traditionally, they facilitate cheese maturation or are used to obtain lipolyzed milk fat, a popular flavoring agent in butter, coffee, cheese, or chocolate (Ardö, 2021).

Milk is a complex water-based dispersion. It contains proteins, fats, carbohydrates, and various salts. Lipids are present in milk as dispersed droplets that stabilize globules with milk fat and prevent coagulation. In milk powder, phospholipid molecules coat the powder particles, thus improving the thermal stability of reconstituted milk. Phospholipids give additional volume and stability to ice-cream, which is actually a foam or emulsion with ice crystals and an unfrozen fraction with proteins (Rathnakumar, 2023).

YieldMAX® (Novozymes A/S) consists of phospholipases of the PLA1, PLA2, and PLB types. In mozzarella cheeses, this commercial product was reported to increase the yield by 0.7–3.8 %. Lysophospholipids in the cheese composition demonstrated greater emulsifying properties than phospholipids; as a result, more milk fat and proteins were captured by the cheese mass and did not remain in the whey. In addition, phospholipases increased the yield of low-fat cheese (<5 % solids) (Lilbaek, 2006).

FoodPro CLEANLINE (Danisco A/S) is another lipase preparation with acyltransferase activity that catalyzes the transfer of acyl groups from the *sn*-2 position of milk cholesterol, leading to the formation of lysophospholipids and esters. These lysophospholipids have high surface activity, which reduces the amount of heat-treatment by-products, e.g., caramelized proteins on container walls, thus reducing the cleaning costs (Berestova, 2014).

Egg Yolk Processing

Emulsions are a separate class of dispersed systems formed from two immiscible liquids, one of which is distributed in the other as tiny droplets. Emulsions can be direct (oil-water) and inverse (water-oil), depending on which phase is in the dispersed state. As for the concentration of the dispersed phase, emulsions can be diluted, concentrated, and highly concentrated. Egg yolk is a good emulsifier. Its emulsifying capacity and consumer properties can be increased by pre-treating it with preparations of phospholipase A2 (Karray, 2012).

Chymosin and Its Improvement Options

Chymosin is an aspartate endopeptidase that catalyzes the break of the Phe105-Met106 peptide bond of κ-casein. Chymosin is a major part of the rennet enzyme complex and has been used in the cheese industry for millennia. Natural chymosin is obtained from young calves' rennet, ground and washed with salt. The resulting enzyme mix is 70 % chymosin and 30 % pepsin (Kumar, 2010).

The earliest industrial method for chymosin purification was invented in 1874 by Ch. Hansen. Since then, chymosin has become one of the best-studied proteins. New methods for producing recombinant bovine chymosin from the fungus *Aspergillus niger* and the yeast *Kluyveromyces lactis* appeared in the 1990s (Bodie, 1994). Although the production involves a genetically modified organism, the finished cheese product contains no foreign genetic material. The global share of recombinant chymosin continues to grow (Kumar, 2010).

Since 2004, scientists have been trying to improve the physicochemical properties of recombinant chymosin using rational protein engineering methods. For instance, recombinant camel chymosin is gaining popularity due to its good thermal stability and specificity to κ-casein (Kappeler, 2006). Recombinant camel chymosin serves as a basis for even more specific and stable variants (Jaeckel, 2016; Pushkarev, 2023).

Transglutaminase

Recombinant enzymes are also used in meat processing. Papain and other proteases tenderize meat raw materials (Makhova, 2019). Transglutaminase provides cross-linking agents to form integral polymer structures (Abril, 2023).

Transglutaminase is an aminotransferase that catalyzes the transamination reaction. It transfers acyl groups between the γ-carboxyamide group to form a peptide bond between the side chains of glutamine and various primary amines. The ε-amino groups of lysine bases lead to the formation of ε-N-(γ-glutamyl)-lysine bonds. Transglutaminase is found in various eukaryotes and prokaryotes, including mTG transglutaminase from *Streptomyces sp*. (Martins, 2014).

The Japanese have used fish paste to form *surimi* fish sticks since the XII century. This fish paste contains natural transglutaminase, which makes the *surimi* method an iconic example of using transglutaminase in the food industry (Petcharat, 2018). The method has recently gained popularity in the food industry. Today, transglutaminase improves the texture of meat and dairy products, as well as the elasticity of candies, and emulsifies gluten in the bakery industry (Bagryantseva, 2021).

Since 2010, more and more information has emerged about the potential harm of transglutaminase in bakery products. It increases the immunogenicity of gluten, and, apparently, leads to the development of such autoimmune diseases as caecalysia (Lerner, 2021). However, microbial transglutaminase is approved for use in the USA (FDA, 2002), in the European Union (EU Regulation No. 1169/2011, October 25, 2011), but the final product is labelled as "formed" or "restructured" (Lerner, 2021). Technical regulation CU 029/2012 "Safety requirements for food additives, flavorings, and processing aids" prohibits the use of transglutaminase in the Eurasian Economic Union[1].

Yet, 600 tons of microbial transglutaminase are imported into the Russian Federation every year[2]. According to Russian Consumer Protection Agency Rospotrebnadzor, this enzyme is found in Russian products, but no approved and accredited methods have been developed to determine its activity in the final product.

Proteins as Flavoring Agents

The first ideas about using proteins as flavor-changing food additives appeared in the 2010s. The so-called sweet proteins have attracted the most attention (Joseph, 2019). Sweet proteins include small proteins (6–22 kDa) of plant origin, which were discovered in the 1970s, e.g., thaumatin, monelin, brazzein, pentadine, mabinlin, curculin, etc. (Table 6).

Thaumatin was first obtained from the fruit of the katemfe tree (*Thaumattococcus danielli*). Natural thaumatin started to be used as a sweetener in the 1970s. The best expression results for recombinant thaumatin belong to the methylotrophic yeast *P. pastoris* (Healey, 2017).

[1] Council of the Eurasian Economic Commission (September 18, 2014). Technical Regulations of the Customs Union TR CU 029/2012 Safety requirements for food additives, flavorings, and processing aids. https://docs.cntd.ru/document/902359401

[2] Transglutaminization of Russia. Factor 13. (August 29, 2016) https://sfera.fm/articles/myasnaya/transglyutaminazatsiya-rossii.-13-i-faktor

Table 6

Proteins as Flavoring Agents

Protein	Natural source	Peptide length in amino acid residues	Sweetness against sucrose	FDA/EFSA status	Application
Thaumatin 22 kDa	*Thaumatococcus daniellii Benth* (Katemfe fruit, West Africa)	One polypeptide chain of 207 amino acid residues with eight intramolecular disulfide bonds (monomer)	1,600 x	Sweetener, approved in 2018; sweetener and flavor enhancer/ modifier, approved in 2020	Flavor enhancer in ice-cream, chewing gum, dairy products, pet food, soft drinks; masks unwanted taste in food and pharmaceuticals (Kelada, 2021)
Monellin 10.7 kDa	*Dioscoreophyllum Cumminsii diels* (Serendipity berry, West Africa)	Two polypeptide chains: chain A of 44 amino acid residues, chain B of 50 amino acid residues (dimer A+B).	3,000 x	No legal status in the USA (FDA / EFSA)	Not used in the food industry due to low stability in thermal or acidic environments
Brazzein 6.4 kDa	*Pentadiplandra brazzeana* (fruit, West Africa)	One chain of 54 amino acid residues with eight disulfide bonds (monomer)	500–2,000 x	Flavor enhancer in citric acid drinks that reduces the off-flavor of other sweeteners (stevioside, acesulfame-K, or aspartame).	Needs more research to improve taste profile
Mabinlin 12.3 kDa	*Capparis masaki* (plant, leaves and flowers, China)	Two polypeptide chains: chain A of 33 amino acid residues, chain B of 72 amino acid residues (dimer A + B).	400 x	Not approved	
Pentadine 12 kDa	*Pentadiplandra brazzeana* (fruit, Africa)	No data	500 x	Not approved	Flavor modifier; as debittering agent with saccharin

Note: Table 6 was compiled based on Farag *et al*. (Farag, 2022).

Recombinant thaumatin is currently approved in the United States as an artificial sweetener in ice-cream, chewing gum, pet food, and other products (Kelada, 2021). Brazzein is smaller in size, which makes it a more convenient object for recombinant production (Bilal, 2022). Some substitutions were reported to enhance the sweet taste of brazzein by 18 times (H31R, E36D, E41A) (Lee, 2013).

Recombinant myoglobin is becoming popular as a beef-flavoring agent in meat substitutes based on plant soy and pea proteins (Yu, 2023). Impossible Foods company produces myoglobin and heme-containing recombinant proteins using *P. pastoris* (Roy-Chaudhuris, 2020). This technology has been approved by the FDA. In the future, new recombinant proteins and peptides are likely to yield more flavor combinations and flavor enhancers.

Protein Substitutes of Staple Foods: Precision Fermentation

Food products with animal proteins may become scarce one day. In 2022, the global population reached 8 billion people and continues to grow rapidly. According to UN forecasts, it will reach 9.7 billion by 2050[1]. The larger the population, the more high-quality food needs to be produced. Humanity has found itself in an "ecological trap": with not enough arable land, the expansion of the agro-industrial complex will lead to air and soil pollution, which are no less dangerous for humanity than famine. The food industry is responsible for a third of greenhouse emissions (Crippa, 2021); the meet industry requires 77 % of arable land while producing 38 % of food protein[2].

[1] https://www.un.org/en/global-issues/population#:~:text=Our %20growing %20population&text=The %20world's %20population %20is %20expected,billion %20in %20the %20mid %2D2080s.

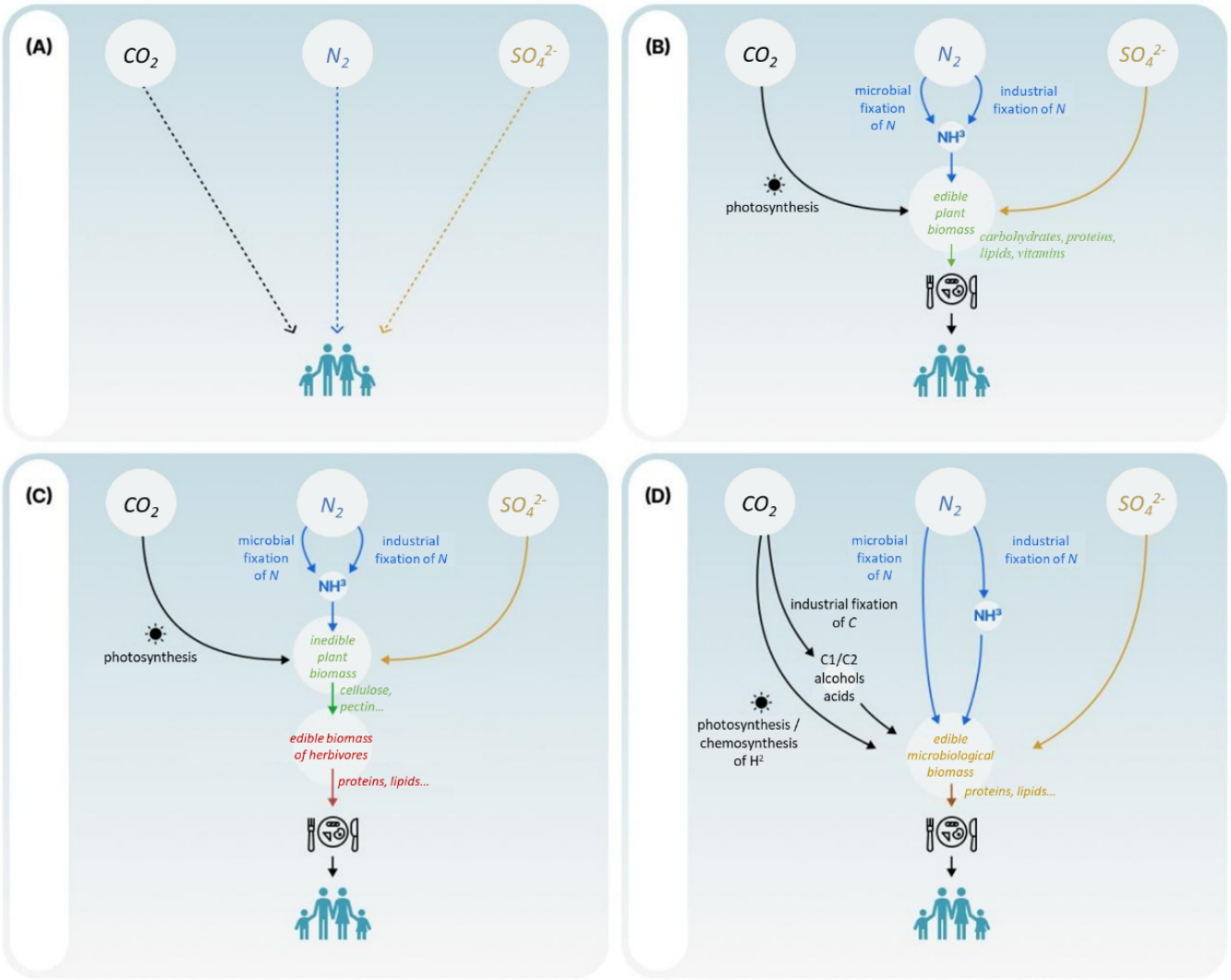
[2] Mridul A. (2023, September 5). 8 Charts That Illustrate the Impact of Food Systems and Our Diets on Climate Change. https://www.greenqueen.com.hk/stats-charts-facts-climate-change-impact-food-systems-diet-agricultu/].

Some technical solutions make it possible to achieve sustainable development in food production. Ephemeralization is the ability of technological progress to produce more and more with less and less using new technologies with greater output of produce, service, or data and less input, i.e., effort, time, materials, etc. (Fuller, 1973). Ephemeralization in food biotechnology faces the problem of obtaining affordable animal protein with low energy costs and environmental load (Linder, 2019). Microorganisms created to produce recombinant animal protein are a potential option to reduce the current anthropogenic load on our planet (Figure 6).

Precision fermentation is a relatively new term. It describes the bioproduction of a particular recombinant protein or other biosynthetic products by an engineered microorganism, followed by purification for food consumption. This process reduces water and energy consumption, as well as CO_2 and CH4 emissions during protein production (Figure 7).

Figure 6

A Biocatalytic View of Food Production and Human Nutrition



Note. Figure 6 was compiled based on (Linder, 2023): (A) People cannot metabolize the most abundant naturally occurring inorganic forms of carbon (carbon dioxide), nitrogen (N2), and sulfur (sulfate). (B) Edible plants allow people to acquire inorganic carbon, nitrogen, and sulfur indirectly. (C) Herbivores provide people with indirect access to nutrients from inedible biomass. (D) Edible microbial biomass provides people with inorganic carbon, nitrogen, and sulfur without photosynthesis.

Figure 7

Biocatalytic View of Food Production and Human Nutrition

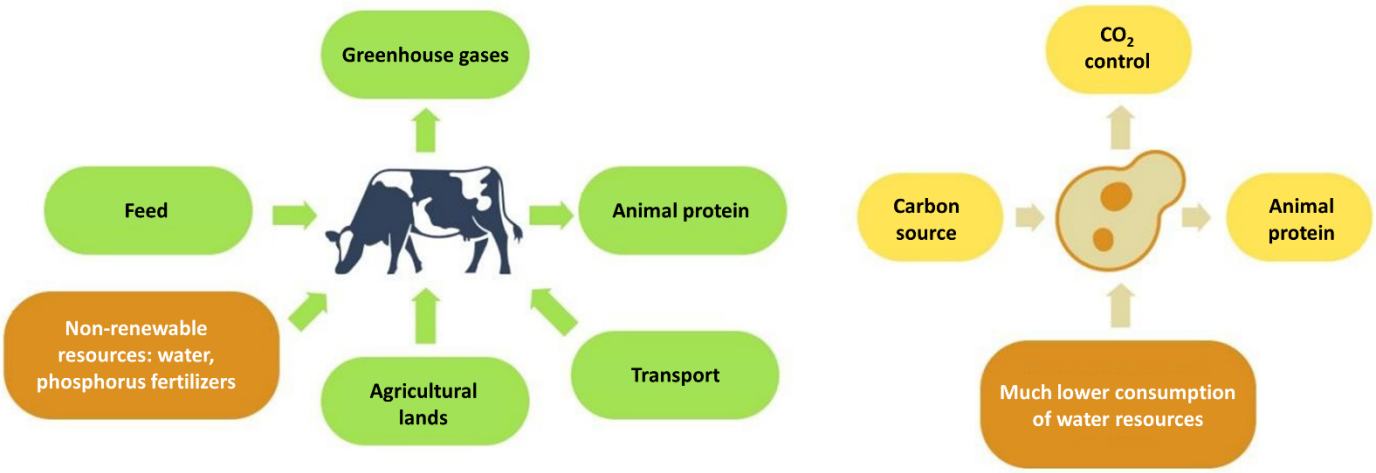


Table 7

Precision Fermentation Products

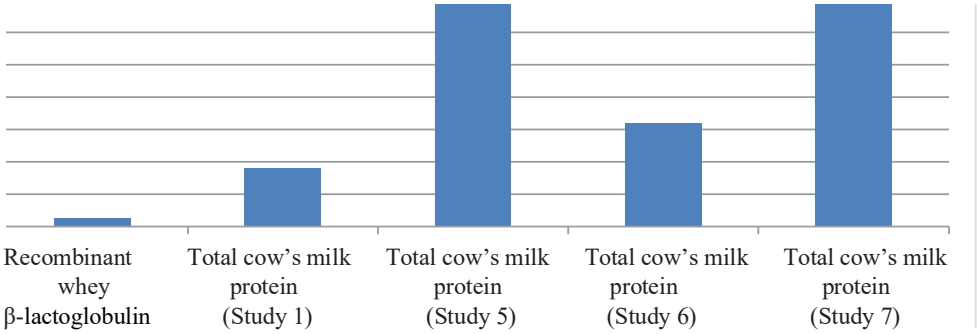
Company	Product	Source of carbon
EVERY Company	Chicken egg whites	Carbohydrates
Liven proteins	Gelatin, collagen	Carbohydrates
New Culture	Milk proteins, caseins	Carbohydrates
OnegoBio	Chicken ovalbumin	Carbohydrates
PerfectDay	β-lactoglobulin	Glucose
Remilk	Milk proteins, β-lactoglobulin, caseins	Methanol, glucose
Better Whey (Nestle)	β-lactoglobulin	No data
TurtleTree	Lactoferrin	No data

The main technologies for recombinant protein production for food purposes focus on a few proteins, e.g., ovalbumin, which is the main protein in chicken eggs, and the main milk proteins, i.e., β-lactoglobulin, α-, β-, and κ-caseins (Table 7) (Hoppenreijs, 2024).

The bioproduction of recombinant ovalbumin by the fungus *T. reesei* yields ovalbumin with a lower environmental impact in terms of life cycle assessment (LCA) and global warming potential (GWP) (Järviö, 2021). The same is true for *T. reesei*-produced recombinant whey protein β-lactoglobulin (Perfect Day, USA) (Figure 8) (Geistlinger, 2018, 2020). Perfect Day has an FDA approval to produce β-lactoglobulin with *T. reesei* and use it in foods. Recombinant β-lactoglobulin is popular in the food industry as an additive to dairy products, e.g., ice-cream, protein bars, etc.

Figure 8

GWP Requirement (CO_2/kg protein) in Recombinant and Natural β-Lactoglobulin Production



Note: Figure 8 was compiled based on information published by Perfect Day, Inc. (August 20, 2021)[1]

Remilk, Israel, is Perfect Day's main competitor. In addition to recombinant β-lactoglobulin, Perfect Day produces recombinant caseins and purifies them to combine them into casein micelles. Recombinant β-lactoglobulin is produced with the help of methylotrophic yeast *P. pastoris* yRMK-66[2] with either methanol or glucose as carbon source. The final product contains no genetic material of its host and has a GRAS status, which makes it possible to use it in the food industry. β-Lactoglobulin makes it possible to obtain lactose-free milk with fully recombinant proteins (Wolff, 2022).

Caseins make it possible to obtain hypoallergenic milk for people allergic to a specific casein protein (Bhatt, 2021) or recombinant mozzarella cheese (Antuma, 2024). As of the end of 2023, Perfet Day entered the markets of the USA3, Canada, and Israel. By using yeast to produce recombinant β-lactoglobulin, the company claims a 97 % reduction in GWP, as well as a reduction in the energy and water consumption required for protein production[3] (Behm, 2022) (Figure 8).

Food Biotechnology in the Russian Federation: Transfer Issues

Russia has several regulatory acts regarding recombinant enzymes. There is a state standard for enzyme preparations used in the food industry, but it concerns only amylases, milk-clotting enzymes[4], and lipases [5]and does not cover all the preparations listed above. Such preparations as glucosidase, catalase, transglutaminase, etc. are subject to no state standard. Russia has no single law that would regulate the list of enzyme preparations permitted for the food industry (Kulev, 2014).

Technical regulation CU 029/2012 "Safety requirements for food additives, flavorings, and processing aids" is currently the only law with the requirements for enzyme preparations in the food industry6. Despite multiple attempts to harmonize it to the actual food enzyme market, the document is outdated as it does not reflect the current

[1] Perfect Day, Inc. (August 20, 2021). Comparative life cycle assessment of Perfect Day whey protein production to dairy protein. https://perfectday.com/wp-content/uploads/2022/01/Comparative-Perfect-Day-Whey-LCA-report-prepared-by-WSP_20AUG2021_Non-Confidential-1.pdf

[2] Sylvester, B. P. (March 4, 2022). GRAS Notice for Non-Animal P-Lactoglobulin Whey Protein from Fermentation by Komagataella phaffi. https:// www.fda.gov/media/168464/download

[3] Perfect Day, Inc. (August 20, 2021). Comparative life cycle assessment of Perfect Day whey protein production to dairy protein. https://perfectday.com/wp-content/uploads/2022/01/Comparative-Perfect-Day-Whey-LCA-report-prepared-by-WSP_20AUG2021_Non-Confidential-1.pdf

[4] State Standard GOST 34353–2017. Dry milk-clotting enzyme preparations of animal origin. (September 1, 2018). https://docs.cntd.ru/document/1200157890

[5] State Standard GOST P 71139–2023 Enzyme preparations for the food industry. Method for determining lipolytic activity. (January 3, 2024).

[6] Council of the Eurasian Economic Commission (September 18, 2014). Technical Regulations of the Customs Union TR CU 029/2012 Safety requirements for food additives, flavorings, and processing aids. https://docs.cntd.ru/document/902359401

diversity of recombinant enzymes and technological platforms used for their production (Aleshkov, 2017; Bagryantseva, 2016; 2021; Kulev, 2014; Khasanova, 2020).

This review revealed a growing scientific interest in the rational design of recombinant enzymes with predesigned catalytic properties (Boukid, 2023; Li, 2024), e.g., a faster catalysis of substrate conversion, a better thermal stability, (Cramer, 2018; Jin, 2023; Zhu, 2023), a lower non-specific activity (Jaeckel, 2019), AI-based analysis systems to design the enzymic structure (Anishchenko, 2021), etc.

The review also highlighted a trend towards using more efficient recombinant protein expression systems created by genome editing (Li, 2022; Salazar-Cerezo, 2023; Yang, 2024), metabolic engineering (Meyer, 2021; Peña, 2018; Zhang, 2023), and advanced recombinant protein production bioprocesses (Fasim, 2021; Mayolo-Deloisa, 2020; Saad, 2023; Yuan, 2024). If the recombinant protein is obtained using microorganisms with GRAS status, its market launch is easier from the legal perspective (Augustin, 2023). The novel bioproduction technologies make old enzyme production methods uncompetitive because the new ones require less raw material, energy, and personnel to produce the same amount of protein.

Precision fermentation is a new direction in recombinant protein production for food purposes (Augustin, 2023; Teng, 2021). Scientists and developers focus not only on the objects for recombinant expression, but also on the expression systems and scalable purification (Amorim, 2021). They provide cost estimates for individual recombinant products (Hettinga, 2022) and techno-economic analyses of environmental burden that show the advantages of precision fermentation compared to traditional methods (Behm, 2022).

The Russian Federation lacks regulatory framework for many enzymes used in the food industry. Technical regulation CU 029/2012 only covers the list of permitted producers and recombinant proteins. This satiation is a paradox: Russian food industry cannot produce its own recombinant enzymes but is free to import and use foreign ones. Technically, the faulty legislation makes the production of recombinant enzymes illegal in the Russian Federation and the countries of the Eurasian Economic Union since it is virtually impossible to distinguish a recombinant enzyme from a natural one1. However, biotechnology is developing fast and is expected to continue to do so up to 2040.2

Some expression platforms for recombinant enzymes that have a GRAS status in the USA and the EU cannot be used in Russian food industry (Bagrantseva, 2020). For example, some methylotrophic yeasts (*P. pastoris*, *Hansenula polymorpha*, *Yarrowia lipolytica*) are not approved as production platforms for food products.

Russian legislation demands the same purity for food recombinant enzymes as for medical recombinant proteins[3]. As a result, companies that produce recombinant enzymes for the food industry have to comply with unreasonably high purity requirements, which makes enzyme production uncompetitive.

The domestic food industry desperately needs a permissive law that will legalize the production of recombinant proteins using the novel genetic technologies and the entire variety of modern expression platforms. This approach will yield modern, competitive, and high-quality food products in sufficient quantities.

[1] Such issues as distinguishing a natural enzyme from a recombinant one and the control and identification methods for impurities of the genetic materials of the producer strain remained outside the scope of this review. For more detail, see (Lensch, 2022), (Sutay Kocabaş, 2019).

[2] McKinsey Global Institute (May 13, 2020) The Bio Revolution Innovations transforming economies, societies, and our lives. https://www.mckinsey.com/industries/life-sciences/our-insights/the-bio-revolution-innovations-transforming-economies-societies-and-our-lives

[3] State quality standard of medicinal product. General pharmacopoeial article OFS 42 "Medicines obtained by recombinant DNA methods": https://base.garant.ru/70457452/5c69d3b8fce87cf1b2bd234a0868ed8c/

CONCLUSION

Modern biotechnology provides an effective use of enzymatic catalysis in many sectors of the food industry. This review of recombinant proteins and enzymes in the modern food industry covered amylases, milk-clotting enzymes, lipases, and transglutaminase. Glucose oxidase, catalase, β-glucosidase, and other enzymes received a general description and need a more detailed analysis. The review was limited to the bioproduction of recombinant proteins for the food industry while bioproduction of vitamins, dyes, and lipids by precision fermentation methods for the food industry remained outside its scope. The results can be used to analyze the relevant development directions in the field of applied biotechnology of effective recombinant proteins and their regulation. Currently, recombinant food protein production needs more effective R&D. Russian legislation in the field of food production fails to keep pace with the new technological capabilities.

CONTRIBUTION

Sergey Yu. Filkin: developed the research concept; designed the methodology; processed the data; drafted the manuscript.

Alexey V. Lipkin: was responsible for data curation; research methodology; proofreading.

Alexey N. Fedorov: developed the research concept; scientific supervision; visualized the results; drafted and proofread the manuscript.

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