

Cellular agriculture: An extension of common production methods for food

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Valentin Waschulin, M.Sc.
Science & Technology Intern,
The Good Food Institute

Liz Specht, Ph.D.
Senior Scientist,
The Good Food Institute



Executive summary

In spite of the sustainability, health, and animal welfare issues associated with industrial production of animal products, global demand for animal protein is rising. Plant-based alternatives to animal proteins are increasing in prevalence and quality, but for many applications they are not currently able to fully emulate the sensory and functional properties of animal proteins. While substantial progress has been made in recent years towards exhaustive plant protein characterization to identify plant-based alternatives that exhibit better mimicry of animal proteins, cellular agriculture seeks to solve this problem directly by producing genuine animal proteins through fermentation. In this process, the encoding genetic material for the desired animal protein is integrated into an efficient host organism (which may be a strain of yeast, other fungi, or bacteria). This host is then cultivated in fermentation tanks where it produces the desired protein in large amounts. The protein is subsequently separated from the host cells and purified. The resulting protein is the same protein as in the original animal-derived product and will exhibit substantially equivalent sensory and functional characteristics in foods in which it is incorporated.

The same technology has been used to safely and effectively produce hundreds of food enzymes for decades, in addition to proteins in other consumer goods and in medicine and therapeutics. This means that fermentation-produced proteins have been part of our diet for a considerable time now. Nowadays, food enzymes are so ubiquitous that in some food processing methods, recombinant proteins satisfy almost the entire market demand for these proteins; for example, 80% of the rennet used to make cheese is produced by fermentation . Cellular agriculture uses the same techniques and processes to make a more diverse set of animal proteins and can therefore be treated as an extension of this common technology. In this paper we outline the long history of safe protein production via fermentation for food applications, as well as address aspects of cellular agriculture that present novel technical challenges. These present opportunities for continued innovation to improve the efficiency of the process.

Table of Contents

1	Introduction	4
2	Protein production via fermentation	6
3	Examples of common proteins made through fermentation	8
3.1	Chymosin, or “vegetarian rennet”, for cheese-making	10
3.2	Amylase, a common enzyme that modifies starch	11
3.3	Lipase, an enzyme that processes fats	12
4	Similarities and differences: today’s protein production versus cellular agriculture	13
4.1	Development and engineering	14
4.2	Production	14
4.3	Nature of the protein	15
4.4	Protein content in the final product	16
4.5	Regulatory approval and safety considerations	16
5	Conclusions	17
6	Additional resources	19

1 Introduction

Cellular agriculture entails the production of genuine animal products without requiring animal breeding, rearing, and slaughter: in other words, farming cells or proteins directly rather than obtaining them from entire animals. The motivation for this approach is to address the enormous public health, animal welfare, and sustainability issues associated with industrial animal agriculture. Cellular agriculture offers an alternative production method that ameliorates all of the issues above while still delivering to the consumer a product that tastes and performs identically to the animal product it seeks to displace.

One product of cellular agriculture is [clean meat](#), genuine meat grown by cultivating animal muscle cells. While large-scale (upwards of 20,000 L reactor volume) animal cell culture has been utilized for decades to manufacture materials for biological research and medical therapies, food is a relatively new application for animal cell culture. This paper will not focus on clean meat but will instead cover other products of cellular agriculture where the protein – rather than the cell – is the relevant functional unit. Examples of such cellular agriculture products include animal-free [dairy products](#), [eggs](#), and [gelatin](#). These products are made using methods that, while also originally deployed in the biomedical field, have been used extensively for many decades in the food industry as well. Cellular agriculture products represent a novel but logical extension of a well established production platform to produce genuine animal proteins and products.

Rather than breeding and feeding animals, cellular agriculture uses large-scale fermentation¹ to produce the main protein components of the respective animal products. These proteins impart specific functional and sensory properties to products like milk and egg white, and recapitulating their full suite of functional properties has thus far been difficult using other ingredients such as plant-derived proteins. Alternative ingredients from plants or algae work quite well for applications that only require one or a few functional properties. For example, the binding properties of egg can be mimicked by some gums while egg's coloring properties are mimicked by pigments like carotenoids, but these alternatives cannot be used interchangeably when both properties are required; furthermore, neither of these alternatives deliver the protein content of egg. Thus, cellular agriculture offers the promise of animal-free proteins that perform identically to their animal-derived counterparts across their entire functional and nutritional repertoire.

Depending on the desired application, these valuable functional proteins can be combined with plant-derived nutrients like fats and sugars to yield a product that is closer to the original animal product than any fully plant-based product. While these products will not be identical to the animal-derived product down to the molecular level, they recapitulate the relevant functional properties of the animal product (for example, the melting and

¹ In the strict scientific sense of the word, fermentation refers to a process of energy generation through the anaerobic breakdown of organic substrates, which occurs in organisms such as yeast and bacteria as well as in human muscle cells. Thus, this term gained additional use as the general method used for making foods like beer, yogurt, or sauerkraut. In this paper, we use the word in its extended "industrial" sense. By this definition, fermentation means any controlled large-scale cultivation of selected microorganisms or cells for the production of a desired product. This product can be a protein, a metabolite, or even the cellular biomass itself. The vessel used for this is called a fermenter or bioreactor, and the process can be aerobic or anaerobic.

stretching of cheese or the binding and emulsifying properties of egg). At the same time, this cleaner, more efficient production process removes undesirable components of animal products like lactose, antibodies, Salmonella, and pus, which will not be present in products made through cellular agriculture.

The method of producing desirable proteins through fermentation has been used to make not only biomedical products like insulin but also food enzymes (a class of proteins) for decades. According to Novozymes, a global market leader in enzyme production, five billion consumers – nearly the entire global population – used or consumed a product made with their enzymes at least once a week in 2016² (see section 4). This is due to the fact that enzymes are not only used in all kinds of foods and beverages, but also in countless consumer products such as textiles, leather, paper, dietary supplements, personal care products, cosmetics, and detergents³.

It is difficult to obtain reliable information regarding the scale of production of various fermented proteins, but leaders in industrial-scale production such as DuPont boast 13 production facilities across four continents⁴ and reactor scales upwards of 300,000 liters⁵. The global capacity of fermentation facilities expanded rapidly in the late 2000s and early 2010s as ethanol fermentation facilities were expanded for biofuels. Many of these facilities can be adapted for protein production to meet the projected growing demand within food applications.

To date, at least 99 generally recognized as safe (GRAS) applications for food enzymes have been answered by the FDA with a “no questions” letter, meaning that the agency does not see reason to question the safety of the enzyme based on the scientific evidence provided.⁶ Eleven enzyme classes from 160 sources for over 300 applications of food enzymes are permitted in Canada⁷ and over 300 applications have been received by the European Commission in order to establish a similar list⁸. Moreover, the market for food enzymes is expected to grow to 2.3 billion USD in 2020⁹. Although fermentation-produced proteins are ubiquitous in our everyday lives and food enzymes have been part of our diet for decades, the average consumer knows relatively little about the technology behind them.

In this paper, we provide an overview of the technology used in recombinant protein production and compare and contrast its historical use with its anticipated use in cellular agriculture. Several examples of contemporary usage, based on publicly available

² [The Novozymes Report 2016](#). 2017, Novozymes A/S, Bagsværd, Denmark

³ Jegannathan K.R., Nielsen P.H. Environmental assessment of enzyme use in industrial production – a literature review. *Journal of Cleaner Production*. 2013;42:228-240. doi.org/10.1016/j.jclepro.2012.11.005.

⁴ <http://biosciences.dupont.com/about-us/operations/production-capabilities/> (retrieved 2018/01/16)

⁵ <http://www.dupont.com/products-and-services/industrial-biotechnology/media-center/media/1B-profile.html> (retrieved 2018/01/16)

⁶ <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices> (retrieved 2018/01/16)

⁷ <http://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted/5-enzymes.html> (retrieved 2018/01/16)

⁸ https://ec.europa.eu/food/safety/food_improvement_agents/enzymes/eu_list_app_en (retrieved 2018/01/16)

⁹ Industrial enzymes market by type (carbohydrases, proteases, non-starch polysaccharides & others), application (food & beverage, cleaning agents, animal feed & others), brands & by region—global trends and forecasts to 2020. <http://www.marketsandmarkets.com/Market-Reports/industrial-enzymes-market-237327836.html> (retrieved 2016/03/24)

knowledge, are used to illustrate the process and show its adaptability to create a wide range of animal proteins. Indeed, the aim of cellular agriculture is simply to expand this technique to encompass proteins that comprise common animal products like milk, eggs, and gelatin.

2 Protein production via fermentation

Proteins are among the most basic building blocks of life. They are found in every organism on earth and exhibit an incredible diversity in both form and function. Structural proteins can give shape and strength (e.g. in spider silk or wool); storage proteins function as reserves for nutrients and energy (e.g. in egg white and milk); while enzymatic proteins, or enzymes, are responsible for many chemical reactions necessary for life (e.g. amylase, which breaks starch down into sugar). Together with medically relevant proteins, enzymes comprise the main products of today's industrial-scale protein production via fermentation.

The long history of use of structural and storage proteins by humankind as foods, garments, and materials is well documented, but enzymes have been used for millennia as well – for example, when brewing beer. The enzymes in malted barley convert starch into sugars. These sugars can then be converted into alcohol through metabolic processes in yeast. With the advent of modern science and technology, enzymes could be identified and extracted in a pure form from microorganisms and animal tissues, giving rise to a multitude of applications ranging from fruit juice manufacturing (1930s) to enzymatic detergents (1950s) to improved glucose production processes (1960s)¹⁰. Figure 1 presents a timeline of relevant events leading up to the commercialization of cellular agriculture.

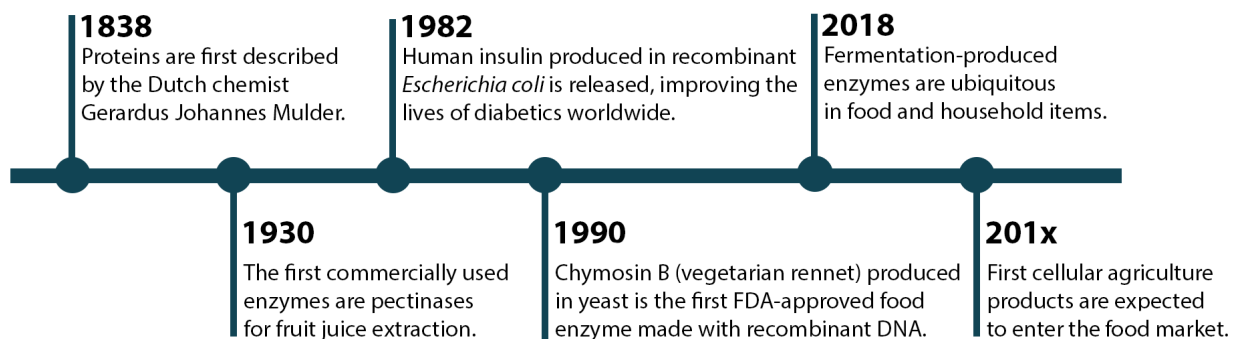


Figure 1. A timeline of selected significant events leading up to the commercialization of cellular agriculture. While one company entered the cosmetics market in 2017 with collagen, cellular agriculture products are expected to enter the food market within the next few years.

Genetic engineering made enzyme and protein production significantly more efficient, since it enabled scientists to produce these proteins independent of their original source. The most well known example – and indeed the first FDA-approved recombinant protein for

¹⁰ Wittmann, C., & Liao, J. C. (2017). *Industrial biotechnology: microorganisms*. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA. doi:10.1002/9783527807796

medical use, in the 1980s - is insulin, a therapeutic protein used to treat diabetes. Instead of extracting porcine insulin from the pancreatic glands of thousands of pigs, one could simply engineer an easy-to-grow microbe to produce the human form. This not only alleviated the animal suffering associated with this process, but provided many direct benefits to consumers as well. The protein was now the genuine human protein, rather than a similar but non-identical porcine form; it eliminated the risk of viral contamination or allergic reaction associated with the purification process; and it significantly reduced the cost and increased the supply of insulin.

Nowadays, most commercially relevant enzymes are produced by genetically modified bacteria, yeast, or other fungi. The process for developing these engineered strains has become routine and is extremely well characterized (Figure 2). First, the gene encoding the desired protein is taken from a donor organism (e.g. human, cow) and inserted into the DNA of the host organism (e.g. bacteria, yeast). Over the past several years, DNA synthesis has become so routine and inexpensive that the donor organism is typically not even needed for this process; the desired gene is identified from a sequence database and a piece of DNA is manufactured to those precise specifications for introduction into the host's genome.

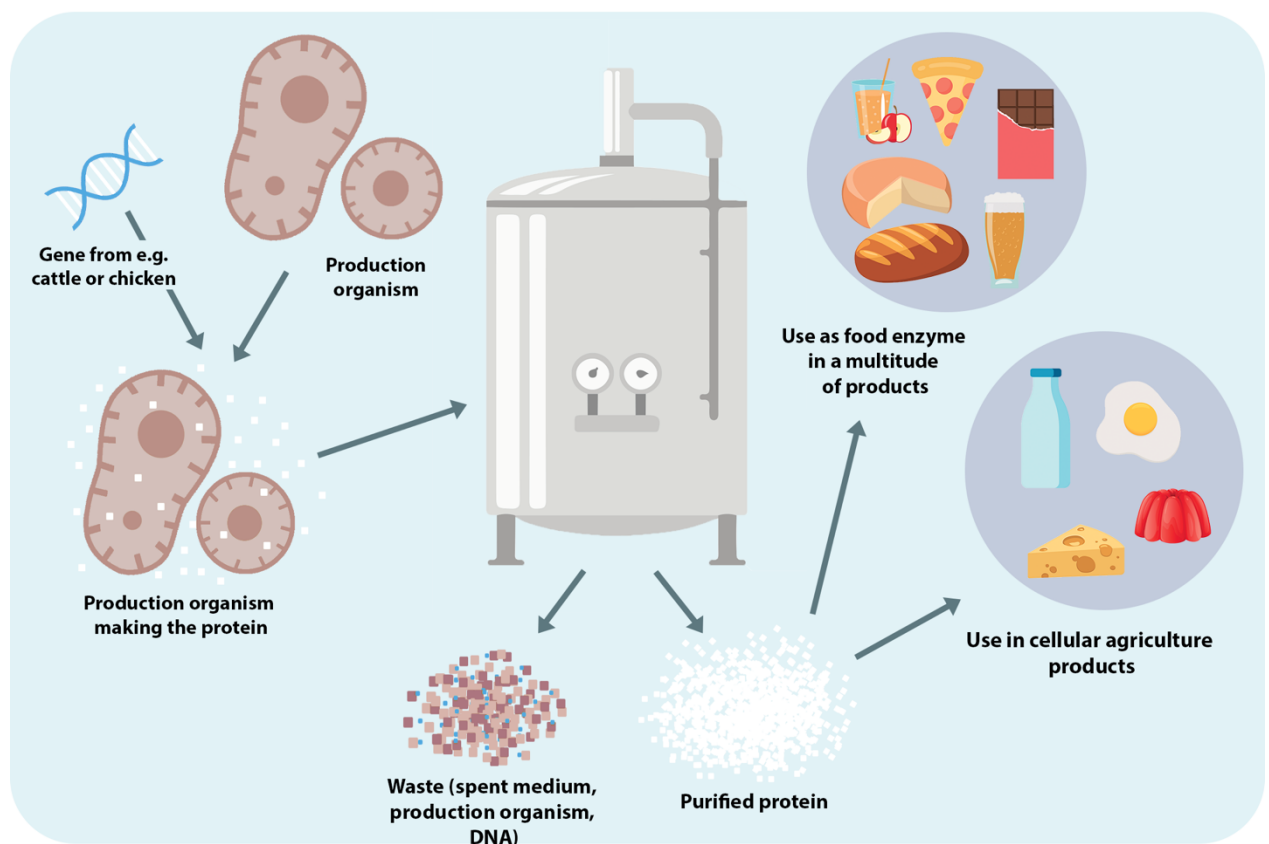


Figure 2. The process for producing animal proteins via cellular agriculture is the same process used for producing food enzymes as well as many other proteins routinely used in household goods and medicines. The genes encoding the desired proteins (in this case, directly from the animal source of the proteins) are introduced into a fast-growing, highly efficient host organism that is grown in fermenters where it expresses the desired proteins. These proteins are then purified from the host for incorporation into consumer goods.

The host organism will then read the gene and produce the protein as if it were its own. This is possible because of the vast similarity among all organisms in the way genes are read and translated into proteins; they all speak the same language, so to speak. In fact, gene transfer among organisms is an extremely common event in nature, even within agriculturally relevant plant species. For example, a recent study found that almost all varieties of sweet potato show recent genetic signatures of gene transfer from soil microbes, all of which occurred naturally¹¹.

The host organism is then grown in large quantities under controlled conditions, producing the protein as it grows. This is often done in a stirred-tank bioreactor, which is a big steel tank filled with a nutrient medium. The tank is inoculated with a pure culture of the production strain cells that produce the protein, which is either secreted directly into the medium or obtained by harvesting and breaking open the cells. The protein can then be separated from the cells and purified to obtain a product free of the host DNA, unnecessary proteins from the host organism, and other impurities. The result is a highly purified form of the same protein that is present in the original source. The nature (for example, the precise structure) and purity of the protein is then rigorously confirmed by modern analytical techniques to ensure that it is identical to the desired product and sufficiently pure.

3 Examples of common proteins made through fermentation

Fermentation-produced enzymes are ubiquitous in the modern world, and it is safe to say that every consumer living in an industrialized country is in contact with and consumes fermentation-produced enzymes on a regular basis. However, since enzymes are considered processing aids and therefore are typically exempt from inclusion on mandated ingredient lists, people are often not aware of their presence in the products they buy. Below we list some common examples of products made with the help of fermented enzymes, and in sections 3.1 through 3.3 we delve deeper into the production methods of three recombinant enzymes. This is far from an exhaustive survey, but rather serves to highlight specific products that frequently use fermented proteins to achieve traits that are beneficial to the consumer or the environment.

In modern bread making, many different enzymes are used and their application is considered the industry standard¹². For example, amylases (starch-digesting enzymes) can be used to supplement the yeast's natural amylases, leading to faster fermentation and a fluffier texture. They also prevent staleness, meaning that the bread remains soft for more than just a few days, thus improving shelf life and reducing food waste. Asparaginase, on the other hand, is added to the dough reduce the amount of acrylamide (a potential carcinogen) that is formed during the baking process from compounds naturally occurring in the dough. Several other enzymes are used to improve properties related to aspects like dough handling and shelf life. Unless a bakery relies solely on artisanal bread making methods, the use of fermented enzymes is highly likely during multiple steps of production.

¹¹ Kyndt T., Quispe D., Zhai H., et al. The genome of cultivated sweet potato contains *Agrobacterium* T-DNAs with expressed genes: An example of a naturally transgenic food crop. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112(18):5844-5849. doi:10.1073/pnas.1419685112.

¹² <http://www.bakingbusiness.com/News/News-Home/Features/2010/11/Enzymes.aspx?cck=1> (retrieved 2018/01/12)

Similar examples abound elsewhere in food. Corn syrup, the well-known sweetener and main constituent of pancake syrup, is made by converting corn starch into sugar using amylases. The enzymatic method has largely replaced the much more inefficient (and chemically harsher) acid-base method used in the past. Clear fruit juices are produced with the aid of pectinases, which break down pectin, a gelatinous substance found in fruits like apples that makes the juice extracted from the fruit cloudy and difficult to filter. Lactose-free milk and dietary supplements for those with lactose intolerance are possible due to the use of lactase, the enzyme that breaks down lactose into glucose and galactose. These examples illustrate consumer benefits (consumers prefer clear juice to cloudy, and consumers with dietary restrictions are able to enjoy foods they previously could not) as well as environmental benefits (enzymatic processes often obviate harsher chemical methods, and increased efficiency means there is less waste throughout production).

Other industrial non-food enzyme applications are associated with reduced use of chemicals and energy. Amylases, for example, are used in environmentally friendly dishwashing detergents, where they replace phosphates. Laundry detergents contain fermented enzymes that have been selected for high functionality at low temperatures, enabling consumers to effectively clean clothes in cold water, reducing waste in water heating. In papermaking, enzymes can significantly reduce the dosages of bleaching chemicals. And in order to produce bioethanol from lignocellulosic biomass (e.g. wood), a whole cocktail of fermentation-produced enzymes is necessary to break down the cellulose, the main component of the plant cell wall, and turn it into sugar.

A fermentation platform can also be used to make ingredients beyond proteins, including ingredients formerly derived from animals. For example, carmine is a widely used red food and textile pigment that is extracted from female cochineal insects¹³. Recently, researchers succeeded in transferring its biosynthetic pathway into filamentous fungi, rendering the labor-intensive farming of millions of insects (which have in many cases become invasive species in areas where carmine is cultivated) no longer necessary. While carmine is not a protein, it helps to illustrate the possibilities that biotechnology offers for a sustainable and humane future.

To further illustrate the development, production, and use of fermentation-produced enzymes, we have selected to explore in greater detail three examples that are abundant in consumer goods. The descriptions below are based on publicly available GRAS (Generally Recognized As Safe) notices^{14,15,16}. GRAS notices are dossiers of documentation, expert opinions, and studies submitted to the FDA to evaluate a new food ingredient. To be granted GRAS status, the applicant must prove that there are no reasonable concerns about safety. All GRAS notices are publicly available and can be found on the FDA website. The Canadian and European food safety authorities have prepared or are preparing similar databases specifically aimed at food enzymes.

¹³ <http://www.fao.org/docrep/v8879e/v8879e09.htm>

¹⁴ [GRAS Notice No. 230](#), date of filing Jul 27, 2007

¹⁵ [GRAS Notice No. 594](#), date of filing Aug 27, 2015

¹⁶ [GRAS Notice No. 238](#), date of filing Dec 27, 2007

3.1 Chymosin, or “vegetarian rennet”, for cheese-making

The primary active component of the enzyme complex rennet is a protein called Chymosin B, a protease naturally found in the lining of calf stomachs. Its biological function is to cleave the milk protein casein at a specific site, causing the milk protein to curdle (separate into clumps) and thereby enabling better protein absorption by the calf. Historically, the stomachs of slaughtered unweaned calves were scraped out to obtain these milk-curdling enzymes in the form of rennet paste, which was then used to curdle milk in an early step of cheese production. Since rennet is both limited in supply and makes cheese unsuitable for vegetarians, various alternatives from plants and microbes were developed. These alternatives, however, all exhibited downsides such as bitter taste. The first fermentation-produced Chymosin B was approved by the FDA in the US in 1990. Being the exact same enzyme as in calf rennet, it did not show the disadvantages seen in vegetable or microbial rennet. By 1999, already 60% of US hard cheeses were made with fermentation-produced rennet¹⁷. Nowadays, 80% of the rennet used worldwide is produced by fermentation¹⁸.



Figure 3. A cheesemaker cuts the curd that has formed after rennet was added to milk during cheese production. “Vegetarian rennet”, or recombinant chymosin, is produced through a process identical to that used in cellular agriculture. Chymosin was one of the first commercial products made using this method, beginning nearly 30 years ago.

In cheesemaking, chymosin is added to the milk at the beginning of the process. The action of the enzyme leads to formation of the curd and the separation of the whey. About 90% of the enzyme goes into the whey while 10% stays in the curd.

The original source of the chymosin enzyme is *Bos taurus*, the species name of cattle and dairy cows. The DNA sequence of the enzyme was elucidated through genetic sequencing

¹⁷ Vogt, D. U., Parish M. 1999. Food Biotechnology in the United States: Science, Regulation, and Issues. U.S. Department of State. (retrieved 2018/01/16)

¹⁸ Johnson, M.E. et al. Major Technological Advances and Trends in Cheese. Journal of Dairy Science , Volume 89 , Issue 4 , 1174 - 1178. doi:10.3168/jds.S0022-0302(06)72186-5

and is publicly available. *Trichoderma reesei* (also known as *Hypocrea jecorina*) is a filamentous fungus belonging to the phylum ascomycetes, a diverse group that also contains morels, truffles, and baker's yeast. It is known for being a highly productive "cell factory" and is widely used for recombinant enzyme production. It is used as a production organism for about 11% of all technical enzyme formulations as of 2014. *T. reesei* is widely considered a safe production organism, is non-pathogenic to humans, and does not produce mycotoxins under the conditions used for enzyme production¹⁹.

Chymosin is produced in a large stirred-tank bioreactor. After a multi-step purification process and quality control checks for production strain DNA, mycotoxins, and any harmful contaminants such as *Salmonella* bacteria, the protein is ready to be used in cheesemaking.

3.2 Amylase, a common enzyme that modifies starch

Amylases are enzymes that cleave chains of glucose (starch) into smaller sugar units. They are found across all domains of life, from humans to bacteria, as this process is necessary in order to extract energy from starch.

Alpha-amylases are widely used enzymes across many different applications, including making starch fermentable for grain alcohol production and beer brewing, producing corn syrup, treating food starch to achieve textures not possible with untreated starch, improving bread quality, and replacing phosphates in dishwashing detergents.



Figure 4. Corn syrup is being used as an ingredient for baking. To produce corn syrup, the starch is broken down into sugar by amylase enzymes. This enzymatic process has completely replaced the older chemical process that used acid and heat to break down the starch. The advantages of the enzymatic process include reduced energy and chemical consumption as well as better yield.

¹⁹ Bischof RH, Ramoni J, Seiboth B. Cellulases and beyond: the first 70 years of the enzyme producer *Trichoderma reesei*. *Microb Cell Fact.* 2016;15:106. doi:10.1186/s12934-016-0507-6

While proteins from mesophilic (i.e. thriving at moderate temperature) organisms get irreversibly damaged at temperatures above approximately 40°C (104°F), proteins from thermophilic (i.e. thriving in heat) organisms remain stable and active at much higher temperatures. This makes them excellent candidates for applications where the product must withstand higher temperatures, e.g. in baking or dishwashing. Thus, since amylases are common across many organisms, amylase genes from thermophilic bacteria like *Geobacillus stearothermophilus*, which is found in habitats such as geothermal hot springs, are ideal for this purpose. *Bacillus licheniformis* is a soil bacterium and a workhorse of industrial biotechnology. It is non-pathogenic and has a long history of safe use in enzyme production²⁰. Producing the *G. stearothermophilus* amylase in *B. licheniformis* combines the heat adaptation of the former with the protein production efficiency of the latter.

Although the host organism in this example is a bacterium rather than a fungus, the production and downstream processing is remarkably similar to that described for Chymosin. The engineered *B. licheniformis* expressing *G. stearothermophilus* amylase is grown in a stirred-tank bioreactor. After a multi-step purification procedure, formulation, and quality control checks for various contaminations and to verify the enzymatic activity level, the liquid amylase solution is ready for use.

3.3 Lipase, an enzyme that processes fats

Lipases are enzymes that break down fat. One unit of triglyceride, which is the main component of dietary fat, consists of one glycerol molecule bound to three fatty acid molecules. The most common lipases cleave the bonds between the fatty acids and the remainder of the molecule. This is a first step in the fat digestion process in humans and other organisms, and thus – like amylases – lipases are naturally made in organisms spanning all domains of life. Various types of lipases can also cleave related structures such as phospholipids, which are found in high concentrations in egg yolk, for example.

Lipases are used for several purposes in food processing. The United States FDA GRAS notice names four different application areas for lipase²¹:

- Improving bread dough handling properties as well as bread volume, crust, and pore formation
- Improving noodle and pasta quality; reducing cooking loss
- Improving emulsification properties of egg yolk to prevent separation during pasteurization
- Removing phospholipids from soybean oil to improve taste

²⁰ Olempska-Beer Z. S., Merker R. I., Ditto M. D., DiNovi M. J. (2006). Food-processing enzymes from recombinant microorganisms—a review. *Regul. Toxicol. Pharmacol.* 45 144-158. doi:10.1016/j.yrtph.2006.05.001

²¹ GRAS Notice No. 238, date of filing Dec 27, 2007



Figure 5. A baker handling dough. As one of the many enzymes used in baking, lipases are added to improve dough handling by e.g. making the dough more elastic and less sticky.

One particularly efficient lipase is found in an organism called *Fusarium heterosporum*, an ascomycete filamentous fungus from the large genus *Fusarium*. Most members are soil-dwelling fungi, while others are plant pathogens that can produce mycotoxins. Some can also cause opportunistic infections in humans. *Hansenula polymorpha* (also called *Ogataea polymorpha*) is a yeast that is related to baker's yeast and is a safe industrial production host for enzymes. By transferring the *F. heterosporum* gene into *H. polymorpha*, the enzyme can be manufactured at large scale without the potential hazards of cultivating *F. heterosporum* itself.

Akin to the processes for chymosin and amylase described above, the host organism is grown in a stirred-tank bioreactor and the protein is purified through several steps to achieve high purity. It is then dried to achieve a powdered product that is more shelf-stable than typical liquid enzyme preparations, like the amylase described in Section 3.2. Before commercial sale, quality control protocols monitor for traces of the production strain, contaminating microorganisms, and heavy metals among other undesirable contaminants.

4 Similarities and differences: today's protein production versus cellular agriculture

Decades of research have rendered the engineering of host organisms for recombinant protein production much more efficient. This makes the expansion of this technology to low-value, high-volume structural and storage proteins a real possibility. Currently, scientists are working on fermentation-produced milk proteins, egg proteins, and collagen, which can be used in food in the form of gelatin or in materials such as leather or other applications like cosmetics and biomedical research. Companies are also exploring other textile and biomaterials applications of cellular agriculture-produced animal proteins like spider silk. In

the following section, we point out similarities and differences in the production, characteristics, and use of these products when compared to contemporary fermentation-produced proteins that have already been widely commercialized.

4.1 Development and engineering

Development of the production strains for cellular agriculture applications is largely the same as for today's enzyme products. Advances in strain development that contribute to higher protein yield, more precise control over protein modifications, and more efficient protein folding are all directly applicable to cellular agriculture applications. The wealth of academic literature and commercial experience with these enhanced strains can accelerate the rate at which cellular agriculture products can achieve meaningful increases in scale and reductions in cost. Moreover, modern genetic tools are much more precise than the technologies used to develop the first wave of recombinant food enzymes, so it is more straightforward to develop host strains for expressing these animal proteins that are immediately suitable for food applications.

4.2 Production

Fermentation of the host organism in large tanks and the subsequent protein purification is extremely similar to today's processes – the target is a pure protein. The required degree of purification depends on the intended end-use application. In the case of therapeutic antibodies that are administered intravenously, cell-derived impurities like proteins or lipids could lead to dangerous adverse effects. Therefore, a high degree of purification is necessary to reduce impurities to the range of parts per million or lower (>99.999% purity). On the other end of the spectrum lie industrial enzymes that are not used in consumer products, where the presence of various proteins and other cell components does not interfere with the application. Food enzymes typically fall in the middle range of this spectrum, depending on whether the presence of other enzymes is acceptable or detrimental for the target application. They additionally need to undergo safety testing and fulfill requirements pertaining to microbiological and heavy metal contaminants. The complete absence of living production organism and antibiotic resistance genes is a requirement in any of these cases. Since the production host organisms currently being explored as workhorses for cellular agriculture, such as yeast and filamentous fungi, have a long history of safe use in food – and a high degree of purification is a significant cost driver – cellular agriculture proteins will most likely fall into the “food enzymes” part of the spectrum with regard to the desired level of purity.

Like for today's recombinant protein products, the production process must fulfill strict requirements pertaining to the containment of genetically modified microorganisms in order to keep them separated from the environment. These requirements are enforced by the Environmental Protection Agency (EPA) in the US, while other countries employ similar agencies. The regulations encompass measures that minimize the risk in each step of the research and production process. Examples include special procedures for transport, handling and hygiene, restricted access to research and production sites, implementation of effective processes for inactivating (killing) the microorganism, as well as safety plans for

emergency cleanups. Furthermore, there are regulations on the kind of microorganism that can be manipulated and the nature of the genetic modification²².

Another difference between cellular agriculture proteins and previous generations of recombinant proteins is that many cellular agriculture proteins accumulate within the host cell rather than being targeted for secretion into the culture media. This makes the purification process to extract the desired protein from the host cell more technically challenging, leading to a greater potential for cellular debris to contaminate the purified protein. However, this could be used advantageously for cellular agriculture applications, if selection of the host organism takes into account its sensory attributes in acknowledgment of the fact that a lower level of purity will facilitate cellular agriculture-produced proteins in approaching price parity with animal proteins. For example, many plant-based cheese recipes utilize the inherent cheesy flavor of certain strains of *Saccharomyces cerevisiae* (colloquially known as nutritional yeast when used as an inert ingredient); if a yeast strain with a desirable sensory profile were used to express animal proteins such as caseins that impart notable functional properties to cheese, the residual yeast components in a crude casein extract could actually contribute favorably to the taste of the final product.

4.3 Nature of the protein

Most proteins currently commercially produced by fermentation are enzymes, which catalyze chemical reactions – for example, converting starch to sugar or breaking down proteins. By contrast, cellular agriculture aims to produce proteins that function predominantly as storage or structural proteins. The primary function of these proteins is storing nutrients and giving structural properties; they do not typically exhibit enzymatic activity.

This distinction comes with advantages and challenges. One notable advantage is that structural and storage proteins are likely to remain inert within the host organism, which may allow the host to tolerate higher expression levels because its native biochemistry will not be compromised by aberrant or off-target activity resulting from a high concentration of the introduced enzyme. Likewise, structural and storage proteins tend to be fairly inert within the food matrix of the product into which they are ultimately incorporated; this reduces complexity in the final food formulation because it is not necessary to quantify the specific activity of the protein from batch to batch – only the yield and purity are measured.

One possible challenge of expressing structural or storage proteins rather than enzymes is that structural and storage proteins can be bulky and comprised of many subunits or repeating units, which can render them difficult to assemble in a non-native host cell that is unaccustomed to properly folding these complex proteins. Indeed, development of host strains that are uniquely capable of expressing properly assembled complex structural proteins has been an intellectual property hallmark of some cellular agriculture start-up companies – for example, Geltor is developing intact collagen proteins as an alternative to collagen derived from animal rendering.

²² Microbial Products of Biotechnology: Summary of Regulations under the Toxic Substances Control Act. United States Environmental Protection Agency, September 2012

4.4 Protein content in the final product

Because most food proteins currently made through fermentation are enzymes, they are typically present in low concentrations in the final product. The function of enzymes as catalysts means that a single enzyme molecule can perform a desired chemical reaction over and over – for example, to convert 100 starch molecules to sugar in an hour. Therefore, enzymes used in food processing are incorporated in low concentrations, typically below 1% or even 0.1% of the final product. A notable example of a non-enzymatic fermentation-produced protein used in food production is the soy leghemoglobin used by Impossible Foods in their Impossible Burger. It is produced in the yeast *Pichia pastoris* and confers a distinctly “meaty” taste to the plant-based burger²³. While this protein is not enzymatic, it is present at relatively low levels (less than 2% of the final product, according to Impossible Foods’ ingredient statement)²⁴.

Cellular agriculture products, on the other hand, are likely to contain greater concentrations of the fermentation-produced protein as these proteins constitute primary components of the final food product. For example, a genuine cellular agriculture-produced egg white would need to contain around 10% fermentation-produced protein, mirroring the protein concentration in animal-derived egg white. The remainder of the food matrix can be comprised of plant-derived ingredients – such as sugars and fats – that mimic the functionality, texture, flavor, and nutritional content of the product’s animal-derived counterpart. Because of the cost of production, it is possible that cellular agriculture-derived products will combine specific recombinantly-produced proteins that are critical for functionality of the final product with other plant proteins, rather than replacing the entire protein component of the product with recombinant animal proteins.

4.5 Regulatory approval and safety considerations

The FDA typically requires premarket approval of food ingredients unless they are generally recognized as safe (GRAS). An ingredient’s GRAS status may be established through a history of safe use prior to 1958 or general recognition by qualified experts that the ingredient is safe.²⁵ The same is true for food contact substances, which are defined to include “component[s] of materials used in manufacturing, packing, packaging, transporting, or holding food if such use is not intended to have any technical effect in such food.”²⁶

A manufacturing process can affect the identity of an ingredient to the extent that it changes the ingredient’s safety or regulatory status. Thus, just because one version of a food ingredient is GRAS, it does not necessarily mean that another version produced using a significantly different manufacturing process would also be GRAS.²⁷

²³ [GRAS Notice No. 737](#), date of filing Oct 10, 2017

²⁴ <https://www.impossiblefoods.com/faq/> (retrieved 2018/01/16)

²⁵ 21 U.S.C. § 321(s).

²⁶ *Id.* § 348(h)(6).

²⁷ FDA, *Guidance for Industry: Assessing the Effects of Significant Manufacturing Process Changes* (June 2014), <https://www.fda.gov/RegulatoryInformation/Guidances/ucm300661.htm>.

In regulatory guidance, the FDA explained that two enzyme preparations of chymosin – one from an animal source, the other from a microbial source – could not be treated identically because consumers would be exposed to different constituents derived from the manufacturing process.²⁸ Specifically, the FDA pointed out that while both substances contain an enzyme component that catalyzes the same chemical reaction, consumers can be exposed to components of the fermentation media or the residues of processing aids. Therefore, a safety assessment must take those differences into account.²⁹

For most producers, the GRAS notification process provides the most efficient regulatory route to market.³⁰ While the FDA permits GRAS determinations without FDA involvement,³¹ in most cases it is advisable to ask the FDA for a “no questions” letter, which indicates that the agency has reviewed the safety determination and does not have concerns.

GRAS status is dependent on safety data specific to the food ingredient in question. Nonetheless, the FDA’s treatment of chymosin is illustrative of how the GRAS process might be used to introduce products of cellular agriculture to the market. In 2007, Genencor submitted a GRAS notice that identified the production microorganism, described the manufacturing process, discussed the composition and specifications, and compared the chymosin it produces to other chymosins – specifically noting that chymosin B from *T. reesei* expressing the bovine prochymosin B gene has an identical amino acid sequence as native bovine chymosin B.³² On the basis of this information, the FDA said that it had “no questions” about “Genencor’s conclusion that chymosin enzyme preparation from *T. reesei* expressing the bovine prochymosin B gene is GRAS under the intended conditions of use.”³³

In all cases, regulators should communicate clear expectations to food producers about what safety data are necessary. Fundamentally, animal proteins produced through cellular agriculture are the same as their animal-sourced counterparts, thus their safety profiles could be expected to be similar to conventional animal proteins. It is important that regulatory oversight not be so onerous as to disadvantage cellular agriculture in the marketplace. Similarly, regulators must adapt labeling conventions to allow cellular agriculture to compete fairly with conventional animal products.

5 Conclusions

Fermentation-produced enzymes have been used safely and effectively for decades to produce and improve our food. They are ubiquitous and used to make a wide variety of foods such as bread, cheese, oil, and fruit juices. The same well-characterized process can now be used for producing animal proteins without the animal.

²⁸ *Id.*

²⁹ *Id.*

³⁰ An alternative approach would be to file a food additive petition with FDA. While the process is more cumbersome and takes more time, it requires only technical evidence of safety and can rely on confidential information.

³¹ Current litigation challenges this practice as an unlawful delegation of FDA’s authority to ensure the food supply is safe.

³² GRAS Notice No. 230

³³ FDA Response Letter to GRAS Notice No. 230

The world's growing population and its ever-increasing appetite for animal protein require efficient and sustainable ways to meet these needs. Current industrial production methods, such as feeding grain and soy to animals in factory farms strain the environment and require large amounts of energy, land, and water. Factory farms are also a point of concern for animal welfare. Using the technology of protein production by fermentation to make animal proteins is a sustainable and humane way to provide people with the animal foods they want to eat. We see this production method as an extension of a safe and common technology, and believe that its success will be of great importance for ensuring a healthy, humane, and sustainable future of our food supply.

6 Additional resources

Below we have linked to several databases that contain recombinant proteins that have been approved for food and other consumer product applications in the United States, European Union, and elsewhere, and we have briefly annotated the content and format of the data in each resource. The vast number of products contained in these resources illustrates how commonly utilized recombinant technology is in food and other consumer goods, and is a reflection of the overwhelming data on the safety and efficiency of producing valuable proteins through this method.

The European Commission's list of 300 food enzymes for which applications have been filed for examination between 11 September 2011 and 11 March 2015. The examination of all the applications is expected to take several years. (Downloadable Excel file available.)
https://ec.europa.eu/food/safety/food_improvement_agents/enzymes/eu_list_app_en

The European Food Safety Authority's publications list. Scientific opinions and supporting data to demonstrate safety and purity for the enzymes listed above are published here; search by enzyme name to find the supporting scientific data.
<http://www.efsa.europa.eu/en/publications>

The Canadian government's list of permitted food enzymes, including specific applications and other conditions of use. <https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted/5-enzymes.html>

The European Chemicals Agency's Inventory of Chemicals. Enzymes used in various industry applications in the European Union can be looked up by searching for the name of the enzyme.
<https://echa.europa.eu/information-on-chemicals/ec-inventory>

The Association of Manufacturers and Formulators of Enzyme Products (AMFEP) maintains a list of commercially produced enzymes supplied voluntarily by enzyme producers. (Downloadable Excel file available.)
<http://www.amfep.org/content/list-enzymes>

The United States Food and Drug Administration (FDA)'s GRAS (Generally Recognized as Safe) notice inventory, in which you can search by enzyme name.
<https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices>

ABOUT THE GOOD FOOD INSTITUTE

The Good Food Institute is a 501(c)(3) nonprofit organization dedicated to creating a healthy, humane, and sustainable food supply. GFI's team of scientists, entrepreneurs, lawyers, and lobbyists are laser focused on using markets and food innovation to transform our food system away from industrial animal agriculture and toward plant-based and clean meat alternatives. To learn more, please visit [GFI.org](https://gfi.org).

ABOUT THE AUTHORS

Valentin Waschulin, M.Sc.

Science & Technology Intern, The Good Food Institute

[✉ valentin.waschulin@gmail.com](mailto:valentin.waschulin@gmail.com) [in Valentin at LinkedIn](#)

Valentin Waschulin, M.S., is a Science & Technology intern at The Good Food Institute, currently based in Denmark. Led by the conviction that a more humane and sustainable future of animal products is possible, he wants to use his expertise and academic training to accelerate the cellular agriculture revolution. Valentin has a bachelor's degree in biology with specializations in microbiology and genetics from the University of Vienna and a master's degree in biotechnology from the University of Copenhagen, where he conducted his master's thesis research in the Technical Industries division at Novozymes.

Liz Specht, Ph.D.

Senior Scientist, The Good Food Institute

[✉ lizs@gfi.org](mailto:lizs@gfi.org) [in Liz at LinkedIn](#)

Liz works to identify and address areas of need for plant-based and clean meat scientific innovation and works with funding agencies to prioritize research that moves this field forward. Liz holds a bachelor's degree in chemical and biomolecular engineering from Johns Hopkins University, a doctorate in biological sciences from the University of California, San Diego, and postdoctoral research experience from the University of Colorado Boulder. Liz is a Fellow with the University of Colorado at Boulder's Sustainability Innovation Lab and has a decade of academic research experience in synthetic biology, recombinant protein expression, and development of genetic engineering tools.

