APPLICATION OF FERMENTATION FOR PROTEIN SUBSTITUTE

Abstract

Growing and expected to continue throughout the coming decade are present-day trends in plant-based foods made from fermentation. A breakthrough biotechnology approach to food preservation, fermentation has been widely used in the world's food supply (fermented foods, probiotics, functional substances, and additives). Consumers may prefer yoghurt and other fermented dairy products over other foods on the market, but fermented products like legumes, vegetables, fruits and cereals have recently piqued their interest and are regaining popularity. With the advancement in the growth of proteins substitute, the collection of tools created via the evolution of fermentation is now ready to transform the food industry. There are three main ways of using fermentation technique by protein industry. Traditionally fermentation influences and manipulates plant-derived nutrients using whole live microbes, resulting in products with distinctive zest and nourishing profiles as well as different touch. In order to efficiently manufacture huge amounts of protein, the process of fermentation for biomass production needs diverse microorganisms' quick growth and high protein content. Precision fermentation makes use microorganisms as cell factories to produce particular bioactive components, which require higher pure concentration than the core protein elements which are used at lesser concentrations. The purpose of sustainable and environment friendly development can be aided through the advancements in food fermentation by increasing the diversity of protein sources and the availability of wholesome, balanced in all the dietary compounds, stable foods that are acceptable for a variety of consumer groups worldwide.

Keywords: Protein Substitute, Fermentation Technology, Precision Fermentation, Single Cell Protein (SCP)

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I. INTRODUCTION

The worldwide population will grow around ten billion people by 2050, necessitating a greater production of food than has been produced today (Molfetta et al., 2022). Rapid growth in population leads to decreased standard of life, hunger, and poverty. The whole world's demand for food is projected to rise by 21%, though the percentage of people at threat of getting malnourished is predicted to be increase 91% by that time (Van et al., 2021). In Europe in 2019, around 55% of the daily requirement for protein was met by eating food with an animal origin. Studies from 2010 predict that by 2050, the demand for meat will have increased by 173% and for dairy products by 158% (Molfetta et al., 2022). Currently, one of the greatest challenges affecting the food industry is the ability to source proteins sustainably, as animal-based proteins may have significant negative effects on the environment and the economy (Karlund et al., 2020). Along with leaving a significant environmental burden, the production and consumption of meat raise important ethical questions, particularly in terms of health (Molfetta et al., 2022). A shift in diet based on animals and animal derived products towards substituting environment friendly options is necessary to accomplish the sustainability goals established forth by the United Nations (Christensen et al., 2022). As a result, the endeavor by humanity to resolve crises by developing technology that can to overcome food insecurity. Subsequently is recommended that the existing dietary patterns can be enhanced by cutting back on excess protein and calorie intake, cutting down on food waste, and switching to plant-based foods. A tendency for eating too much protein is especially prominent in the diet, where it has been declared that by 2050, the protein intake per person should be reduced by 10-15%. At the same time, the percentage of plant-based proteins in the diet should rise from 40% to 60% (Christensen et al., 2022). The overall commercial revenue of meat substitutes and other protein sources from plants products, was expected to be Euros 368 million in 2017, rendering to statistics based on the market analysis firm IRI (Tziva et al., 2020). Such objectives establish standards for protein sources to demonstrate significant biodiversity, preserve ecosystems, be nutritionally sufficient, secure, and wholesome, stay to cultural norms; be accessible, moderately affordable, and equitable economical to meet the various levels of sustainability (Karlund et al., 2020). Both meat and dairy products have a major adverse impact by the land use for protein production by contributing in greenhouse gases. Vegetable proteins can take the place of animal proteins to minimize consumption's greenhouse gas emissions (Blonk et al., 2008). The necessity to create new approaches for food development by increasing the nutritious content of proteins from plants, which also provide high quantities of critical micronutrients, and replace some animal proteins with those derived from plants. Unlike animal-based proteins, plant-based proteins pass via the gut together with unique cell wall elements found in plant whole foods such dietary fibers and phytochemicals, which can cause problems with protein digestion (Karlund et al., 2020). Products that are used in place of meat in the human diet should resemble meat in look, texture, and flavor are known as meat substitutes (Tziva et al., 2020). Recently, the demand for meat substitutes has increased across Europe at an unprecedented rate. Rendering to Euromonitor data, the supply and demand for meat substitutes rose from 15 to and 20% annually in Germany and Denmark in 2016 and by 5-10% in the Netherlands, Sweden, and the UK (Tziva et al., 2020). Antinutritional factors (ANFs) are substances sourced from the plants typically act as nutrient-availability-impairing agents and, as a result, as a target of elimination in the food production process. Recently, however, a debate on the possibility of health-promoting biological functions of ANFs has been progressively initiated. Additionally, although the amino acid composition regarded as the most important deficiency of plant proteins from the perspective of nutritional quality. Food processing techniques can be used to change the structures and components of plant cells and thereby increase the accessibility of plant proteins to digestive enzymes (Karlund et al., 2020). According to epidemiological studies, diets rich in meat products are related to a higher risk of colorectal cancer (CRC), as is the case in Western culture. Furthermore, ingesting meat raises the amount of lipids, heme, and heterocyclic amines consumed, all of which may help develop CRC. Meat consumption also causes an increase in the fermentation of proteins (Windey et al., 2012). One of the oldest and most widely used biotechnological technique for food preparation and preservation is fermentation (Sun et al., 2022). Fermentation as a process is illustrated as the biochemical change of any carbon-based material via the microbial breakdown and gets carried out using multiple biocatalysts (Sun et al., 2022). One of these techniques that has been used the longest is fermentation, which relies on the biochemical processes of microbes to generate several kinds of chemicals that may impede the development and continued existence of undesirable microflora in meals (Ross et al., 2002). Animal proteins have undergone extensive research into their structure-functionality relationships and are applied in the food processing industry; however, the expanding demand for protein from plants as a sustainable substitute has revealed difficulties with regard to their physiochemical and functional properties. Additionally, some plant proteins are challenging to be applied in in high-quality foods due to their negative associations with harmfulness and low nutritional content, among other factors. Both raw components' and microbial enzymes, responsible for hydrolysis reactions like proteolysis, are present in these intricate biosystems (Christensen et al., 2022). Hydrolysis of proteins is one method for extending the range of applications for proteins because the process of proteolysis liberates peptides with useful bioactivities or cleave allergenic molecules. The selected protease and the determined reaction conditions have a momentous influence on the characteristics of the hydrolyzed plant protein. Through targeted hydrolysis, the proteins' emulsification, foaming, and gel-forming characteristics can also be released (Christensen et al., 2022). This chapter focuses on how fermentation technology could help to improve the quality elements of these substitute protein sources, toward making them easier to use as food products, additives, or supplements that give customers dietary proteins other than animal-based protein.

II. FERMENTATION TECHNOLOGY

1. **Introduction of Fermentation:** A biotechnological process called fermentation uses microbes to produce a wide range of industrial commodities. Biotechnology and fermentation technologies have combined to create products with additional value, such as hormones, antibiotics, enzymes, and other metabolites (Dwivedi, S. P. 2023). Fermented foods have special features like probiotics, antibacterial, antioxidant, peptide synthesis, etc. due to the presence of functional microorganisms, which provide consumers with health advantages. Additionally, fermentation of waste from agriculture and food industry could result in value-added nutritious meals and sustainable food supplies. Furthermore, fermented food items can be a vital source of generating livelihoods by encouraging and marketing traditional fermented recipes that are also healthful and also by producing important food ingredients through the process of fermentation of frequently produced organic wastes in rural regions (Van et al., 2021). Humans have come to embrace this process as a result of beneficial sensory and technological developments in food, which have facilitated the emergence of a variety of food items via the fermentative pathways of several microbes. (Christensen et al., 2022).

When pasteurization process came into existence in 1861 AD, the essential role that microorganisms serving in fermentation was recognized for the first time (Ross et al., 2002). At this time industrial revolution took place that leads to a radical change from small-scale to the large-scale production of food. In order to produce fermented foods and alcoholic beverages, large-scale fermentation procedures were created. The most widely used microorganisms for these procedures are lactic acid bacteria (LAB) for a variety of dairy, vegetable, and meat fermentations as well as yeast for the creation of beer, wine, and spirits (Ross et al., 2002).

2. **Process of Fermentation:** An enzyme-catalyzed metabolic process known as fermentation releases energy while anaerobic conversion of starch or sugar into alcohol or an acid. Zymology is a field that studies fermentation. The fermentation procedure is an anaerobic biological process. The creation of pyruvic acid through the process of glycolysis which results in the production of net 2 ATP molecules, is the first step in fermentation, similar to how it occurs in the process of cellular respiration. In the following stage, pyruvate is changed to either lactic acid, ethanol, or other substances. NAD+ is generated here and used once more all throughout the glycolysis process. The metabolic process of fermentation produces energy from sugar or perhaps other organic substrates through the activity of many enzymes (Dwivedi, S. P. 2023).

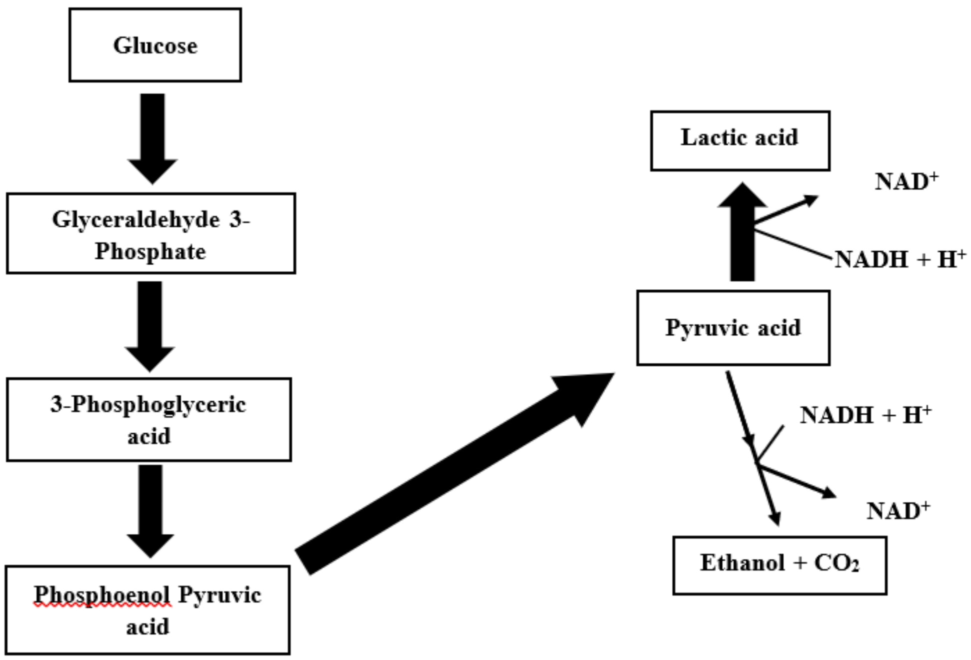

Figure 1: Process of Fermentation

A group of bacteria generally known as the lactic acid bacteria (LAB) is principally accountable for many of the microbial alterations found in the extra typical fermented food items while thinking about the fermentation of foods (as compared to yeast-based alcoholic fermentations). This group, which is made up of several genera, mostly produces lactic acid as ts main byproduct. These genera include *Streptococcus, Enterococcus, Pediococcus, Lactococcus, Lactobacillus, and Leuconostoc.* and the most frequently used individuals in the group for food purposes are *lactococci* for the production of cheese, *Streptococcus salivarius* subsp. *thermophilus* for the production of yogurt and cheese, and various individuals from the Lactobacillus genus for a variety of meat, vegetable and dairy fermentations (Ross et al., 2002). *Thermotoga* species, *Candida sake, Enterococcus faecalis, L. sakei, L. plantarum, Leuconostoc carnosum, Bifidobacterium dentium, Weissella confusa, Rhodotorula rubra, R. oryzae* and *Rhizopus oligosporus* are among the microorganisms that participate in the fermentation of herbal products (Sun et al., 2022).

3. Types of Fermentation

- **Production of Alcohol:** The following chemical equation illustrates the alcohol fermentation of glucose, which has the molecular formula $C_6H_{12}O_6$. Two ethanol molecules and two carbon dioxide molecules are produced from one glucose molecule:

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2$$

  Prior to fermentation, single glucose molecule is divided into double pyruvate molecules. The name of the procedure is glycolysis (Dwivedi, S. P. 2023).

- **Production of Lactic Acid:** The metabolite lactate, or cellular energy, is produced while the fermentation of lactic acid from the fermentation of glucose and other carbohydrates with six carbon atoms. This particular sort of anaerobic fermentation frequently takes place in bacteria, mammalian red blood cells, some animal cells, or occasional skeletal muscles when there is not enough oxygen present to sustain aerobic respiration. Pyruvate, a byproduct of glycolysis, is converted into lactic acid during this process. Lactate dehydrogenase catalyses the conversion of NADH into NAD+. The accumulation of lactic acid brought on by anaerobic respiration during exercise causes fatigue (Dwivedi, S. P. 2023).

- **Production of Acetic Acid:** Vinegar is the final product of the acetic acid fermentation. The double step process includes the following steps: Ethyl alcohol is produced by anaerobical conversion from sugar with the help of yeast. Through aerobic respiration, acetobacter converts ethyl alcohol to acetic acid (Dwivedi, S. P. 2023).

- **Production of Butyric Acid:** Pyruvate is created at the first stage of glycolysis through the sugar oxidation. Following stage includes the oxidation of pyruvate to produce acetyl-CoA through the use of the oxidoreductase enzyme, which also produces CO_2 or H_2. Finally, more acetyl-CoA reduction results in the production of butyric acid. Butyric acid fermentation produces a net 3 molecules of ATP, which results in an increase in energy (Dwivedi, S. P. 2023).

III. SOURCES OF PROTEIN AVAILABLE FOR FERMENTATION

1. **Single Cell Protein:** Parched cells of microorganisms including fungi, bacteria, and algae are referred to as SCPs and are added as a protein supplement to food for people or animals. Microorganisms can be employed to create biomass or protein concentrates by using waste products and inexpensive feedstock as the sources of carbon and energy. (Reihani et al., 2019). Microbes have the ability to thrive on a diverse range of materials,

including low-cost residues like food leftovers, byproducts from food production, various types of wastewaters, along with agricultural and forestry remains, as well as substances like CO_2 or methane. This makes the production of Single Cell Proteins (SCPs) an eco-friendly approach. Using microbial biomass as an option can be seen as available alternative to guarantee food security in the future while also reducing the overall strain on global sustainability (Molfetta et al., 2022). Typically, the kind of microorganism utilized, the temperature and the duration of incubation, agitation speed, chemical makeup, and the ability to acquire carbon from diverse materials, along with factors like inoculum quantity, pH, and aeration rate, all play crucial roles in influencing the production of SCPs (Reihani et al., 2019). Contrasting with animal or plant proteins, SCPs are regarded as having a high nutritional value. The primary drawback is the high nucleic acid (NA) content (6–12% dry weight) of yeast and bacterial SCPs. Humans who consume large amounts of NA may have uric acid precipitation, which can result in health problems such kidney stones or symptoms of gout. Additional treatment is required for application of SCPs as food. SCPs are employed either straight as food products or as flavor, culinary or nutraceutical components. SCPs can be exploited as protein substitute, produced from "precise fermentation". Fermentation can be effective in improving some end product characteristics like appearance, taste or ability to digest. The design of microbial complexes or the application of microorganisms with anticipated bioconversion functions (e.g., aromatization by amino acid catabolism, proteolysis), can be a promising approach and effective to make SCP more palatable. Methionine-rich SCP's amino acid composition can also be employed to manufacture volatile sulphur compounds (VSCs), which give fermented goods a cheese-like flavour. Apricot can give dairy products well-known organoleptic qualities (Molfetta et al., 2022). A fresh method is suggested to yield the amino acid-loaded xylooligosaccharide and SCP as a useful use of paper mulberry. The paper mulberry's carbohydrate is successfully transformed into high-value goods (Gu et al., 2022).

Table 1: SCP Manufacturing Substrates and Microorganisms (Reihani et al., 2019)

<table>
<tr><th>Substrate</th><th colspan="3">Microorganism</th></tr>
<tr><th>Food waste, Petroleum by-products, Natural gas</th><th>Bacteria</th><th>Fungi</th><th>Yeast</th></tr>
<tr><td>Lactose</td><td>Aeromonas</td><td>Aspergillus</td><td>Amoco</td></tr>
<tr><td>n-Alkane</td><td>Achromobacter</td><td>Cephalosporium</td><td>Candida</td></tr>
<tr><td>Methanol</td><td>Acinetobacter</td><td>Chaetomium</td><td>Saccharomyces</td></tr>
<tr><td>Ethanol</td><td rowspan="2">Bacillus</td><td rowspan="2">Penicillium</td><td rowspan="2">Trichoderma</td></tr>
<tr><td>Hemicellulose</td></tr>
<tr><td>Cellulose</td><td>Flavobacterium</td><td rowspan="2">Rhizopus</td><td>Kluyveromyces</td></tr>
<tr><td>Maltose</td><td rowspan="2">Lactobacillus</td><td>Thermomyces</td></tr>
<tr><td>Glucose</td><td>Scytalidium</td><td>Methylomonas</td></tr>
<tr><td>Galactose</td><td>Methylomonas</td><td></td><td>Rhodotorula</td></tr>
<tr><td>Pentose</td><td>Pseudomonas</td><td>Trichoderma</td><td>Trichosporon</td></tr>
<tr><td>Uric acid and other nonprotein nitrogenous compounds</td><td>Rhodopseudomonas</td><td>Fusarium</td><td>Mucor</td></tr>
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2. **Filamentous Fungi:** Food ingredients such as enzymes, itaconic, kojic, fumaric, gluconic and citric, as well as sesquiterpenoids, alkaloids, terpenes, and nutraceuticals along with

pharmaceuticals such as zeranol, taxol, lovastatin, grisefulvin, ergot alkaloids, contraceptives, statins, and immunosuppressants are all produced by filamentous fungi. (Molfetta et al., 2022). Food nutrition can be enhanced through fermentation utilizing edible filamentous fungus. By cultivating filamentous fungi in solid state or submerged fermentation processes, fungal SCP can be produced. *A. oryzae* is used in the brewing of sake, a traditional Japanese fermented alcoholic beverage, which turns rice's starch into sugar before ethanol is produced by yeast fermentation. Oilcake, which is produced when oil from peanut seeds, soybeans, rapeseeds and sunflower seeds, and rapeseeds is pressed that is also fermented using *A. oryzae*, which results in a higher protein matter and a lower concentration in terms of lipids. The Japanese fermented dish known as hamanatto (also known as soy nuggets) is another product of *A. oryzae*. It is made from fermented, cooked soybeans that have been dried after being marinated in brine or soy sauce. Traditional fermented delicacies from Indonesia's West Java region include back and red oncom. Red oncom is made by growing *N. intermedia var. oncomensis* or *Neurospora* on a blend of powdered cassava and leftover peanut dregs. Tofu waste is fermented to produce black oncom by *Mucor* spp., *R. oligosporus,* and *Rhizopus microsporus var. oligosporus*. Gari is a flake manufactured from fermented and roasted cassava utilizing, *Aspergillus fumigatus*, *Fusarium* spp., *Rhizopus* spp., or *Penicillium* spp, and *Aspergillus niger*. It is a staple food among several ethnic groups in Liberia, Cameroon, Nigeria, Guinea, Benin Republic, Ghana and Togo. Foods that have undergone fungal fermentation contain enzymes that may aid in the better digestion of proteins and carbs. Fungi engage in a complicated metabolic transformation throughout the fermentative process and generate secondary metabolites, not necessary for microbial development. Furu is a Chinese cheese-like food prepared from soybean curd that has been fermented by the mould *Actinomucor elegans* (Molfetta et al., 2022).

Since filamentous fungi have evolved a wide array of extracellular digestive enzymes and glandular routes to get nutrition and sustain life in a variety of environments, they are particularly well designed for SSF, especially those occurring on lignin-based substrates with relatively low water content. (Strong et al., 2022)

3. **Microalgae:** Microalgae can transform inorganic phosphorus, nitrogen, and carbon resources into biomass. Thesy are eukaryotic or prokaryotic photosynthetic microorganisms that can be generated on an industrial scale with little greenhouse gas emissions. Only a small number of microalgae strains are generated industrially and sold commercially, despite the great biodiversity of these organisms (Molfetta et al., 2022). There aren't many reports of microalgae being used in fermented foods. In a study, a soy beverage was created and fermented using a probiotic strain *Levilactobacillus brevis* OCK 0944 in addition to *C. vulgaris*. Spirulina *(Arthrospira platensis)* was added to yoghurt to improve the process of fermentation, texture, nutritional value, and sensory qualities. The authors discovered that 0.25 percent of *A. platensis* sped up fermentation. Additionally, it was hypothesized that the microalgal biomass's high dietary fibre and protein concentrations acted as physical stabilisers, enhancing syneresis and apparent viscosity and enhancing mouthfeel. Another study examined the effects on the physicochemical properties and dietary value of Spanish "chorizo" sausages that have undergone fermentation that contained 3% *Chlorella* or *Spirulina* (Molfetta et al., 2022).

4. **Plant Protein:** Cereals (barley, millet, maize, sorghum, wheat and rice), legumes (cowpea, fava bean, soybean, lupin, chickpea, and pean bean), and pseudocereals (buckwheat, quinoa, and amaranth) have all been suggested as dietary proteins sources. The production of bioactive peptides with antibacterial, antihypertensive, hypocholesterolemic, immunomodulatory, antioxidant, antithrombotic, and anticancer properties is another way that the proteins of these vegetable matrices have health advantages. Additionally, plant-based proteins have useful qualities that make them appropriate for use in gluten-free, food fermentation or protein-enhanced goods, or bio-fortified cereal-based goods (Molfetta et al., 2022). The most common kind of cereal-based foods ingested, frequently after fermentation, may generally be used to discriminate between different world dietary trends. Large amounts of cereals and legumes are fermented throughout the Indian subcontinent, frequently in tandem, to create foods like pupadum, vada, adai, idli and dosa. *Rhizopus* spp., *Mucor* spp., and *Aspergillus* spp. are filamentous fungi, as well as bacteria (such as *Levilactobacillus* spp *Lactiplantibacillus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., and *Streptococcus* spp.) and yeast (such as *S. cerevisiae*) (Molfetta et al., 2022). Researchers and manufacturers have been shifting increasingly in recent years towards the fermentation-based conception of innovative foods like minor cereals, legumes, and pseudocereals in an effort to boost the nutritious content of everyday foods like bread and pasta. Additionally, a wide variety of microorganisms alter the profiles of amino acids. For instance, greater levels of critical amino acids like lysine were produced when soybeans were fermented with *Lactiplantibacillus plantarum* (Molfetta et al., 2022).

Table 2: Plant resulting materials and features for microbial proteolysis Abbreviation: Angiotensin-converting enzyme (ACE) (Christensen et al., 2022)

Plant Substrate	Properties of proteolysis	Proteolytic microbial species
Cereals	Increases Anti-oxidant function Increases ACE inhibitory activity Increases essential amino acids bioavailability Increases Protein Absorption Decreases Allergenic epitopes Decreases Toxins	*Levilactobacillus brevis, Mucor pusillus, Lactiplantibacillus plantarum, A. oryzae, Latilactobacillus sakei, Aspergillus niger, L. curvatus, Lactobacillus helveticus, Torulaspora delbrueckii Rhizopus oryzae, Loigolactobacillus coryniformis, Lacticaseibacillus rhamnosus, Weissella cibaria, Pediococcus pentosaceus, P. acidilactici, Saccharomyces cerevisiae, Saccharomycopsis fibuligera*
Seeds	Increases Flavor Decreases formation of Haze	*Aspergillus oryzae, Rhizopus oligosporus*
Fruits	Increases Protein solubility Increases Flavor Decreases formation of Haze	*Wickerhamomyces anomalus, Metschnikowia pulcherrima, Aspergillus niger, Trichoderma viride*, Mixed fungual culture, *Candida utilis, Oenococcus oeni*

Legumes	Increases growth of Microorganisms Increases Anti-oxidant function Increases ACE inhibitory behaviour Increases Anticancer activity Increases revalorization of byproduct Increases Flavor Decreases Off-flavor Decreases Immunoreactivity/allergen	*Lactobacillus helveticus, Lacticaseibacillus casei, Yarrowia lipolytica* *Rhizopus* spp., *R. oligosporus, Mucor flavus, M. wutungkiao, Aspergillus egyptiacus, Actinomucor elegans, R. oryzae, Streptococcus thermophilus*

Meat substitutes can be prepared from the materials from sources of plant origin. In a study, the karkade (*Hibiscus sabdariffa* L.) seed is cooked, then fermented meant for nine days to make indigenous furundu. The minerals in karkade seeds, furundu ferments, and their physicochemical characteristics and HCl extractability were examined. Additionally, fermentation-related microbial proliferation was examined. The karkade seed's total protein content increased after furundu fermentation, showing that the dry matter composition had changed. On fermentation, it was discovered that the protein fractions of karkade seeds, albumin plus globulin, and prolamin significantly decreased, while G1, G2, and G3 significantly increased. Additionally, a reduction in pH and overall acidity was seen. Glutamic acid, aspartic acid, and arginine dominated the karkade seed's amino acid profile (Abu et al., 2008). In a 12-week feeding study with a completely random design, 220 Black Harco breed laying hens were fed experimental layer diets with fermented palm kernel meal (PKM) and copra meal (CM) substituted for soybean meal (SBM) at 0%, 25%, 50%, and 75% of the protein content, respectively. The study demonstrated that either PKM or CM protein could optimally replace SBM protein at a 50% substitution level (Dairo at al., 2008). There are four primary steps in the supply chains for plant-based meat substitutes. Around the world, the first phase of planting involves a variety of protein crop kinds. In the subsequent stage, crops are harvested and transformed into protein-containing products like protein isolates and concentrates. Companies in the food sector purchase protein sources, formulate them, and then process them into intermediate texturized goods to create completed meat substitutes during the third phase. In the final step, customers receive things through eateries and retail establishments (Tziva et al., 2020).

5. **Hybridized Protein from Plant and Animal Sources (Mixed Fermented Poteins):** Consuming mixed fermented products (MFPs), which are formed of vegetable and milk mixtures, may be one potential remedy for such a dietary problem. Food's normal sensory characteristics, increased nutritious value (e.g., through a reduction in the quantity of ANFs), and increased health aspects (e.g., through the production of bioactive chemicals like immunomodulating peptides) are all influenced by fermentation. The most traditional fermented foods from Turkey is tarhana, created by combining wheat flour and yoghurt are often combined in a 2:1 ratio, along with yeast, salt, and other cooked vegetables (such as green pepper, onions, and tomatoes). The combination is fermented for one to seven days at 25 to 30°C, and then dried at ambient temperature or under controlled conditions to lower the moisture content to around 10% and increase shelf life (Molfetta et al., 2022). By choosing the right microbial strains, one may regulate the fermentation of foods containing milk and vegetables, standardising the procedure and preventing the

growth of undesirable bacterial genera (such *Bacillus* and *Clostridium)*. Comparatively few of the typical legume notes (such as pea, wood, dried fruit, or grass) could be detected in fermented pea gel-milk mixtures, in contrast to pea gels. In fermented skim milk, the use of plant protein additions was investigated increasing the nutritional value and enhancing the physico-chemical, sensory, and quality properties. (Molfetta et al., 2022).

6. **Insects:** Insects initially exploited as a food source since the very beginning of human history. The impending need for new food sources and worries about feeding the world's population to alleviate the food crisis, and the need to address dietary deficits in microelements are what make insects appealing as alternative food sources (Molfetta et al., 2022). In comparison to conventional products, the manufacture of meat replacements and protein powder derived from insects is two to five times more environmentally friendly (Smetana et al., 2016). Insect-based protein is quickly gaining popularity as a potential replacement for soy-based protein as an alternate protein source to animal-based protein for both direct human consumption and usage in animal feed. Insect protein offers the greatest potential to reduce European consumers' carbon footprints when consumed directly as food. When it comes to reducing the Global Warming Potential of grill production systems, using insects as animal feed can make a significant difference (Vauterin et al., 2021). A study shows that younger males with a weak commitment to meat, the most likely supporters of insects as an innovative and more sustainable protein source will be those who eat meat, who are more willing to try new foods and concerned with the effects their dietary choices have on the environment in Western nations (Verbeke, 2015). In contrast to less sustainable and often utilised sources of protein and lipid from plant origin, the studied full-fat insect meals appear to have promise as an intriguing protein and lipid source for ruminants. Being high in n-6 polyunsaturated FA, the fatty acid (FA) profile of the rumen digesta of *Tenebrio molitor, Musca domestica, Alphitobius diaperinus, Gryllus bimaculatus, Hermetia illucens, Grylloides sygillatus, Blatta lateralis* and *Acheta domesticus* may be useful to enhance the standard of foods originating from ruminants (Renna et al., 2022).

IV. MICROBIAL PROTEASES INVOLVED IN PROTEIN FERMENTATION

In order to transform complicated substrates into simple compounds, the fermentation process uses a variety of reactions, including those that are physical, enzymatic, chemical, biochemical, and microbiological. Both raw materials' and microorganisms' enzymes, responsible for hydrolysis reactions like proteolysis, are present in these intricate biosystems. Traditional food microorganisms like LAB, yeast, and mould species, which have origins in a vast range of food matrixes based on plants and animals, contain versatile extracellular proteases. LAB are widely used and show a significant part in numerous processes of fermenting food products. LAB are often selective, complex-requirement microorganisms that have specific requirement for a range of amino acids. Cell envelope proteinase (CEP), a component of the system of hydrolyzing proteins in some strains of LAB, enables the breakdown of extracellular proteins into peptides that are small sufficient for peptide transport systems to ingest. Another category in microorganism frequently employed in the procedure of fermenting food is fungi. A system for hydrolysis of protrein seen in fungi uses proteases to produce tiny peptides and amino acids that may then be transported into cells. Aspartic proteases, often referred to several filamentous fungi produce a group of proteolytic enzymes known as aspartic acid proteases generated by a variety of filamentous fungi and several yeast species. To guard against proteolysis, fungus aspartic proteases are created in inactive form known as zymogen. These enzymes are activated by the binding of two aspartic residues at the catalytic site when the pH is altered. Aspartyl proteinases are classified as pepsin related and rennin related enzymes and, generated by the fungi *Endothia* spp., *Mucor* spp., *Penicillium* spp., *Neurospora* spp., *Rhizopus* spp., *and Aspergillus* spp. Historically, food bioprocessing has been linked to fungi that produce GRAS [Generally Recognised as Safe] proteases. Due to their functionality and durability at small pH levels, aspartic proteases had a significant character in the cheese manufacturing business, particularly as agents for milk coagulation in cheese production. Although many fungi have been researched as potential sources (such as *Penicillium oxalicum, Saccharomycopsis fibuligera and Endothia parasitica*), mucorpepsins generated by *Mucor miehei*, *Mucor pusillus* or recombinant mucorpepsins synthesized by *Aspergillus oryzae* are the most commonly used commercial proteases in the dairy industry (Christensen et al., 2022).

V. IMPACT OF LACTIC ACID FERMENTATION ON PROTEIN

Protein molecule alterations generated by the proteolytic hydrolysis by Lactic acid bacteria in the application of fermentation encouraged this process of forming gel in soybeans, this leads to the increase in number of hydrophobic peptides and amino acids. According to the descriptive sensory analysis, pea protein fermentation by applying lactic acid bacteria was intensified by reducing unpleasant flavors; fermentation caused the digestion of bigger peptides, bringing out the decrement of protein solubility; and lactic acid fermentation raised the palate of pea proteins. Conferring to reports, the primary method for extracting leaf proteins uses lactic acid fermentation, and to maximize process yields, plant maturity should be taken into account when using a feedstock to make protein concentrates for animal feeding. Methionine, lysine and isoleucine lacking in wheat bread, were improved by the LAB fermentation of rapeseed protein concentrate. Goat milk was used to create oil-in-water emulsions, which were then fermented with lactic acid bacteria to reduce the size of the oil droplets. At the conclusion of the processing, caseins dominated the protein species at the interface. Lupin protein functional components were dramatically increased through lacto-fermentation, and adding fermented lupin flour to wheat-lupine bread improved the texture. The flavor of baijiu was improved by the coexistence of lactic acid bacteria and yeast, which boosted ester formation and decreased acid generation. Lactic acid bacteria at different phases demonstrated varying utilization abilities to carbon sources. Fermentation enhanced the faba bean's overall nutritional quality and allowed for sophisticated protein and amino acid bioavailability. Fermentation with lactic acid bacteria has the potential to significantly improve soy protein isolate's ability to gel. Technology, flavour, protein composition, nutritional value, health and well-being, and preservation are all impacted by lactic acid fermentation of legume protein (Sun et al., 2022).

VI. PROTEIN MASS EXPRESSION TECHNIQUE

The set of technologies developed through the advancement in fermentation are now able to transform the nutriment industry by fast-tracking the development of novel substitute proteins.

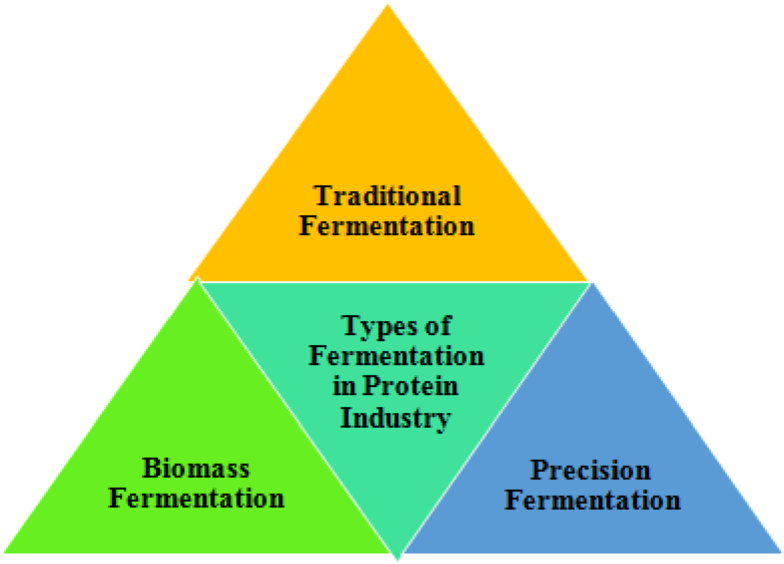

Figure 2: Types of Fermentation used in the Protein Industry (Sun et al., 2022)

In the protein sector, the fermentation procedure is mainly employed in three distinct manners:

1. Traditional fermentation utilizes whole live microorganisms which control and alter plant-derived constituents, leads to food with distinctive flavor and nutritional profiles as well as altered textures. One of the examples is the application of the fungus Rhizopus for fermenting soybeans into a fermented product "tempeh" and the use of various lactic acid bacteria to produce yoghurt and cheese. Recent variations in this strategy include the fermentation of plant-based proteins by Myco Technology to progress flavonoid content.

2. To efficiently manufacture huge amounts of protein, biomass fermentation exploits diverse microorganisms' spontaneous growth and elevated protein content feature. The biomass produced from microbes itself is presented as a component with the cells intact or hardly processed, for instance, the cells are being broken open to better digestion or to elevate the protein content even further, comparable to processing plant flours into protein isolates and concentrates. This biomass aids as either the main element or one of the principal ingredients in a intermingling of a food product. Quron's and Meati's of filamentous fungus, which serve as the basis for their meat analogues, are examples of biomass fermentation.

3. Microbial hosts are applied for precision fermentation in cellular factories to produce particular functional components, which typically necessitate higher purity than the core protein constituents which are useful even at lower levels. These functional components are key enhancers of the enhanced sensory qualities and functional traits of plant-based goods or animal products that have been raised (Sun et al., 2022).

   The appropriate options to establish fermentation are grouped into five main extents that span the value chain: target assortment and design; strain development; bioprocess design; feedstock optimization; and end-product formulation and manufacturing ingredients (Sun et al., 2022).

Protein fermentation by the microbes in the gut significantly leads to the collection of metabolites in large intestine that may result in amino acid homeostasis in the host. The highly networked process of proteolytic fermentation can have a variety of effects on the host, and the variations in proteolytic fermentation based on the availability of fiber suggest that when analyzing the effect of protein fermentation on health, it is also important to take the gut microbiota's need for carbohydrates into account (Sun et al., 2022).

VII. BENEFITS OF PROTEINS VIA FERMENTATION

1. **Ensuring Sustainable Food Production:** Whole grain cereals, vegetables, fruits, and berries are more frequently chosen for sustainability, while plant-based protein sources are more frequently favored to cut down on the use of meat and other animal-based protein sources. Additionally, this process has been sped up by the growing scientific interest in gut bacteria. Consumers are gazing for another digestive health products in addition to the classic and renowned fermented dietary products like yoghurt, kefir, sauerkraut, kimchi, and miso that have previously made their way back to consumers' dinner tables. Some of the solutions to this expanding demand include artisan breads with longer fermentation times, protein- and cereal-based juices, and fermented fruit juices. Public interest in nondairy alternatives has been sparked by the rise the growing acceptance of vegan and plant-based diets, the rise of vegetarianism, lactose intolerance, allergies to milk proteins, the high fat and cholesterol content of fermented dairy products, and consumers' general willingness to try a diet comprised of plants for environmental or health benefits. The millennial generation is especially drawn to kombucha and other fermented drinks because they like negligibly processed goods and are intense to try innovative flavors. If implemented improperly, the nutritional sufficiency of environmentally sustainable meals could put some groups in low-income nations and Western civilizations, including newborns, children, and the elderly, at danger of nutritional deficiency. With the exception of yoghurt and other fermented dairy products, fermented products are not specifically listed and advised in dietary plans despite their acknowledged nutritional qualities and health advantages. In addition to serving as food, the encouragement of fermentation and the recovery of conventional fermented foods may have societal benefits. role, supporting, for instance, the reduction of infections and diarrhoea and the development of underprivileged communities. In several social development initiatives, probiotic *Lactobacillus* or *S. thermophilus*-enriched yoghurts have been used, but the creation of fermented meals made from grains and vegetables has also been promoted (Karlund et al., 2020).

2. **Reducing Antinutritional Factors to Enhance the Nourishing Value of Sustainable Protein Sources:** Condensed tannins, phytic acid, phenolic compounds, and protease inhibitors are the ANFs that have been investigated the most for their effects on the availability of protein and minerals. Phytic acid found in plant-based foods may combine with metal ions to create insoluble complexes that lower the biological availability of vital micronutrients like calcium (Ca), iron (Fe), magnesium (Mg), and zinc (Zn) in the physiological pH of the gastrointestinal tract. In general, novel and conventional food technical approaches are proactively evaluated for phytic acid reduction to prevent mineral shortages in populations eating diets rich in phytic acid and/or vulnerable to malnutrition. Phytases are enzymes that are formed naturally by both plants and

fermenting organisms. The phytic acid reduction in fermented vegan matrices is normally greatly aided by endogenous phytases, which are frequently activated by the pH drop that typically occurs during food fermentation. A desired trait of starter culture bacteria is phyto-selectivity, particularly if it can be sustained in the anatomical settings of the human digestive tract. Genetic alterations may provide fresh ways in the future to boost the phytase functions of food-fermenting bacteria and to continue developing food products with greater nourishing superiority and functionality because there are significant variances in the phytic-acid-diminishing capacity of LAB (Karlund et al., 2020).

In gastrointestinal circumstances, phenolic chemicals have the probability to form complexes with protein molecules, impairing protein absorption. With the addition to the phenolic compounds' and proteins' structural similarities, environmental factors like pH and temperature also play a role in the relationship and affinity of phenolic chemicals with proteins, including dietary proteins and digestive enzymes. Proanthocyanidins, or condensed tannins, are a subtype of tannins that may be harmful to a variety of organisms and have a comparatively high propensity to precipitate protein molecules. Microbial tannase enzymes were developed to biodegrade tannins for energy production and to lessen toxicity, and they are frequently produced during bacterial and fungal fermentation processes. Tannase enzyme is capable of tannin compound degradation produced by Lactobacillus plantarum strains that are commonly isolated from fermented plant materials (Karlund et al., 2020).

Inhibitors of trypsin and chymotrypsin from the Kunitz and Bowman-Birk families, which are naturally protein-based and include spontaneous inhibitory points in their amino acid chains, are found in cereals and legumes. Molecules acting as inhibitors bind to the active sites of digestive enzymes via these reactive sites, preventing them from acting on proteins generated from food. The effectiveness of removing trypsin inhibitors by fermentation depends on the protease's microbial source, the conditions of the process, and the type and composition of the food matrix. Proteolytic enzymes produced by fermenting bacteria have the power to reduce protease inhibitors (Karlund et al., 2020).

VIII. FUTURE SCOPE

Animal sourced foods are causing catastrophic situation for the environment. As it is a great concern for humans to reduce their carbon footprints, it necessitates us to take steps to a greener and sustainable way of consumption of our diets. Especially, meat and meat products have shown that their consumption is not suitable for environment as well as for health. Because of this, humans have diverted their focus towards healthy and sustainable living by switching to protein substitutes. Plant based protein, Single cell protein, Fungi, Insects, Mixed fermented proteins all these substitutes have drawn a great attention towards them. Fermentation has helped in production of these proteins as well it improves the nourishment profile of the product. Novel products can be develop using different sources of protein substitutes through fermentation as a process. Sensory assessment of SCP is still lacking, but should be accomplish prior to usage as a food or food ingredient to assess consumer acceptability.

REFERENCES

[1] Abu El Gasim, A. Y., & Mohammed, M. A. (2008). Fururndu, a meat substitute from fermented Roselle (*Hibiscus sabdariffa* L.) seed: Investigation on amino acids composition, protein fractions, minerals content and HCl-extractability and microbial growth. *Pakistan Journal of Nutrition*, *7*(2), 352-358.
[2] Blonk, H., Kool, A., Luske, B., De Waart, S., Blonk Milieuadvies, G., & Vegetariërsbond, N. (2008). Environmental effects of protein-rich food products in the Netherlands: consequences of animal protein substitutes. *Gouda (NL): Blonk Consulktants*.
[3] Christensen, L. F., García-Béjar, B., Bang-Berthelsen, C. H., & Hansen, E. B. (2022). Extracellular microbial proteases with specificity for plant proteins in food fermentation. *International Journal of Food Microbiology*, 109889
[4] Dairo, F. A. S., & Fasuyi, A. O. (2008). Evaluation of fermented palm kernel meal and fermented copra meal proteins as substitute for soybean meal protein in laying hens diets. *Journal of Central European Agriculture*, *9*(1), 35-44.
[5] Dwivedi, S. P. (2023). FUNDAMENTALS OF FERMENTATION TECHNOLOGY.
[6] Gu, Y., Hu, Y., Huang, C., Lai, C., Ling, Z., & Yong, Q. (2022). Co-production of amino acid-rich xylooligosaccharide and single-cell protein from paper mulberry by autohydrolysis and fermentation technologies. *Biotechnology for Biofuels and Bioproducts*, *15*(1), 1-10.
[7] Kårlund, A., Gómez-Gallego, C., Korhonen, J., Palo-Oja, O. M., El-Nezami, H., & Kolehmainen, M. (2020). Harnessing microbes for sustainable development: Food fermentation as a tool for improving the nutritional quality of alternative protein sources. *Nutrients*, *12*(4), 1020.
[8] Molfetta, M., Morais, E. G., Barreira, L., Bruno, G. L., Porcelli, F., Dugat-Bony, E., ... & Minervini, F. (2022). Protein sources alternative to meat: state of the art and involvement of fermentation. *Foods*, *11*(14), 2065.
[9] Reihani, S. F. S., & Khosravi-Darani, K. (2019). Influencing factors on single-cell protein production by submerged fermentation: A review. *Electronic journal of biotechnology*, *37*, 34-40.
[10] Renna, M., Coppa, M., Lussiana, C., Le Morvan, A., Gasco, L., & Maxin, G. (2022). Full-fat insect meals in ruminant nutrition: in vitro rumen fermentation characteristics and lipid biohydrogenation. *Journal of Animal Science and Biotechnology*, *13*(1), 138.
[11] Ross, R. P., Morgan, S., & Hill, C. (2002). Preservation and fermentation: past, present and future. *International journal of food microbiology*, *7Reihani et al., 2019*(1-2), 3-16.
[12] Smetana, S., Palanisamy, M., Mathys, A., & Heinz, V. (2016). Sustainability of insect use for feed and food: Life Cycle Assessment perspective. *Journal of cleaner production*, *137*, 741-751.
[13] Strong, P. J., Self, R., Allikian, K., Szewczyk, E., Speight, R., O'Hara, I., & Harrison, M. D. (2022). Filamentous fungi for future functional food and feed. *Current Opinion in Biotechnology*, *76*, 102729.
[14] Sun, W., Shahrajabian, M. H., & Lin, M. (2022). Research progress of fermented functional foods and protein factory-microbial fermentation technology. *Fermentation*, *8*(12), 688.
[15] Tziva, M., Negro, S. O., Kalfagianni, A., & Hekkert, M. P. (2020). Understanding the protein transition: The rise of plant-based meat substitutes. *Environmental innovation and societal transitions*, *35*, 217-231.
[16] Van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nature Food*, *2*(7), 494-501.
[17] Vauterin, A., Steiner, B., Sillman, J., & Kahiluoto, H. (2021). The potential of insect protein to reduce food-based carbon footprints in Europe: The case of broiler meat production. *Journal of Cleaner Production*, *320*, 128799.
[18] Verbeke, W. (2015). Profiling consumers who are ready to adopt insects as a meat substitute in a Western society. *Food quality and preference*, *39*, 147-155.
[19] Windey, K., De Preter, V., & Verbeke, K. (2012). Relevance of protein fermentation to gut health. *Molecular nutrition & food research*, *56*(1), 184-196.

