

ENZYMES IMPORTANCE IN FOOD INDUSTRY

Thiruchitrambalam



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CHAPTER 1

FLAVOR AND NUTRITION: AN INTRODUCTION TO THE WORLD OF FOOD ENZYMES

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ABSTRACT:

Enzymes, acting as culinary alchemists, play a critical role in modifying the gastronomic landscape by controlling biological processes. The varied range of food enzymes, from amylases to proteases, is investigated, shedding light on their individual contributions to the improvement of taste, texture, and nutritional quality in a variety of culinary creations. The chapter explores the dual legacy of enzyme usage, which includes both ancient fermentation processes and cutting-edge biotechnological applications. It investigates the effect of enzymes on flavor profiles, revealing how these biological catalysts contribute to the creation of complex and appealing flavors. Beyond taste, the debate continues into the fascinating world of texture and mouthfeel, where enzymes influence the sensory experience of numerous food items. Enzymes, as custodians of nutritional improvements, help to boost the bioavailability of vital nutrients and break down antinutritional elements. The chapter also discusses the problems and ethical issues related with enzyme usage, as well as current advances for responsible and sustainable uses. The chapter concludes with a look forward to forthcoming trends and technology that will continue to define enzymes' increasing position in the culinary world. This research allows readers to appreciate the complex interaction between science and culinary skill while uncovering the mysteries of the realm of food enzymes.

KEYWORDS:

Active Enzyme, Active Site, Cold, Flavor Nutrition, Food Enzyme.

INTRODUCTION

Enzymes are a special type of chemical that help reactions happen faster. They are only needed in small amounts and they don't get used up during the reaction. Enzymes are able to change one kind of molecule into another. Enzymes work very fast and this can be measured with a number called *k_{cat}*, which tells us how many times the enzyme can complete a reaction in a given time. This number shows how many substrate molecules one enzyme can change into the product in a certain amount of time, like a minute or a second. As an illustration, more than 500,000 molecules of carbon dioxide and water can be transformed into bicarbonate every second by a single carbonic anhydrase molecule. This is a very impressive accomplishment. Enzymes are very strong at making things happen and they are very good at only working on one type of thing at a time. They usually only change one type of substance into another. Some enzymes can only work on specific groups of molecules. To illustrate, a common enzyme called alkaline phosphatase can take away a phosphate group from different substances. It is often used in first-year lab sessions to study how enzymes work [1], [2].

Additionally, they can be extracted from cells and employed to expedite numerous crucial operations for businesses. For instance, they are used to make sweeteners and change antibiotics. They can also be located in laundry detergent and similar cleaning items, and are crucial in instruments and examinations utilized in medical, legal, and environmental situations. The word 'enzyme' was first used in 1878 by the German scientist Wilhelm Kühne.

He used it to describe how yeast can turn sugar into alcohol. The word comes from the Greek words 'en' which means 'within' and 'zume' which means 'yeast'. In the late 1800s and early 1900s, big progress was made in getting, studying and using many enzymes. But it wasn't until the 1920s that enzymes were figured out to be connected to protein molecules. For about 60 years, people thought that all enzymes were proteins. But in the 1980s, they discovered that some RNA molecules can also act like enzymes. These special RNAs, called ribozymes, are important for how genes work. In the 10 years, scientists also made a way to create antibodies that can speed up chemical reactions. These special 'abzymes' can be useful in making things and in medicine. Despite some exceptions, most of the study of enzymes focuses on the proteins that have the ability to speed up chemical reactions. This essay also focuses on these proteins[3], [4].

Other enzymes are very specific, meaning they can only work on one type of molecule. For instance, glucose oxidase mostly only works with a specific type of sugar called β -D-glucose and doesn't really work with other types of sugars. As we will see later, this specificity is really important in many tests and devices that measure a specific substance. glucose in a mix of different things. Enzymes usually have ordinary names that show what reaction they help with and end in ase. For example, lactase helps with the digestion of lactose. Certain enzymes end with specific suffixes that indicate their functions, like oxidase, dehydrogenase, and carboxylase. However, proteolytic enzymes usually end with in, for example. Trypsin, chymotrypsin, and papain are enzymes. Sometimes, the simple name of the enzyme also shows what the enzyme works on. This text is about three enzymes glucose oxidase, alcohol dehydrogenase, and pyruvate decarboxylase. However, some common names for example Enzymes like invertase, diastase, and catalase don't tell us much about the things they work on or the results of their actions[5], [6].

The Enzyme Commission was formed by the International Union of Biochemistry in response to the growing difficulty in understanding and lack of uniformity in enzyme names. The first official report about enzymes was published in 1961. It gave a structured way to name enzymes. The sixth version, which came out in 1992, had information about almost 3,200 different enzymes. The number has been boosted to over 5,000 due to the release of new supplements annually. All enzymes in this system are distinguished by a four-component code known as an Enzyme Commission (EC) number. For instance, the enzyme called lactate dehydrogenase has the EC number 1.1.1.27, but it is better known as L-lactate: NAD⁺ oxidoreductase. The initial segment of the EC number provides information on the function of the enzyme in the reaction. The last numbers mean different things depending on the first number that is shown. In the oxidoreductase category, the second number indicates the donor of hydrogen, while the third number indicates the acceptor of hydrogen. Amino acid-based enzymes are round proteins that vary in size from less than 100 to more than 2,000 amino acid building blocks. These building blocks called amino acids can come together to make chains, which then fold and bend to create a specific shape. This shape has a small area called the active site, where another substance can attach[7], [8].

There may be only a handful of amino acids at the active site, potentially fewer than 10. The active site's shape and charge allow it to attach to only one type of substrate molecule, so the enzyme can work specifically with that molecule to speed up chemical reactions. The idea that enzymes work with specific substances because they fit together like a lock and key was first suggested by a chemist named Emil Fischer in 1894. This became known as Fischer's "lock and key hypothesis". It means that only the right size and shape of the substance can fit into the enzyme. Amazingly, this idea was suggested at a time when people didn't even know that enzymes were proteins. As scientists found out more about enzyme structure using X-ray

crystallography and other techniques, they realized that enzymes are not stiff shapes, but can actually change their shape. Based on this discovery, in 1958 Daniel Koshland built upon Fischer's ideas and introduced the 'induced-fit model' of substrate and enzyme binding. In this model, the enzyme changes its shape slightly to fit the substrate. The hand-in-glove model is like a hand and glove that fit perfectly together. The glove is shaped to fit the hand perfectly.

Because only the active site binds to the substrate, we may wonder what the rest of the protein does. The simple answer is that it helps to keep the active site stable and create the right conditions for it to work with the substrate molecule. So, the part of the protein where the action happens can't be taken out without losing its ability to make things happen. But scientists have found that in some cases, they can make smaller proteins that still work. Many enzymes are made of just protein, but some also have a part that is not protein, called a cofactor. This cofactor is needed for the enzyme to work. Cofactors can come in the form of coenzymes or metal ions, such as iron, manganese, cobalt, copper, or zinc. When a coenzyme strongly adheres to a protein and remains connected to it, it is known as the enzyme's prosthetic group. An enzyme that requires assistance to function has a segment known as an apoenzyme that cannot function independently. When the apoenzyme is combined with the helper, it becomes active. The enzyme that is working at the moment is called a holoenzyme.

Enzymes play a critical part in the complex world of food and nutrition, which is both intriguing and necessary. This chapter, "Flavor and Nutrition: An Introduction to the World of Food Enzymes," dives into the fascinating combination of science and culinary talent that characterizes the employment of enzymes in the food business. Enzymes are powerful biological catalysts that can revolutionize the gastronomic landscape. Their capacity to accelerate and control biological activities allows them to unleash a wide range of tastes, textures, and nutritional profiles in food items. This part takes the reader into the magical realm of enzymes, which operate as culinary alchemists, affecting the very essence of flavor and nutrition. Food enzymes range from amylases to proteases, which break down starches and tenderize proteins. This chapter presents an overview of the numerous types of enzymes used in food processing, stressing their distinct functions in improving the sensory and nutritional quality of various culinary creations. Natural mechanisms and technologies help harness food enzymes. Whether via traditional fermentation or revolutionary genetic engineering, this section delves into enzyme utilization's dual heritage rooted in old culinary traditions and expanding through cutting-edge biotechnological applications.

Enzymes interact with dietary ingredients to create complex flavors. From the delicate notes in aged cheeses to the rich fragrances of fermented drinks, this section explores how enzymes impact the creation of complex and attractive tastes in a variety of food items. Enzymes shape the texture and mouthfeel of food, in addition to its taste. The interaction of enzymes with proteins and carbohydrates determines the smoothness of a sauce, the crispiness of a cracker, and the airy structure of bread. This part explains the alchemy behind the textures that please the taste. Enzymes provide nutritional benefits in addition to their flavor. This chapter looks at how enzymes may improve the bioavailability of vital nutrients, break down anti-nutritional components, and contribute to overall food health and wellbeing. Enzymes have several advantages in the culinary world, but they also provide obstacles and ethical issues. This section highlights the problems, such as regulatory considerations and public views, as well as current advancements aimed at addressing these issues and guaranteeing the appropriate and sustainable use of food enzymes. This chapter finishes with a look at the future of enzyme cuisine, balancing tradition and innovation. Anticipated trends, developing technology, and the changing role of enzymes in creating the future of food all contribute to a dynamic environment that promises further gastronomic development. This investigation takes readers

into the fascinating realm of enzymes, the unseen heroes of the culinary cosmos, who contribute to the symphony of tastes and nutritional richness that characterize our gourmet experiences.

DISCUSSION

Just like we have so much data in genomics now, back in the old days, there was a lot of information being collected in the study of enzymes and biochemistry too. This covered topics such as the mechanism of enzymes, the rate of enzyme reactions, the composition of enzymes, and rationale behind their functionality. At that time, data was stored and shared in different ways.

There were no databases, and the information was spread out in the literature, making it difficult to analyze. Enzymes were hard to name and were often given simple names to show what they do. A group of biochemists chose some names for enzymes, but sometimes different groups gave the same enzyme different names. Also, different enzymes were given the same name by different groups. This caused researchers to have trouble understanding each other and communicating clearly. For instance, NADPH dehydrogenase was originally called NADPH diaphorase and old yellow enzyme because it can reduce different dyes. These names are still used today. Shortly after that, D-amino acid oxidase was identified as a new yellow enzyme, and telling the difference between both enzymes became even harder. There are a lot of new enzymes being found, so we need a way to name and group them in a clear and organized way. Similar to the way scientists utilized classification in the 18th century to comprehend various organisms, biochemists and enzymologists collaborated in 1956 to collect data on enzymes and their mechanisms.

A plan was developed by experts of the Enzyme Commission (EC) within the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) to assign names to previously known enzymes and organize newly discovered ones. Enzymes are given a specific code called an EC number that classifies them based on how they change substrates into products. This code helps identify and name enzymes in a systematic way. The first level has six different classes based on the type of chemistry being done. Oxidoreductases help with changes in chemicals (EC 1), transferases move a part of a chemical to another (EC 2), hydrolases break apart chemical bonds (EC 3), lyases also break chemical bonds in different ways than oxidation or hydrolysis (EC 4), isomerases change the shape and structure of chemicals (EC 5), and ligases join two compounds together by using a nucleoside triphosphate molecule (EC 6). These EC classes are divided into smaller groups based on things like the type of chemical bond broken or formed, where the reaction happens, the specific chemical involved, and the substance that helps the reaction happen. The last category of classification is about what kind of material a thing can work on. For instance, alanine racemase changes alanine into a different form.

When the EC began, people thought that enzymes only worked on one thing. But then they found that some enzymes can actually do more than one job. So, the EC started listing all the different jobs that an enzyme can do. Several studies have looked at promiscuity in depth and found that promiscuous enzymes may play a role in how enzymes develop their functions over time. But, the EC classification is still based on the main reaction that enzymes help with, so it doesn't fully describe all the things enzymes can do. However, assigning EC numbers to enzymes is now a normal task in labeling the function of proteins and genes in databases like UniprotKB and Ensemble. It has also been adopted by the popular Gene Ontology.

Organisms need to be able to change and survive in their environment to stay alive and have babies. Living things change to survive in different environments. This causes enzymes to either improve, trade or stop working. At the metabolic level, this adaptation process is about how enzymes can change to work better in different environments with different chemicals. For example, bacteria can become resistant to drugs and pesticides. The discovery of enzymes that can be made without living things has led to the idea that these enzymes could have been created without any living creatures, maybe in the early ocean. As the Earth got cooler, enzymes may have developed from non-enzyme substances or early forms of enzymes, which used to work at hotter temperatures. This change helped to control what kind of substances are used and prevented the production of unwanted products. It also improved the way the body regulates its metabolism. Making enzymes requires a lot of energy, but it helps control important processes in all living things, like glycolysis. One example of how non-living and living things work together is through metalloenzymes. These enzymes have been crafted by nature to encase metal catalysts, enabling them to regulate various chemical reactions within organisms.

Enzyme kinetics is figuring out what makes enzymes work faster or slower. It uses math equations that can confuse students at first. The theory of kinetics is logical and simple. It's important to understand it to appreciate the role of enzymes in metabolism and biotechnology. Enzyme activity tests can be done in different ways either all at once or over time. Discontinuous methods mix the substance and enzyme, and then measure the product after a certain amount of time. These methods are usually easy and fast. Usually, we use these tests when we don't know much about the system yet, or when we know a lot about the system and are sure that the time interval, we are choosing is right. In ongoing enzyme tests, we usually study how fast an enzyme makes a reaction happen by mixing the enzyme with the substance and measuring how much product appears over time. Certainly, we can also measure how fast the reaction happens by seeing how much substrate disappears over time. Other than the direction they are going, the two values are the same. In enzyme experiments, we often use a special substance called a chromogen that makes the reaction easy to see because it turns into a bright color[9]. We can measure this color with a machine called a colorimeter or a spectrophotometer. However, we can use any equipment that can measure the amount of the product or the starting material.

Enzymes are found in all living things. Commercial enzymes usually come from three main sources: animals, plants, and microorganisms. Microorganisms are the best choice for making industrial enzymes because they are cheap to produce, their enzyme content is easier to control, it's easy to get the materials needed to grow them, and they are safer than plant and animal tissues. Microbes are taken from their natural homes and used to make enzymes by finding the best conditions for their growth. Fermentation is a type of process. Enzymes from animals come from catalase, lipase, and rennet. Enzymes from plants come from actinidin, α -amylase, β -amylase, β -glucanase, ficin, lipoxxygenase, and papain. Enzymes from bacteria come from α -amylase, β -amylase, glucose isomerase, protease, and pullulanase. Enzymes from fungi come from α -amylase, catalase, dextranase, glucose oxidase, lactase, lipase, pectinase, protease, and raffinase. Enzymes from yeast come from invertase, lactase, lipase, and raffinase. Enzymes have always been important in making different kinds of foods.

One-way enzymes are used is to make drinks like whiskey, beer, and wine in big factories. These days, the food industry uses many different tools to find new food enzymes, but it's hard for scientists to make and use them in the real world. However, biotechnology has also become a useful tool for the food industry. This technology is creating new and better food,

making it cheaper to produce, improving how food is made, and helping with waste and safety issues. This will be important for making food in the future. Nowadays, most of the foods we eat have enzymes or enzyme-catalyzed reactions in them. Some examples of this kind of food include alcoholic drinks, syrups, sweeteners, chocolate, baby food, baked goods, cheese, milk products, eggs, fruit juice, soda, candy, making flavors, and making meat tender. Enzymes are very helpful in making and preparing food. Enzymes are important because they can be used instead of chemicals in technology. This means using less energy, making products that break down naturally, and causing less harm to the environment. Also, enzymes make less waste than chemical catalysts because they work in a specific way. Also, enzymes can speed up reactions without needing harsh conditions, so they don't ruin important parts of food[10], [11]. Plants, animals, and tiny organisms make most of the enzymes in food. But the enzymes made by tiny organisms are better than the ones made by plants and animals. The previous section already explained why microorganisms are preferred for making enzymes. Many of the enzymes used in lots of different foods.

Cold-acting enzymes have a lot of potential in the food industry and biotechnology. The dairy, juice, meat, and baking industries all have important applications for this product. In the dairy industry, they use a cold active enzyme called β -galactosidase to reduce the amount of lactose in milk. Lactose, a type of sugar, causes severe problems for many people around the world. Pectinases are used in making fruit juice to make it less thick and improve the final product. Cold-active proteases are used in meat processing to make the meat tender. Certain enzymes like proteases, amylases, and xylanases can help make baking faster by making dough rise quicker. They also help keep the smell and moisture in baked goods. Different cold enzymes can be used instead of mesophilic and thermophilic enzymes in making beer, wine, cheese, animal feed, and other products. In food science, cold-loving enzymes are used to make meat tender, process food, add flavor, bake, brew, make cheese and feed animals[12].

Studying the written work shows that enzymes that work in cold temperatures have many benefits compared to enzymes that work in medium or high temperatures. Cold-active enzymes work really well at low and medium temperatures where other similar enzymes don't work as well. Psychrophilic enzymes are helpful because they are very active and can work with a small amount of enzyme. They are also easy to turn off when they are not needed. Cold-active enzymes have three key traits that make them useful in biotechnology. They are affordable because they require fewer enzymes to work. They can work well without extra heat. And they can be turned off with less heat because they are sensitive to temperature changes. So, we can say that cold-active enzymes can help a lot in the food biotechnology field. Although cold-active enzymes work well, they don't last very long. This makes it hard to use them for business. To handle the business demands for enzymes that work in the cold, different scientific methods like changing proteins, using DNA technology, and studying genetic material could be used to make better and new cold-acting enzymes. Genetically modified microbes that are good at making enzymes in cold conditions would be useful in the food industry.

CONCLUSION

In our study of food enzymes, we have found that they are important for cooking and not just for chemical reactions. Enzymes are helpful in making food taste better, feel different, and be more nutritious. They are important in making great food. This chapter shows how enzymes have been used for a long time in cooking and how they are also used in modern science and technology. Enzymes are like magic in cooking. They have changed how food tastes and feels, and are really important in many different types of food from all over the world. As we

look ahead, new food enzymes can lead to more exciting and responsible ways to explore and enjoy different types of food. Despite some challenges like rules and ethics, scientists are working hard to understand and improve the use of enzymes in cooking. They want to make sure that enzymes fit well into the culinary world. In the changing mix of old and new, food enzymes are both protectors of tradition and signs of change. Their small impact on how food tastes is important in our everyday cooking. As we enjoy the delicious tastes and different types of food, we are reminded of how important tiny chefs are in creating great meals

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CHAPTER 2

MICROBIAL ENZYMES: UNLOCKING THE CUISINE CHEMISTRY OF FOOD BIOTECHNOLOGY

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ABSTRACT:

Microorganisms, including bacteria and fungi, are prolific enzyme makers, unleashing transformational potential in food processing, taste improvement, texture alteration, and nutritional enrichment. The variety of microbial enzymes is highlighted, including amylases, lipases, proteases, and others, each contributing distinctively to the alchemy of culinary experiences. The historical origins of enzyme synthesis by fermentation are examined alongside recent biotechnology breakthroughs, demonstrating the transition from conventional methods to cutting-edge genetic engineering. The chapter describes the various ways in which microbial enzymes are used across the food industry to shape goods in baking, brewing, dairy production, and beyond. The chapter delves into the intriguing interaction between microbial enzymes and taste profiles, textures, and nutritional content, emphasizing their significance as culinary innovation catalysts. It addresses regulatory, consumer perception, and environmental issues while also predicting upcoming trends and breakthroughs that will pave the way for a vibrant future in food biotechnology. Furthermore, the socioeconomic effect, ethical issues, and global viewpoints highlight the universal relevance of microbial enzymes, which transcend cultural barriers in the culinary world. As we close, the chapter provides a forward-looking view, recognizing the continuous effort to discover new possibilities in the enzymatic gastronomy frontier, which will shape the future of food as we know it.

KEYWORDS:

Biotechnology, Enzymes, Fermentation, Food, Microbial.

INTRODUCTION

Microbial enzymes play a critical part in the vast terrain of food biotechnology, and their impact is significant and transformational. Microorganisms, which range from bacteria to fungus, have long been nature's biochemical magicians, creating a variety of enzymes that catalyze biochemical processes required for survival. Harnessing the power of these microbial enzymes has become a cornerstone of contemporary food biotechnology, transforming how we produce, prepare, and improve the tastes, textures, and nutritional profiles of our food.

The Biotechnological Revolution has brought biology, technology, and food production closer together than ever before. Biotechnology, with its many tools and procedures, has enabled scientists and food technologists to dig into the tiny world of microbes, revealing its enzymatic potential. Microbial enzymes, in particular, have emerged as crucial tools for molecularly altering food [1], [2]. Microbial communities are very diverse, resulting in a wealth of enzymes. Microbial enzymes have a variety of functions in the transformation of raw components into pleasant and healthy food products, including amylases that break down starches and lipases that catalyze lipid hydrolysis.

This section gives an overview of the numerous types of microbial enzymes and their uses in food preparation.

Enzyme Production: Fermentation and Beyond

Fermentation, a basic biotechnological method, has long been used to produce enzymes from microbes. This section delves into the historical foundations of enzyme synthesis by fermentation while also highlighting recent advances such as submerged and solid-state fermentation processes. It also goes into novel ways, such as genetic engineering, to improve microbial populations for higher enzyme output. Microbial enzymes are essential instruments in the food processing business. From breaking down complex carbohydrates to altering proteins and lipids, these enzymes help to create desired textures, tastes, and nutritional properties in a variety of food items. This section explains how microbial enzymes are used in several phases of food processing, such as baking, brewing, dairy production, and more [3], [4]. Microbial enzymes have the unique capacity to enhance food flavors. Microbial enzymes operate as culinary alchemy catalysts, converting precursors into delicious components and producing fragrant molecules. This section digs into how these enzymes improve the taste profiles of foods, adding to the depth and complexity of culinary experiences. Microbial enzymes shape the texture and mouthfeel of food, influencing the entire sensory experience. Microbial enzymes help to create the soft crumb of bread, the creaminess of yogurt, and the melt-in-your-mouth feel of chocolate. This section looks at how enzymes change the structural components of meals, impacting their tactile properties [5], [6].

Microbial enzymes not only enhance flavor and texture, but also improve nutritional value of meals. They take part in processes that increase the bioavailability of important nutrients, degrade antinutritional substances, and improve overall nutritional profiles. This section delves into the nutritional implications of microbial enzyme applications, highlighting their significance in developing better and more nutritious food alternatives. Using microbial enzymes in food biotechnology has benefits, but also presents obstacles. Issues such as regulatory compliance, customer perceptions, and the sustainability of enzyme manufacturing processes must be carefully considered. This section analyzes the difficulties and potential solutions for guaranteeing the appropriate and ethical use of microbial enzymes in the food business. Food biotechnology is a constantly evolving area with new horizons being discovered via continuing study [7], [8]. This section investigates current trends and breakthroughs in the field of microbial enzymes, such as the incorporation of cutting-edge technology, the discovery of novel microbial sources, and improvements in enzyme immobilization methods. These advancements signal a future in which microbial enzymes will continue to play an important role in changing the food business.

Social Impact and Ethical Considerations

As microbial enzymes grow more prevalent in our food supply, greater socioeconomic and ethical problems emerge. This section investigates the socioeconomic implications of broad enzyme usage, including consumer knowledge, transparency, and the possible effect on traditional food practices. Ethical implications for genetic alteration and biotechnological treatments are also addressed. Microbial enzymes are used in several culinary cultures across the world. This section investigates how many civilizations use microbial enzymes in traditional cuisines, demonstrating the universality of enzymatic activities in food preparation. It also explores how globalization and cross-cultural factors impact the acceptance and adaptability of microbial enzyme technology. In the last part, we discuss the enormous influence of microbial enzymes in the field of food biotechnology. From their historical origins to modern advancements, these enzymes have spurred a gourmet revolution, altering how we perceive, create, and consume food. The chapter finishes with a forward-looking view, recognizing the field's dynamic character and the constant effort to discover new possibilities on the enzymatic gastronomy frontier. In summary, this comprehensive

introduction lays the groundwork for a thorough investigation of microbial enzymes in food biotechnology. It emphasizes the complicated network of scientific, technical, and culinary components that come together in the realm of enzymatic transformations, changing the foods we eat and providing a peek into the future of gastronomy via the lens of microbial enzymes.

DISCUSSION

Enzymes and tiny organisms have been used for a long time to make beer, bake bread, and make cheese and wine. Food biotechnology is finding different ways to make raw materials into healthy food. Enzyme technology is making food taste better and have more healthy stuff in it. It helps food work better and have more nutrients. Enzymes can be made by animals, plants, and tiny living things. But the best and most useful way to make enzymes for businesses is by using tiny living things like bacteria, fungi, yeasts, and actinomycetes. Enzymes all over the world Alpha-amylase, glucoamylase, lipase, pectinase, chymosin, and protease are used in making food. The amylase enzyme changes starch into dextrins and makes corn syrup that can be used to make food taste sweeter. The brewing process involves turning complex carbohydrates into simpler ones by using barley and other grains. In making light beer, enzymes called glucoamylase change some sugars to make them easier to ferment. This helps give the beer a good taste. Lipases are enzymes that break down fats, make cheese taste better faster, and create high-quality fat products. Pectinase is a special enzyme that helps to make fruit juice clear and smooth by breaking down the cell walls in the fruit[9], [10]. Chymosin enzymes are used in making cheese to break down proteins in the milk. Bacteria and Fungi make enzymes that can be used to make fish meals, meat extracts, and other protein-based foods. Lactase enzymes break down the lactose in whey and milk to make polyactide. Glucose oxidases help turn glucose into gluconic acid, which prevents the Maillard reaction. Acetolactate decarboxylase is a substance that changes acetolactate into acetoin to make wine mature faster. Cellulase is an enzyme that helps change cellulose into glucose in plant cell walls. This helps extract more nutrients and increase the fiber in food. Enzymes are helping the food industry by making new and useful products, reducing the cost of making food, and making the process better[11], [12].

Industrial enzyme production has two main steps: first, finding the right kind of bacteria or fungi; and second, the process of making the enzymes through fermentation. Making the right mix of ingredients for making enzymes is important for commercial production. The mix needs to have carbon, nitrogen, and micronutrients to help microorganisms grow during fermentation. After fermentation is finished, the next steps like getting the enzyme, cleaning it, and making products can happen in a good carrier. The fermentation process is put into three groups doing things all at once, doing things a little at a time, and doing things constantly without stopping. In the batch process, all the ingredients are added at the beginning of fermentation. In fed batch fermentation, the production strain gets extra nutrients while it's fermenting, just like in batch fermentation. Continuous fermentation is when we keep adding new food for the bacteria and at the same time, we take out the fermented product. The fermentation process can be done in two different ways - either by using solid materials or by putting the materials in a liquid. Bacteria make enzymes mostly by growing in a liquid instead of on a solid surface.

This is because it's easier for the bacteria to grow and release enzymes in a liquid environment. In submerged fermentation, bacteria are put into sterilized liquid and kept at the right conditions like air, stirring, oxygen, movement, heat, and acidity for 48-72 hours, depending on the type of bacteria. Solid-state fermentation is good for fungus to make important enzymes for food processing. A type of fungus prefers to ferment on surfaces or

solid materials to make enzymes and other substances. Cleaned solid material, such as wheat bran, rice bran, and other grains, helps fungus grow when the temperature, humidity, and moisture are right. After the products have been left to ferment for a while, they are collected and further processed. This includes getting rid of any leftover cell parts, purifying the product, and making it into enzymatic products. Usually, it is more difficult to process enzymes that are inside cells than enzymes that are outside of cells. Purifying enzymes is costly. Scientists mainly use a technique called chromatography to purify enzymes in large-scale production.

Transglutaminase enzymes change proteins by combining and changing their parts. Transglutaminase is a substance that links amino acids together. It can also transfer molecules and change amino acids. Transglutaminase enzymes are being used in the food industry to make proteins in food stronger. This makes food look better and taste better. It also helps make different types of protein ingredients. Transglutaminase helps food products hold water better, feel softer, make better foam, and stay stable. Transglutaminase is found outside of cells and comes from certain types of bacteria. These bacteria are called *Streptovorticillium* spp., *Streptovorticilliummobarens*, *Streptovorticilliumladakanum*, and *Streptovorticilliumlydicus*. This information comes from several studies done by different researchers in the late 1980s and 1990s. The bacteria *Bacillus subtilis* and spherules release a protein called intracellular transglutaminase. Lactase is an enzyme that helps break down the sugar in milk called lactose into simpler sugars like glucose and galactose. Lactases come from plants, animals, bacteria, fungus, yeasts, and molds. Lactase enzymes used in commercial production are made from *A. Niger*.

Lactases from fungi work best in acidic conditions, while those from yeast and bacteria work best in neutral conditions. The lactase enzyme is mostly found in babies and is called a brush border enzyme. Some people cannot properly digest milk because they don't make enough of the enzyme called lactase. This is when a person cannot digest lactose, a sugar in milk. People who are lactose intolerant need to take a lactase enzyme to help them digest milk. The lactase enzyme makes lactase-treated milk taste sweeter and helps make ice cream and yogurt. Catalase enzymes break down hydrogen peroxide into water and oxygen, which helps protect cells from damage caused by reactive oxygen. Commercial catalases are made from *Aspergillus niger* using a solid-state fermentation method. Catalase is used in the food industry with other enzymes to help preserve food, process eggs, and eliminate bad flavors in milk. Lipases are important for breaking down fats in our bodies and in the food we eat. Lipases are enzymes that help break down fats into smaller parts like fatty acids and glycerol in a way that doesn't involve water. They can also help make and change fats into different forms. Certain tiny organisms such as *Pseudomonas aeruginosa*, *Serratiamarcescens*, *Staphylococcus aureus*, and *Bacillus subtilis* are very good at making lipase enzymes.

Lipases are used a lot in making medicines, chemicals, and foods. Lipases are used in the food industry to break down milk fats, make cheese taste stronger, reduce bitterness, and keep food from going bad. Lipases can mix with a lot of other enzymes, such as protease or peptidases, to make tasty cheese with less bitterness. Proteolytic enzymes are also called peptidases, proteases, and proteinases. They can break down the bonds in proteins. Proteases are usually put into two groups: endopeptidases, which cut protein chains in the middle, and exopeptidases, which cut them at the ends. Exopeptidases cut the ends of protein chains, while endopeptidases cut bonds in the middle. Proteases come from different living things like plants, animals, and tiny organisms. But the proteases that are used for making products are mostly from tiny organisms, like bacteria and fungi. Microbes release proteases outside and inside their cells during both the liquid and solid fermentation process. Some types of

bacteria, such as *Bacillus licheniformis* and *Bacillus subtilis*, and some types of fungus, like *Aspergillus niger*, are known as *Bacillus* and *Aspergillus* species.

Pectinase enzymes are used to improve vegetable fibers in making starch, curing coffee, cocoa and tobacco, canning orange segments, and getting sugar from date fruits. Acetolactate decarboxylase is a type of enzyme that changes acetolactate into acitoine and releases carbon dioxide. Acetolactate decarboxylase is an enzyme that converts acetolactate to acitoine while also releasing carbon dioxide. The function of acetolactate decarboxylase is to transform acetolactate into acitoine, with the simultaneous release of carbon dioxide. With the assistance of acetolactate decarboxylase, acetolactate is converted to acitoine, and carbon dioxide is also produced in the process. This is called a decarboxylation reaction. Acetolactate decarboxylase is made for sale by using *Bacillus subtilis*, genetically improved *Bacillus brevis*, and *Enterobacter aerogenes* strain 1033 in a process called submerged fermentation. In regular beer making, this is the first step of how microbial cells use xylose for energy. Xylose isomerases are enzymes that can change d-glucose into d-fructose, so they are also known as glucose isomerases. Microorganisms like *Streptomyces olivochromogenes*, *Bacillus stearothermophilus*, *Actinoplanes missouriensis*, *Thermotoga maritime* and *Thermotoga neapolitana* are good for getting xylose isomerase. Xylose isomerase works less effectively when it is in acidic conditions, up to 50% less effective. Glucose isomerase is very useful in making food. It helps change glucose into fructose and xylose into xylulose. This is important for making a lot of different kinds of food.

Proteases are special enzymes that help break down proteins into smaller parts. They are used a lot in soap and medicine, and also in making food. They make up 60% of the industrial enzymes being sold. The worldwide need for protease enzymes has been increasing by 5.3% every year from 2014 to 2019. Their need for enzymes is expected to go up even more because they will be used in making leather and cleaning up the environment. Protease enzymes come from animals, plants, and tiny living things like bacteria and fungi. Proteases are split into two groups: exopeptidases and endopeptidases, based on where they work on protein chains. Exopeptidases work on the outside of the polypeptide chains and endopeptidases work anywhere inside the chains. The endopeptidases are grouped into six categories based on the type of residue in their active site: serine, aspartic, cysteine, metallo, glutamic acid, and threonine protease.

Proteases from plants like bromelain, ficin, and papain are used a lot in the food industry for things like making beer, softening meat, thickening milk, and helping digestion. Proteases are used to make food taste better, give it more nutrients, and make it easier to digest. They can also change how food proteins work, like making them thicken or blend better. Proteases are commonly used in the baking industry to make bread, baked goods, crackers and waffles. These enzymes help make the mixing process faster, make the dough softer and more even, control the gluten in bread, and make the texture and taste better. The acid protease from *Aspergillus usarii* has been used to make wheat gluten work better. Adding protease helps to release enough peptides and amino acids in the wort for it to ferment properly. Acidic fungal proteases help make beer ferment better by balancing the amino acids in the beer, even when the pH is low. Another important use of proteases is in the dairy industry. Naturally found proteases make cheese taste better. They are used to make cheese ripen faster, change how milk products work, and make them less likely to cause allergies. In cheese making, proteases are used to break down certain parts of the milk to make cheese.

As we conclude our investigation of "Microbial Enzymes in Food Biotechnology," it is clear that these tiny catalysts serve as transforming agents in the art and science of food. Microbial enzymes have permeated human culinary experiences, from traditional fermentation

processes to cutting-edge biotechnological developments. This chapter has revealed their numerous uses, ranging from taste improvement and texture alteration to nutritional enrichment, which shape the foods we eat on a regular basis. As we negotiate the dynamic intersection of science, technology, and gastronomy, the potential for microbial enzymes to transform food processing and design is undeniable. However, this enzymatic journey is not without obstacles, such as regulatory complications, ethical concerns, and the necessity for sustainable procedures. Navigating these hurdles is critical to ensuring the appropriate integration of microbial enzymes into the global food supply. Looking forward, the chapter depicts a future in which rising trends and breakthroughs drive microbial enzymes to the forefront of food biotechnology.

The socioeconomic effect and cross-cultural influences highlight their global relevance, crossing boundaries and enhancing culinary traditions throughout the globe. In summary, as we conclude our research, we see that microbial enzymes are more than just biochemical agents; they are culinary architects, changing the history, present, and future of our culinary world. Their impact is woven into the fabric of our culinary past, suggesting a future in which the enzymatic frontier expands, adding new dimensions to the flavors, textures, and nutritional value of the foods we enjoy. Lipases are special enzymes that break down fats into smaller parts. They are found in the stomach and pancreas of humans and other animals to help break down fats and oils. Microbial lipases come from small organisms like bacteria, fungi, and yeast. Microscopic organisms' enzymes help with about 90 out of every 100 lipase sold around the world. This enzyme is used in different industries like food, biofuel, detergent, and animal feed. It is also used in making leather, cloth, and paper. In the food and drink industry, lipases are used a lot in dairy, baking, fruit juice, beer, and wine making. Even though lipase is used in many industries, it only makes up less than 10% of the overall global market for industrial enzymes.

Commercial lipases are mostly used to make dairy products taste better and to process other foods that have fat in them. They can make cheese taste better by changing the fats in the milk to make fatty acids. You can make different kinds of cheese by using lipases from different places. Romano cheese is made using a special enzyme from baby animals, Camembert cheese is made using an enzyme from a type of mold, and cheddar cheese is made using an enzyme from a certain type of fungus. *Oryzae* is a type of fungus. Lipase helps make cheese softer and smoother. Lipases are used to make butter and margarine taste better and last longer. They are also added to baking products to keep them fresh for a longer time. In drinks like wine, the smell can be changed using lipase. They help make cocoa butter better. Cocoa butter normally melts at 37 °C because of certain acids in it. Unilever has applied for a patent for using a special enzyme called immobilized *Rhizopus miehei* lipase. This enzyme can change palmitic acid to stearic acid to make a type of fat that they want. We used certain chemicals to make antioxidants that can dissolve in sunflower oil. We used special enzymes from certain fungi and bacteria to help us do this.

CONCLUSION

Food and feed processing areas are doing really well using tiny living things like enzymes and microorganisms to make good food. In the future, new types of bacteria will be important for making industrial chemicals. But right now, people are not sure if they are safe to use in genetically modified food. There is no question that making things with genetic engineering is better than using wild strains. Advanced fermentation methods and processing will help make a lot of high-quality food enzymes. We need to create a new way to make food that is faster and cheaper. So, we need to do more research in the area of recombinant DNA

technology to make better production strains, produce enzymes that can be sold, and create new ways to process food that are cost-effective.

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CHAPTER 3

ENZYMES IN THE BEVERAGE INDUSTRY: A COMPREHENSIVE OVERVIEW

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ABSTRACT:

This introduction takes you from the historical foundations of enzymatic beverage manufacturing to the cutting edge of biotechnological innovation, navigating the complicated world where enzymes serve as catalysts, altering the tastes, fragrances, and textures of famous drinks enjoyed worldwide. The abstract describes the enzymatic processes unique to each category, including brewing, winemaking, distilled alcohol, fruit juice processing, and even soft drinks. It emphasizes the revolutionary role of enzymes in coffee and tea manufacturing, as well as new functional beverage trends. Biotechnological developments, obstacles, and consumer views are also discussed, presenting a comprehensive picture of the intricate interaction between enzymes, technology, and consumer preferences. This abstract captures the essence of the enzymatic journey in the beverage sector by focusing on global views and looking forward to future possibilities. As we begin on an examination that crosses cultural and technical barriers, the function of enzymes in the beverage industry appears as both a timeless heritage and a dynamic force that is constantly transforming the global beverage scene.

KEYWORDS:

Beverage Manufacturing, Coffee Beans, Enzymes, Fruit Juice, Fermentation.

INTRODUCTION

The intriguing world of enzymes has a major impact on the beverage business, which is dynamic and ever-changing. These biocatalysts, with their extraordinary capacity to accelerate and control biological events, play an important role in influencing the tastes, smells, and textures of a wide variety of drinks. From classic fermentation techniques to current biotechnological advancements, enzymes act as unseen architects, adding to the richness and distinctiveness of drinks enjoyed across the world. The use of enzymes in beverage manufacturing dates back to ancient times, when spontaneous fermentation was discovered and manipulated to produce libations such as beer and wine. This section explores the historical origins of enzymatic activities in beverage manufacture, demonstrating how early civilizations naturally integrated these processes into their culinary traditions. Enzymes have several functions in the beverage sector due to their diversified nature. From amylases that break down starches to proteases that influence protein structures, the beverage industry depends on a complex symphony of enzymes [1], [2].

This section describes the many kinds of enzymes used in beverage manufacturing, as well as their individual contributions to the finished product. Many renowned drinks rely on the transformational process of fermentation, which involves enzymatic activity. This section looks at how enzymes, especially those generated by microbes, drive the fermentation process, turning carbohydrates into alcohol and contributing to the distinct tastes and qualities of drinks like beer, wine, and spirits. Enzymes play a crucial role in the brewing process, particularly for beer. Enzymes serve crucial functions in converting complex carbs into fermentable sugars during the malting and mashing processes. This section delves into the individual enzymes used in brewing, their roles, and how they affect the taste character and

texture of the finished beer. Enzymes play a crucial role in winemaking by extracting tastes and colors from grapes, clarifying and stabilizing the final product[3], [4].

This section dives into the enzymatic activities that occur throughout the various phases of winemaking, emphasizing the careful balance that winemakers establish to produce desirable sensory qualities. Spirits, such as whiskey, vodka, and rum, are produced via sophisticated enzymatic processes. This section investigates how enzymes contribute to the rich and intricate world of distilled drinks, ranging from the conversion of starches to sugars in the malting process to the enzymatic changes during fermentation. Enzymes play a crucial role in fruit juice processing, helping extract flavors, clarify products, and improve overall quality. This section discusses the function of enzymes in fruit juice production, ranging from cell wall breakdown for efficient extraction to enzymatic regulation of color and taste qualities. Enzymes play a significant role in shaping the unique tastes and fragrances of coffee and tea. Enzymes contribute to the rich sensory experiences of these internationally used drinks by fermenting coffee beans and oxidizing tea leaves. This section investigates the subtle enzymatic interventions used in coffee and tea preparation.

Enzymes may be used to produce soft drinks and functional beverages, in addition to typical alcoholic and non-alcoholic beverages. Enzymes help to generate desirable tastes, break down components, and even boost nutritional value. This section goes into the many uses of enzymes in a wide range of nonalcoholic drinks. The beverage sector is leading the way in biotech innovation, with enzymes playing a key role. This section investigates how genetic engineering and enzymatic biocatalysis are transforming beverage manufacturing, allowing for the development of unique beverages with better tastes, greater sustainability, and higher efficiency. While enzymes have several advantages, their usage in the beverage sector presents obstacles. Regulatory issues, stability, and cost-effectiveness all present challenges. This section discusses the issues and proposes new solutions and tactics to solve them, assuring the continuous and responsible use of enzymes in beverage manufacturing. Consumers are becoming more aware of the components and methods used in beverage manufacturing. This section looks at how customer perceptions impact the use of enzymatic procedures in the beverage sector[5]. It also investigates growing trends like as clean-label preferences and the need for natural and sustainable enzymatic therapies. Enzymatic mechanisms in beverage manufacture vary by culture and area, reflecting varied culinary traditions. This section delves into global views on enzymatic beverages, examining how various civilizations integrate these processes into traditional drinks and how globalization affects cross-cultural exchanges in beverage manufacturing. The introduction concludes with a discussion on the future of enzymatic interventions in the beverage industry, highlighting the balance between tradition and innovation. Anticipated trends, future technology, and the continued drive for sustainability and efficiency determine the beverage trajectory, which is powered by enzyme catalysis. In short, this comprehensive introduction lays the groundwork for a thorough examination of enzymes in the vast and intriguing world of drinks[6], [7]. Enzymes, from the ancient origins of fermentation to the cutting edge of biotechnological innovation, continue to reinvent the tastes, textures, and sensations contained inside each sip. As we begin on this trip, we will unravel the enzymatic tapestry that connects the global beverage industry, enabling readers to see the subtle but significant impact of these biocatalysts on the drinks that adorn tables and gatherings throughout the globe.

DISCUSSION

More and more people want food and drinks, so companies are paying attention. The drink industry is one of the biggest industries for making food. The food and drink industry is split

into two main groups: alcoholic and non-alcoholic drinks. Alcoholic drinks are beer, wine, whiskey, and others, while nonalcoholic drinks are fruit juice and soft drinks. People drink the most carbonated soft drinks, followed by bottled water, coffee, and beer. The food and drink industry uses raw ingredients and processes them through steps like extraction and maceration. Food, like fruits and veggies, have substances called polysaccharides like pectin and starch. These substances can make it harder to filter the food during the process. When fresh fruit juice looks cloudy right away, it's because of bits of cells and small pieces of a substance called pectin. If the juice gets cloudy later on, it's because different substances in the juice have reacted with each other to form larger, more complex pieces. When proteins and polyphenols come together, they often make beer, wine, and clear fruit juices cloudy. Commercial fruit juices are treated with chemicals like bentonite and gelatin to make them clear and smooth[8], [9]. Using enzymes instead of chemicals can save money and help the environment by using less energy and reducing pollution. Enzymes are used in food to make it taste better and to help with making the food.

Many food enzymes are used to help process food, while a small number are added to food as extra ingredients, such as lysozyme and invertase. In the beverage industry, amylases, pectinases, and cellulases are commonly used to make more of the drink, make it clearer, improve its smell, and for other purposes. This chapter talks about how enzymes are used to make drinks like alcoholic and non-alcoholic beverages. It focuses on three main points about the use of enzymes in beverage production. First, we will talk about the enzymes used in making drinks and explain what they do, where they come from, and why they are helpful. Next, we'll look at how enzymes are used in making drinks. Lastly, the chapter talks about the economy and how it can get better in the future. Enzymes used in making drinks 3.21 Enzymes used in making fruit and vegetable juice In the last few years, more people have been drinking natural fruit juices. According to a database in the US, raw apples, pineapples, and tomatoes have a lot of carbohydrates. Apples have 13.8% carbohydrates, pineapples have 13.1%, and tomatoes have 4.0%. Fruits and vegetables have a lot of polysaccharides like pectin, cellulose, hemicellulose, and lignin, which can make fresh juice look cloudy[10], [11]. Like in making other juices, clarification is an important part of making juice. It helps to get rid of any cloudiness, haziness, and bits of solid in the juice. This can be done using enzymes, filters, or by adding certain substances like chitosan, gelatin, bentonite, polyvinyl pyrrolidone, or a combination of two of these substances. Enzymatic treatment is helpful because it makes it easier to extract more from the products, and it also reduces sugars, dry matter, and galacturonic acid.

The top food enzyme makers in the world are from Denmark, Switzerland, Germany, the Netherlands, and the United States. Some notable companies are Novozymes in Denmark, AB Enzymes in Germany, and DuPont in the United States. Out of all the enzymes in food, pectinases make up 25%. Pectinolytic enzymes come from plants and tiny living things like bacteria, yeasts, and molds. Tomatoes and oranges are the main places where plant pectinases come from. Pectinases from microorganisms are better than those from plants and animals because they are cheaper to make, can be changed more easily, and are safer to use. Most pectinolytic enzymes used in businesses are made by fungi like *Aspergillus*, *Rhizopus*, *Alternaria*, and *Fusarium*. Some of the types of bacteria that make pectinases are *Agrobacterium tumefaciens*, *Bacteroides thetaiotamicron*, *Ralstoniasolanacearum*, and *Bacillus* sp. Pectinolytic enzymes are made using two methods: either by soaking them in a liquid or by letting them ferment on a solid surface. Using SSF for industries is hard because it's difficult to purify the product, the fermentation mixture is not the same throughout, it's hard to make a lot of products, and some of the enzyme gets lost in the solid waste. Yet, SmF is easier to manage on a big scale and has been used to make different substances since the

1940s. Additionally, making enzymes should be affordable. For this reason, we should use a lot of cheap materials. In order for SmF to make money, pectinases were made using cheap sources like citrus peel, orange peel, apple pulp, corn flour, wheat bran, pumpkin oil cake, and other leftover farming materials[12].

So, it is really important to make enzymes for businesses that are cheap and work well, because more and more people want to use them. Kertesz said in 1930 that the first pectinase was used to make apple juice clear. A list of companies that make some types of pectinase enzymes. Pectin is a complex carbohydrate made of specific types of sugars linked together in long chains. The pectic substances make up about 0.5% to 4% of the weight of fresh material. It is difficult to get juice by squeezing because the fruit purees are very thick after the fruits are crushed by a machine. Pectin helps to thicken the juice and separate the liquid from the pulp. This makes it easier for the juice to hold onto water. To make more juice with better smell and nutrition, we need to break down the pectin. Pectic enzymes break down pectin. Pectins hold onto water because they have hydrophilic groups. Pectic enzymes break the bonds to let out the water during the extraction process. Also, this enzyme breaks down the plant cell wall to let out these natural chemicals like flavonoids and other phenolics that are found in the cell walls. Pectinolytic enzymes are enzymes that break down pectic substances. They are also called pectinases and are very important in industry.

Pectinases are enzymes that break down pectin into smaller pieces. They are divided into different types based on their specific role in this process. Pectinesterases are enzymes that help to break down pectin by removing methyl ester groups, creating pectic acid. Enzymes that break down large molecules into smaller ones by breaking the bonds between sugar molecules. Breaking down sugar molecules using PMG and PG enzymes needs to happen through hydrolysis. PMG speeds up the breaking of certain chemical bonds in a substance called pectin. There are two types of PMG: endo-PMG which breaks the bonds randomly, and exo-PMG which breaks the bonds in a specific order. The enzyme PG breaks down a specific type of bond in pectic acid and is the most common enzyme that does this. This enzyme is also called endo-PG or poly galacturonohydrolase. It breaks down pectic acid in a random way. There is also exo-PG, which breaks down pectic acid in a sequential way. Breaking of certain bonds in pectic acid and pectin, forming a specific kind of acid, is helped by PMGL and PGL enzymes. PMGL helps to break down pectin by cutting it into smaller pieces. It can be categorized as endo-PMGL or exo-PMGL. Endo-PMGL, or poly lyase, breaks down pectin by cutting random places in its structure.

Exo-PMGL helps break down pectin step by step by cutting it into smaller pieces. Alternatively, PGL breaks down a certain type of bond in pectic acid. It can be divided into two categories: endo-PGL and exo-PGL. Endo-PGL is an enzyme that breaks apart pectic acid by cutting its connections in a random order. The enzyme Exo-PGL breaks down pectic acid by cutting specific types of bonds. The temperature is 3.1 degrees Celsius. Protopectinases break down protopectin into a soluble and highly polymerized form of pectin (Pectinases are mostly used to help fruit juices filter better and become clear. They are also used to break down and extract juices from vegetables. Many research studies found that using pectinases to remove pectin from juices can help the food industry make juice faster, get more juice, make filtering easier, and make the juice clearer.

They tried different amounts of the enzyme, different levels of acidity, and different amounts of time. They found that using 0.5% of the enzyme, an acidity level of 7.0, and letting it sit for 6 hours made the juice 100% clear. In another research, using pectinase from *Bacillus* sp. increased juice yields from carrot pulp to 45.3 and 499% MBRL576 and commercial pectinase were used for 2 hours at a temperature of 40 degrees Celsius. In 2010, researchers

showed that using a certain enzyme from a bacteria called *Bacillus subtilis* CM5, can help make more carrot juice. The juice made with this enzyme had 13.1% more yield compared to using another enzyme called Pectinex. Sin, and others. In 2006, a study looked at how different amounts of pectinase enzyme, how long the juice was left, and how warm it was affected Sapodilla juice. They measured how cloudy the juice was, how clear it was, how thick it was, and its color. They looked at pectinase concentrations of 0.03% to 0.10%, incubation times of 30 to 120 minutes, and temperatures of 30 to 50°C. Sin and his team found that. In 2006, it was found that the best conditions for clarifying sapodilla juice with pectinase enzyme were using 0.1% of the enzyme, a temperature of 40 degrees Celsius, and a time of 120 minutes.

Also, certain enzymes in cocktails can break down the walls of cells and make more juice. Researchers studied how using certain enzymes from the store (like pectinase, cellulase, and α -amylase) can help make more jicama juice. They found that a mix of pectinase, cellulase, and α -amylase enzymes boosted the amount of juice produced by 92.7%. Using pectinase, cellulase and amylase when getting fruit juice makes more juice. Using a mix of pectinase, amylase and cellulase in kiwi made a lot more juice (78.5%) compared to a sample without these enzymes (58.4%). In 1984, it was discovered that using pectinase and cellulase together can recover 74.8% of pineapple juice. Cellulase is an enzyme that breaks down cellulose into glucose. Cellulase has three enzymes: β -1,4-endoglucanase, cellobiohydrolase, and β -glucosidase. Bacteria and fungi make cellulases. Fungi like *Aspergillus niger*, *Aspergillus nidulans*, and *Aspergillus oryzae* make microbial cellulases. Cellulase helps to extract and clarify juices in fruit processing and also helps to break down plant tissues in fruit nectars. Cellulases help to increase the amount of juice produced during pressing. Amylase and pectin can make fruit juices cloudy. Another reason for cloudiness in fruit juice is starch. Starch makes it harder for liquid to pass through and causes the filter to get dirty, cloudy, and thick.

Amylase breaks down starch. Amylases are a type of enzyme that make up about 25% to 33% of the enzyme market. They break down the bonds in starch to make sugars. Bacteria and fungi are used to make amylase. Amylase helps to make fruit juice clear. Cellulase, xylanase, pectinase, and amylase are enzymes that help get more juice from fruits and vegetables. They also make the juice clearer. α -Amylase is a type of enzyme that breaks down starch in fruits, like apples, to stop them from getting cloudy. This is especially helpful in the early stages of harvesting the fruit. Researchers found that there was 1.143 grams of starch per liter in unfiltered apple juice. When we put in a little bit of amylase (0.4%), the amount of starch went down to 0.33g/L. Fruits and vegetables have a substance called hemicellulose that makes up 15% to 19% of their dry weight. Xylan is the most common type of hemicellulose. Xylanases are enzymes that break down a type of sugar called xylose in plants called Xylan. They help make xylose for other uses. Xylanases are often used with cellulases to clear fruit juices and wine. Industrial xylanase comes from a type of fungus called *Aspergillus*.

Enzymes in wine making help make the wine clear, bring out its color, and keep it stable. Red and white wines are made by fermenting grapes and they contain special compounds like polyphenols, anthocyanins, and other chemicals. Enzymes help make the wine taste better, and also make it clearer and more stable. They are used during different steps in making wine. To get more juice after squeezing, we need special enzymes like pectinases, cellulases, and hemicelluloses. Furthermore, enzymes are also useful in making wine faster and clearer, and in filtering and stabilizing it. Two enzymes, β -glucosidase and pectinase, play a role in the creation of wine. In order to enhance the quality and stability of the wine, they break down the sugars in cell walls. The skin and pulp of grapes have pectic substances,

hemicellulose, cellulose, and lignin. Pectin is released from grapes during the process of making white wine, resulting in the formation of small particles in the juice. Tiny wine particles make the alcohol level go up and cover up the fruity smell. They also make the bad taste from sulfur worse. So, we need to use enzymes to break down pectic substances into smaller particles. Normally, pectic enzymes are added in the beginning of making white wine to help extract aroma compounds from the skin. High alcohol levels can stop the enzymes from working, so it's best to add them early on.

Pectinases are important for making coffee and tea taste good. Green tea is the most popular drink in the world and about 1.3 million tons of it is made every year. Green tea will grow 4 times faster than black tea because it is better for your health. Tannase is an enzyme that is commonly used to process tea. This enzyme comes from a type of fungus and bacteria. EGCG and ECG are found in new tea leaves as special types of catechins. EGCG is broken down into EGC and gallic acid, while ECG is broken down into EC and gallic acid using tannase that breaks ester bonds. A big problem in making tea is that it can become cloudy and form cream, which makes the color change and stuff fall to the bottom. Usually, the caffeine and polyphenols in tea cause green tea cream. Pectinases are used to get rid of the foam that forms on instant tea by breaking down the pectin. Insoluble tea cream can be turned into soluble form in cold water by using tannase. This helps to make the tea less cloudy and easier to dissolve in cold water. Special enzymes that break down complex sugars found in cell walls, like cellulose, hemicellulose, and pectin, are used to help with digestion. Enzymes are used at three different times when making green tea: before treating the leaves, when extracting the tea, and when treating the extract. The goal of using enzymes to get green tea is to collect as much of the tea as possible. Then, using enzymes on the extract helps to make the catechins stronger without changing how it tastes. By using tannase and enzymes that break down carbohydrates, we can make green tea taste better and dissolve more easily in cold water during the process of making it.

According to a study in 1997, using certain enzymes (pectinase and cellulase) can make tea taste better. This is because it increases the number of certain substances in the tea. Every year, more and more coffee are being made and drunk. In general, people drink coffee as either ground coffee or instant coffee. Green coffee beans are made up of arabinogalactan, mannan, and cellulose. Green coffee beans are made of arabinogalactan, mannan, and cellulose. The main type of sugar in coffee extract is called mannan. It makes the extract thick and gooey. Mannan has a straight line of mannose units linked together in a specific way. When coffee beans are roasted, some of the natural sugars in them dissolve. Around 7% to 30% of the glucan, 45% to 60% of the mannan, and up to 75% of the galactan in green coffee beans become soluble. Mannan is a big molecule that makes coffee extract thick. The mannan in coffee beans is broken down by mannanase to make the coffee extract less thick. Also, pectic enzymes are used to take off the slippery coating from the coffee beans. Breaking down the mucilage into sugars makes the coffee bean better. Adding pectinases helps break down the gooey stuff, which make sugars come out more and shorten the time taken to get rid of the gooey stuff. A mix of enzymes helps break down mucilage during digestion.

The food and drink industry is one of the main industries that provides for the needs of the growing population. There are many different types of packaged foods like milk, drinks, meat, chicken and fish, but they are not all made by the same company. When businesses pack food, they usually use enzymes to make the process easier. Enzymes are becoming more popular in the food and drink industry. Food enzymes, like lipases and proteases, help to reduce pollution and waste less material. Also, by using enzymes in food and drinks, it helps

make products that are high-quality and cost less, and always satisfy what customers like. **Benefits of Enzymes in the Food and Drink Industry** Enzymes are helpful in making food and drinks. Not every food can be eaten right away; some need to be kept for later, and a few need to be made ready to last a long time. Enzymes make food taste better, like cheese and wine. Additionally, it helps to replenish energy and resources that make food items work better overall. Enzymes have been used in the food industry for a very long time. Enzymes help keep milk fresh and tasting good for people to drink. Enzymes are now being used in the food industry for making meat, fish, plant proteins, and vegetable oils. The ability to prevent things helps enzymes work better in the food industry.

Enzymes are used in making meat and dairy products. Enzymes used in making meat better. It also helps to get rid of extra hydrogen peroxide in dairy products. The lactase enzyme can lower the amount of lactose in dairy products to make them easier to digest. Enzymes can also make food taste better, sweeter, and more nutritious. Enzymes are helpful in making drinks, just like they are in making food. These enzymes are mainly used to make fruit juice clean and less thick. Also, it helps to keep vegetables and fruit juice fresh for longer without losing their vitamins and minerals. Besides fruits and vegetables, enzymes are also used to make tea in a special way. It helps the tea dissolve in cold water. Cellulase and pectinase break apart the cell wall of the tea, allowing the active ingredients to easily dissolve. Also, it makes the flavor better. Enzymes are really important in the food and drink industry. Enzymes are being used more and more in food and drinks because they are really helpful. Because they make the products healthier, they become more valuable in the market. It also makes the products last longer. In business, enzymes are very important for making food and drinks more valuable. Several enzymes are used to make the food taste better and stop it from going bad. Various enzymes are used to make different things. Cellulase is a very important enzyme for making food and drinks. It is used to break down a substance called cellulose and change it into a sugar called glucose. This enzyme is used to make fruit juices and wine. It helps get more juice out and makes it clearer. Enzymes like lipase make bread better by helping the dough ferment and stay stable. This leads to high-quality bread. It is also used to make noodles not stick together when they are boiled. Protease is an enzyme used in the food and drink industry to make products last longer and have a better texture. In addition, it also improves the way bakery products are baked.

CONCLUSION

Natural fruit drinks have become more popular in recent years. Specific enzymes are employed to increase color stability and turbidity in juices and alcoholic drinks. Pectinases play an important role in clarifying fruit and vegetable liquids by eliminating colloidal pectin. In addition to their primary function in clarity phases, the use of pectinase, cellulose, and amylase in fruit juice extraction boosts juice output. Winemakers utilize β -Gucosidase and pectinase to boost clarity and maceration yield. Pectinases, cellulases, and tannase are important enzymes in coffee and tea fermentation. Furthermore, enzymes when combined improve juice output, clarity, and reduce viscosity and turbidity. During the advancement of enzyme technology, more beverage production has been achieved while maintaining higher product quality. Approximately 20 enzymes are commercially available. Enzymes are mostly manufactured by large corporations such as Novozymes in Denmark and DuPont in the United States. The worldwide industrial enzyme market is very competitive, with narrow profit margins. However, there is still a need to enhance creative, sustainable, and economically competitive manufacturing procedures since naturally occurring enzymes are often ineffective. Microbial fermentation used to create enzymes in industrial processes must be both cost efficient and highly active in order to compete with traditional procedures. The

latest technical breakthroughs in the enzyme sector provide great production while improving the quality of final goods. Thus, recombinant DNA technology, such as microbial genomes and diversity, is still employed to screen for and isolate novel enzymes from microbial resources for use in food and beverage processing. Novel enzyme activity and stability should also be constructed and tested under difficult processing conditions. Furthermore, speedy and effective enzyme purification procedures and techniques are still required for future advancement. However, innovative approaches to the marketing of tropical drinks or goods should be encouraged.

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CHAPTER 4

FRUITFUL TRANSFORMATIONS: THE VITAL ROLE OF ENZYMES IN FRUIT JUICE PROCESSING

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ABSTRACT:

This abstract goes into the magic of enzymes, specifically how they maximize juice output, ensure clarity, and enhance scent via enzymatic release. It emphasizes the delicate balance needed for enzymatic inactivation and preservation, which is critical for preserving the quality and shelf life of fruit juices. The introduction delves further into developing technologies and creative enzymatic treatments that are transforming the sector, providing insights into the issues encountered and sustainable methods used. As we complete this initial voyage, the abstract considers global views, consumer patterns, and the long-term importance of enzymatic treatments in creating sensory-appealing fruit juices. This introduction lays the foundation for a more in-depth examination, enabling readers to discover the enzymatic symphony inside each sip and enjoy the delicate interaction between enzymes and the lively world of fruit juice production.

KEYWORDS:

Enzyme, Fruit Juice, Juice Extraction, Juice Processing, Pectin.

INTRODUCTION

As individuals become more health conscious, the demand for fruit juices grows. Most juice extraction technologies do not provide sufficient quantities or quality juices. Enzymatic juice extraction yields a greater yield. The most common enzymes utilized are pectinase, cellulase, and hemicellulose. Enzyme addition not only promotes juice extraction but also stimulates the release of different phenolic and other nutritionally significant components from the juice. Enzymatic clearing also helps to enhance the look of juice. The quality of the juice is enhanced in terms of viscosity, turbidity, and filterability. Enzymes are also used to thicken citrus fruit juices and prevent them from browning. Because of their perishable nature, many fruits and vegetables have a relatively short shelf life after harvest. In India, more than 20-25% of fruits and vegetables decay before consumption. Despite being the world's second greatest producer of fruits and vegetables, India processes just 1.5% of its entire production [1], [2]. Fruits are mostly water (75-90%), with the majority contained in vacuoles, generating turgor in the fruit tissue. Fruit juice is made by physically pressing or macerating fresh fruits without the use of heat or solvents. Fruit cell walls are made up of crystalline cellulose microfibrils embedded in an amorphous matrix of pectin and hemicellulose. Juice contains water, soluble solids, fragrance and flavor components, vitamins and minerals, pectic substances, pigments, and, to a lesser extent, proteins and lipids. Fruits lose acidity and starch as they mature, while increasing sugar content. Juices are direct-consumption goods made by extracting cellular juice from fruit, which may be done by pressing or diffusion [3], [4].

Fruit juice intake has been linked to a healthier diet. Beyond their nutritional value, these juices include a variety of chemicals that exhibit biological activity. The current interest in phenolic compounds stems from their antioxidant ability, which helps to preserve human health from the negative consequences of oxidative stress. In the vivid world of fruit juice processing, enzymes orchestrate alterations that go beyond the commonplace and enrich the

sensory experience of one of the world's most popular drinks. From the citrus rush of orange juice to the delicate sweetness of apple nectar, enzymes act as invisible maestros, sculpting the tastes, scents, and textures that pleasure our taste buds. This thorough introduction takes readers on a voyage into the fascinating world of enzymes in fruit juice processing, revealing the science, creativity, and technical advances that distinguish this vibrant business. Fruit juices contain the essence of nature's gift, including tastes, vitamins, and antioxidants found in the pulp of fruits. This section dives into the cultural and nutritional importance of fruit juices, tracing their origins from ancient civilizations to the present day, when they have become staple drinks valued for their refreshing properties and health advantages[5], [6].

Enzymes play a crucial role in fruit juice production, acting as natural catalysts. This section introduces the enzymatic protagonists amylases, pectinases, cellulases, and others and emphasizes their roles in breaking down complex components such as starches, pectins, and cellulose into simpler forms, so revealing the rich palette of tastes and nutrients found in fruits. Understanding the architecture of fruits is crucial for understanding how enzymes work during juice extraction. This section delves into the structural makeup of fruits, from the peel to the pulp and seeds, offering insight on how enzymes are located in various fruit tissues. The interaction between enzyme activity and fruit shape provides the foundation for effective juice extraction. Enzymatic softening and maceration are crucial stages in fruit juice processing, affecting the mouthfeel and texture of the finished product. Enzymes such as pectinases and cellulases help to break down cell walls, allowing juice to be released from the fruit matrix. This section discusses the enzymatic softening process and its effects on the overall quality of fruit juices[7].

Pectinases play a crucial role in maintaining the clarity of quality fruit juices. Enzymes help to clarify juices by decomposing pectins, which prevents haze development and sedimentation. This section delves into the enzymatic systems that produce crystal-clear fruit juices that fascinate customers. Enzymes help maximize juice output, making extraction more efficient and cost-effective. This section goes into the enzymatic processes used to extract every drop of juice from fruits, hence reducing waste and improving resource usage in the fruit juice business. Enzymatic release of volatile chemicals from fruit precursors has a significant role in enhancing the scent of fruit juices. Enzymes, such as esterases and lipases, catalyze processes that produce aromatic molecules responsible for the distinct fragrances of different fruits. This section investigates the enzymatic processes that increase the scent of fruit juices, resulting in a multisensory experience.

Enzymes play a crucial role in juice extraction, but their activity must be carefully controlled to avoid unwanted changes after processing. This section investigates the enzymatic inactivation techniques used to protect the quality and shelf life of fruit juices, balancing freshness and avoiding enzymatic deterioration. Enzyme monitoring is essential for maintaining consistent quality in the fruit juice business. This section examines the analytical tools and approaches used to monitor enzyme activity, offering insight into how manufacturers maintain high quality requirements while meeting customer expectations. Fruit juice processing is constantly developing due to new technology and enzymatic innovations. This section investigates how advances in enzymatic biocatalysis, genetic engineering, and enzymatic immobilization are altering the sector, opening up new avenues for increasing both the efficiency and sustainability of fruit juice production.

While enzymes make significant contributions to fruit juice processing, there are certain limitations, such as substrate selectivity, enzyme stability, and regulatory issues. This section addresses these obstacles and proposes creative methods, such as enzyme engineering and immobilization techniques, to overcome them and assure the continuous growth of enzymatic

fruit juice processing. The fruit juice market is evolving to meet customer desires for natural, clean-label, and minimally processed goods. This section investigates how consumer trends and needs affect the usage of enzymes in fruit juice processing, highlighting the industry's reaction to changing customer expectations. Enzymatic fruit juice processing is a worldwide activity, with regional differences in fruit availability, tastes, and processing methods contributing to a variety of viewpoints. This section explores how various civilizations use enzymatic techniques in fruit juice manufacturing, reflecting the diverse tapestry of worldwide fruit juice consumption.

Enzymatic fruit juice processing is ecologically friendly and conforms with contemporary food processing processes that prioritize sustainability. This section examines how enzymes help to sustainability by lowering water use, energy consumption, and waste, demonstrating the industry's dedication to environmentally sensitive practices. On the last part, we discuss the enormous role of enzymes on fruit juice processing. Enzymes choreograph a symphony of changes, from softening the fruit to boosting scent and maintaining quality, culminating in each delicious sip. This result stresses the long-term importance of enzymatic treatments in producing fruit juices that engage the senses and contribute to the worldwide tapestry of beverage consumption. In essence, this broad introduction lays the groundwork for a thorough examination of the diverse function of enzymes in fruit juice processing. It reveals the enzymatic complexities that characterize the business, from orchard to glass, and encourages readers to appreciate the symbiotic link between enzymes and the beautiful, delicious world of fruit juices. As we go on this journey, we discover the science, creativity, and invention that come together in the enzymatic alchemy that transforms fruits into the wonderful beverages we enjoy.

DISCUSSION

There are markets around the world for both common and uncommon fruits. In some countries, they have started to process fruits for food. Fruit juices, drinks, and nectars are the most popular fruit products. People are drinking more fruit juice, so countries that grow fruit are making more juice to sell to other countries. Enzymes are important in making food because they make it easier to produce different parts of the food. Enzymes were first used in businesses in 1930 to make FJ. Industrial enzymes are divided into different types, and the most important ones for fruit processing are pectinases, cellulases, and tannases. These enzymes help in breaking down fruit components. Using enzymes to treat FJ is better than the usual way of processing it. These benefits include more juice, clearer juice, more fruit flavor in juice, smoother pulp, and less cloudiness and thickness in juice. Macerating enzymes are used in two steps of making fruit juice. First, after crushing the fruit, the pulp is broken down, which makes more juice, saves time, and gets more healthy parts from the fruit. Then, after getting the juice, it is clarified to make it more stable. The foggy look of FJ comes from pectin, cellulose, starch, proteins, tannins, and lignin in it. Using enzyme mixtures with pectinases, cellulases, and tannases helps the fruit juice industry make better products. These enzymes are called macerating enzymes [8], [9].

They are widely used in various industrial processes. Pectinase breaks down pectin by removing its ester groups and breaking it into smaller parts. Cellulases are getting a lot of attention around the world because they can turn plant materials into useful products. The combination of cellulases is important for breaking down cellulose into useful products with the help of certain microorganisms. Tannases are important enzymes used in making things like juice and tea. They are used in many industries. Tannases work in many different temperatures and levels of acidity, and are made by tiny organisms like *Aspergillus*, *Paecilomyces*, *Lactobacillus*, and *Bacillus*. Enzymes are very important for making food and

drinks. They help save money and make things better. The growth of the FJ industry was closely linked to the enzyme industry. In recent years, there have been many improvements made to the process of making FJ. Specific enzymes have been used to make FJ better. This chapter talks about how pectinases, cellulases, and tanninases are used in making FJ.

Pectic materials are made up of complex acid and sugar molecules that are linked together in a specific way. Potassium, sodium, or ammonium ions, along with methyl groups, partially neutralize and esterify the carboxyl groups of galacturonic acid. Pectin's side chains are made up of arabinose, galactose, l-rhamnose, and xylose. Pectic substances come in four different types: protopectin, pectic acids a type of polygalacturonan with very little methoxyl groups, pectinic acids, and pectins. 75% of the galacturonate units are methylated. Protopectines cannot dissolve in water, but other pectic substances can partly or completely dissolve in water. Pectin is made up of different molecules, with pectinic acid as the most important part. Pectin is a substance in the cell wall that can be connected to other molecules to make protopectin. Pectin is the main part of the cell wall of fruits. Percentage of pectin in different fresh fruits. Out of all the fresh fruits, currants have the most pectin, which is about 0.9% to 15%. Pectins are useful in making healthy products and food. Pectin is used in food to make it thick, add fiber to it, and replace fats and sugars in low-calorie foods. Also, pectins make FJ thicker and cloudy.

When you crush fruit with a lot of pectin, it makes a thick juice that sticks to the fruit. It is hard to remove this FJ using other machines or by pressing. Pectin helps stick cellulose and hemicellulose fibers together. Pectinases help cellulases get to their targets more easily. Pectinases make FJ less thick, break down the jelly structure, make the pulp easier to press, and give more FJ. Enzymes that break down pectin are called pectinases, pectic enzymes, or pectinolytic enzymes. Pectinolytic enzymes were first used in 1930 to make FJ and wine. In the 1960s, scientists figured out how plant tissues are made, and started using enzymes more effectively. Pectinolytic enzymes are really important enzymes in the food industry, especially when processing fruit juice. They help make the juice stable and clear. Many studies have looked at how these enzymes work and how they can be used effectively. Pectinases are made when fruits ripen. They break down a substance called polygalacturonic acid in the fruit into a simpler form called monogalacturonic acid by breaking chemical bonds. This process makes the cell walls of the fruit softer and increases the amount of juice that can be taken out. Acidic pectinases are mostly used in fruit juice processing, while alkaline pectinases have practical and eco-friendly uses in industry.

The enzymes are divided into two groups based on how they work: exo if they work at the end of a process, and endo- if they work randomly. Pectinases are made up of different types, such as pectin esterases, polygalacturonases, pectin lyases, and pectate lyases. They work on different kinds of substances. Pectin lyases break down pectin into smaller sugar molecules called oligosaccharides. These molecules have a specific structure at their ends. Pectin-destroying enzymes break down plant cell walls, making the inside of the cells less sticky and the tissue less rigid. Microbes and plants make pectinases. Many fungi and bacteria make pectinolytic enzymes, like pectin methyl esterase, that have been found in all the plants scientists have studied so far. Microbes that break down pectin were used in industry to make environmentally friendly enzymes called pectinases. Tiny living things can make special substances outside their bodies called enzymes. One such organism called *Streptomyces GHBA10* can make pectinases which can help clarify and extract juice. We collected samples from places where fruit waste is processed, leftovers from farming, soil with a lot of leftover fruit, wastewater from different places, and waste from the pectin industry. Fungal-pectinases are enzymes that are found outside the cells, and polygalacturonase is the main type among

them. Some types of fungi, like *Botrytis cinerea*, *Aspergillus*, and *Penicillium*, make pectinases[10], [11]. Pectinases are important in the FJ industry and are used for making different types of food.

Microbial pectinases make up around 10% of all the enzymes produced worldwide. Pectinases break down the long and complicated pectin in fruit pulp, which makes the pulp cloudy. Pectinases work best at certain pH levels. Acidic enzymes are used in the beverage industry to clear up and extract materials in fruit juices. They help remove the stuff that makes the juice cloudy and thick. Pectinases are needed to clarify fruit juice because they help reduce bitterness and cloudiness, make the juice less thick, and make it easier to squeeze out the juice from the fruit. They also help break down the jelly-like substance in the fruit. Pectinase treatment was used on various fruits like raspberry, strawberry, orange, blackberry, and grape juice, as well as apple pomace. This made the fruit juice look and stay better. Pectinases are used to help get fruit juice and to separate the solid parts that are left over. They can also help with filtering and settling the juice. If you need a cloudy FJ, it is heated to stop enzymes from working. Centrifugation separates big stuff and keeps small stuff in the liquid. If you need a clear FJ, you need to remove the suspended particles. To do this, we use a special mix of enzymes from a store to break down the fruit juice, and then spin it in a machine to make it clear. Breaking down the pectin in mashed fruit helps to get more juice and make it clearer, while also making it less thick. Also, pectinase mixtures in applications could help with filtering. Tropical fruits with a lot of pectin are too juicy to make fruit juice by pressing or spinning them. These methods need a lot of energy and don't produce much food. It's hard to find FJ in fruits like banana, guava, mangoes, and papaya using the usual methods[12], [13]. In grapes and apples, not all of the juice can be taken out because some of it stays in the leftover fruit. In the old way of making fruit juice from soft fruits, the pulp is boiled and the juice is processed.

Using enzymes allows FJ to be made without major changes, and also helps to make FJ clear. The process involves mashing the fruits and heating them to 65°C for 15 minutes to stop enzymes inside the fruits from working. The pulp is cooled down, pectinases are added, and left for some time to sit. Then the FJ is separated by either pressing it through cheesecloth or using a centrifuge. The FJ is put in a cold place for 24 to 48 hours, and during that time all the floating bits sink to the bottom. The clear liquid on top can be made clear and saved after heating it up. Pectinases are used in the apple juice industry to help press the juice and separate the solid bits using a machine called a centrifuge. Schols and others. In 1990, a study was done on an enzyme called rhamnogalacturonase from a certain type of bacteria. Rewrite: We studied the sea urchin (*Strongylocentrotus purpuratus*) and how it affects the apple tissue. Using pectinases to treat something takes 15 to 120 minutes. The time depends on the type of enzyme used, how much is used, the temperature of the reaction, and the type of apple being treated. Adding pectinase made the grape FJ produce more juice, up to 30% more. The amount of FJ produced went up when the levels of pectinases went up from 0.05% to 1.

De-pectinizing actions do two things: they make cloud particles clump together and they break down thick, sticky pectin. In acid, pectin has a negative charge and wraps around proteins in liquid, making them push away from each other. Pectinases break down pectin chains, which allows positively charged proteins to show. The force that pushes cloud particles apart gets weaker, so they stick together. To make FJ clear, we can use cellulase, hemicellulose, and pectinase to make it less thick and easier to filter. Pectinases have made apple juice clearer by 35%, as well as pineapple juice, tangerine juice, and also peach, plum, pear, and apricot juice before filtering. In 1995, there was a big decrease of around 50%. The viscosity of guava FJ increased by 63% when Clarex-L concentrate was used. Sharma and

colleagues also found. In 2005, it was found that the thickness of carrot juice decreased by 41% when they used Pectinex Smash XXL, an enzyme, at a certain concentration, temperature, and time. The thickness and cloudiness of banana FJ are mostly because of the pectins in the banana. Pectin makes it harder to make the juice clear because of its fiber-like structure. They said that using pectinases can make banana pulp clearer. The enzymes often used in making apple juice are the ones that break down a type of pectin found in apples. People found a way to make apple juice using special methods. They used pectic enzymes to make a fizzy apple juice and were able to get 20% of the pectin back [14], [15]. Some researchers wrote about this in 1978 and 2014. Apple juice can be made by mashing up apples and using enzymes to break them down.

The FJ industry has created ways to fix these problems because consumers care about FJ quality and how it looks. They tested how pectinolytic and proteolytic methods can make pomegranate FJ clearer and get rid of substances that make it hazy. When protease and pectinase were used together, they had a strong effect. This led to very good results in reducing cloudiness and preventing haze in the juice. Even though using certain enzymes didn't change the levels of protein, pectin, and phenolics, it did affect how cloudy molecules form in the liquid. Also, this type of enzymatic treatment did not change the color or amount of anthocyanin in the FJ. In the orange FJ, pectin esterases are found, which make the pectins partially methylated. Polygalacturonases are often used for this FJ. When making orange juice, you can add pectinases to the pulp at the end to make the juice less thick. This results in getting a lot of FJ, getting more TSS, and having lower viscosity. Pectinases make fruit juice less thick without breaking down the solid parts, so the juice stays cloudy. Enzymes should contain as little pectin methyl-esterases as possible to prevent the juice from becoming clear. We studied the characteristics of lime juice that was clarified using enzymes at different moisture levels.

The thickness of enzymatic-clarified lime FJ and its ability to flow easily decreased when there was more water. However, the ability to conduct heat and its capacity to store heat increased with more moisture and water. The ability to disperse heat also increased a little. Researchers found a connection between the way lime FJ responds to heat and its moisture content after being treated with enzymes. Also, there was a strong connection between the physical and thermal features that was not good. Cellulose is made by tiny living things like some types of algae, plants, and animals. Cellulose is made up of layers of small bundles of fibers called microfibrils. Every small fiber contains 36 to 1200 cellulose chains that stick together to make a strong structure using forces and bonds. A cellulose chain is a straight line of d-glucose parts that can be as short as 100 or as long as 20,000 glucose units linked by β -glycosidic bonds. Weak hydrogen bonds in the shapeless areas can be easily broken by enzymes and water. Usually, breaking down cellulose into glucose is done by working together of different enzymes called exoglucanases, endoglucanases, and β -glucosidases.

Breaking down cellulose using cellulases is a good way to use a lot of leftover plant materials to make useful things. Cellulases make up 8% of the enzymes used in industries around the world. Cellulases are helpful in food technology because they can be used in many different ways to make food. Cellulases are used all around the world because they have potential for helping with different food processes like clarifying fruit juice and making nectar less thick. Making apple and pear juice (FJ) involves crushing the fruit into a pulp, which is then separated into clear juice and solid remains using machines. By adding special enzymes, the amount of juice and the speed of the process were improved. The thickness of passion FJ went down by half when cellulases, pectinases, and amylases were used together. Using exo-enzymes in making black carrot FJ made it have more antioxidants because it increased the

amounts of phenolic compounds and flavonoids. Fruit drinks are made by mixing fruit juice with sugar syrup and citric acid to make drinks that are ready to be enjoyed. The important thing about these drinks is that they stay cloudy. Applying external enzymes made nectars more stable in the clouds.

Special enzyme mixtures like Pectinex Ultra, Rohapect, or mixtures with both pectinases and cellulases can make nectar less thick. The enzyme mixtures were tested to see if they could make mango puree smoother, and RapidasePomaliq and Rapidase were the most effective. The enzyme products have a lot of cellulases, pectinases, and xylanases that can make the puree less thick quickly so that it can be used for business. They found this in the P. Peruvian FJ was changed by using Ultrazym AFP-L, which has enzymes that break down cellulose and pectin. Enzyme treatments help increase the amount of P. obtained. The amount of peruviana FJ is higher, and there are also more macro- and micro-components. Fruit juice has certain qualities like the way it smells, feels in your mouth, and tastes. These qualities are important in making food and other products. The way fruits taste and feel could change by adding enzymes like cellulases to them. Using enzymes makes fruits better for you and smell better. When you add Glucosidase to tea, it makes the smell better because it increases the number of essential oils. Tea can stay fresher if we use immobilized glucosidase because it can still work well at low temperatures. This is different from the free enzyme. Using enzymes to treat FJ was found to make the color better, increase the amount of FJ made, and improve its health benefits. The carrot juice became more acidic and sweeter with more beta-carotene when enzymes were used to process it. This made the juice taste better and look more appealing.

Enzymes are often found in food, the most significant of which being oxidoreductases and hydrolases. The food business is interested in enzymes such as phenolases, peroxidases, catalases, peroxidases, and lipoxygenases. The use of enzymes in food processing is one of the most significant effects of biotechnology on the industry. Hydrolases and oxidoreductases are the most common enzyme families in the food business, and they are used to produce novel products, improve organoleptic features, and increase production. Pectinases and amylases are the two most common types of enzymes utilized in the fruit juice business. Amylases have several biotechnological uses, including textiles, pulp and paper, leather, detergents, beer, spirits, bread manufacture, children's cereals, starch liquefaction and saccharification, animal feed, and chemical pharmaceuticals. Since 1970, the juice business has begun to prepare edible fruits in huge numbers; such fruits are gathered when unripe and preserved for relatively lengthy periods of time at low temperatures. Under these circumstances, fruit pulp contains enough starch to produce turbidity or gelatinization during processing, making productive methods difficult. As a result, the need for amylolytic enzymes, particularly glucoamylase, has surged in the industry.

Pectinase was one of the first enzymes used commercially to prepare juices and wines. Its use in the juice business is attributed to a variety of characteristics, including clarity, maceration, and extraction, color stabilization during storage, and higher production. Currently, plants, fungus, yeast, and bacteria naturally generate these enzymes, which account for around 20% of the global enzyme market. These enzymes degrade lengthy, complicated polymers known as pectin; they now play an important role in the juice business. Pectinases are categorized into three classes using the following criteria: Hydrolytic or trans eliminative glycosidic bond cleavage; endo or exo (from the molecule end) method of action; and substrate preference for pectic acid or pectin. There are three kinds of pectinases: pectinesterase, depolymerising enzymes, and protopectinase.

In addition to pectinases, additional enzymes such as hemicellulases and cellulase are utilized in fruit juice manufacturing to optimize product processing. These enzymes are part of the maceration enzymes, which work on soluble pectin hydrolysis as well as cell wall components to reduce viscosity and maintain texture. Enzymatic liquefaction technique degrades cell wall polysaccharides, releasing soluble substances such as D-galacturonic acid and neutral sugars. Pectin and cellulose are hydrolyzed during pulp liquefaction by polygalacturonases, pectin lyases, pectinesterases, and cellulases, respectively. The impact of these enzymes on the cell wall causes neutral sugars such as D-arabinose, D-galactose, L-rhamnose, and D-xylose, which are bound to pectic compounds, to be liberated and soluble.

High juice output is an essential aim for juice manufacturers. Many current procedures for producing fruit and vegetable juice include enzymes as essential processing aids to increase yields and clarity. The use of maceration enzymes boosts extraction yield and improves processing without incurring additional expenditures. These enzymes are employed after the raw material has been chopped to macerate the pulp, resulting in whole or partial fruit liquefaction, which reduces processing time and improves fruit extraction. Pectinases are added after extraction to clarify the solution and reduce viscosity, allowing for easier filtering and concentration. The occurrence of late bitterness in various citrus fruit juices is a significant economic obstacle to citriculture globally, particularly in terms of consumer appeal. The bitter taste is primarily caused by the synthesis of limonin, a highly oxygenated derivative of triterpene that belongs to the limonoids class.

The use of microorganisms to transform bitter citrus juices into non-bitter products necessitates the presence of enzymes from bacterial cells capable of metabolizing limonin. Limonoate dehydrogenase is an enzyme present in many microorganisms that catalyzes the oxidation of limonite A-ring lactone to 17-dehydrolimonoate, a non-bitter derivative that cannot be transformed into limonin. Limonoate dehydrogenase has been identified from *Arthrobacter globiformis*, *Pseudomonas* sp. strains, and *Rhodococcus fascians* drolimonoate, a non-bitter derivative that cannot be transformed into limonin. Enzymes in fruit juice are very valuable to the juice business. Their usage increases the output of fruit juice while also improving physical quality attributes such as clarity, viscosity, filterability, and color. Enzymatic juice extraction provides nutraceutical nutrients while also improving organoleptic qualities, which minimize bitterness and prevent juice darkening.

CONCLUSION

Enzymes play a crucial part in the symphony of fruit juice processing, creating a beautiful combination of science, nature, and creativity. This research has shown the critical role of enzymes in creating the delicious elixirs we enjoy across the world, from softening fruit structures to improving smells and maintaining quality. As we explore the complexities of enzymatic interventions, it becomes evident that these biocatalysts are more than just tools; they are maestros, orchestrating transformational processes that characterize the sensory experience of fruit juice. The industry's response to difficulties, acceptance of developing technology, and adherence to sustainable practices demonstrate its dedication to innovation and excellence. Every delicious sip carries the long heritage of enzymatic fruit juice processing, transporting customers to a realm where enzyme technology meets the natural essence of fruits. Looking forward, this conclusion envisions a future in which enzymatic alchemy continues to advance, pushing the frontiers of efficiency, sustainability, and customer happiness in the changing terrain of fruit juice manufacturing. As we relish the rewards of enzymatic effort, we celebrate not just a beverage, but also the convergence of scientific creativity and nature's richness in a glass—a tribute to the everlasting charm of fruit juice made with enzyme precision. More people are drinking FJ instead of drinks with

caffeine because they want to be healthier. Fruits are often juicy and soft, so we can make fruit juice easily by simple methods. The enzymes cellulases and tanninases are used in FJ biotechnology and FJ processing to break down pectin. Enzymes break down pectins and help the particles in the juice to settle, while also keeping the juice the right color and stable. Enzymatically-clarified FJ may be cheaper to make and could be produced in large amounts compared to other methods.

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CHAPTER 5

USING MICROBIAL ENZYMES TO OPTIMIZE TEXTURE, FLAVOR, AND QUALITY IN PRODUCTION

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ABSTRACT:

From ancient cheese-making to current lactose-free inventions, this thorough introduction dives into the critical role enzymes play in sculpting the vast variety of dairy pleasures. Proteases, lipases, and lactases, among others, operate as molecular builders, shaping textures, tastes, and nutritional profiles. The abstract describes the enzymatic symphony of cheese-making, yogurt fermentation, and butter manufacture, demonstrating how microbial enzymes convert liquid milk into a diverse array of dairy products. Beyond typical applications, the research focuses on lactose-free alternatives, sweets, and tackling enzyme stability and regulatory concerns. As customer tastes shift, the abstract charts the industry's reaction to issues like clean-label demands and sustainability. Global viewpoints reveal regional differences in enzymatic dairy processing, but a forward-looking vision predicts future horizons by investigating new technologies and active research topics. This abstract captures the essence of microbial enzymes as catalysts for innovation, sustainability, and quality in the dairy industry's ever-changing context.

KEYWORDS:

Cheese, Dairy Processing, Dairy Business, Enzyme, Microbial Enzyme, Milk Products.

INTRODUCTION

Barley yeast microorganisms have been used by the Sumerians and Babylonians since 6000 BCE to make alcoholic drinks. Around the world, tiny organisms called microbes have enzymes that are used in many industries, like farming, making chemicals, producing energy, making food, and medical treatments. People are really interested in microbial enzymes because they use less energy, work quickly, are better for the environment, not harmful, and save money. With protein engineering and DNA technology, we can grow and change tiny organisms in large numbers to meet the needs of industry, reduce costs, make products for consumers, and use natural resources more wisely. In 2014, the global market for tiny organisms' enzymes was worth about \$4.2 billion. It's expected to grow by 7% by 2020. Enzymes are special kinds of proteins in living things that help speed up chemical reactions in the body. Enzymes are special because they help to make chemical reactions happen more easily without using up energy. Usually, enzymes need gentle conditions like pressure and temperature to speed up reactions, instead of needing dangerous materials and pollutants [1], [2].

The best pH and temperature for mammalian enzymes are 7.4 and 37 degrees Celsius. Also, when the pH is higher than 7.4 and the temperature is over 40°C, the enzymes change shape and can't work well, which means they can't be used in certain conditions. Additionally, enzymes from mammals can be affected by certain things and can cause allergies. It is also more expensive and harder to purify, isolate, and use them again. Enzymes are big molecules made of amino acids. Their size is measured in Dalton. The enzyme's active site is often deep inside a pocket, which makes it specific for a certain substance. Many enzymes were identified and separated, which led to the creation of enzyme names. The IUBMB and IUPAC worked together to make a guide for organizing and naming enzymes called EC.

Small living things are a good choice for making industrial enzymes because they grow quickly and are easy to find. Changing the genes of tiny organisms using DNA technology can help make more enzymes and advance science. In industries, making microbial enzymes is very important. The importance of microbial enzymes comes from their ability to work really well in different chemical and physical situations. Some say that the enzymes from tiny organisms can help treat the lack of human enzymes caused by genetic disorders. For instance, people who can't digest sugar were given sacrosidase medicine to help them digest sugar[3], [4]. This treatment was given by mouth, not as a shot. Researchers found a way to treat phenyl ketonuria by using ammonia phenylalanine lyase to break down phenylalanine. Microbes' enzymes are used in many industries like dairy, medicine, food, paper, clothes, and leather. Furthermore, people are using them more and more because they work better, are higher quality, and are better for the environment than other methods. This chapter will talk about how tiny organisms' enzymes are used in making milk products.

The dairy sector, a dynamic terrain where tradition meets innovation, has undergone a transformation driven by the strategic use of microbial enzymes. From the modest origins of artisanal cheesemaking to the vast expanse of contemporary dairy production, enzymes have emerged as silent architects, carefully molding the textures, tastes, and overall quality of a wide range of dairy products. This comprehensive introduction delves into the fascinating realm of microbial enzymes in the dairy business, revealing the complicated interaction of science, handicraft, and technical innovations that defines this ever-changing sector. The dairy business is characterized by a rich tapestry of creamy cheeses, silky yogurts, and versatile dairy proteins. This section goes into the history of dairy processing, tracking its growth from ancient techniques to today's technologically driven dairy sector. From the undulating hills of ancient cheese caves to the glittering stainless-steel tanks of modern dairy plants, the dairy sector exemplifies the long-standing human bond with milk and its many modifications[3], [5].

Enzymes, as molecular catalysts, have been used in dairy processes for ages. This section exposes the reader to the many functions of microbial enzymes in the dairy business. Enzymes are the hidden heroes of dairy processing, doing everything from coagulating milk proteins for cheese manufacturing to enabling lactose breakdown for lactose-free products. The enzymatic trip proceeds with precision, finely modifying milk's components to create a variety of textures and tastes. Dairy processing requires a wide set of microbial enzymes, each with a specific purpose. This section delves into the many enzyme types used in the dairy business, including proteases, lipases, lactases, and more. The focus on their unique activities lays the groundwork for understanding how these enzymes contribute to the varied properties of dairy products. Cheesemaking, a centuries-old art form, is an excellent example of enzymatic prowess. This section delves deep into the enzymatic symphony that occurs during cheese formation. From rennet's involvement in coagulation to lipases imparting unique tastes, the cheesemaking process is deconstructed to illustrate how microbial enzymes convert liquid milk into a variety of solid, tasty cheeses.

Yogurt's rich texture and sour taste are the result of a careful balance of microbial enzymes and bacteria. This section discusses the function of enzymes in yogurt fermentation, including how lactases break down lactose into simpler sugars, adding to the product's sweetness, and how proteases impact texture by changing milk proteins. The symbiotic relationship between bacteria and enzymes produces the creamy enjoyment that people throughout the world enjoy. Enzymes play a crucial role in the creation of butter and cream, contributing to their richness. This section looks at how lipases contribute to taste development in butter and how proteases affect cream viscosity. The enzymatic intricacies in the churning and ripening

processes are shown, showing their effect on the taste properties of these dairy products. Many customers struggle with lactose intolerance, and enzymatic therapies may help them overcome this obstacle. This section goes into the manufacture of lactose-free and low-lactose dairy products, where lactase enzymes play a critical role in converting lactose into more readily digested glucose [6], [7]. The section looks into enzymatic advancements that appeal to a larger audience with various lactose tolerance levels.

Enzymatic inventions enhance dairy sweets, including velvety puddings and luscious ice creams. This section examines how enzymes influence the texture, mouthfeel, and stability of dairy desserts. Enzymes play an important role in the production of decadent dairy products, from preventing crystallization in ice cream to improving the silkiness of custards. Enzymatic interventions may improve the shelf life and quality of dairy products, but maintaining a precise balance is crucial. This section analyzes how enzymes are strategically used to extend the shelf life and freshness of dairy products. Enzymatic treatments, such as protease inhibitors and lipase control, are crucial in maintaining the sensory properties of dairy products. Despite their various benefits, enzymatic treatments in the dairy business present obstacles. This section covers topics such as enzyme stability, regulatory concerns, and consumer perception. Innovative ideas and tactics are investigated, stressing the industry's tenacity in overcoming challenges in order to leverage the advantages of enzymatic dairy processing [8], [9].

In an age of shifting consumer preferences, this section investigates how clean-label demands, plant-based alternatives, and a rising interest in functional dairy all influence dairy product choices. The role of enzymes in addressing these needs is discussed, demonstrating the industry's capacity to adapt to shifting consumer environments. Enzymatic interventions in the dairy business vary by location, reflecting different culinary traditions and customer preferences. This section explains how various cultures use microbial enzymes in dairy processing, which influences the kinds of products produced and the techniques used. Sustainability is an important factor in modern food production, and the dairy business is no exception. This section investigates how enzymatic dairy processing matches with sustainability objectives, ranging from maximizing resource efficiency on farms to decreasing waste during manufacturing. Enzymes emerge as partners in the industry's dedication to environmentally friendly operations.

As we stand at the crossroads of tradition and innovation, the last segment provides an outlook on the future of enzymatic treatments in the dairy business. Anticipated trends, upcoming technologies, and current research areas drive the trajectory of dairy innovation, which is fueled by the catalytic power of microbial enzymes. In summary, this broad introduction lays the groundwork for a thorough investigation of microbial enzymes in the dairy sector. From cheese caves to yogurt fermentations, butter churns to lactose-free breakthroughs, the enzymatic trip through dairy processing is a symphony of science, craftsmanship, and consumer-centric innovation. As we begin on this journey, we will uncover the molecular complexities that change milk into a variety of dairy pleasures, allowing readers to understand the delicate role of microbial enzymes in every creamy, velvety, and sensual dairy experience.

DISCUSSION

In the dairy industry, tiny organisms are used to make milk products taste and look better and to make more of them. Many different tiny organisms make enzymes that are used in the dairy business, like catalase, aminopeptidase, proteases, lactoperoxidase, lipases, transglutaminase, and more. They are experts in this area and they are different from

coagulants because they help make things last longer. Microbes' enzymes are used to make yogurt and cheese. Pepsin and chymosin are mixed together and used to make milk turn into cheese and whey. Every day, about 33% of the cheese made in the world comes from rennet microorganisms. In the dairy industry, tiny organisms are used to make a variety of products like yogurt, cheese, syrup, bread, and more. to make them better. Old-time methods like brewing, making cheese, and tenderizing meat with papaya leaves were made before we knew about enzymes. In the past, the beginning stages of making milk products involved the breakdown of proteins due to the action of enzymes. These are the most important tiny organisms' enzymes used in making dairy products. Rennet is a well-known enzyme from outside the body that is used in making dairy products. It has been used since 6000 BCE. Cheese making in the US went up a lot, from 8000 to 471,434 metric tons by April 2017, the USDA/NASS says. This led to a high need for the external enzyme rennet from various places. Cheese makers use rennet enzyme a lot when they make cheese[10], [11].

This is one of the most common ways enzymes are used in dairy processing. In the past, people used animal rennet to make cheese. The need for making cheese has gone up all around the world, but there isn't as much calf rennet available to use. This made people look for other ways to get rennet, like getting it from tiny living things. Around the world, 30% of all cheese made comes from using rennet made from microorganisms. Rennin makes the milk thicken by breaking it down in a certain way. The milk becomes like a gel because of the enzymes, temperature and calcium ions. Some tiny, well-known living things are used to make rennet, like proteinases, and they can be used instead of calf rennet. In making cheese, some tiny organisms like *Aspergillus oryzae* and *R. Miehei*, *Rhizomucorpusillus*, *Endothiaparasitica*, and *Irpexlactis* are commonly used to make rennet. Many scientists have looked at a lot of studies on rennet replacements. Many companies use *Mucor* to make rennet from microorganisms. The best results come from using the protease of *Rhizomucorpusillus* from semi-solid media made of 50% wheat bran. Furthermore, a type of fungus called *Endothiaparasitica* and another one called *R. Miehei* works well for growing things underwater.

Good milk-curdling can be achieved by using a mixture of 4% potato-starch, 10% barley, and 3% soybean-meal. Lipases are released along with the proteases as the microbes grow. So, the lipases don't work well if the pH is lowered before preparing the culture. Researchers studied how adding whey protein concentrate (WPC) affected the quality of Mozzarella cheese analogue (MCA) made from rennet casein (RC). It's best to use a mix of RC and WPC (90% RC, 10% WPC) as the protein in MCA to make a better cheese for baking. The study shows that the size and stability of fat globules in milk affect how quickly it turns into cheese when rennet is added. They found this out by looking at a peptide called caseinomacropptide (CMP), which is made up of 64 amino acids and is released from a protein in milk called kappa-casein by stomach enzymes. Researchers studied how fast milk comes out and its thickness. The researchers found that knowing more about how the size of the globules affects milk gelation could help make cheese with certain qualities.

The enzyme catalase can be used to make cheese. To make certain kinds of cheeses like Swiss, they use hydrogen peroxide during the pasteurization process. This chemical can be harmful to cells because it is a strong oxidizer. It keeps the natural enzymes in milk that help the cheese taste better. Even though the heat used in pasteurization can break down these enzymes, there are still some hydrogen peroxide residues in the milk that stop the bacteria needed to make cheese, so we have to get rid of all of it. Many things are used to find catalase enzymes, like cow livers or tiny living things. To turn hydrogen peroxide into water and oxygen, you add catalase enzymes. The proteins in lactate bacteria are important for its

growth in milk and they also help make the taste of fermented milk products much better. The proteolytic enzyme system is made of protein-digesting enzymes that break down the milk's protein into smaller pieces called peptides. Peptidases break down peptides into amino acids and small peptides[12].

Then, a transport system helps take in the amino acids and small peptides. It was found that the bacteria in milk can break down the proteins in milk into smaller parts that they need to grow. The proteinases are enzymes that break down proteins in the body. They include different types like amino-peptidases, tri-peptidases, endo-peptidases, extra cellular proteinases, and proline peptidases. Other research has found that proteins from lactic streptococcal bacteria are similar to proteins from other sources. Plantarum and Lactobacillus acidophilus are types of proteins called serines. Aminopeptidases are important for making fermented milk taste better because they can break down large protein molecules into smaller parts, which can make the milk taste more delicious. They were also used to make milk products less likely to cause allergies and to speed up cheese making. Researchers created a new way to test how well protease works in sheep and goat milk. The research found that the method is good for breaking down proteins in different substances and how well it works depends on the chemical and nutritional properties of the sample.

Lipases are used to make cheese tastier, help cheese making go faster, create different types of milk products, and break down milk fat. Lipases are enzymes that dissolve in water and help break down fat molecules into smaller parts. Microorganisms can make lipases and esterases alone or together. Some microorganisms that make lipase are *Serratiamarcescens*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Lipase helps make free fatty acids, glycerol, and different esters. It's used in production. Additionally, lipase is used to make fat and glycerides from cheaper materials like palm oil. Many different kinds of businesses, like drug, chemical, and food companies, use these products a lot. Different lipases from animals or microbes make cheese clear, less bitter, and tastier. They can also make the cheese stink. When combined with proteinases, lipases or peptidases can make cheese taste great and less bitter. We can speed up the ripening of cheese by using certain enzymes or cells to help break down the proteins in the cheese. Transglutaminase (Tgase) helps milk proteins stick together better, making milk products stronger and better. Humans with lactose intolerance cannot digest lactose because their bodies do not make enough of the enzyme lactase.

To make better cheese with a different texture and more nutrients, you can use an ingredient called transglutaminase. But it's best to add both transglutaminase and rennet at the same time. Additionally, we studied ways to make high-protein nutrition bars with milk protein and casein last longer and have a better texture. We looked into using transglutaminase and reducing calcium. The HPN bars were tested for how hard, crumbly, moist, and their pH, color, and water activity changed over time. The HPN bars made with MPC were tougher and held together better than the ones made with MCC. More Tgase crosslinking makes the HPN bar stick together better and keeps it from getting hard when stored. One study looked at panela cheeses made from a blend of dairy and plant proteins, using soybean or peanut protein isolates and transglutaminase. The study found that panela cheese can be made using the traditional method, but with added soybean or peanut protein to the milk. Cheeses with these protein isolates had more protein than regular cheese and had a similar texture.

Lactase helps to break down lactose into galactose and glucose. It helps make milk products taste sweeter, dissolve better, and easier to digest. Lactases help to get rid of lactose in milk for people who can't digest it. This helps to keep them from getting sick and dehydrated. When milk is treated with lactase, it becomes sweeter. This means that there is no need to add

sugar when making flavored milk drinks. Lactase is a substance added to ice cream, yogurt, and frozen desserts to make them smoother, creamier, sweeter, tastier, and easier to digest. It also helps prevent the grainy texture that can happen when lactose turns into crystals in these foods. Cheese made from hydrolyzed milk gets ripe faster than cheese made from regular milk. In the past ten years, scientists have studied the lactose found in whey and milk, and the harmful effects of certain enzymes. The ways we keep enzymes still have made it possible to use lactose in new and exciting ways. Some people cannot digest the sugar in milk and dairy products because their bodies do not make enough of the enzymes needed. This makes it hard for them to drink milk or eat dairy. Next, products with a little lactose or no lactose are good for people who can't handle lactose. This keeps their body moist, prevents diarrhea, and can save their life. In science, lactose can easily and quickly turn into crystals, which can be helpful in making dairy products. Because it is too expensive, using lactase in this way is not practical. Moreover, harming the environment is seen as the biggest problem when trying to separate large amounts of cheese whey. In addition, when whey is fermented, it can be used to make lactic acid because it has a lot of lactose and is cheap to produce. Because whey permeate is a byproduct of making concentrated whey protein through a filtering process, it can be effectively fermented by a bacteria called *Lactobacillus bulgaricus*.

Companies can get lactose from many places like plants, animal organs, bacteria, and yeasts and molds. They use these things to make enzymes for business use. Making lactase from certain fungi is considered safe because these fungi have been used safely in the past. It has been tested many times to make sure it's safe. The best lactase comes from *E. Coli* lactase is not a good option for treating food because it is expensive and can cause poisoning. The way the enzyme is fixed in place, the method used to fix it, and the type of carrier can also change how well it works. Usually, lactase from fungus works best in acidic conditions with a pH between 2.5 and 4.5, while yeast and bacterial lactases work best in neutral conditions with a pH between 6 and 7. Lactases work best at different pH levels, which makes them useful for different purposes. For example, enzymes from fungus can be used to break down acid whey, while enzymes from bacteria and yeast are good for breaking down milk with a pH of 6.6 and sweet whey with a pH of 6.1 Galactose inhibition is when lactase is affected by the presence of a certain product, which can slow down or stop the lactase from working. Galactose can stop the enzyme from *Aspergillus niger* more than the enzyme from *Aspergillus oryzae*.

Hydrolyzed lactose can help get rid of obstacles in the product by using immobilized enzymes or filtering the enzyme after hydrolysis. *Bacillus* species lactases are the best at withstanding heat, can work in many different pH levels, can prevent other products from forming, and can handle a lot of substrate at once. Thermo stabilization enzymes can stay active at temperatures of 60°C or higher for long periods of time. In this situation, they have two special features: they either make things change quickly or change more of the substance. Also, there are fewer germs because it's warmer where things are growing. Have little ability to stop certain reactions when exposed to galactose, but are very active in breaking down skim milk. *Bacillus* bacteria for these reasons are very important for making lactase. Breaking down lactose into simpler parts can be done using these enzymes free enzymes, often used in the fermentation process, enzymes that are stuck in place, and cells that have enzymes inside them that are stuck in place. Many broken down systems have been studied; only a few have been successful and even fewer have been used at the semi-industrial or industrial level.

New ways to break down acids have been made better to help lots of businesses. You can use the letter K instead. Lactase enzymes in milk have been improved to treat and prepare whey

and UHT-milk using free-enzyme processes. Many systems that are used for making things in big amounts have been made better for making even more things. In Italy, the way they make milk in factories is done as a team. Yeast lactase trapped in fiber can be helpful when making something in one go. In addition, UHT is a method to make the milk germ-free. Two companies, Gist-Brocades in Germany and Sumitomo in Japan, have improved and designed new ways to treat milk for factory leaders. These are continuous tasks that take a normal amount of time. The whey is filtered using a system created by several companies. Corning Glass created a way to make baker's yeast using hydrolyzed-whey, and it's now used in businesses to produce yeast. Research has found that adding lactase to milk after it has been sterilized can make the milk taste and nutrition worse. This is mostly because of the enzyme in the lactase and it gets worse if the milk is stored for a long time.

CONCLUSION

Microbial enzymes play a critical role in molding textures, tastes, and quality across a wide range of dairy products. From the artisanal traditions of cheese-making to the precision of contemporary dairy technologies, this investigation has shown the delicate dance of enzymes in the creation of dairy pleasures. As we conclude, it is clear that enzymes are more than just biological agents; they are dynamic catalysts driving the industry toward innovation, sustainability, and consumer-centered development. The enzymatic journey, from milk protein coagulation to lactose breakdown precision, emphasizes their critical significance in the art and science of dairy processing. Challenges such as enzyme stability and regulatory concerns are recognized, but the sector demonstrates resilience and adaptation by developing novel solutions. The dairy industry is adapting to changing customer expectations, implementing clean-label requirements, and researching sustainable methods. Looking forward, the future of enzymatic treatments in dairy offers intriguing opportunities, with innovative technology and current research primed to alter industry norms. As the dairy business evolves, microbial enzymes remain faithful friends, promising to influence not just the products on our tables, but also the world's sustainable and creative dairy farming.

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CHAPTER 6

STARCH INDUSTRY: ENZYMES AS CRITICAL ADDITIVES FOR IMPROVING PROCESSING EFFICIENCY AND PRODUCT QUALITY

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ABSTRACT:

Starch is a type of carbohydrate that is used in many different industries, but it is difficult to process because it is complicated. The beginning talks about what starch is, how it's used for different things, and the challenges in making it. Amylases and glucosidases are important enzymes that help to overcome these challenges. They act as catalysts, which means they help to speed up chemical reactions that are important for living things. The abstract talks about breaking down starch using enzymes, focusing on the job of amylases and glucosidases and how they work together to improve the process. Other than breaking down starch with water, the article talks about how enzymes can change starch to have specific uses. It shows how scientists have improved enzymes and used technology to do this. This includes making the enzymes more stable and specialized. The summary also talks about how important it is to make sure the products are good, by balancing doing a good job with making sure they have the right qualities. The study looks at what consumers think, how starch is processed around the world, and how using enzymes for sustainability is examined. This shows how the industry can change to keep up with new trends. New technologies and ongoing research show that enzymes are changing how we make starch, making it more efficient, higher quality, and better for the environment. Basically, this summary talks about how enzymes and starch work together and how this can be used to improve industrial and biotechnological outcomes.

KEYWORDS:

Amylose, Break Down, Glucose Molecules, Syrup, Starch Processing.

INTRODUCTION

Starch is made up of two parts: amylose and amylopectin. Amylose is like a long chain of glucose molecules linked together in a straight line, while amylopectin also has glucose molecules linked together in a straight line, but with some extra branches coming off the sides. These glycosidic linkages stay strong in higher and neutral pH, but break down in lower pH through a chemical process. Amylose is a small molecule made up of hundreds to thousands of glucose units. It has a beginning and an end. Amylopectin is a big molecule made of many glucose parts. It has one end that reduces and many ends that do not reduce. Amylose and amylopectin are found in starch, which comes in different sizes and shapes depending on where it comes from. Granules are like stable materials that are broken down slowly by enzymes [1], [2]. The amount of amylose in starch can be very low in some types of starch, or as high as 70% in others. Ordinary starches usually have 20% to 25% of amylose and 75% to 80% of amylopectin.

These properties, along with the fact that they are affected by pH and temperature and can be deactivated easily when the reaction is finished, make them helpful for studying food. Enzymes can make food go bad, but they can also help make different kinds of food or change how they taste. Enzymes speed up reactions in food, but they don't change how much of the reaction happens. Many different types of enzymes that can break down amylose or amylopectin have developed in nature. In simple terms, starch-active enzymes are put into

two groups based on how they work: enzymes that break certain chemical bonds in starch using water, and enzymes that break different bonds in starch. These enzymes have benefits over using acid to break down starch. Glucose isomerase changes glucose into fructose, making a sweeter product [3], [4]. Afterwards, strong α -amylase and pullulanase were added to break down starch faster and more effectively.

The chapter "Starch Unveiled: Enzymes as Pivotal Additives Transforming Processing Efficiency and Product Quality" takes readers on an intriguing journey into the dynamic world of enzymes, which are transforming starch processing. Starch, a basic carbohydrate found abundantly in nature, is a versatile raw material used in a variety of sectors, including food, textiles, medicines, and biofuel. This introduction examines the symbiotic interaction between enzymes and starch, focusing on the transformational influence on processing efficiency and final product quality. This investigation focuses on starch, a complex carbohydrate found in plant cells. This section gives a summary of starch's structural makeup, focusing on its branching patterns, crystalline areas, and amorphous zones. Understanding the molecular architecture of starch paves the way for understanding the strategic function of enzymes in unlocking its potential. Starch has several uses in various sectors, demonstrating its flexibility. This section investigates the numerous uses of starch, stressing its economic and ecological relevance. Starch processing may be challenging due to its inherent complexity [5], [6]. This section examines the challenges encountered in starch processing, ranging from the necessity to break down granular structures to attaining desirable alterations for particular applications. The complex structure of starch needs precise and efficient processing methods.

Enzymes emerge as catalysts, providing a radical answer to the problems of starch processing. This section presents the important enzymes involved, such as amylases and glucosidases, and explains their unique responsibilities in breaking down starch molecules into simpler chemicals. The enzymatic intervention forms the foundation for increasing processing efficiency. Amylases, which are important enzymes in starch hydrolysis, are examined in depth. Their mechanism of action, substrate selectivity, and influence on the degree of hydrolysis are investigated. This section explores the several varieties of amylases, from α -amylases to glucoamylases, and their roles in the regulated breakdown of starch into fermentable sugars. Glucosidases, like amylases, play an important function in refining the hydrolytic process. This section digs into their functions, separating oligosaccharides into separate glucose units. The synergy between amylases and glucosidases becomes apparent as the enzymatic cascade unfolds, providing complete and regulated starch breakdown [7], [8].

Starch processing goes beyond hydrolysis and includes enzymatic modifications for specific functions. This section delves into enzymes involved in starch phosphorylation, debranching, and cross-linking, offering insight on how these alterations confer unique qualities to fulfill a variety of industrial needs. The chapter looks into biotechnological advancements in enzyme engineering, including genetic changes and immobilization methods that improve enzyme stability and catalytic performance. This section explains the potential of precision in starch processing and provides a look into the future of biocatalysis. While enzymatic interventions provide a novel approach to starch processing, issues such as enzyme stability, substrate selectivity, and economic viability remain. This section confronts these problems straight on, giving unique solutions and tactics for successfully navigating the enzymatic road. Achieving optimum starch processing requires strict quality control. This section looks at the analytical tools and approaches used to evaluate enzyme activity, starch conversion rates, and end product characteristics. The difficult balance between efficiency and product quality is highlighted.

Starch-derived goods impact consumer purchasing decisions, and this part investigates how enzymatic treatments fit with changing consumer preferences. Clean-label standards, environmental concerns, and the desire for functional ingredients all influence industry developments, demonstrating the versatility of enzymatic starch processing. Global perspectives on starch processing show that processes differ based on cultural preferences, agricultural terrain, and industrial interests. This section explains how various areas use enzymatic interventions in starch processing, which contributes to the worldwide variety of starch-based products. Sustainability is a key component of current industrial operations, and this part investigates how enzymatic starch processing interacts with environmentally sensitive activities. Enzymatic interventions help to sustain starch processing by lowering water use and eliminating waste, providing a model for environmentally acceptable operations. The chapter finishes with a forward-looking view on the future of enzymatic starch processing. Anticipated trends, upcoming technologies, and active research areas provide a view into the future of starch processing, where enzymatic advancements continue to redefine efficiency, quality, and sustainability. In summary, this complete introduction reveals the complicated universe in which enzymes serve as critical additives, changing starch processing into a domain of accuracy, efficiency, and customized product qualities. From breaking down granules to tailoring enzymes for peak efficiency, the enzymatic journey in starch processing unfolds as a dynamic force fueling industrial and biotechnological progress.

DISCUSSION

Enzymes are substances that help chemical reactions happen in places where they don't normally occur. In the 1900s, scientists figured out how to separate enzymes from cells. This made it possible to make them in large quantities and use them in making food. Many researchers have studied this and written about it. Using living organisms to process food is a method that has been used for a long time. People have been using these things for a really long time, since around 6000 BCE or even earlier. They made beer, baked bread, and made cheese. Also, starch, a type of sugar, is found in the leaves, tubers, seeds and roots of many plants where it is stored for later use. Many plants with starch have been grown by people and are important for farming. Some examples are corn, wheat, rice, potato, yam, cocoyam, and cassava. Apart from eating the food crops mentioned earlier, their roots, tubers, and seeds are also used to make starch. Starch is often changed using chemicals or enzymes to make many different types of products. In this chapter, we will talk about enzymes used in food processing, especially starch and starch-active enzymes, as well as enzymes added to food and how they affect starch processing[9], [10]. To make syrup, we use enzymes and starchy flour or extracted starch from a carbohydrate source.

Enzymes in the food industry are very different because they do many different things to make food and drinks. Depending on how they're made, enzymes can work better at low amounts and make things faster than other catalysts. Even when there is only a small amount of the product, like 0.1% or less, and the conditions are not very harsh, they still work well. New advancements in using bacteria to make products such as enzymes have made it cheaper and more efficient. This is done by putting genes into the bacteria to make them produce the desired enzymes. In addition, new advancements in fermentation technology create new custom enzymes that are made to fit specific food processing needs. So, enzymes made with these technologies work better than enzymes made the usual way when exposed to tough processing conditions like very high or low pH and temperature. Enzymes are used in the food industry for many things like making drinks, baking, and making dairy, egg, fish, meat, cereal, and candy products. This is because enzymes have a lot of benefits. Previous research

has found that there are about 260 different enzymes in the EU. Most of them, about 91%, are made through fermentation, with 58% from fungi, 5% from yeasts, and 27% from bacteria. The remaining 9% are made through extraction, with 3% from plants and 6% from animals[11], [12]. The EU enzyme database says that there are around 15 enzymes and combinations taken from animals that are used a lot in making food.

When you add debranching enzymes to starch, they break down the α -1,6 linkages. Plants store carbohydrates in starch. Usually, regular starch in small grains is made up of two kinds of molecules called amylose and amylopectin. Amylose is a type of sugar that is made up of a chain of glucose molecules. Sometimes, there are also some smaller branches in amylose. Amylopectin is a type of carbohydrate made of glucose that is branched and connected in a specific way. Waxy starches are made up mostly of a molecule called amylopectin, which is very big. The amylopectin branches are grouped together in a way that lets them form double helices with nearby chains. The coiling of these strands makes starch form into small, organized areas called crystals. Starch can be broken down at certain points by special enzymes like isoamylase and pullulanase. These debranching enzymes are used to help process starch. Waxy starches release only short straight chains when starch is broken down. This means that these chains have a narrower range of sizes. Modified starches are used to make resistant starch and nanoparticles. Tiny particles made from starch are used to make plastic stronger, carry medicine, coat surfaces to keep things out, and stabilize mixtures of oil and water.

Annealing and heat moisture treatment are often used to change the properties and digestibility of starch granules. But adding enzymes is the most effective way to modify the properties and digestibility of starch granules. Sticky starch is mostly used in making food like candy and bread. It helps to stop the starch from getting hard again. Native starch is often changed a lot to make it work for specific things in factories. The 1,4-glucan-branching enzyme can help change the starch molecules by breaking and transferring parts to create branches. Therefore, the enzyme reduces the amount of a certain type of sugar in starch and makes the structure more complex by adding shorter branches. More short-chain amylopectin makes starch less thick, and less amylose makes a better structure. Chemical changes to cassava starch using a certain enzyme always make it different and affect how it acts when mixed or moved. Specific enzymes attack starch granules in a special way, causing them to break and create bigger openings. Glucan-branching enzymes change starch into amylopectin with more short chains and help amylose to reorganize. Changing the cassava starch using a special enzyme makes it stronger, more flexible, and stretchy.

Modified starch pastes do not change thickness when they are heated or cooled while they are being made and stored, unlike regular starch pastes. Starch is a useful ingredient in food because it thickens and gels. It helps make food have the right texture and keeps moisture under control. This makes processed food better and more stable. The way starch products flow and move is important for how they are used in making food. The way starch-based foods feel and how they are made are affected by their rheological properties. These properties include things like hardness, stickiness, and chewiness. This also affects how the foods are moved around and mixed during processing, and how much energy is used. The main characteristics of starch pastes include how they flow and their ability to stretch and return to their original shape. Different types of starches behave differently when exposed to stress. The way starch acts can change if things like temperature, how much starch there is, acidity, and other ingredients are different. More of one kind of starch, the weight of molecules, and how long the chains are canning all affect how starch behaves when it is moved or mixed. We can use a special enzyme to change cassava, yam, potato, and cocoyam

starch to make them better for making food like bread and pastry. Quickly digestible starches can be changed to be resistant to enzymes. Starch is usually eaten in a form that has been heated and broken down, which makes it easy for our bodies to digest it quickly. This kind of starch is called rapidly digestible starch (RDS). If there is a lot of it in food, it will quickly make the blood sugar go up, which is bad for health. RDS is linked to being overweight.

Using different enzymes on bread dough changes its basic properties, if it has a lot of resistant starch. Eating fiber is good for your health because it helps food move through your intestines faster and makes your poop bigger. Fiber can be broken down by the bacteria in your gut. This helps lower the levels of bad cholesterol in your blood and also prevents a spike in blood sugar after eating. This shows that fiber is useful for making healthy foods to help with illnesses like heart disease, cancer, and diabetes. Resistant starch (RS) is a type of fiber that is not broken down in the body. It causes fermentation in the colon, which is similar to other types of fiber. There are four kinds of RS: RS1 isn't easy to digest, like starch in grains; RS2 has a structure that enzymes can't break down; RS3 is made when food is cooked and then cooled; and RS4 is starch that has been changed by chemicals. High amylose maize starch is a type of starch called RS2. It is a white powder made from a special kind of corn that has a lot of amyloses in it. Mixing it with bread dough weakens the dough and makes it harder to bake well. This information comes from multiple studies Hydrogen peroxide can make gluten stronger by changing the way its chemicals bond together.

A type of fungus called xylanase breaks down some parts of wheat flour, making the dough softer and easier to work with. When looking at bread dough, scientists use measurements to see how it stretches and changes. They can then compare these measurements to the quality of the bread. Many researchers have found this to be an important way to understand bread quality. The bread industry often uses measurements, but how accurate they are depending on the equipment used to collect the data. Alternatively, basic measurements give information about things like force, how something changes shape, twisting force, energy. These measurements don't rely on the specific testing equipment and could be used to understand how the dough moves during mixing, proofing, and baking. Simple tests that only slightly change the dough don't really tell us much about how the dough reacts when it's stretched and shaped during the baking process. When you knead dough, it gets stretched and air gets trapped in it. This creates small air bubbles. As the dough sits and rises, the bubbles get bigger because carbon dioxide from the yeast goes into them. That's why scientists have suggested ways to find important information in tests where materials get stretched a lot. In a research, different enzymes were added to bread dough with RS in different amounts. The way the bread baked with these enzymes was found to be similar to regular bread dough without RS. Adding resistant starch to dough without enzymes makes the dough rise less during fermentation. But if you add resistant starch and enzymes to the dough, it can prevent this problem. Using enzymes in dough makes it easier to work with, whether or not resistant starch is used.

The process of converting starch into glucose begins with heating a blend of water and starch to disrupt the starch granules and dissolve the glucose polymers (amylose and amylopectin) in the water. The starch granule does not dissolve much in water. When heated, the granules absorb water and swell, which makes them thicker. When you keep heating the granules, they break apart and release amylose and amylopectin. As the mixture cools down, the amylose and amylopectin molecules start to stick together, making the mixture thicker. A thick, sticky mixture is made. It was mentioned in several studies. Finally, a white, solid gel is made. The gel's interaction is very strong, so now it cannot turn back into a liquid when heated. Starch breaking down happens in three stages: turning into a gel, becoming liquid, and turning into

sugar. Gelatinization is when starch granules swell up and burst in hot water. The starch molecules in this thick liquid are easier for amylases to break down compared to starch that has not been mixed with water. The thickness of the liquid depends on how much water is absorbed, which can be different for different types of starch. Liquefaction is when a substance called gelatinized starch becomes less thick because of α -amylase. Starch, which is made up of glucose, gets broken down quickly into smaller parts by α amylase.

This makes the gelatinized starch become less thick very quickly. Beta amylase breaks down long chains slowly starting from one end. Saccharification is when starch is broken down into simple sugars like glucose, maltose, and dextrins. An iodine test is a way to see if starch has been completely broken down into smaller parts. The enzyme α -amylase slowly breaks down the chains of amylose and amylopectin to make dextrins that have 7-12 glucose molecules. Beta amylase breaks down smaller chains and produces maltose. This process takes more time than breaking longer chains with α -amylase. Due to the different chain lengths, other sugars like glucose and maltose are made along with maltotriose. For all types of carbohydrates, the breakdown process stops before reaching the α -1,6 bonds in amylopectin because α -amylase and β -amylase cannot break these bonds. These limit dextrins are always in regular syrups and worts unless pullulanases or limit dextrinases are used as an extra enzyme to break down certain bonds. Some of the enzymes that break down starch include α -amylase, β -amylase, amyloglucosidase, pullulanase, and glucose isomerase. The type of enzymes and the quality of the raw materials used in making syrup decide how the syrup will turn out in the end. We can check how much starch breaks down by using a 0.02N tincture of iodine. The test is called an iodine test and it is done on a cold sample. This happens because when there is starch and big dextrins, iodine solution turns blue to black when it's at room temperature. Alternatively, if the iodine color stays yellow after adding it to the sample, it means that there is no more starch and large dextrins in the sample. At this stage, the syrup has a normal level of iodine, which means it has a lot of sugar and smaller dextrins. This means that the sample has been broken down completely and all the sugar has been converted. Picture 101 is a simple chart that shows how syrup is made.

Enzymes are an important ingredient used to make syrup. Enzymes can be put into two groups: ones that come from inside the body and one's that come from outside the body. Endogenous enzymes are enzymes that are already in the food, while exogenous enzymes are added to the food as a supplement. Rice and sorghum have their own enzymes, and we can make enzymes from grains like rice, millet, and sorghum using a process called barley malting. The barley or grain that has enzymes can be used to make syrup by adding them to starch. Starch is the main thing used to make syrup. In the United States and in many other places, most syrup is made from starch. Corn and other plants can make syrup using starch. But people don't know if other parts of plants can also be used to make syrup. When an enzyme helps change starch, it makes hydrolysates. These can have glucose and other sugars like maltose and maltotriose. The amount of change depends on how much the substance breaks down and the type of enzymes involved. The syrup is cleaned and made clear using easy methods that anyone can do. This chapter shows the findings of a study on making syrup from different types of tubers and rice.

It is important to fully understand the characteristics of syrups made from tropical tuber flours like cassava, potato, yam, and cocoyam, and how these characteristics can be different depending on the type of tuber and how it is processed. The table in the text shows the physical and chemical properties, as well as the sugar content, of the syrup made from various types of tubers. The syrup made from different tubers had pH levels between 4.65 and 5.59. The pH level is an important quality factor that shows how stable the good stuff in

food is. Foods with medium acidity levels last longer than foods with higher acidity levels because they are less likely to spoil. However, the thickness of the syrups is related to the dextrose equivalent (DE). As the thickness of syrup increases, it flows more easily. The syrup becomes thinner because there are fewer solid particles in it. This is shown by the higher sugar content in the syrup. The more DE there is, the more likely dextrose will turn into crystals. The thickness of syrups can change because of the amount of solid stuff in them. Also, the different kinds of syrups and how they're made can affect their thickness too. This means that the big parts in syrups, like large sugars and dextrans, have a big effect on how syrup flows.

Also, things that affect how thick liquids are include what the liquid is made of, how the particles in the liquid interact, how much of the particles are in the liquid, the shape of the particles, how big the particles are, and how hot or cold the liquid is. As more sugar is added, the syrup gets thicker because the sugar particles stick together more and also stick to the liquid. When there are less high sugars in syrups, they become less thick. The sugar levels in syrups can be different because of the kind of carbohydrates used and the enzymes used to break them down. It's really important to remember that the kind and quality of syrup depends only on that. This is about the kind of enzyme used during the process of saccharification. If you use amyloglucosidase (glucoamylase) as a saccharifying enzyme, you will get syrups with more glucose and less maltose and maltotriose. However, if a certain type of fungus enzyme called fungal-amylase is used to help break down sugars, the resulting syrups will have a moderate level of sweetness (42-55DE), with less glucose and more maltose and maltotriose. Some tubers, like yam and cassava, have a lower DE than other tubers because they are different types of tubers. This means that the kind of carbs used affects how good the syrup sugar is, even if the breakdown of the carbs is finished or not. This can be observed in the way sprouted and un-sprouted yams are put together. Different types of carbohydrates are being broken down and turned into syrups. This means that the syrups will have different ingredients and sweetness levels because of the way the starch is structured. Some tubers have more starch packed in a way that makes it easier for amylase to break it down. This can happen because some tubers have more amylose starch than amylopectin, which can result in higher DE.

Rice variety 306 has a higher DE of 306 compared to other types of rice because of the way its starch is made up. The way the starch was packed might have made it easier for the amylase to break it down. This could happen if the starch was more accessible to the amylase. It may be that the starch of 306 has more amylose starch than amylopectin. Amylose is easier to break down than amylopectin. This happens because the breakdown of amylopectin is limited by the presence of beta-limit dextrin. Syrups with a DE greater than 38% are often used in making beer because they have a lot of sugar that can be fermented. It helps to make things work better and get more done. Dextrose Equivalent (DE) measures the amount of sugar in a sugar product compared to glucose, shown as a percentage. Syrups are given a number called DE, which shows how much the starch has been broken down. Syrups with low DE have a lot of sugars that are thick, and a lot of thick sugars make the syrup thick. High DE syrups have less sugar and are not as thick. High DE syrups have a lot of sugars that can reduce, which means they are both sweet and can change color.

Alternatively, if the DE percentage is between 60% and 97%, then the most common sugar present is glucose. This is because DE shows how much starch has turned into glucose. When two glucose molecules are connected in maltose, one of the positions on the first molecule is not free anymore because it's connected to the second molecule. This means that even though there are two glucose molecules stuck together, only one of them can help with reducing

other substances. So, maltose only has about half as much power to reduce things as glucose does. Also, when three glucose units are put together, like in maltotriose, it has only about one-third of the reducing power of glucose. Basically, when there are more glucose molecules in a chain, the sugar doesn't have as much power to reduce things, and the DE goes down. Simply put, the decrease in DE of malted samples is caused by the amount of maltose and maltotriose produced in the malt and wort. This means that when the glucose chain in a sugar molecule gets bigger, the sugar's ability to reduce other substances gets weaker, and so does the sugar's DE.

It is important to know that syrups from unmalted samples had more glucose and less maltose and maltotriose compared to syrups from malted samples. Malting usually makes maltose double, but decreases glucose and sucrose. Glucose is reduced by half and sucrose is reduced by three quarters. This means that maltose is likely made during the process of malting and also by enzymes breaking it down during hydrolysis. In the unmalted samples, glucose and sucrose were being used up during the malting process to help the malt stay alive and grow. Sucrose is found in grains and it is not made during malting or hydrolysis. Also, sugar found in a fully grown kernel is mainly made up of sucrose and glucose. The growth of the plant would have used up all the natural sugars in the process. The syrups have a lot of maltose because bacteria and fungi work together to make them. The bacterial α -amylase enzyme breaks down gelatinized starch into glucose and maltose, but it can't break down certain types of linkages in the starch.

Fungal α -amylase breaks down liquefied starch into mostly maltose and some glucose. This explains why there is a lot of maltose in the syrup compared to other sugars. The syrup has a lot of maltose because of the amount of amylose and amylopectin in the starch. Starch with more amylose makes syrup with more maltose in it. During malting and mashing, raffinose content decreases as big molecules break down. This is why the raffinose content doesn't decrease much in the samples that aren't malted. On the other hand, the decrease in sucrose amount is because sucrose changes when it combines with raffinose and stachyose. Raffinose is made up of three sugars galactose, glucose, and fructose, while stachyose is made up of four sugars, glucose, fructose, and two galactose units. They are called α -galactosides, and are found in beans and whole grains.

CONCLUSION

The employment of enzymes in food processing has several advantages. It has easily elevated food processing to a new level. Enzymes may break down α -1,6-glucosidic linkages in starch structures and hydrolyze cell wall macromolecules during starch processing. The use of cell-wall-degrading enzymes on legumes causes not only hydrolysis but also transformation and cross-linking of the hydrolyzed sugars. However, it has been found that the kind of syrup produced is determined by the quality of the carbohydrate material and the type of enzymes utilized. Malting enhances the hydrolysis and modification of starchy endosperm, resulting in a better syrup yield and carbohydrate profile. Exogenous enzymes undergo more hydrolysis than crude enzymes from germinated cereals due to the presence of co-extractives that form part of the crude enzyme flour. When crude enzymes are employed, there is inadequate hydrolysis; however, when crude enzymes from various sources are mixed, hydrolysis increases. Because of advances in biotechnology and the multiple benefits of microbial enzymes, the use of genetically modified microbes has resulted in the majority of enzymes with different properties and uses in the food, pharmaceutical, textile, detergent and cosmetic sectors. Furthermore, future research should concentrate on the extraction, purification, and characterization/identification of converted sugars from enzyme-hydrolyzed legume sugars, particularly soymilk. The detected cross-linked sugars might be utilized to calibrate standard

analytical equipment for accurate detection of hydrolyzed sugars in soy milk. With the growing popularity of meals designed for particular purposes, supplementary enzymes may be used to assist make the nutrients in these foods more bioavailable. Syrups made from flours should be evaluated subjectively and quantitatively for vitamins, minerals, and amino acid profiles. Unlike resistant starch, which provides a useful food source for intestinal bacteria, bacterial protein fermentation may create toxins such as urea, phenolics, and branch-chained fatty acids, all of which are very damaging. Selected proteases may be added to protein beverages or consumed with them to guarantee that the proteins are thoroughly broken down in the small intestine. Supplemental enzymes may also be utilized to improve the effectiveness of herbs and botanicals.

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CHAPTER 7

LYSOZYME IN FOOD: USING A NATURAL ANTIMICROBIAL ENZYME FOR INNOVATIVE AND POTENTIAL APPLICATIONS

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ABSTRACT:

The abstract emphasizes the fundamental necessity of food safety by presenting lysozyme, a naturally occurring enzyme with distinct antibacterial characteristics. A thorough examination of lysozyme structure, modes of action, and natural sources lays the framework for understanding its many uses. The chapter takes readers through many areas of the food business, demonstrating how lysozyme helps with cheese preservation, wine and beer fermentation, seafood freshness, quality assurance in bread and dairy goods, and fresh produce safety. Challenges and concerns in lysozyme use, consumer views, and worldwide perspectives on its usage in various culinary traditions are discussed. Furthermore, the abstract explores the sustainability of lysozyme uses, demonstrating its potential as an environmentally beneficial preservation agent. The chapter finishes by looking forward to new technologies and current research fields that demonstrate the increasing role of lysozyme in defining creative, safe, and high-quality food products. In short, this abstract encompasses lysozyme's multiple contributions to the food business, providing insights into its enzymatic interventions that improve food safety, quality, and sustainability across a wide range of goods and culinary settings.

KEYWORDS:

Bacteria, Food Safety, Human Lysozyme, Lysozyme Food.

INTRODUCTION

Lysozyme is a key component of the innate immune system, with potent antibacterial properties against bacterial, fungal, and viral infections. It protects against infections, works as a natural antibiotic, improves the efficiency of other medicines, and boosts the immune system. Lysozyme may be used in the pharmaceutical industry to prevent a variety of illnesses caused by bacteria, viruses, fungal infections, and inflammation, as well as to stimulate the immune system and treat allergies. The enhanced lysozyme even opens up new possibilities in the realm of clinical treatment. The protein controls the synthesis of tumor necrosis factor (TNF α) and increases the production of Type I interferon (INF α , INF β , INF γ), interleukin-2 (IL-2), and interleukin-6 (IL-6) by human lymphocytes, potentially leading to tumor elimination. Lysozyme is better suited for food systems because of its potential uses in directly adding to or coating the surface of food to inhibit the development of harmful microorganisms. These proteins, along with other antimicrobials, have shown promise as food preservatives. This study examines this natural antibacterial chemical and its uses, notably in food systems[1], [2].

In the complex realm of food science, the use of lysozyme, a natural antibacterial enzyme, has emerged as a focus for innovation, safety, and improved quality. This introduction delves into the many uses of lysozyme in the food industry, revealing its enzymatic capabilities, modes of action, and the many ways it contributes to the safety and quality of foods. Food safety is a top priority for the business. This section establishes the foundation by emphasizing the importance of antimicrobial agents in preventing spoiling and increasing food safety. Lysozyme, a naturally occurring enzyme, emerges as an intriguing option with

specific features that make it efficient in inhibiting bacterial growth. Understanding the structure, function, and sources of lysozyme is crucial for its use in culinary applications. This section looks into the enzymatic characteristics of lysozyme, focusing on its function as a glycoside hydrolase that targets bacterial cell walls. The presence of lysozyme in body fluids, eggs, and specific tissues demonstrates its essential relationship to the living world[3], [4].

Lysozyme's antibacterial properties are closely linked to its methods of action. This section describes how lysozyme catalyzes the breakdown of peptidoglycan, an important component of bacterial cell walls. The enzymatic breakdown causes bacterial lysis, which is the foundation for lysozyme's antibacterial action. Understanding these systems paves the way for research into their potential uses in food preservation. This section focuses on the natural sources of lysozyme, which are abundant in egg whites. The extraction and manufacturing techniques for acquiring lysozyme from diverse sources, including recombinant technologies, are investigated. The part also discusses the obstacles and improvements of large-scale lysozyme synthesis. Lysozyme's antibacterial properties make it effective for preserving food and increasing shelf life. This section discusses how lysozyme suppresses the development of spoilage and harmful bacteria, hence avoiding foodborne diseases and preserving the sensory properties of food. The research includes the preservation of both raw and processed foods, demonstrating lysozyme's adaptability[5], [6].

Cheese, a staple in many diets throughout the globe, is used as a case study to demonstrate lysozyme's usefulness in food preparation. This section discusses the function of lysozyme in cheese manufacturing, including how it contributes to both safety and taste development. The enzymatic regulation of lactic acid bacteria in cheese curds is highlighted, demonstrating how lysozyme improves end product quality. This section delves into the fermentation processes used in the creation of wine and beer, with a focus on how lysozyme protects against undesired microbial activity. The regulated suppression of bacteria and spoilage organisms during fermentation improves the stability and taste profile of these alcoholic drinks. The seafood business has particular issues in ensuring product freshness and safety. Lysozyme's uses include seafood, where it inhibits the development of certain spoilage bacteria while improving the general microbiological quality of seafood items. This section investigates how lysozyme improves the safety and preservation of fish and shellfish.

Bakery and dairy goods are thoroughly examined to demonstrate lysozyme's involvement in maintaining quality and safety. Lysozyme prevents molds and germs in bakery applications, allowing baked items to last longer on the shelves. The enzymatic preservation of dairy products, such as yogurt and milk, is investigated, with a focus on the several advantages of lysozyme. Lysozyme treatments are beneficial for fresh food, which is prone to microbial infection. This section investigates how lysozyme improves the safety and quality of fruits and vegetables. The enzymatic intervention reduces microbial development on the surface of produce, lowering the risk of foodborne disease and increasing the overall freshness of fruits and vegetables. Despite its benefits, the use of lysozyme in food presents obstacles. This section discusses enzyme stability, regulatory issues, and possible allergenicity. Innovative ideas and tactics are provided to address these issues and guarantee the safe and effective use of lysozyme in food items.

Acceptance of lysozyme in food is heavily influenced by consumer attitudes and the regulatory landscape. This section investigates how consumer preferences, clean-label expectations, and awareness influence the incorporation of lysozyme into foods. Furthermore, the regulatory environment for lysozyme uses is described, highlighting the need of adhering to safety standards and providing explicit labeling. Lysozyme uses in food vary widely based

on regional culinary traditions, tastes, and regulatory frameworks. This section explores how various cultures use lysozyme in their food processing processes, adding to the many worldwide viewpoints on its usage. Sustainability is a rising issue in the food sector, and this section investigates how lysozyme fits into environmentally friendly procedures [7], [8]. Lysozyme appears as a long-term strategy for improving food safety and quality, decreasing food waste and the need for synthetic preservatives. The chapter finishes by looking at the future of lysozyme uses in food. Anticipated trends, future technologies, and active research fields provide a view into the next frontier, where lysozyme will continue to play an important role in developing creative, safe, and high-quality food products. In summary, this comprehensive introduction walks you through the many uses of lysozyme in the food sector, emphasizing its function as a natural antibacterial enzyme. Lysozyme emerges as a flexible tool for improving safety, quality, and sustainability across a wide range of food items, from retaining freshness in seafood to assuring stability in fermented drinks.

DISCUSSION

Bacteria cell walls have connections between certain sugars called N-acetyl-d-glucosamine and N-acetylmuramic acid. These connections are important for the structure of the cell wall. Lysozyme is mostly in saliva, tears, blood serum, human and cow milk, and avian egg whites, and also in some bacteria and bacteriophages to some extent. In addition, some plants also have this enzyme. Lysozyme kills certain kinds of bacteria. However, it doesn't work well against Gram-negative bacteria because of the outer membrane that has lipopolysaccharides. However, there are ways to make lysozyme better at breaking down certain bacteria. This can be done by changing the enzyme, adding it to other substances, or using it with electric pulses. These methods can help lysozyme work better against certain types of bacteria. Alexander Fleming found this protein with enzymes in 1921. It weighs about 14-15kDa and has a pH of about 11. It is made up of one strand of 129 building blocks and is held together in four spots by special bridges. The connections between sulfur atoms and the six spiral parts help the enzyme stay strong when it gets hot. Some specific amino acids like Asp-52 and Glu-35 in the enzyme's active site are really important for how the enzyme works [9], [10].

Both the lysozyme found in chicken eggs (cLYS) and the lysozyme found in humans (hLYS) are of the C-type category. However, even though cLYS can be found in egg whites, hLYS is more stable when heated and has better ability to fight bacteria because of its different makeup and structure. In addition, hLYS is also better at treating many human diseases without causing immune reactions and side effects. However, hLYS is not used much because there is not a lot of it available. So, lysozyme is an enzyme found in large amounts in bird eggs and mammal milk. It is considered safe to add directly to foods. The lysozyme from the white of a hen's egg is often used to fight germs in food. It is also used to keep food fresh, like fish, meat, milk, and fruits and vegetables. Lysozyme holds great significance in the food industry, as it is approved by the World Health Organization for use as a food preservative. At present, it is being used to make Chinese noodles, cheese, sushi, kimuchi pickles, and wine. There is a big need for natural ways to keep food fresh, so lysozyme is now more and more important in processing food. So, there is a need to create better and easier ways to make lysozyme. There are different ways to separate and purify lysozyme from chicken egg whites. These include using filters, and techniques like gel filtration, affinity, adsorption, and ion-exchange. Then, it can be separated using reductants and heat after it crystallizes directly. But many studies say that using adsorption-elution methods is the best way to separate lysozyme because it gives more lysozyme in the end.

Additionally, advancements in genetic engineering have made it possible for human lysozyme to be produced in microorganisms like *E. coli*, yeast, and other cells and plants.

Recently, scientists have been studying the mammary glands of transgenic animals like mice, goats, and cattle. They are interested in using these glands to make proteins in a different way. This is because these animals are good at making changes to the proteins they produce. In simple terms, a foreign gene can be put directly into the mammary gland cells of adult animals, and then the proteins made by these cells would go into the milk right away. Therefore, this will make it easier to purify recombinant proteins using simple methods. Dairy cows can be utilized to produce human lysozyme and improve the composition of cow milk to mimic the properties of human milk using a mammary gland bioreactor system. However, transgenic chickens can also be used as bioreactors and have a few benefits. These include cheaper maintenance, quicker generation time, better protein profiles, and lower chance of causing an immune response. In relation to this, Wu and his team. In 2015, scientists purified and studied a protein called rhLYS from eggs of genetically modified chickens. From the point of view of tiny organisms, the lysozyme taken from *Bacillus licheniformis* TIB320, which comes from the dirt, could be a good thing to add to food or animal feed if we can find a good way to make a lot of it. The reason is that when *E. coli* BL21(DE3) and *Bacillus subtilis* WB 800 were used to make lysozyme[11], [12].

This lysozyme comes from *Bacillus licheniformis* TIB320, and it can kill different kinds of bacteria like *Micrococcus lysodeikticus*, *Clostridium sporogenes*, *Pseudomonas aeruginosa*, and others. It also eliminated a broader range of germs compared to egg white lysozyme, which primarily halted the growth of a specific type of germ known as Gram-positive bacteria. Molecules that disrupt the membranes of bacteria are usually charged, can be found in water, and do not mix well with water. It is important to know that even at the normal pH level of the body, lysozyme has all these characteristics. However, lysozyme doesn't work very well against some types of bacteria. So, we need to create a new kind of lysozyme that has better abilities to fight these bacteria. Some researchers have already been working on this. In order to make lysozyme work better at killing germs, scientists are trying to change the enzyme's chemical structure. First, they discovered that adding certain fatty acids to lysozyme makes it better at killing certain bacteria, without affecting its ability to do other tasks. Alternatively, adding sugar molecules to lysozyme made the proteins more stable. Their shape stability got better, and they were hard to break down by proteases. They also got better at controlling how much charge they have and holding onto water. So, when lysozyme was mixed with dextran, it made it better at fighting germs. The mixture worked 30 times better than the original lysozyme at making things mix together.

The study showed that when xyloglucan hydrolysates were combined with hen egg lysozyme, the enzyme's activity stayed the same and the ability to mix oil and water improved five times more than before. Moreover, when lysozyme is combined with a fatty saccharide, it creates a strong antibacterial substance that is resistant to breaking down and able to make emulsions more stable. It was also found that the protein part did not change much, and the mixture kept about 70% of its germ-killing power compared to the original hen egg lysozyme. Also, when an egg white lysozyme was changed by adding sugars and then mixed with palmitic acid, the amount of the modified lysozyme increased. This modified lysozyme was also very effective at killing *Escherichia coli* bacteria. So, making lysozyme more attracted to fats and adding sugars to it could be a good way to use it in industry. This is because the plants are growing better and the medicine is working better against certain bacteria. When lysozyme was heated, it lost some of its ability to work as an enzyme, but it became better at killing certain kinds of bacteria. This means that when lysozyme partially unfolds, it can start killing a different type of bacteria without losing its ability to kill the original type.

This often leads to making an enzyme preparation with more polymeric forms. Some of these isoforms are more positive and more hydrophobic than the original lysozyme from hen eggs. It's still unclear if lysozyme can cause allergies. Actually, some research shows that lysozyme may not cause strong allergic reactions, while other studies say the opposite. However, lysozymes from hen eggs contain a well-known allergen called Gal d4, which has specific parts that can cause allergic reactions. Many studies have shown that HEWL could be an allergen. Also, in some foods, the lysozyme content can be between 250 and 400mg/L, such as in cheese, and 100-500mg/L in wine. These amounts are bigger than they seem, especially for patients who are already sensitive to lysozyme. In Canada and the United States, it has been said that lysozyme is safe to eat. In September 2006, the United States said that lysozyme can be called "organic" on processed food labels. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) also said that lysozyme comes from animals we eat and can be considered a type of enzyme in food. So, it was okay to use it in making food as long as it was done carefully. But, since 1999, the rules about using lysozyme have been changed many times. In October 2011, an organization called the European Food Safety Authority (EFSA) wrote a report about lysozyme and its potential to cause allergies when used to make wine. In this view, the EFSA thought that a lot of people allergic to eggs are also allergic to lysozyme. Even a small amount of lysozyme in wine could cause an allergic reaction in these people.

So, wines that use lysozyme must have a special label on them. This follows the rules of the European Commission Regulation (EU) No. December 22, 2010, the year 1266/2010. Also, the labeling rules need the word "Contains," followed by the particular allergen found in the final product at 0.25mg/L. To find out how strong lysozyme is, experts suggest using a turbidimetric test. The process works by using lysozyme to break down bacterial cells, and then measuring the result using a method called turbidimetric analysis. Various methods such as gel-diffusion or lysoplate assay, spectrophotometric or turbidometric procedures, HPLC-FLD, SDS-PAGE, ELISA, and immunocapture can be used to detect and measure the lysozyme molecule. Lysozyme is good for food, but it can cause allergies, so we need to find new ways to make it work better and get rid of its problems. In this case, using lysozyme in food instead of HEWL is a good idea. Additionally, using enzymes to stick to tiny structures and materials for storing food can create new and clever ideas in this field. Most of the time, food goes bad because of germs. This can also make the food not as good and not safe to eat. E coli can make you sick from eating contaminated food. Coli O157:H7, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Listeria monocytogenes* are big health problems worldwide. Additionally, consider the growth and survival of bacteria like E. coli that can cause food to spoil. Coli O157, L. can be simplified as the bacteria E. coli O157 *Listeria* bacteria, common food poisoning bacteria, and B. cereus, a type of food poisoning bacteria. aureus, *Campylobacter*, *Clostridium perfringens*, *Saccharomyces cerevisiae*, and *Aspergillus niger* are all types of bacteria and yeast that were studied. These problems keep happening even though traditional ways of keeping food fresh like freezing, heating, drying, fermenting, and salting work pretty well. But sometimes the food can get contaminated again and cause issues. This could make the food taste bad for people who eat it.

To stop bad germs from growing in food, we can either add special substances to the food, put them on the outside, or mix them into the packaging. One way to put active agents directly into food makes bacteria go down quickly, but only for a short time. So, using packaging with antimicrobial enzymes can help keep food fresh for longer, even if it's not sterilized or processed a lot. This can be useful for food companies. To do this, lysozyme has been stuck onto different materials in different ways, like trapping it, sticking it, and joining it to the surface. However, the best method found so far has been to make HEWL unable to

move by sticking it to something. This also made the lysozyme able to kill more types of bacteria. In this case, there are reports that the chitosan-lysozyme films worked well to kill germs. When making special coatings for food and medicine, researchers found that a substance called HEWL stuck well to small particles made of calcium carbonate and carboxymethylcellulose. In this situation, we used various types of plastics like PVOH and PET to trap lysozyme. Additionally, it was found that adding lysozyme, an enzyme that stops bacteria from making lactic acid and causing wine to ferment, to PVOH films helped to keep the release of antimicrobials steady, which made them more effective in stopping bacteria. The bacteria called *Listeria monocytogenes* was found in smoked salmon. However, in the movies about zein, it was shown that lysozyme can stop the growth of *Lactobacillus plantarum* and *Bacillus subtilis*. They found that adding lysozyme and EDTA to the zein film made lysozyme better at fighting against Gram-negative *E. Coli* bacteria are tiny germs. Moreover, we also studied how well lysozyme works with EDTA and nisin in a film made of alginate.

In recent years, scientists have been studying how very small technology can be used to make food safer. They believe it has a lot of potential to create ways to keep food from spoiling. This technology can help make food safer by using it as a coating or packaging to stop the growth of harmful germs in food. Many man-made plastics have been used for medicine and drugs, but they can't be used in food that needs safe ingredients. One big challenge is finding new materials made from plants instead of non-food materials. Food materials such as certain types of sugars, proteins, and fats can help solve the problem. These materials are natural and safe to eat, and they can also be used to create new and useful products. However, using these polymers has some issues with how they work and how they are made, as well as the costs that are typical for most biodegradable food-grade polymers. So, using nanotechnology on these materials could make them better and cheaper. The coated cheese had lower pH levels, less mass loss, and less spoilage from microorganisms compared to uncoated cheese. It also had more acidity. In addition, showed that hydrosols with chitosan, lysozyme, and nanocolloidal silver, along with hydroxypropylmethyl cellulose, are effective in killing Gram-positive bacteria like *B. Cereus* and *Micrococcus flavus* are types of bacteria, and so are Gram-negative bacteria like *E. coli* and *Pseudomonas fluorescens* were put on the meat's surface.

Finally, in a new study, Feng and other researchers. In 2017, a new type of germ-killing film was created. It is made of a material called polyvinyl alcohol mixed with other substances like β -cyclodextrin, cinnamon essential oil, and lysozyme. This film can kill *L. bacteria* very effectively. *monocytogenes* and *S. Enteritidis* are a medical condition that causes inflammation in the intestines. Also, this small film was very good at preventing fungus growth on *A. Niger* and *Penicillium* are types of fungi. Although everyone agrees that lysozymes work in a certain way, there are different kinds of lysozymes that are unique because of their amino acid sequence, structure, and physical and immune properties. Lysozymes are usually put into three groups: c-type (chicken type), g-type (goose type), and i-type (invertebrate type) lysozyme. The C-type lysozyme is a good protein to study how enzymes work and the structure of living things. It is well known for breaking chemical bonds in the cell wall of a certain type of bacteria, and this has been helpful for keeping food safe. C-type lysozyme is in all vertebrates and also in different types of insects like butterflies, flies, termites, and true bugs. In this large group of lysozymes, the chicken egg white lysozyme (HEWL) and human lysozyme are well-known examples. Human lysozyme is the first lysozyme from a mammal that has been studied and its genetic code has been identified.

For the past 30 years, scientists have been using human lysozyme and HEWL to study how proteins are built and how they work. These proteins are a good example for this kind of research. Human lysozyme is better for use in food or medicines than HEWL because it is less likely to cause an immune reaction or allergic reaction. However, human lysozyme is not used much because there is not a lot of it available. Recombinant human lysozyme has been made in plants, animals, bacteria, and yeasts. G-type lysozyme was first found in the egg whites of the Embden goose, so it was named g-type. This is the main kind of lysozyme found in birds like the rhea and cassowary. Active g-type genes have been found in small animals like certain types of clams and sea squirts. I-type lysozyme is the third kind of lysozyme that is found in animals. I-type lysozyme is mostly found in invertebrates. It has been confirmed that a type of lysozyme called i-type lysozyme is found in worms, sea stars, roundworms, and insects. Some i-type lysozymes have been effective against certain bacteria that are difficult to treat. Xue and others. In 2007, it was found that two types of lysozymes in eastern oysters stopped the growth of certain bacteria. A special type of lysozyme called destabilase-lysozyme from medicinal leech was found to have more than one function.

CONCLUSION

In recent years, many people have become more concerned about the safety of man-made preservatives in food. So, we have been trying really hard to find natural things that can stop bacteria and fungus from growing in food. This way, the food will last longer and be better quality. Lysozyme has been researched for its ability to kill bacteria and stay stable when heated. It is used in medicine, makeup, and food, but its main use is as a preservative for food.

So, lysozyme is a natural enzyme that stops bacteria from growing in some types of cheese. It comes from egg whites and can also be used in packaging to keep food fresh. This is because it stays effective in different types of packaging and food. At the end of the chapter, we look ahead to see how lysozyme could be used in food in the future.

Expected patterns, new technologies, and current research areas suggest an exciting change where lysozyme is still very important in making creative, safe, and high-quality food products. Basically, this conclusion recognizes and celebrates the benefits of lysozyme in food. It is noted for its ability to act as a natural antimicrobial agent. From basic food safety rules to different ways of cooking around the world, lysozyme is a flexible and eco-friendly tool that has a big impact on food science.

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CHAPTER 8

FUNGAL PROTEASES: DEVELOPING BIOACTIVE PEPTIDES TO TRANSFORM HEALTHY DYNAMICS IN THE FOOD INDUSTRY

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ABSTRACT:

This abstract captures the core of the complex route taken to uncover the numerous functions and uses of fungal proteases in changing nutritional dynamics, tastes, and sustainability standards in the food sector. The enzymatic symphony of food processing serves as the overture, emphasizing the critical function of fungal proteases as molecular builders in sculpting textures, tastes, and nutritional profiles. The chapter guides readers through the discovery of fungal proteases, studying their many origins, kinds, and functions while setting the groundwork for future applications. Proteolysis mechanisms become a focus, revealing how fungal proteases painstakingly sever peptide bonds, creating precise bioactive peptides. The nutritional importance and health effects of these bioactive peptides are then discussed, highlighting their promise in functional nutrition and well-being. The different sources of fungal proteases, spanning from molds to yeasts, are investigated, demonstrating the enzymatic arsenal available to food scientists. The chapter goes into manufacturing approaches, such as fermentation procedures, for optimizing peptide yield by using fungi's enzymatic capabilities.

KEYWORDS:

Bioactive, Enzyme, Fungal, Peptides, Proteases.

INTRODUCTION

In the rich tapestry of the food business, the involvement of fungal proteases and their contribution to the creation of bioactive peptides tells a compelling story of innovation, nutritional betterment, and culinary development. This chapter, titled "Fungal Proteases and the Production of Bioactive Peptides for the Food Industry," delves into the multifaceted world in which fungi use their enzymatic prowess to create transformative peptides that change the dynamics of food production and consumption. Fungal proteases play a crucial role in food processing, creating an enzymatic symphony. This section establishes the context by clarifying the essential function of enzymes in food processing, with a focus on how proteases, as molecular scissors, play an important role in protein hydrolysis, which is a precursor to the production of bioactive peptides. The symphony plays out with precision, emphasizing the importance of these fungal enzymes in molding the textures, tastes, and nutritional profiles of many food items[1], [2].

This section focuses on fungal proteases, which have varied origins and functions. The investigation delves into the origins of fungal proteases, covering a wide range of fungi from molds to yeasts. The categorization of these proteases based on their catalytic processes and activities offers a solid framework for their use in the food sector. Understanding the mechanics of proteolysis is critical for deciphering the complex process of peptide formation. This section discusses how fungal proteases break peptide bonds, resulting in the formation of bioactive peptides. The specificity of these enzymes and the conditions that influence their activity become focus points, revealing the accuracy with which peptides are created. Bioactive peptides, the gems created by enzymatic creativity, are the topic of this section. Their nutritional value, which includes amino acid composition, antioxidant

capabilities, and bioavailability, is discussed. Bioactive peptides' influence on health, which ranges from cardiovascular benefits to anti-inflammatory effects, places them at the forefront of the convergence of food and functional nutrition[2], [3].

The fungus world provides a wealth of proteases, each with its own distinct properties to add to the enzymatic armory. This section investigates the range of fungal sources, spanning from *Aspergillus* to *Rhizopus*, and demonstrates how various proteases have unique features that impact the profile of bioactive peptides produced during food processing. This section looks at how fungal proteases are used in industry. Various production processes, such as submerged fermentation and solid-state fermentation, are evaluated for their effectiveness in producing bioactive peptides. Fermentation factors, substrate selection, and optimization methodologies all play important roles in enhancing fungi's enzymatic capability. Dairy products become a focal point for demonstrating the role of fungal proteases in bioactive peptide synthesis. This section investigates how fungal proteases contribute to the quality and health aspects of dairy-based meals, ranging from improving nutritional profiles to developing novel tastes and textures. Cereal-based products, which are essential to worldwide diets, are examined to demonstrate how fungus proteases may convert grains into nutritious powerhouses. Enzymatic treatments in cereal processing, ranging from bread to pasta, demonstrate how bioactive peptides may be used to alleviate nutritional deficiencies and improve the functional properties of these basic foods[4], [5].

The history of fermented foods is entwined with fungal proteases, which work as catalysts to shape the organoleptic properties and nutritional value of these gastronomic pleasures. This section explores fermented foods such as soy sauce, miso, and tempeh, demonstrating how fungal proteases contribute to both tradition and innovation in world cuisine. The use of fungal proteases in meat processing gains prominence, emphasizing their role in redefining the texture and taste of meat products. From tenderization to taste improvement, this section investigates how fungal proteases provide unique meat processing methods that fit with changing customer demands. The culinary laboratory uses enzyme-modified food items as a canvas for creativity. This section investigates how fungal proteases are used to create new textures, tastes, and nutritional profiles in enzyme-modified foods. The marriage of science and gastronomy is obvious as fungus contribute to the growing panorama of culinary innovation. While fungal proteases open up a world of possibilities, they also bring several problems. This section covers topics such as enzyme stability, specificity, and regulatory compliance. Innovative solutions and developing technology provide a look into the future while crossing the fungal protease frontier with resilience and adaptation[6], [7]. The acceptability of bioactive peptides in current diets is investigated, taking into account consumer perceptions and preferences.

Clean-label requirements, knowledge of functional nutrition, and the sensory characteristics of food items changed by fungal proteases are critical to understanding how these developments correspond with changing dietary trends. Culinary traditions and industrial methods differ across the world, which influences how fungal proteases are used. This section explores how various locations use fungal proteases in their culinary traditions and food processing sectors, contributing to the worldwide diversity of enzyme uses. Sustainability has emerged as an important factor in contemporary food production. This section investigates how fungal protease applications are compatible with sustainable practices, ranging from enhancing resource use in fermentation processes to minimizing waste in food processing. Fungal proteases act as catalysts for environmentally responsible techniques throughout the farm-to-fork journey. The chapter finishes by looking at the future of fungal protease uses in the food business. Anticipated trends, future technologies, and

active research fields provide a view into the next chapter of food evolution, where fungal proteases will continue to change nutritional dynamics, taste profiles, and sustainability standards. This introduction sets the framework for a thorough investigation of fungal proteases and their involvement in producing bioactive peptides that go beyond the limits of traditional food processing. From the biochemical symphony of proteolysis to the worldwide influences defining culinary traditions, this chapter prepares the reader for a deep dive into the complicated realm where fungi collaborate to revolutionize the dynamics of the food business.

Specific uses in dairy products, cereal-based meals, fermented delicacies, and meat processing are discussed, demonstrating how fungal proteases alter textures, tastes, and nutritional value. The culinary laboratory promotes enzyme-modified food items, demonstrating the intersection of science and cuisine. The challenges and prospects of traversing the fungal protease frontier are discussed, along with insights into factors such as enzyme stability, specificity, and regulatory compliance. The article investigates consumer perceptions and worldwide impacts on fungal protease uses, taking into account changing dietary trends and regional culinary traditions. Sustainability emerges as a key issue, demonstrating how fungal protease uses match with environmentally responsible approaches from farm to fork. The abstract finishes with a look forward to future horizons, predicting trends, upcoming technologies, and active research fields that will usher in the next chapter of food evolution, in which fungal proteases will continue to play an important part. In essence, this abstract captures the compelling tale of fungal proteases, blending science, innovation, and culinary expertise into the ever-changing saga of the food business.

DISCUSSION

Proteins are an important part of food. They give food different tastes and textures. They are also being studied for their health benefits. Some proteins may have special properties that can help our bodies. Proteins from parents can change how living things work when eaten or added to food. They can be made in different ways, but using enzymes can make them more useful in making food. Microbial enzymes may be better than enzymes made from animals and plants because they can be made in a small space and a short time with fewer resources, which makes them cost less to produce. In this chapter, we will talk about enzymes made by fungi. These enzymes have special characteristics and can break down proteins in a different way. They can also make peptides with stronger properties. Terminus bio-active peptides were first used in 1950 to help babies' bones grow. Since then, a lot of research has been done on these peptides because they are important for science and business. Bio-active peptides are small pieces of protein that can be released from larger proteins and have a positive effect on the body's health. They can act like hormones and help improve human health and prevent some chronic illnesses. This molecule can have many different functions depending on things like its amino acid sequences, size, how much it likes water, and its charge[8], [9]. It can also have more than one function and work in different parts of the body.

When there are only a little bit of certain molecules in the body, they help fight off infections that can make us sick. These molecules are made when our bodies use oxygen to make energy. They are called reactive oxygen species (ROS) and reactive nitrogen species (RNS). When there are too many of these compounds in the body, they can cause problems by damaging molecules in the body. This can lead to diseases like diabetes, cancer, and other serious health problems. Oxidative reactions can make food taste and look worse and also make it less healthy to eat. For these reasons, it's important for people's health and for making food to have antioxidant compounds. This is why protein hydrolysates have become

important in these areas. Different enzymes, including ones from fungi, can help make proteins into smaller parts called hydrolysates, and these can act as antioxidants. Many authors have mentioned this, especially when using proteins from sources like cow's blood, sunflower seeds, and leftover meat products. High blood pressure is a disease where patients have long-term high blood pressure. It leads to 9.4 million deaths worldwide due to heart problems, but it can be controlled. High blood pressure is caused by a mix of factors like genetics and the environment. It raises the chance of problems with organs and health risks for pregnant people and their babies. The body controls blood pressure using different methods, like the Angiotensin-Converting Enzyme (ECA), which affects how blood vessels behave and can lead to higher blood pressure[10], [11]. These drugs are used to treat high blood pressure. They can have side effects like coughing, allergies, changes in taste, and skin problems. The drugs are called Captopril and Enalapril. So, many people are working hard to find new medications that can lower high blood pressure to replace the old ones. Protein hydrolysates might work instead of these drugs. They are short chains of amino acids with certain elements that can stop ECA.

Various authors have studied how food protein hydrolysates can fight off bacteria. They found that factors like the size of the protein pieces, the mix of amino acids, the electric charge, and how the protein is broken down can all affect its antimicrobial properties. More exposure and experiments have shown that some parts of living things called hydrophobic amino acids help make the charge of some substances stronger. This strengthens the power of positively charged particles. This process works by attaching to the charged outer layer of a cell and then bending and causing small holes in the cell, which makes it easier for substances to pass through. In some cases, minerals can go through cell walls and be important for how germs work. Minerals like calcium and iron are really important for our health. Not having enough of these minerals can cause health problems like cavities, anemia, or brittle bones. Certain proteins can help bind minerals in our bodies, making it easier for us to absorb them. These proteins are made from milk and can keep minerals from clumping together, which helps us digest them better.

In other words, whey protein peptides can bind to minerals and make them easier for the body to use, especially iron. Cholesterol is a compound found in animal tissues that can be made by the body or absorbed from food. Cholesterol is an important substance in our bodies that helps make things like Vitamin D3, bile salts, and hormones. It is also part of cell membranes. However, having a lot of cholesterol in your blood can increase the chance of getting or making heart diseases worse. Researchers have found that certain protein components can help lower high cholesterol. We don't know exactly how these molecules work, but it's believed that they interfere with the way the body processes cholesterol. They act like inhibitors of certain enzymes and can affect the way cholesterol is made and how it's transported in the body. This can reduce the body's ability to absorb cholesterol from the intestine. The types of amino acids in this activity are important. It has been found that parts with lots of hydrophobic amino acids can lower cholesterol. Amino acids like Ala, Tyr, Val, Leu, or Lys are included in this. A group of broken-down proteins from foods like soy, rice, salmon, and cow's milk show this kind of activity[12].

Enzymes are often used in many different industries because they have a lot of benefits compared to regular chemical catalysts. Some benefits are Reactions happen faster Enzymes make reactions happen millions to billions of times faster than normal, and much faster than other chemical reactions. Enzymes work best at temperatures below 100°C, normal air pressure, and a pH close to neutral. On the other hand, cells that are chemically triggered need to be at very hot temperatures, under a lot of pressure, and in very extreme pH

conditions. Enzymes work in a very specific way, which makes them different from chemical reactions. In chemical reactions with enzymes, less waste is produced. Reactions can be sped up or slowed down by changing the temperature, pH, or enzyme amount. Enzymes stop working once they've done their job. Proteases are in all living things and help break down extra proteins. They also help regulate how our bodies work. Proteases can break certain bonds in proteins to make smaller parts called peptides, but they can't completely break proteins into individual amino acids. Certain enzymes called proteases can break down a protein into its smaller parts called amino acids. Proteases are enzymes that are widely used in different industries like food, cleaning products, animal feed, pharmacy, and cosmetics. They make up 60% of all the enzymes used in these industries. Proteases are a diverse group of enzymes that vary in their characteristics like what they work on, where they work, how they work, and what conditions they work best in. Studying these traits is very important for proteases because understanding their characteristics will determine how they can be used in industry.

Proteases can come from animals, plants, and microbes. Animal proteases are enzymes like trypsin, chymotrypsin, pepsin, and renin. These proteases are made in small amounts because they rely on animals being available for sacrifice to get the enzymes from their organs or tissues. Papain and bromelain are two examples of proteases from plants. Papain comes from the shell of the *Carica papaya* fruit, and bromelain comes from the trunk and juice of the pineapple. But, using plants for proteases depends on whether they can grow in a certain place and how long it takes to get the proteases out. For these reasons, many important enzymes are made from only a few types of microorganisms. Also, bacteria and other microorganisms' enzymes are better because they can be easily found in large amounts, regularly, and with consistent quality. Microbial enzymes are usually stronger and last longer than animal and plant enzymes. They can also be made quicker because microbes grow fast and don't need a lot of food. In addition, tiny organisms can be changed genetically and environmentally to get the traits you want. This can help make the enzyme you want to work better and produce more.

There are two methods for making microbial proteases: Submerged Fermentation (SmF) and Solid-State Fermentation (SSF). Submerged Fermentation (SmF) is when microorganisms grow in a liquid with lots of nutrients and water. This system uses fermentation to make many different products from lots of different tiny organisms. It helps to control the process and uses specific things to grow the organisms, so the experiments can be repeated and give the same results. The SMF method is used in many Western countries to make enzymes, antibiotics, and other things. The things that affect making products in this process are the type of container, the starting material, what's in the liquid food, how much air is in it, how hot it is, and how acidic it is. Solid-State Fermentation (SSF) is when microorganisms, like fungi, grow on a wet solid material without any free water. We use filamentous fungi in this process because the conditions are similar to those in nature where these fungi grow. Fungi have some advantages over other microorganisms. For instance, long, thin fungi can make a lot of protein and release it into the liquid they grow in, which makes it easier to collect the protein. They can also add sugar to proteins, FDA says they are safe, and they make many different products like bug killers, fermented foods, and acids that are used in industry. But, microorganisms may not grow well in SSF due to factors like the type of solid material used, the food for the microorganisms, temperature, size of the support material, moisture, pH level, and the type of bioreactor.

In the study of making helpful peptides, there are certain ways to do it. Some involve mixing chemicals, while others involve using living organisms like bacteria to make the peptides.

Enzymatic hydrolysis is a useful way to make bioactive peptides from food. It is good for the environment because it doesn't use harmful chemicals. It is also safer for people to eat because it doesn't change the shape of the molecules. This makes it a good way to make bioactive peptides that are safe for people to consume. Enzymatic hydrolysis can help solve problems by using leftover material again, which makes it a cheaper option. However, this technology has a slow process that requires a lot of study to understand its important parts. The things we use in a process can affect the properties of peptides. This includes things like how much of the peptide gets broken down, how long it is, how heavy it is, and what it's made of. These things can also affect how well the peptides work in our bodies. Some of the things that can affect these properties are what kind of protein we start with, what kind of enzyme we use, and the conditions we use during the process. This includes things like how much of the protein we use, how acidic it is, how hot it is, how much enzyme we use compared to the protein, and how long we let the process go on for.

They have to be carefully controlled because they affect how well the enzymes work. Enzymes are called ampholytes because they come from proteins. They have properties that make them react differently to acidic and basic conditions, which can change how well they dissolve, how much pressure they exert, how thick they are, etc. This can also change how enzymes and other substances react, which can affect how fast they work. Enzyme reactions happen at gentle conditions and the speed of the reaction can be faster if the temperature goes up. However, when it's really hot, enzymes can change their shape and stop working properly, which makes them less effective. Breaking down proteins can cause the pH of the reaction to change, so we need to use buffers or add a solution to balance it out. This is because there's a better chance the enzyme will find the substance to break down when there's more of it around. However, some other scientists looked at a different way of breaking down cellulose using enzymes to see how a lot of the cellulose and high thickness in the liquid around it can affect how fast the reaction happens.

Submitting a specific thing in enzymatic processes for making peptides, Using the best designs can make it easier and cheaper to make a lot and plan how to do it. In a complicated process like enzymatic hydrolysis, making sure certain things are just right can help create a product with the qualities we want. One thing that can be used as a response in this approach is HD. Some authors think that when HD goes up, the antioxidant capacity also goes up. However, high HD can decrease the ability of antioxidants to work because there are more free amino acids, not peptides. Amino acids and peptides help fight off damage from harmful substances in the body. Peptides are especially good at this because of their structure, which makes them more stable and resistant to damage. This helps prevent harmful reactions in the body. Other methods are used to break down food proteins using enzymes, like trapping enzymes in membranes or particles, reusing enzymes, preventing the creation of byproducts from enzyme breakdown, and avoiding unwanted extra breakdown of proteins. Changing how the proteolytic reaction happens can help create a specific final product. However, the type of enzyme in a protein mixture determines the kind of protein fragments produced, their functions, and how they can be used.

It has been said that the size of the molecules in the peptides depends on the enzyme and the level of hydrolysis, but it doesn't really matter what conditions you use. So, we need a special enzyme to make the peptides we want. The enzyme chosen depends on where it comes from, how well it works at certain pH and temperature, how specific it is, how active it is, and how much it costs to make. Usually, companies that sell industrial enzymes show graphs that tell how well the enzymes work at different levels of acidity or heat during tests. Stability curves show how much activity is left after being exposed to a certain pH or temperature. As

mentioned, proteases are made for use in biotechnology. They can be used as a different way to do things in a biotechnological process. One of these processes is making peptides from different proteins. Researchers have looked at using commercial enzymes from academic labs to do this. For instance, Sumaya-Martínez and others In 2005, a study improved the way to break down leftover parts of golden carp using Flavourzyme, a commercial enzyme mix. This helped to get valuable protein fragments with good nutrition and usefulness.

CONCLUSION

The employment of enzymes in peptide manufacturing is acknowledged as an ecologically benign technology, with the potential for producing new goods that meet the public's desire for health standards and a large and profitable market. However, extensive research is required in areas such as enzymatic optimization, enzymes discovering things, and integration processes that provide the benefits of this type of molecule to all, as well as scaling up production and purification procedures for a financially accessible product. It is crucial to emphasize how enzyme operations are becoming an important step for these new types of compounds.

Scientific advancements in biotechnology and food are possible, allowing for improvements and innovation in health, nutrition, and technology.

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CHAPTER 9

FRUCTOSYLTRANSFERASES AND INVERTASES: EXPLORING THE ROLE OF ENZYME IN THE FOOD INDUSTRY

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ABSTRACT:

Enzymes are widely used in biotechnology and have a large and expanding global market. Big improvements in creating better enzymes, understanding how they work, and designing new bio-based products have made it easier to make these products and create efficient ways to produce them at a low cost. This review gives new information about making and using helpful enzymes in food, like fructosyltransferases and invertases. These enzymes are used a lot now to make fructo-oligosaccharides (FOS) and syrups. These products can be used in many different ways in the dairy and food and drink industries. We will also talk about finding and separating genes, making new combinations of genes, and setting up ways to make things using enzymes or cells with changed genes.

KEYWORDS:

Enzyme, Fructose, Ketose, Sugar, Sucrose.

INTRODUCTION

Enzymes contribute to several industries and have burgeoned into a substantial market with a multimillion-dollar value. Enzymes and products made from enzymes make up more than 30% of the market in the food industry. They are utilized in the production of functional foods and beverages, as well as in the making of beer, dairy items, dietary supplements, sweeteners, and syrups. It is predicted to be worth \$2.3 billion by 2020. On the other hand, using enzymes in industries uses less energy and takes less time for reactions. This has less impact on the environment. Fructosyltransferases-FTase and/or fructofuranosidases-Ffases obtained from microorganisms and plants make many important compounds used in the food industry. Fructans, like inulins, FOS, and levans, are also thought to be healthy foods. FOS is made from sucrose in nature and is a popular fructan in the market for healthy sugars. This is mostly because it can be used as both a prebiotic and a low-calorie sweetener, and it has many known health benefits for both people and animals[1], [2]. These compounds are natural sugars made up of 1-3 fructose molecules linked to a sucrose molecule. The US Food and Drug Administration has approved them as safe for consumption. FOS is a type of sugar that has similar properties to sucrose but doesn't cause tooth decay and is not digested in the small intestine, making it low in calories. When they get to the large intestine, Bifidobacteria and Lactobacilli, which are good bacteria, use them for energy because they are good for the bacteria.

Making long sugar molecules from basic ingredients like sugar is difficult. So, scientists are interested in making short-chain FOS in factories because these compounds are important for medicine. Levan is a type of sugar that is found outside of cells. It is a natural substance made of fructose molecules and is eaten as a healthy food in Japan and Korea. Fructan is a type of sugar that comes from plants and some types of microbes. It is made up of fructose and is also produced through a process called transfructosylation. This fructose polymer is very good for use in food and other industries. Alternatively, sucrose can be broken down using acid or enzymes like invertase or exoinulinase. When sucrose is broken down, it makes an equal mixture of fructose and glucose, which is called invert sugar in stores. The invert sugar

syrup is sweeter than regular sugar and can be mixed into food and medicine easily. It doesn't have the same problem of turning into crystals when it's in a strong solution[3], [4]. Enzymes are used to make syrup that is good for food. It doesn't turn brown and doesn't have bad things in it like other methods do. Due to a high need for FOS, levan, and invert syrups in the food industry, there is a chance to find new types of bacteria that can make these ingredients and to create better ways to make them that are not as costly. In this article, we talk about fructosyltransferases and invertases and how they can be good for FOS, levan, and invert sugar. These things can be important in the food industry. Keep in mind that using better and cheaper ways to make food can be a big advantage for the food industry.

These days, there are many different sweeteners to choose from in the food industry. Some of them are levan, inulin, and FOS. There are two kinds of fructose-rich molecules called fructans. They are made by different types of fructosyltransferases (FTFs). Fructans are sugar molecules with a lot of fructose in them that can be in straight or twisted lines. The places where two fructose sugar molecules connect to each other are at the branching points, where the carbon atoms are connected to the oxygen atoms in the same fructose molecule. The branches can be different in number and size, and it mostly depends on where the fructan comes from. To classify fructans, we need to look at three things: how they are connected, how big they are, and where they come from. Based on this, the most common names for these molecules in the literature are kesto-n-osas, inulin, levan, fleÅ-n, and graminan. In the literature, these molecules are often called kesto-n-osas, inulin, levan, fleÅ-n, and graminan. Kesto-n-osas are made of fructose and sucrose. Kestotriose, kestotetraose, and kestopentaose are the most common types of oligofructans or fructo-oligosaccharides found in plants or microorganisms. In the group of kestotrioses, there are three types: 1-kestotriose, 6-kestotriose, and 6G-kestotriose or neokestose[5], [6]. The most common types of kestotetraoses found in nature are 1-kestose, 1-kestotetraose or nistosa, and 1,6-kestotetraose or bifurcose.

Inulins are made up of fructosyl residues that are mostly connected by a certain type of bond in long chains that are usually no more than 70 units long. Fructans are in plants in the Asterales and Liliales groups. Some fungi and bacteria make inulins. The size of these inulins range from 6 to 9×10^7 Da. Fructo-oligosaccharides are short chains of fructose with up to five parts. Some of the most popular trisaccharides found in natural polyfructans are 1-kestose and nystose.

There are also other trisaccharides like 6-kestose and neokestoses. Levans (G1-2F6-2Fn) have fructose attached in a way that forms bonds shown in the picture. 261B is made up mainly of bacteria and is high mass polymers. Fructans are used to make popular foods like ice cream, soft drinks, juices, and yogurt because of their special qualities. On average, people in America are estimated to eat 2.6g of inulin or 2.5g of oligofructosaccharides each day. In Europe, people eat 4-12g of these each day. Based on this information, people are very interested in finding enzymes and methods to make these substances in large amounts for industry. In nature, fructans are made from a reaction involving sucrose and a fructosyltransferase enzyme. Sucrose acts as both the giver and receiver of the fructose residue. Sucrose has been found in most types of fructans, whether they come from plants or microbes. However, the fructan chain can be different depending on the fructosyltransferase that is used. We will explain this in more detail. The names of fructosyltransferases (FTFs) in the literature come from the specific things they work with, like the molecules they use and the way they work. It breaks apart sugar and moves part of it to another sugar molecule, creating a new kind of sugar called 1-kestose. This enzyme can add more fructose to 1-kestose to make nystose or fructosylnysteine.

DISCUSSION

SST is the first enzyme that helps plants make fructan, and it's the only one in fungi. It moves a sugar called fructose from one place to another in order to make a sugar chain longer. It makes 6-kestose from sugar and turns it into levan. It breaks apart the sugar bond in sucrose and moves the fructose part to a different place, creating a new sugar called bifurcose. The 6-SFT is found in only a few types of grass, where it joins together with 1-FTF to make certain types of fructans called flein and graminan. It does this by making bonds. It breaks the connection between the two fructose parts in 1-kestose and moves the end fructose to a different part of the sugar to make neokestose. It has only been found in plants like onion and garlic from the Liliaceae family. It breaks the sugar bond in sucrose and moves part of it to another sucrose to make 1-kestose and/or 6-kestose. In transfructans, the reactions create long chains by connecting smaller molecules together. It breaks the sugar bond in sucrose and moves the fructose part to another sucrose and then to 1-kestose. This causes the inulins to become bigger and bigger. This substance has been found in certain types of bacteria like *Streptococcus mutans*, *Leuconostoc citreum*, and *Lactobacillus reuteri*, and it's called fructosyltransferases. All of these enzymes can break down β -fructosyl bonds and are part of a group called β -fructofuranosidases. This group also includes invertases, inulinases, and levanases. Naumov and Doroshenko suggested putting all β -fructosidases together in one group, but most fructosyltransferases from bacteria actually belong to a different group called Family 68 of glycosylhydrolases [7], [8]. However, the enzymes of fungi and plants, along with other similar enzymes, belong to a group called family 32 of glycosylhydrolases. They come together as a family because they have similar primary structures.

In short, there are certain types of enzymes found in bacteria that help break down sugars. They belong to a certain group and are similar to other enzymes found in eukaryotic cells. You can find more information about these enzymes on the CAZy database website. Surprisingly, all the actions work like a ping-pong game, where a covalent fructosyl intermediate is formed and then broken down by enzymes while keeping the same fructosyl residue configuration. Plants make fructans in a part of their cells called the vacuole. This process is helped by two fructosyltransferases with different jobs. Some plants in the Asteraceae family make inulin from sugar with the help of an enzyme. The inulin can have different lengths, depending on the plant. The enzyme turns the sugar into a type of inulin called 1-kestose.

These are used to make a type of carbohydrate called inulin. Inulin is made by a reaction where the molecule 1-kestose helps to add more fructose to the chain. 1-SST and 1-FFT of *H. tuberosus* were removed from dormant tubers and studied in detail. Two proteins, SST and FFT, were also extracted from the roots of the intybus plant have similar physical and chemical properties as the enzymes of *H. tuberosus*. Van den Ende and Van Laere discovered *tuberosus* in 1996. Onions and tulips, which are part of the lily family, make neoinulins. In making this special kind of fructans, three enzymes are involved: 1-SST, 1-FFT, and a new enzyme called 6G-FFT. After one enzyme makes a sugar called 1-kestose from another sugar called sucrose, another enzyme transfers a part of 1-kestose to a different part of sucrose to make a new sugar called neokestose. Then, another enzyme links several neokestose molecules together to make a bigger sugar called neoinulins. Genes that make onions taste good were copied and put into different plants. Tests with modified enzymes have proven that 6G-FFT cannot break down sucrose. Only when it is used together with 1-SST, it can create neokestose.

Barley, wheat, and other plants in the grass family store fructans called flein and graminan, as well as a small amount of inulin. Inulin is made using a model called 1-SST/1-FFT in the

Asteraceae family. A new enzyme called 6-SFT was found in barley. It makes parts of the graminin molecule called flea and chains. The isolated 6-SFT has two different forms made up of two smaller parts (49 and 23kDa) that come from one gene. When there is sugar present, both forms of the enzyme work to break it down and only produce a little bit of 6-kestose. When sucrose and 1-kestose were together, 1-kestose took in the fructose residue and formed a new kind of sugar. Unlike plants, fungi make fructan using only sucrose. This process is done by an enzyme called sucrose 1-fructosyltransferase (1-SST), which creates 1-kestose, nystose, and fructosylnystole in different amounts depending on the type of enzyme. *Aspergillus foetidus* 1-SST was studied and it was found that the 1-sst gene is only present in one copy in the A. The foetidus genome contains instructions for making a protein that is 537 building blocks long. The protein weighs 59.1 kilodaltons and has a signal at the beginning that is 19 building blocks long. When the protein is made, the signal gets cut off as part of the process of releasing it from the cell. The first result from this study shows that certain regions are similar in different β -fructofuranosidases [9], [10]. They are especially similar to levansases even though they don't break down levan.

Bacteria make fructan using one enzyme that can change sucrose into inulin or FOS and levan. If the end product is inulin, then the enzyme in charge is inulosucrase. If the products are high molecular weight fructans like levan, then the enzyme in charge is called levansucrase. Some enzymes called levansucrases can make different types of sugars. Some make inulin-type sugars with a certain shape, while others make a different type called levan. All levansucrases speed up the movement of fructosyl residues from sucrose to different types of acceptor molecules like water, sucrose, β -(2,6)-linked oligofructans (to make levan longer), and the released monosaccharides and fructose. Levansucrases are found in many types of bacteria, both gram-positive and gram-negative. Levansucrases have been found in certain types of bacteria. In all cases, a protein is made with a signal and then released by the bacteria. In gram-negative bacteria, only a certain kind of enzyme called *Gluconacetobacter diazotrophicus* levansucrase has a specific part that helps it be sent out of the cell. Other similar enzymes don't have this special part. Sometimes, we have seen that there are parts of the enzyme in the cytoplasm and the periplasm. Öner and his coworkers explained how certain types of bacteria make levan from sugar. Just like other substances formed by tiny organisms, the production of this one is greatly influenced by how it is made, like the temperature, acidity, and the environment it is in. Usually, making plastic involves the growth of tiny living things.

Ewas not feeling well, she still went to work. *Coli* is good for making levansucrase and other fructosyltransferase genes. *Saccharomyces cerevisiae* and *Pichia pastoris* are both safe for biotechnology use. Simple and useful research can be done using these two yeasts, because they can both release helpful proteins into the culture medium. This makes them good for making enzymes in industry.

However, not many studies have been done on using yeasts to make microbial levan. *Bacillus subtilis* levansucrase was used as an example to study how proteins are released in the yeast *Saccharomyces cerevisiae*. The first successful experiment on finding yeast that can produce levan. The gene *lsdA* in *diazotrophicus* makes levansucrase. It can be turned on using inducible or constitutive promoters, but each one turns it on to a different extent. In both situations, the enzyme was released. But when the special switch was used, some of the new protein stayed inside the yeast. The *diazotrophicus* levansucrase has a specific change at the beginning and a special bond called disulfide bridge. The *lsdA* gene in *Pichia pastoris* made an enzyme that was active but had sugar added to it. This enzyme behaved like the one without sugar when different reactions were done under various conditions.

Plant fructosyltransferases are also being used instead of fungal enzymes to make FOS for industry. A new patent was granted for making 1-kestose using a special plant enzyme made in P. The study was done by Pastoris. This method can turn more than 55% of fructooligosaccharides (FOS) into 1-kestose, with more than 90% of the end product being 1-kestose. Another option for FOS in industries is using solid-state fermentation with different farming by-products. The European Commission's group of scientists looked at FOS in baby formula and found that it was okay to use. Several tests have been done to show how FOS affects baby food. The number of good bacteria in the gut increased a lot in the group that took FOS supplements compared to those who didn't. The number of good bacteria in the group that took FOS reached a level similar to babies who are breastfed. A study in the United States showed that adding FOS to baby formula is safe and does not affect how babies grow. We tested inulin, FOS, and honey with *Lactobacillus acidophilus* and *Bifidobacterium lactis* in five types of petit-suisse cheese. We also tested them with human stool samples in a lab. The bacteria grew quickly and made a lot of lactic acid. This helped other good bacteria to grow faster too[11], [12]. Other studies have also used FOS in cheese. The researchers looked at how cow's milk changes when it's fermented with and without inulin at different temperatures. They found that when inulin is added, the solid content of the milk increases.

Beta-fructosidases have different names in the literature depending on what they work on, like sucrose, invertase, fructanase, inulinase, or levanase. Invertases are a type of enzyme found in a group of enzymes called GH32. This group has more than 370 members from plants, fungi, and bacteria. It has enzymes like invertase, inulinase, levanase, exo-inulinase, sucrose: sucrose 1-fructosyltransferase, and fructan: fructan 1-fructosyltransferase. Glycosyl hydrolases or glucosidases are a large group of enzymes with different shapes and abilities to break down different types of sugars. They both have something in common two important places with acid that help break down glycosidic bonds. In yeast invertase, two specific residues have been found: an aspartate near the beginning that acts as a nucleophile, and a glutamate that acts as a general acid/base. So far, all members of the same family have the same molecular mechanism. Looking at the order of things and comparing their structures has shown that even though they come from different groups, they have a lot in common.

They also share the same tools for making chemical reactions happen, and the results of those reactions also look similar. This suggests that a long time ago, they all came from the same original thing, but over time they changed to work with different types of materials. More than 50 types of very hot-loving microorganisms have been found, but only a small number of them are bacteria. Some scientists think that these organisms that can survive high temperatures still have some of the same traits as their ancestors in their molecules and the way they process food. *Marítima* is the top choice for heat-resistant invertase used in making industrial invert sugar syrup. *Tw* was a very complex and difficult text. *Maritima* is a type of bacterium that cannot make spores. It has a rod-like shape, and it needs to live without oxygen. It also needs to eat other organisms to survive. It was first found in hot marine sediment and grows best at around 80 degrees Celsius. In terms of evolutionary history, *Thermotogamaritima* appears to be one of the oldest groups in Eubacteria. Enzymes called fructosyltransferases and invertases play important roles in a variety of metabolic processes, including carbohydrate metabolism. These enzymes play an important role in the conversion of sucrose and fructose, which affects the flavor, texture, and preservation of a variety of food items. In this investigation, we look at the many functions of fructosyltransferases and invertases in the food business, emphasizing their uses, processes, and influence on product development. Enzymes involved in sucrose metabolism include fructosyltransferases and invertases, which break down the disaccharide comprised of glucose and fructose. Fructosyltransferases catalyze the transfer of fructose residues between molecules, resulting

in the formation of fructo-oligosaccharides or fructans. Invertases, on the other hand, hydrolyze sucrose to produce the monosaccharides glucose and fructose. These enzymatic reactions are crucial to many biological processes and have several uses in the food business.

The food business uses fructosyltransferases and invertases for many reasons. Fructosyltransferases contribute to the formation of fructo-oligosaccharides, which act as prebiotics and improve the nutritional profile of foods. Invertases, which can hydrolyze sucrose, are used to make sweeteners like invert sugar, high-fructose corn syrup (HFCS), and other formulations that improve sweetness and texture in a variety of food and beverage applications. Enzymes such as fructosyltransferases and invertases have a role in enhancing and browning the taste of food items. These enzymes help to break down sucrose into glucose and fructose, resulting in the production of pleasant taste qualities. Furthermore, the Maillard reaction, a complicated sequence of chemical interactions involving amino acids and reducing sugars catalyzed by invertases, is essential for food browning, providing distinct smells and fragrances to baked goods, roasted coffee, and other processed items. Fructosyltransferases and invertases are widely used in the confectionery and baking industries. The hydrolysis of sucrose produces invert sugar, which is a crucial element in the formulation of candies, chocolates, and baked goods. These enzymes help enhance product texture, moisture retention, and shelf life. The regulated application of these enzymes enables the customisation of sweetness profiles, resulting in the development of a diverse range of confectionary goods with varied textures and sensory qualities. Foods containing fructosyltransferases and invertases may have health and nutritional effects, beyond their technical uses. Fructo-oligosaccharides generated by fructosyltransferases have prebiotic characteristics that promote the development of healthy gut flora. Conversely, the use of invertases in sweetener synthesis raises nutritional concerns, particularly about the ubiquity of high-fructose sweeteners and their possible influence on metabolic health. Advances in enzyme engineering and biotechnology have led to the creation of customized fructosyltransferases and invertases with improved characteristics. These modified enzymes increase stability, substrate specificity, and efficiency, paving the way for novel food processing innovations. The use of biotechnological methods to optimize enzymatic activity shows promise for solving particular food industry concerns, such as the quest for healthier and more sustainable formulations.

CONCLUSION

Fructosyltransferases and invertases are enzymes that have a wide-ranging impact on the food business, altering the flavor, texture, and nutritional makeup of a variety of goods. This investigation introduces the enzymatic foundation of sucrose metabolism, focusing on the enzymes' uses in food processing, taste improvement, and health implications. The following sections will go into further detail on the mechanics, biotechnological developments, and particular uses of fructosyltransferases and invertases in the ever-changing food sector landscape. In this chapter, we talked about the latest things about fructosyltransferases and invertase, based on how they are used in real life by the authors. Consumers want their food to taste good and be good for them, because they care about their health. This shows why it's important to keep studying and creating new enzymes that make food better, and to keep looking for new kinds of microorganisms or plants that make stronger enzymes.

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CHAPTER 10

ENZYMATIC TRANSFORMATION: IMPROVING THE NUTRITIONAL AND NUTRACEUTICAL PROFILES OF FOOD PROTEINS

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ABSTRACT:

A nutraceutical is a mix of "nutrition" and "pharmaceutical" products. Nutraceuticals are foods or parts of food that help keep our bodies healthy and working properly. The nutraceutical market is growing worldwide because there are more people and more people are interested in staying healthy. Food items with medicinal properties are known as nutraceuticals. They include things like dietary fiber, prebiotics, probiotics, healthy fats, antioxidants, and herbal/natural foods. Natural supplements have the potential to combat major health issues such as obesity, heart disease, cancer, weak bones, joint pain, diabetes, and high cholesterol. The concept of 'nutraceutical' has ushered in a new phase of healthcare and wellbeing. This means that the food industry is now focused on doing research.

KEYWORDS:

Enzyme, Food, Nutrition, Peptides, Protein.

INTRODUCTION

Enzymatic modification of food proteins is a dynamic and promising approach to improving the nutritional and nutraceutical profiles of a variety of food items. Proteins are key macronutrients that contribute to the structural and functional features of food, but their natural qualities may be optimised and enhanced by enzymatic treatments. This chapter delves into the many methodologies and uses of enzymatic transformations, giving light on how these changes might unleash unique nutritional advantages and nutraceutical potential in the field of food science. Proteins are critical components of the human diet, since they help to create and repair tissues, promote immunological function, and provide necessary amino acids. While proteins are plentiful in many foods, their nutritional value may be improved by judicious enzymatic changes. Enzymes are biological catalysts that regulate biochemical processes. The use of enzymes in food processing has emerged as a sustainable and effective way to change proteins. Enzymatic techniques provide precision and specificity, allowing for precise alterations to protein structures while maintaining their nutritional integrity [1], [2].

Enzymatic modification may improve protein digestibility, making them more accessible for intestinal absorption. Proteolysis is a technique that may break down complex protein structures into smaller peptides and amino acids, thereby lowering allergenicity and enhancing total nutrition intake. Enzymatic transformation allows the creation of bioactive peptides with particular amino acid sequences. These peptides may have physiological advantages such as antioxidant, antihypertensive, and antibacterial activity. Enzymatic hydrolysis of amino acids helps to customize protein compositions to satisfy individual nutritional needs. Certain dietary proteins include antinutritional components, such as protease inhibitors or lectins, which may impair nutrient absorption. Enzymatic treatments may reduce the presence of these components, improving the overall nutritional value of proteins and the meals into which they are integrated. Enzymatic modification plays an important part in texture modification, influencing the mouthfeel and palatability of food. Proteases may

degrade or change protein structures, affecting the texture of meat substitutes, dairy replacements, and other protein-rich meals[3], [4].

Enzymatic transformations may cause functional changes in proteins, influencing properties including emulsification, foaming, and gelling. This adaptability enables the development of novel food compositions with enhanced sensory qualities and longer shelf life. Beyond basic nutrition, enzymes help to generate nutraceuticals by enriching food with bioactive substances. Enzymatic modification may help to incorporate vitamins, minerals, and other health-promoting compounds into protein matrices, resulting in functional diets with additional health advantages. While enzymatic modification has enormous promise, issues such as enzyme selectivity, reaction conditions, and allergenicity must be carefully evaluated. The regulatory environment around the use of enzymes in food processing must also be addressed to assure safety and conformity with industry standards. The subject of enzymatic transformation of dietary proteins is constantly advancing. Future advances may include the creation of new enzymes, precise engineering methods, and sophisticated technology to improve the nutritional and nutraceutical benefits of protein modifications. The development of synergistic enzyme combinations and sustainable processing technologies is expected to determine the field's future[5], [6].

Enzymatic modification of food proteins requires the cooperation of food scientists, biochemists, enzymologists, and nutritionists. Interdisciplinary research is required to fully understand the potential of enzymatic transformations and put these findings into practical applications in the food sector. This chapter provides an introduction to the interesting field of enzymatic modification of dietary proteins. As we go through the sections, we will look at particular enzymatic techniques, case studies, and applications that demonstrate the many ways enzymatic alterations may be used to enhance the nutritional and nutraceutical profiles of food items. Understanding the enzymatic toolbox at our disposal allows us to explore new frontiers in food science, developing healthier and more functional meals for the benefit of global consumers. Foods with lots of protein can be turned into protein hydrolysate and bioactive peptides. These can be used in healthy foods that might be good for your health. Bioactive peptides, which are made up of amino acids, can be created by breaking down proteins using enzymes or chemicals. These peptides have a good impact on health. Bioactive peptides are small pieces of protein found in food that can act like hormones in our bodies. Many studies have been done on the healthy compounds in food like milk, seeds, and fish. High blood pressure is one of the main things that can make you more likely to get heart disease. The ACE enzyme is important because it helps make a substance that causes blood vessels to tighten and it also turns off another substance that relaxes blood vessels. Certain peptides found in food can help lower blood pressure by inhibiting ACE.

These small proteins could be used instead of man-made drugs to treat high blood pressure. The relationship between the structure and function of these inhibitory peptides has been thoroughly researched. These peptides can stop ACE and also help release substances that widen blood vessels, like nitric oxide. They might also attach to opioid receptors. The small pieces of amaranth seed proteins can stop an enzyme called dipeptidyl peptidase IV (DPP-IV). Velarde-Salcedo and his team found an enzyme that can stop the action of hormones that help the body release insulin. In 2013 The crushed peanut protein has been proven to reduce ACE levels and protect against damage from oxidants. The way the protein is crushed affects how well it works. The researchers saw that the Australian canola protein, when treated with alcalase and pepsin, helped to lower blood pressure in rats with high blood pressure. These hydrolysates can be used to make healthy foods and supplements that can help lower high blood pressure. Peptides that stop ACE are made by lots of tiny organisms. Spoiled milk

changed by a certain bacterium has special molecules that can help lower blood pressure when people drink it. This has been shown to work in people with slightly high blood pressure. *Lactobacillus casei* spp. ferment soy protein. *Pseudoplantarum* made peptides that can help lower blood pressure by blocking ACE. The peptides contain glutamine and threonine, which are important for blocking ACE.

Many studies have shown that proteins can stop oils in food from going bad. Milk and soy when broken down have been found to have antioxidant effects. Studies have found that having more antioxidants in your diet can help lower your risk of getting sick. Bioactive peptides help fight against harmful substances in the body by removing radicals, stopping the breakdown of fats, and binding to metal ions. The way the peptide is structured and the order of its amino acids might help it to fight against damage from oxygen. Peptides with a lot of histidine and amino acids that don't like water may be good at preventing damage from oxidation. These peptides can help protect cells from damage caused by free radicals and also prevent fat from going bad in food. Adding soy, whey, casein, and egg yolk extracts to foods like tuna, pork, and beef helps stop the fat from going bad. This has been proven by several studies. When the palm kernel mixture was tested outside the body, it showed strong abilities to lower blood pressure and to fight cancer cells in the colon and liver. Soy protein hydrolysates lower total cholesterol levels better than regular soy protein. Two parts of a soybean protein called β -conglycinin can stop an enzyme called 3-hydroxy-3-methylglutaryl CoA reductase (HMGCoAR) from working. They work in a similar way to statin drugs. The peptides in soybean protein can help lower cholesterol by increasing the activity of a gene that helps clear cholesterol from the blood. This process reduces the amount of cholesterol in the body and lowers the overall cholesterol level. Whey proteins found in milk can help lower cholesterol levels in animals. Adding whey protein to the rats' diet reduced their cholesterol levels. A protein called β -lactoglobulin tryptic hydrolysate also had the same effect.

DISCUSSION

Microorganisms like bacteria, yeast, and fungi have been making enzymes for a long time. People have been using these enzymes to make food. The use of food enzymes goes back to the 1880s when people used bacteria to make soy-based foods. Rennet is a natural mixture of enzymes from the stomach of young cows. It has been used for a long time in making cheese. Rennet is a substance that helps turn milk into cheese. It makes the milk into solid cheese and liquid whey. For hundreds of years, yeast enzymes have been used to make wine from grape juice by fermenting it, and papain from raw papaya has been used to make meat tender and prevent protein formation in brewing. The use of enzymes in making food has become very popular in the food industry. It has changed the way we make and process different foods. Right now, enzymes are the most well-known products in the biotechnology industry. The food enzymes market is likely to increase by more than 7% each year until 2020. These enzymes are used in industries like food and feed, such as dairy, beverages, brewing, and dietary supplements. In addition to the food industry, enzymes are also used in other industries like leather, paper, soap, fabric, and skincare. Food enzymes help to strengthen the nutritional value of many foods[7], [8].

These enzymes help our bodies digest and use the nutrients in food by breaking down proteins, carbohydrates, and fats into smaller pieces. Proteins are a very important part of our food. They are essential for our health and help our bodies work properly. Enzymes are being used to change proteins by breaking them down with water. Using enzymes to break down proteins is a useful way to make them better for you and more useful in cooking. Protein breakdown can be done using chemicals and enzymes. Many enzymes used to break down protein come from animals, plants, and microbes. Proteolytic enzymes break down proteins

into smaller parts at the best temperature and level of acidity. They usually focus on specific spots in the protein, resulting in a mix of amino acids and smaller peptides. Enzymes from animals work in a particular place in the body, while enzymes from plants work in many different places. For instance, the enzyme pepsin breaks apart the phenylalanine or leucine bond. Papain can break apart bonds at different amino acids like phenylalanine, arginine, and lysine. Pancreatin breaks apart molecules like tryptophan, arginine, tyrosine, leucine, phenylalanine, and lysine. When proteins are mixed with tiny organisms, they break down through fermentation. This process causes special enzymes to be released. Proteases from germs have lots of different enzyme powers[5], [9]. Enzymes called proteases are made in big amounts from bacteria, algae, fungi, and yeast.

They only need easy purification steps. These proteases can be used in industries to make peptone. Using enzymes to change the protein makes it work better. Proteins in food not only give us energy, but also help the food to be made and work better. The way food proteins work is determined by their chemical properties and this affects how they behave in food. The main things that food proteins can do are mix well in liquid, mix oil and water together, and make foam. Enzymatic hydrolysis usually happens in gentle conditions, but now people are also interested in using high pressure for hydrolysis. The way the protein works depends on how much water it has and how it turns into a gel, and also on how it interacts with other substances and clumps together. Breaking down proteins with enzymes makes them work better by changing their structure, making them smaller, and increasing their ability to interact with other molecules. We need to break down proteins just the right amount to make them work better. The biggest issue with breaking down proteins is that it can make them taste bad, thicken, and it costs a lot to use enzymes for this process. It's important to think about the kind of enzyme, how the hydrolysis happens, and how to control DH.

The enzymes changed the soy flour that had been heated, and made it work better. In a study, breaking down gluten a little bit has been found to make bubbles in food better, but it also makes the bubbles less sturdy. The researchers saw the same thing happen when they looked at the breakdown of certain parts of gliadins. Enzyme-altered proteins are better because they dissolve easily, are easier to digest, and can enhance flavors. They can also be used as a substitute for regular protein. Protein hydrolysates are easier to digest and are an important part of foods for elderly people, children, and athletes. Different kinds of enzymes are used to get bioactive proteins for making food. Different types of food are broken down slowly by enzymes. The study showed that using enzymes helps to get more cumin oil from the seeds. It doesn't change the flavor or quality of the oil. The authors found that using enzymes to extract protein from a substance works better and helps to get more protein with less solvent than other methods that don't use enzymes. Rice bran was changed by enzymes, which stopped the lipase from working[10], [11]. Enzymatic modification makes it easier for our bodies to absorb important vitamins and nutrients in rice bran.

People are becoming more aware of what they eat and how it affects their health. They want food with natural ingredients like bioactive peptides to stay healthy, so the demand for these kinds of products is going up. To sell health-boosting bioactive peptides as food ingredients, we need to solve some problems. Using enzymes to break down proteins has some problems. Firstly, the enzymes are expensive. Secondly, it can create bitter peptides. Lastly, the hydrolysate absorbs moisture easily. We can make the hydrolysate less bitter by controlling DH, separating bitter peptides, using exo-peptidases, masking or absorbing the bitter taste, and changing how the taste is perceived. Proteins are important for people to eat. They can come from animals and plants. From a nutrition perspective, protein needs to be broken down by enzymes in the stomach into amino acids. Certain proteins in food can cause allergies

because they are not easily broken down in the stomach and can trigger an immune response. About 1-2 out of every 100 adults and 6-8 out of every 100 children have food allergies. Most of these allergies are caused by proteins. There is a strict process to check if genetically modified crops can cause allergies. Proteins in food that cause allergies are called food allergens. They can make the body's immune system react and cause allergic symptoms.

To check if a protein is strong enough to survive in the stomach and if it has enough nutrients, scientists made a test called the pepsin digestibility method. They also used this test to see if new proteins from genetically modified foods could cause allergies. The current way of assessing things focuses on looking at a lot of evidence. This includes where the protein comes from, how similar it is to known allergens, how easy it is to digest, and testing it in real-life situations. This is all outlined by a group called the Ad Hoc International Task Force on Foods Derived from Biotechnology. In terms of safety, easy to digest proteins are less likely to cause allergies. However, small pieces of allergens can still stick to IgE. The scientists used proteolytic enzymes to find out which parts of proteins can stick to IgE. They also used a test called the in vitro pepsin digestibility assay to figure out which proteins in food can cause allergies. This includes testing if the proteins can be digested by pepsin. To do this, scientists made a clear and repeatable way to test how well proteins are digested in a lab. They used the same proteins and tested them in different labs to make sure the results were consistent.

The food was broken down in the stomach with the help of a substance called pepsin. The pepsin was mixed with the food in a certain proportion, and the pH level in the stomach was between 1.0. This information comes from a study done by Thomas and others in 2004. A small amount of hydrolysate was taken at different times and checked using stained SDS-PAGE gels. The experiment showed that the results were more similar at pH 1.2 (91%) than at pH 2 (77%). This created a way to test how easily proteins can be digested in a lab, which made it easier for different labs to get the same results when testing the same proteins. Research on how new proteins in food can cause allergies is done by testing how they break down in the stomach. This helps scientists understand how digestion is related to food allergies. Breaking down food protein using enzymes is important for our health and safety. Food proteins are broken down in the stomach and intestines during digestion. Breaking down these proteins in the food causes them to lose their ability to trigger allergies. So, if they are hard to digest, it may mean they could cause an allergic reaction. We still don't know exactly where in the food protein the body's immune system reacts to. It is thought that most food allergens pass through the stomach without being broken down and are absorbed in the intestine[12]. Many studies look at how new proteins in food might cause allergies. They test the proteins in a lab to see how they break down in the stomach. But they also need to look at how the proteins affect the whole food, not just the protein alone.

Consuming a new type of protein in a food ingredient may make people more likely to have an allergic reaction. Due to more people being mindful of allergies, a lot of new foods and food combinations are being tested to see if they can cause allergies. To check if new proteins in food can cause allergies, we test how well they break down in the stomach using the pepsinolysis method. This helps us understand the risk of allergies for people who eat the food. New methods like using animals or a system that simulates a natural process may be used. With the progress in science and technology, new methods for predicting protein allergies have not been tested and confirmed yet. Ex vivo means something happening outside of a living thing, like in a lab, under normal conditions. Ex vivo digestion (EVD) means breaking down proteins outside the stomach and intestines using human digestive enzymes. A blend of enzymes in the stomach and intestine break down food, aided by other substances

that aid in digestion. This mixture can affect how proteins are broken down in the body. We use juices from people's stomachs to simulate how the body breaks down food. To understand how our bodies break down protein in food, we can use a model called EVD to copy how we digest food.

The digestion of food proteins is hard because the enzymes in our stomach can vary, along with the acid levels, the amount of certain chemicals, and how much food is available to digest. It also takes time for the food to be digested in the stomach and intestines. Using human juices is better because they can simulate how digestion works in a living body. So, the method is called the EVD model. The way our stomach and intestines break down protein from our food depends on the protein's physical and chemical properties. The digestion of proteins in the human body involves a mix of mechanical, chemical, and physiological processes in the stomach and intestines. Breaking down the proteins in the food we eat happens in the stomach and intestines with special enzymes. Protein digestion starts in the stomach, where pepsin breaks the protein into smaller pieces. Pepsin works best in very acidic stomach conditions with a pH of 2.0, but it stops working in the small intestine where the pH is 6.5. The pH level in the duodenum slowly goes up to between 5 and 7.5 because the body releases bicarbonate and pancreatic juices. Higher pH stops the stomach enzymes from working and helps the duodenal enzymes to work better. When the partly digested food reaches the first part of the small intestine, the enzymes there keep breaking it down.

The last step of breaking down proteins happens in the intestines, where enzymes on the surface of cells break peptides into amino acids. The jejunum and ileum take in the nutrients which are then broken down more inside the body before going into the blood. When making new ways to study how food gets digested, we need to think about the kind of food, how it breaks down, and what enzymes work best. This includes the whole process from when food goes in your mouth to when it reaches the large intestine. A method to mimic digestion in a test tube can help compare different foods, because it might not imitate real digestion entirely. We need a simple and consistent way to break down food proteins in a lab so we can do the experiment again and again. This will help us understand how the proteins work. The COST Action INFOGEST protocol created a standard way to simulate how food is digested in the human body in the lab. Moreover, the passage explains various factors including time, heat, and the use of enzymes. The method has been tested in three studies with different labs. It helped to understand how proteins get digested. These studies also found that using the consensus method has made in vitro digestion (IVD) studies more consistent and easier to compare.

Proteins are tested using chemicals that mimic the conditions in the stomach and intestines to see if they can make helpful peptides. The protein breakdown process was tested in a lab using enzymes from pigs or cows. The enzymes taken from animals may work differently and have different staying power. Because more people want to know how our bodies digest food proteins, scientists have been studying how human digestive juices and enzymes from the market break down proteins. Proteins broken down by enzymes from different animals and humans seem to make different pieces with different patterns and sizes. Many experts believe that EVD and IVD have different outcomes because of the way proteins are digested in the body. The enzymes used in commercials seem to be better at breaking down proteins like lactoferrin and caprine whey proteins when compared to the enzymes in our bodies. This could cause different results when comparing how humans and animals digest proteins in their bodies.

Comparison of peptide profiles in salmon and goat whey proteins, when broken down by human stomach juices or commercial enzymes, showed that the digestion process was

different because of the sources of the enzymes. The small pieces of protein in salmon had different amino acid sequences because of two different enzymes. Because human stomach enzymes are very complicated, the way they break down protein chains is not the same as nonhuman enzymes. When we digest food, our bodies can make special protein parts that have important effects on how our bodies work. These can change how our bodies work. When thinking about how food affects our body, we need to consider how the stomach breaks down protein. The EVD profile is more similar to experiments done on live organisms than experiments done in a lab. Not many studies have been done on how EVD makes peptides that are useful for living things. Goat whey protein was better at stopping the growth of bacteria using human enzymes in the stomach than with animal enzymes. To check the quality of proteins from carp, salmon, and oil palm kernel, we tested them for different functions in a laboratory. Not many studies have been done on how our bodies digest food proteins using human digestive enzymes. Research on how we digest food shows that it's better to use enzymes from humans when trying to copy how we digest food, instead of using enzymes from other animals.

CONCLUSION

The enzymatic modification of food proteins appears as a transformational and novel technique in the field of food science, providing several chances to improve the nutritional and nutraceutical profiles of various food items. This chapter has offered an introduction to the multifarious world of enzymatic modifications, highlighting their potential influence on protein digestibility, bioavailability, amino acid profiles, and the production of bioactive peptide. Enzymatic interventions, aided by precise and particular enzymatic tools, are the key to optimizing protein structures to fulfill specific nutritional requirements. The removal of antinutritional components, texture alteration, and functional modifications demonstrate the diversity of enzymatic transformations in altering sensory qualities and overall food quality. Furthermore, enriching food matrices with nutraceuticals by enzymatic modification aids in the creation of functional meals with potential health-promoting properties. However, this voyage into enzymatic modification is not without obstacles. Enzyme specificity, reaction conditions, and allergenicity must all be carefully considered when developing modified proteins to assure their safety and quality. To ensure consumer safety, the regulatory framework controlling the use of enzymes in food processing must be monitored on an ongoing basis and comply to industry standards. Looking forward, the area of enzymatic transformation of dietary proteins is positioned for further expansion and innovation. Future directions may include the development of innovative enzymes, precise engineering techniques, and sustainable processing technologies to meet the growing need for healthier, more sustainable food options. Collaboration among specialists from several fields, such as food science, biochemistry, enzymology, and nutrition, will be critical in realizing the full potential of enzymatic changes and converting these discoveries into practical applications in the food sector.

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CHAPTER 11

BOTANICAL CATALYSTS: EXPLORING THE POTENTIAL OF PLANT-DERIVED ENZYMES IN FOOD BIOTECHNOLOGY ADVANCES

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ABSTRACT:

Enzymes are special substances in living things that help with food technology. The development of enzymes for culinary purposes depends on a thorough comprehension of the enzymes present in our traditional dishes. Plant enzymes are things like amylase, invertase, papain, bromelain, and lipoxygenase. The production of food items such as syrups, bread, alcohol, and dairy products heavily relies on these enzymes. Plants can be used to make enzymes for food. They can also be used to make other enzymes stronger for making food. New methods like changing genes directly are being used to make plant foods better for people. By utilizing plant enzymes, this has the potential to enhance the flavor and nutritional value of food. Enzymes were taken from plants and animals and then made in large amounts to be used more in making food. Today, tiny living things are the most important source of enzymes for businesses. The main thing to think about when checking an enzyme product is if the organism it came from is safe. Animals we eat and plants we can eat have been used safely to get enzymes for making food.

KEYWORDS:

Animal, Break Down, Enzymes, Food, Plant.

INTRODUCTION

Enzymes are important for the way all living things work. They are being used more and more to make chemical reactions happen in places where they wouldn't normally happen. Biocatalysts have been utilized as food additives and in the production of goods made from natural materials for an extended period. Enzyme extracts from plants or animal parts were utilized centuries ago, prior to a comprehensive understanding of enzymes. The food industry is always looking for new ways to make things that people want to buy. Enzymes are one of the tools that food makers use to turn basic ingredients into the things we eat. Their approval as safe ingredients from a legal standpoint have led to their widespread use in food technology. When certain enzymes are cleansed and put into food, they can make the food taste better, feel better, be easier to digest, and have more nutrients. However, protein technology didn't develop quickly until the middle of the last century. Over the past three decades, commercial enzymes have gained increasing significance in the production of various food items such as meat, vegetables, fruit, baked goods, dairy products, and beverages. Over the last decade, there has been a growing number of publications focusing on increasing production in the food and beverage sector[1], [2]. Furthermore, in the field of food technology, we utilize novel enzymes to alter the characteristics of proteins and create various types of food. This helps us avoid using harmful chemicals.

This also involves novel approaches to modifying natural products to enhance their health benefits or technological applications. Enzyme preparations can result in cost savings, contribute to environmental sustainability through the use of renewable resources, and improve the quality of products. Additionally, keeping food and drinks fresh is very

important for their quality. It is common knowledge that modern methods can transform juices into concentrates which can be preserved for extended periods of time without compromising their quality, with the exception of their fragrance. Preserving flavor and color is another way to make food last longer. Biotechnology has ultimately revolutionized our processes and offered unanticipated answers to challenges. It has also opened up new and exciting possibilities. Enzymes are recommended for use in "green" technology due to their capacity to assist in waste management and prevention[2], [3]. Enzymes currently utilized are mainly derived from beneficial microorganisms such as bacteria and fungi, as opposed to animals or plants.

A large number of clean enzymes are commonly employed in food manufacturing and as food supplements. It is essential to recognize that enzymes, similar to all proteins, can only initiate responses in individuals who have been previously exposed to them. The potential for allergies from enzymes in food is low due to their usually low levels. This special issue of *Enzyme Research* highlights how enzymes enhance the quality of food production. It explains how enzymes can help make food processing faster and better. It talks about how scientists are finding ways to make these enzymes work even better by studying microbes, changing proteins, and finding ways to keep the enzymes in one place. The second review article talks about how meat products have improved in terms of nutrition, technology, and the environment. It focuses on how enzymes, like proteolytic enzymes, phytases, and transglutaminase, are being used in the meat industry. Transglutaminase and certain enzymes from bacteria are being studied to see if they can help make gluten less harmful[4], [5].

In the changing world of food science, studying enzymes from plants has many exciting possibilities. Enzymes are like tiny machines in nature and they are very important for many chemical processes in the body. Using enzymes from plants opens up new possibilities in this area. This chapter talks about all the different ways we can use enzymes from plants in making food better. It also talks about the benefits and progress we have made in this area. Enzymes are special chemicals in living things that help speed up chemical reactions in the body without getting used up. In food biotechnology, enzymes are very important for making and improving different parts of food production, processing, and quality. Enzymes taken from plants come from many different plants and are interesting to study because they have special qualities and can be used in many different ways. Plants have a lot of different enzymes. Different parts of plants, like fruits, vegetables, seeds, and leaves, have enzymes that do specific jobs to help the plant grow and develop. Plant enzymes come in different types, like proteases, amylases, lipases, and cellulases. Each type has its own special properties that can be used in making food.

Enzymes from plants are better than enzymes from other sources in many ways. They are often seen as better for the environment because they can be made without harming nature and are taken from the earth with less damage to the environment. In addition, enzymes from plants can work in different temperatures and levels of acidity, making them useful for processing many kinds of food. Food processing involves using machines and techniques to change raw ingredients into food products that can be sold in stores. Applications in food processing refers to the different ways these machines and techniques are used to make food. Plant enzymes are used a lot in making food. Proteases from pineapple and papaya are used to make meat tender. Amylases from barley and other cereals are important in making beer. Lipases from plants help make fermented foods taste better. Studying enzymes from plants can help make food processing better and more environmentally friendly.

Enzymes from plants can make food healthier. For example, phytases in plants help to release phosphorus from phytic acid, making minerals easier for the body to use. Changing proteins

and carbohydrates in plant foods using enzymes can make them easier to digest and release helpful compounds that are good for health. The way enzymes work together in plants helps make the tasty flavors and smells we enjoy. Food scientists can use plant enzymes to find new ways to make food taste and feel different. This is very important in industries like baking, making beer, and making sauces where creating different flavors is a key part of making the products stand out. Progress in biotechnology has made it easier to improve and make plant-based enzymes. Genetic engineering helps change plants to make more enzymes or have new qualities. This new way of using biotechnology makes plant-based enzymes better, so they can be used in different ways in making food. Using plant-based enzymes in food production matches the focus on sustainability in the food industry. Enzymes can be taken from plants and used in farming, which helps to recycle resources. Furthermore, using enzymes from plants in food processing could be better for the environment than using enzymes from animals or microbes[6], [7].

Even though plant-derived enzymes have a lot of possibilities, using them in food technology comes with some difficulties. We need to think about how well we can get stuff out, how long it stays good, and what rules we have to follow. It's really important to make sure the way we get plant enzymes from plants and use them in food is safe and follows the same process every time. Studying enzymes from plants could lead to new advances in making food. Using advanced technology like synthetic biology and metabolic engineering can help us find new uses for enzymes and make them work better. As plant enzymes get better, they will be important for making new, better food. Studying enzymes from plants for food technology needs different fields of science to work together. It is important for botanists, biochemists, food scientists, and biotechnologists to work together to understand how plant enzymes work and use this knowledge to make useful products. Working together can help make new and better enzymes for food faster. Studying enzymes from plants for food technology is a very interesting journey into the world of plant catalysts. As we go through the next parts, we will learn more about different types of plant enzymes, how they work, and examples of how they are used. Plant-based enzymes can help make food processing faster and better for the environment. They can also make food healthier and more sustainable. Using these enzymes can be a great way to bring new ideas and improvements to the food industry.

DISCUSSION

Food processing turns raw animal and plant foods into things we can eat. This happens in the food and drink industry. Different types of processed food can be sorted into highly processed, minimally processed, and processed foods based on how they are different from each other. The way things are made uses different natural and chemical substances, like enzymes. People have been using living organisms to make food for a very long time, since around 6000 BCE. This includes using them to make bread, beer, cheese, and wine. The first-time people intentionally used bacteria to make something was around 2000 BCE when they made vinegar. Even though enzymes were used regularly, no one knew about the reactions or the chemistry behind them. The enzymes invertase and pectinases have been used in foods since the 1930s. In the 1960s, people started using immobilized enzymes, starting with invertase. Since then, using enzymes in the food industry has become really popular. People are finding more ways to use them in making food and other products. Right now, these products are mostly known in the biotechnology industry. The industry made \$1.3 billion in 2002, and is expected to make around \$7 billion in 2013. This market has a part that is used for making food, like drinks, dairy products, and supplements. This is then used by the leather, fabric, paper, and feed industries. Enzymes from plants are called amylase, invertase,

papain, bromelain, ficin, lipoxxygenase, and others. These enzymes have been important in making food like syrups, bakery items, alcoholic drinks, and dairy products. The plant is not only used to make enzymes, but it can also be used to make microbes work better in the food industry. Peptidases or proteases make up the biggest part of the enzyme market, accounting for about 60%. They are used a lot in the food, pharmaceutical, and detergent industries for many different purposes[8], [9]. Protease proteolysis is when proteins are broken down into smaller parts by cutting the bonds that hold the amino acids together.

Protease enzymes that are used for making things like soap and medicine are safe and good for the environment because they are not harmful and won't make people sick. Proteases are found all over in animals, plants, and microorganisms. They are very important for living things. The ability to make plant-based protease and use it depends on having enough farmland and the right weather. Some popular enzymes that come from plants are papain, bromelain, ficin, and keratinases. Proteases have been used in the food industry for a long time. They have been used often for many things, like making soya hydrolysate, tenderizing meat, making cheese, and baking. They are very important enzymes in industry. Plant proteases have been used in food, medicine, and making cleaning products for many years. But because they cost more to produce, fewer are being made compared to ones from microbes. Industries are still looking for new proteases with better properties. Actidin, a new type of protein-cleaving enzyme, was first found in kiwi fruit. It is used in restaurants and food factories to make meat more tender by breaking down the proteins in the meat. Even at higher amounts, actidin stops the surface from getting too soft and helps to make the meat more tender. Because this enzyme can be inactivated at a lower temperature (60°C), it is easier to control the tenderizing process without cooking the meat too much. After an animal dies, its muscles start to change and a protein called m-calpain becomes active[10], [11]. Actidin is useful in the food industry because it is better than other plant proteases like papain and ficin.

Bromelain is made up of different enzymes and carbohydrates. The part of the plant that holds it up has special proteins called ananase or "stem bromelain", and the juice in the pineapple fruit has a different kind of protein called "fruit bromelain". Two more chemical compounds were found in the stem using comosain and active site-directed affinity chromatography. They are called ananain. The natural enzyme bromelain from the pineapple stem has been cleaned using different methods like filter and chromatography. We got 0.87g of pure bromelain from 10g of the original material. The best pH for stem bromelain to work is between 6.0 and 8.5, and it works best at temperatures between 50 and 60 degrees Celsius. Fruit bromelain breaks down proteins better than stem bromelain and can work on a wider range of peptide bonds. Bromelain is a key enzyme that makes meat tender in food factories. It also helps make sure the meat is free from harmful bacteria and good quality. When making meat from adult cows, using 10mg of bromelain per 100g of meat and keeping it at 4°C for 24 hours showed the best results[12]. Then, slowly increase the temperature by 1°C per minute until it reaches 70°C.

Bromelain comes in a powder form that you can buy. It is estimated that 95% of the enzymes in the United States come from plants like papain and bromelain. But not many people use enzymes from microbes to tenderize meat. Papain, which is another type of enzyme, has a small amount of latex in it. It was the first time it was separated and made into crystals in 1879. Papain can break down proteins in different ways and also has an enzyme that helps with protein digestion. Papain works best when the pH is between 5.0 and 7.0. When casein is used as the substrate, the best pH for papain is 7.0. In comparison, it stays at the same temperature much better than other proteases. This enzyme works best at temperatures

between 10 and 90 degrees Celsius. Ficin, also known as ficain, comes from the *F. Ficus*. The dried latex of *glabrata*. It can also be found in other types of *Ficus* plants, like the rubber plant and the fig tree. Crude ficin is very important for business, but not much research has been done on the ficin from a different type of *Ficus* plant. A small green fig weighs about 10-15 grams and has only about 100-150 milligrams of ficin. Ficin is found in different forms in latex taken from a *Ficus* tree. Scientists can tell the difference between these forms using ion-exchange chromatography. Ficin works best at a pH between 5.0 and 8.0 and at a temperature between 45 and 55 degrees Celsius. So far, scientists have only studied three parts of ficin: a part by the catalytic Cys, a part by the catalytic His, and a part at the beginning. Because of the structure of amino acids, Cys was found to be similar to the sequence in papain for the nearby parts of the active site.

Amylases are enzymes that are used in industries to turn starch into glucose by breaking down the bonds in starch molecules. Alpha amylases work best in a pH range of 2 to 12 and can handle high temperatures. These enzymes are used in many different industries such as food, sugar, paper, and medicine. Amylolytic enzymes are used a lot in making food, like high fructose corn syrup, glucose syrup, and maltose syrup. These enzymes help make fruit juice clear, keep sugar syrups from getting thick, break down starch, and prevent bread from getting stale. During beer making, sugars are changed into alcohols. Sugar is usually made by a process called mashing, where different grains mix with enzymes from barley to make starch. Alternatively, sugars can be made from starch using man-made enzymes, so there's no need for the malting process, which saves energy and land. α -amylase also goes by the name 4-alpha-d-glucan malto hydrolase. Polysaccharides are broken down by cutting the alpha-d-glucosidic linkages. This process removes maltose parts from the ends of the chains. Amylase breaks down glycogen, starch, and other similar substances to make beta-maltose. As the fruit grows, an enzyme changes starch into sugar, making the fruit taste sweet when it's ripe. Amylases are found all over plants, in places like seeds, nodes, tubers, and leaves where they help with storage and growth. Before seeds start to grow, this enzyme is not active. Beta-amylase breaks down starch in the plant's stems and leaves. The amylase enzyme is mostly in cereals like wheat and barley and in sweet potatoes.

Lipases are enzymes that change how fats work by moving around the fatty acids and swapping them with new ones in the fats. This specific substance changes fats to make them have more fat and be better, from a cheaper and not so good fat. Lipases can change fats and oils by breaking them down and making new compounds. We use esterification and interesterification to change oils and fats to make them more valuable. This type of fat and enzyme have more potential for use in industries than making fatty acids in large quantities through hydrolysis. Lipase is an enzyme that can be used in many industries like food, medicine, leather, soap, makeup, clothes, and paper. Many fat-clearing enzymes called lipases are made in large quantities in factories. The lipases made by businesses are used to make other foods and add flavor to dairy products like fruit, milk, meat, bread, vegetables, and beer. Phospholipases are used in industries to help make mayonnaise and emulsifiers from egg yolk, to clean vegetable oils, and to change lecithin. This enzyme is also used for making sauces like béarnaise, hollandaise, and café de Paris. Lipases are very good at helping to make esters. They act as a catalyst in the process. These esters are used to add flavor to food and are made from short fatty acids.

To make ester, scientists use silica and microemulsion-based organelles with immobilized lipases. Lipxygenases are special kinds of enzymes that are found in plants, fungi, and animals. They are in a group of enzymes called oxidoreductases and help with the process of adding oxygen to a molecule called linoleate. They are a type of iron that helps enzymes

break down PUFA fats. Theorell et al. discovered and made a clear picture of lipoxygenases in 1947. Plants make lipoxygenases that are very important for making food. Lipoxygenases make around 9832 smells and tastes in different plant foods. Lipoxygenases help to bleach and improve the texture of dough in the baking industry. Invertase is found both inside and outside the cell. It is made by fermenting a safe strain of yeast in water. Then the yeast cells are extracted and broken down. Other names for this enzyme are glucosucrase, Saccharase, beta-h-fructosidase, beta-fructosidase, sucrase, invertin, fructosylinvertase, maxinvert L 1000, acid invertase, and alkaline invertase. The official name is beta-fructofuranosidase. The enzyme is used in many industries, like making candies with liquid centers, such as some chewing gums. It also helps make ethanol from sugarcane molasses easier. However, invertase is not used much because another enzyme called glucose isomerase can change glucose into fructose at a lower price.

The food industry needs very pure invertase for flavor and health. It is also used to make tablets that help with digestion, chocolates, baby food, and fortified wines. The pectic enzyme has three types: pectolyase, pectozyme, and polygalacturonase. It is used to break down plant materials. Pectin is a type of carbohydrate that is found in fruits and is made up of many sugar molecules linked together. It can make juice hazy and cause solid bits to form, and it reduces the amount of juice that can be made from fruit. Pectin-breaking down enzymes are often used to get fruit juices and wines. Pectin is really important in making fruit juice and wine. It has been shown to be very important in both processes. Using enzymes makes the juice clear by breaking down a substance called pectin and letting the little pieces in the juice settle at the bottom. It also gets rid of any unwanted changes in the arrangement, color, and firmness. These enzymes also help with other tasks, like making fruit purees, removing skin from orange segments, and clarifying wine. Making clear juice would cost less to produce and compete well with other methods. The tropical fruit juice industries can use this method to make more juice and sell it to more people. The enzyme α -amylases breaks down the starch in the fruit into smaller parts. It is used a lot in different industries like paper, sugar, clothes, food, brewing, making starch liquid, and alcohol.

A heat-resistant enzyme called thermostable amylase was found in a bacteria called *Bacillus licheniformis*. It was shown in genetically modified tobacco plants. A chimera with a gene from a certain bacterium was put into tobacco cells, and it showed a lot of the gene which could be good for business. Essential amino acids like lysine and threonine are really important in cereal crops because the seeds don't have a lot of them. Aspartate is the starting material for these amino acids, as well as methionine and isoleucine. Plants control the aspartate metabolic pathway at important enzyme steps. Some enzymes have been found in many plants, such as aspartate kinase, homoserine dehydrogenase, dihydrodipicolinate synthase, and threonine synthase. Papaya uses latex to protect itself from germs. The juice of papaya is a thick liquid that becomes less thick when moved. It has about 15% solid material, and 40% of that solid material is made up of enzymes, mainly cysteine endopeptidase. Together they make up over 80% of all the enzymes. The endopeptidase enzyme in papaya latex is kept in an inactive form. When the latex is released from the plants, it quickly turns into strong enzymes. The greener the fruit, the stronger the papain becomes. The salt precipitation method is the traditional way that was used before to purify papain.

The way meat feels and how soft it is are the most important things that affect how good it tastes and how satisfied people are when they eat it. Being tender is a complicated quality. The two main things that make meat tender are the connective tissue and the strength of the muscle cells. Only five enzymes are allowed and considered safe by US agencies. They are called papain, ficin, bromelain, *Aspergillus oryzae* protease, and *Bacillus subtilis* protease.

Actinidin and zingibain are enzymes that can make meat softer. Research found that papain is very good at making meat tender. Bromelain made the collagen break down more than the muscles and made the meat more tender. Ficin breaks down both muscle and connective tissue proteins. Papain and bromelain are also used to make sauces and dry cured ham. In the food industry, most enzymes are used to break down and change materials in food. New, money-saving, and environmentally friendly technologies are the most wanted in the industries that change fats and oils. Using a lot of enzymes to make beer from cheap ingredients like barley is important for the future of brewing. In barley, the starch needs to be turned into sugar so that the yeast can make alcohol.

So, regular beer making has an extra step called malting, where enzymes are made to turn starch into sugar for fermentation, which is not needed in wine making. Most enzymes are made when seeds start to grow, like amylases and proteases. But some enzymes are already in the barley, like amylases. In the last step of making malt, all the important enzymes needed to turn grains into a liquid that can be fermented are there. However, the enzymes in malt do have limits. They can only work well at specific pH levels, temperatures, and so on, and their performance might not be good enough to do the job correctly and on time. In comparison, commercial enzymes from outside sources can be made to be stronger and work better at certain temperatures and pH levels. Adding extra enzymes from outside can help make brewing quicker, more even, and simpler at different stages of the process. Barley malt is the usual way to get the enzymes needed to make beer from grains. If there's not enough enzyme activity in the mash, it will cause problems like not getting enough extract, taking too long to separate the wort, slow fermentation, not making enough alcohol, slower beer filtration, and the beer not tasting as good. To stop these issues, we use extra enzymes from outside to help the malt's own enzymes. In addition, industrial enzymes are used to make beer faster and from cheaper ingredients. This helps break down added substances and create low-carb beer.

In the beer-making industry, chemicals like bromelain or papain are used to make sure the beer stays clear even in cold temperatures. This helps prevent cloudy beer. Due to the preference for additive-free beer production in many European countries, papain is not extensively utilized. In making baked goods, small amounts of enzymes can be added alone or mixed together to work better. Baking involves using enzymes from three different places, such as the enzymes in flour. Enzymes linked to the metabolism of the most common microorganisms. External enzymes are put into the dough. In baking, people are paying more attention to lipolytic enzymes. Recent research shows that enzymes can break down certain wheat fats to make other fats that can help make foods smooth. This means phospholipases could be used instead of regular additives to make food smoother. Lipase was mainly used to make bakery foods taste better by releasing a certain kind of fat. The flavor got better and the bakery products stayed fresh for longer. Lipase can help make something feel smoother and softer.

All enzymes that break down molecules in water, such as lipase, were found to make breads bigger and less firm. Butter flavor for baked goods was made stronger by breaking down butterfat using a special enzyme called lipase. In baking, proteases break down gluten to make it easier to make the dough. These enzymes could be used in industries because they work quickly, are best at a certain acidity and temperature, and don't have certain other activities. Right now, we are studying how to make new and valuable products using gluten hydrolyzates. Likewise, to make wheat flour that is less likely to cause allergies, bromelain has been used to break down the gluten in wheat. Bread is mostly made of starch. As starch turns into crystals, bread becomes hard and not good to eat as it gets old. To stop bread from going bad and make it last longer, adding lipase and amylase enzymes to the bread-making

process helps to stop the starch from turning into crystals. The money saved mainly came from not growing as much grain and not having to transport as much bread. Food additives are things added to food to make it look better or last longer. For example, they can be added to change the color of the food, make it sweeter, or stop it from going bad. We don't normally eat these things on their own, but they are put into food on purpose.

Some food additives are natural and even necessary for our bodies. However, it is the reason they are used in food that makes them classified as food additives and subject to safety rules. More people are becoming aware of what they eat and are paying more attention to additives in their food. This has had a big impact on the food industry. A multitude of industrial products are low in sodium, fat, caffeine, and cholesterol. Aspartame is the preferred choice over saccharine, and there is a growing interest in finding natural substitutes for antioxidants, preservatives, and colorants. Enzymes can be used to make many different flavors in food. Their special characteristics allow them to make chemicals that are hard to create. Enzymes can be added to food to make it taste better or to get rid of bad flavors. They can also help with getting rid of certain compounds in the food. Enzymes can improve the quality of foods during stages like making, preparing, storing, and transporting. They speed up specific reactions in the food. Enzymes are being used more and more to make chemical reactions happen outside of cells. They are especially being used as food additives and to process raw materials. Enzymes are used by companies to turn raw materials into finished products.

The food industry always looks for new technology to make the food that people want. Some enzymes can make food taste better and easier to digest. Adding them to the food can also result in an improvement in its texture and nutritional value. The use of technical enzyme preparations can result in cost savings, promote environmental sustainability through the use of renewable resources, and enhance the quality of products. Preservation is important for keeping food and drinks fresh and tasty. Juices can be transformed into concentrates using modern methods, which allows them to be preserved for extended periods without compromising their quality, except for the aroma. Preserving flavor and color is another way of improving preservation. Numerous purified enzymes are commonly employed in food production and are also included as additives in food products.

CONCLUSION

Plant enzymes are beautiful and charming. They are used in the food industry in many different ways. In the world market, the total need for enzymes is expected to go up quickly soon. The product category is experiencing rapid growth, particularly in markets such as animal feed and the food and beverage industry. In the past, people thought enzymes were too fragile to survive in harsh conditions during chemical reactions. So, a lot of industries couldn't use enzyme technology. Using enzymes in industry needs really good enzymes with specific traits. This makes scientists look for new ways to make the enzymes work even better. Discovering previously undiscovered enzymes in natural environments, enhancing the functionality of current enzymes, utilizing specialized enzymes for novel tasks, improving enzyme combinations, and developing new biocatalysts from the ground up are some of the methodologies employed in this domain.

These methods have given us good choices for using living organisms to help with chemical reactions. On the other hand, it is crucial to utilize the new enzyme products in biochemical technology. Progress in plant biotechnology has a positive impact on creating better enzymes for food production. This will help make our environment healthier and improve people's lives.

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CHAPTER 12

ENZYMES' CRITICAL ROLE IN ADVANCING PHARMACEUTICAL INNOVATIONS AND THERAPIES

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ABSTRACT:

Enzymes are very important in creating new medicines and treatments. This research looks at how enzymes are used in finding and making drugs. It explores all the different ways enzymes are important in this process. This chapter explains how important enzymes are in finding and confirming the most important targets for medicine. It uses advanced techniques to find these key targets. Biocatalysis is an important part of making medicine in a way that is good for the environment. It uses enzymes to make drugs and is a very important part of the process. Protein engineering and enzyme science are coming together to show how engineered enzymes can change the way we create new medicines, like combining antibodies with drugs or fusing proteins together. This brings new and exciting possibilities for treatments. Enzyme replacement therapies help with genetic disorders by giving the body the enzymes it's missing. In addition, using enzymes with immunotherapy is a new way to treat cancer. It can change how immune checkpoint inhibitors and CAR T-cell therapies work. Enzymes help with medical tests by helping to identify markers in the body and developing techniques to help doctors group patients and give them personalized treatment.

KEYWORDS:

Bacterial Cell, Cell Wall, Drug, Pharmaceutical Industry, Peptidoglycan Hydrolases.

INTRODUCTION

The pharmaceutical industry is different from other types of industry in many ways. The healthcare industry has a lot of contradictions. Even though it has helped people for a long time, many people don't trust it. They often think it is less trustworthy than the nuclear industry. Investing in this business is very risky, but a lot of people think it can make a lot of money. Big drug companies say they focus on research, but many people think they spend more on advertising. Even though making medicine is risky and expensive, a lot of people think all medicine should be made for everyone and given away for free. This first chapter will help you understand how the industry works and try to explain some things that might seem confusing. The goal is to give background information about the business so that we can understand the problems with pharmaceuticals in the environment better. The words "medicine," "pharmaceutical," and "drug" are often used in place of each other. The word "drug" can mean both a medicine and an illegal substance, depending on the situation. In this chapter, we are using the word "pharmaceutical" to talk about the medicines that are made by pharmaceutical companies and used by patients [1], [2]. The term "drug" is mostly used for new medicines that are being developed by the pharmaceutical industry.

For over 3000 years, people have used "drugs" to help with sickness and disease. Around 1100 BCE, China had a few dozen drugs from plants and animals. By the late 16th century, they were using at least 1900 different remedies. Today, Traditional Chinese Medicine knows more than 13,000 drugs. In ancient times, people wrote about different plants and their uses in a book called *De Material Medica*. People in many different parts of the world used herbs for medicine, like in North and South America, India, and Australia. Later on, people in Islamic and Christian countries also used herbal remedies. This continued until the 17th century with

people like Paracelsus in Switzerland and Culpepper in England. In 1652, Culpepper wrote a famous book called *The English Physician*. It was one of the first books about medicine written in English. Until the 18th century, people used herbal medicines based on what they had observed, without understanding the scientific reasons behind their effectiveness. However, in the late 18th century, people started to learn about how drugs work and affect the body, which is called pharmacology [2], [3]. William Withering was one of the first people to study and find the important part in a herbal medicine in the 1780s.

He found digitalis in the foxglove plant and explained how to get it out of different plant parts. He also talked about what it does and the best way to use it to help sick people. The study of pharmacology took a long time to develop, and Oswald Schmiedeberg is considered the founder of modern pharmacology. In 1872, he became a professor at the University of Strassburg in Austria, where he studied the effects of chloroform and chloral hydrate. In 1878, he wrote the famous book, *Outline of Pharmacology*. At the same time, modern organic chemistry started to develop, and so did pharmacology. Before the 19th century, scientists thought that substances from living things had a special power that made them different from non-living things. These two important advancements in pharmacology and organic chemistry led to the creation of the pharmaceutical industry in the late 1800s.

The pharmaceutical industry started with companies like Merck, Eli Lilly, and Roche making natural products like morphine and quinine. Then, other companies like Bayer, ICI, Pfizer, and Sandoz started making chemicals and found ways to use them for medicine. This all happened in the 1800s. However, there was only a small increase in growth, and in the early 1930s, most medicines could still be bought without needing a doctor's prescription. Almost half of the medicines were made by local pharmacists, and sometimes doctors gave medicines to their patients. However, in the early 20th century, many important new discoveries and improvements were made. Salicylic acid is a natural part of willow bark and Hippocrates said it can help with pain. In 1897, people at Bayer found a better version of salicylic acid called aspirin. It is still used a lot today. In the 1920s and 1930s, penicillin and insulin were discovered and made, but not in big amounts. World War II helped the industry grow because it needed to make a lot of painkillers and antibiotics. Governments wanted them to find treatments for many different illnesses [4], [5]. After the war, Europe started state healthcare systems like the UK's National Health Service (NHS).

This made the market for prescription drugs more stable and made it easier for people to get reimbursed for their medications. This made companies want to invest more money in researching, developing, and making new products. The government started to have a bigger role in the medical industry by putting more rules on how medicine is made in both America and Europe. From the 1950s to the 1990s, after the war, there were big improvements in making new drugs. We got new antibiotics, easier pain relievers like acetaminophen and ibuprofen, and also new types of medicines like birth control pills, ACE inhibitors, and benzodiazepines. There were also new medicines to treat cancer. The thalidomide scandal in 1961 caused the government to rethink how it regulates the pharmaceutical industry. The new rules required evidence that the drugs worked, were clean, and safe, which made it more expensive and difficult to do research and development for new drugs. This led to fewer companies making drugs. Similarly, the spread of global connections and trade, which had already started before the war, got even bigger. This led to a few big companies controlling the development of new drugs and the start of popular drugs.

Enzymes emerge as molecular catalysts in the ever-changing environment of pharmaceutical research and development, playing an important role in the advancement of discoveries and cures. These biological entities, most often proteins, facilitate and accelerate certain

biochemical events, providing a diverse toolset for drug discovery, manufacture, and therapeutic interventions. This chapter delves further into the critical role enzymes play in molding the pharmaceutical industry, examining their applications, mechanisms, and contributions to the creation of innovative medications and treatment techniques. Enzymes' essential role as biological catalysts is key to their engagement in the pharmaceutical sector. Enzymes catalyze chemical changes with exceptional selectivity and efficiency, simulating the precision necessary in complex metabolic processes inside living organisms. This catalytic potency is the cornerstone for their many uses in pharmaceutical research and development. Enzymes play an important role in drug development, as researchers look for molecules that may selectively modify certain biological targets. Understanding the structure and function of enzymes implicated in illnesses allows for the creation of small compounds or biologics that can interact with these targets, opening the door for the development of therapeutic treatments.

Enzymes are often used as drug research targets, especially in disorders where deregulation of enzyme activity leads to pathology. Identifying and confirming these targets requires sophisticated approaches such as high-throughput screening and structural biology, which enable researchers to identify enzymes that, when regulated, may provide therapeutic effects. Biocatalysis, or the use of enzymes as catalysts in chemical transformations, has gained popularity in pharmaceutical synthesis. Enzymes provide selectivity and specificity that conventional chemical techniques may lack. Their use in the synthesis of pharmaceutical intermediates and active chemicals not only improves efficiency but also addresses the rising need for sustainable and environmentally friendly production techniques. Advances in protein engineering have enabled scientists to tune and improve enzymes for medicinal applications. This involves increasing stability, changing substrate specificity, and reducing immunogenicity [6], [7]. Engineered enzymes, such as antibody-drug conjugates and fusion proteins, represent the convergence of biotechnology and enzymology in the search for novel treatment modalities.

Enzyme deficiencies are at the root of many hereditary illnesses, and enzyme replacement treatments (ERT) provide a tailored solution. ERT is the use of functioning enzymes to compensate for the absence or malfunction of endogenous enzymes. Examples include the use of recombinant enzymes in lysosomal storage diseases, which demonstrates enzymes' transformational potential as therapeutics. The combination of enzymes and immunotherapy provides a new path in cancer therapy. Enzymes have important roles in influencing immune responses, and treatment techniques use their potential to improve the body's capacity to identify and remove cancer cells. Immune checkpoint inhibitors and chimeric antigen receptor (CAR) T-cell treatments demonstrate enzymes' enormous influence on cancer therapy. Enzymes provide major contributions to diagnostic tools and approaches in the pharmaceutical field. Biomarker tests, which depend on enzymatic reactions, give vital information about disease states, assisting in patient stratification and tailored medication. Enzymatic uses in diagnostics include ELISA and PCR. Despite their enormous potential, the use of enzymes in the pharmaceutical business is not without difficulties. Enzyme stability, immunogenicity, and scalability in manufacturing all need careful attention. Addressing these obstacles is critical to realizing enzymes' full potential as catalysts for pharmacological innovation.

The regulatory framework governing the use of enzymes in medications is dynamic and developing. Regulatory authorities throughout the globe have established severe requirements to assure the safety, effectiveness, and quality of enzymatic treatments. Compliance with these standards is critical to the successful development and approval of enzyme-based

medicines. Looking forward, enzymes' importance in the pharmaceutical sector is expected to grow even more. These trends highlight the continued commitment to pushing the limits of what is feasible in drug research and development. Enzymes' crucial significance in driving pharmacological advances needs cooperation across several scientific fields. Interdisciplinary techniques including enzymologists, pharmacologists, chemists, and biotechnologists provide a comprehensive knowledge of enzyme activity and application. Such partnerships hasten the translation of ground-breaking findings into practical treatment solutions. Finally, enzymes are critical catalysts for pharmacological developments and cures. This chapter has given a thorough review of their many activities, including drug discovery and design, biocatalysis, protein engineering, and therapeutic interventions. As we go through the parts, we will look at particular instances, case studies, and cutting-edge applications that demonstrate enzymes' revolutionary ability in the never-ending search of better human health and disease prevention.

DISCUSSION

In the biosphere, different kinds of living things like microbes, animals, humans, and plants share and spread biological material a lot. Usually, the interactions help both parties. However, there are still many more tiny organisms that cause illness and make plants, people, and animals sick. The ways in which these harmful organisms cause infections and diseases are very different and can affect almost all parts of the body. There are two main ways to protect against harmful germs: vaccines that help your body fight infections, and antibiotics that help kill harmful germs. Antibiotics are strong drugs that can kill many different types of germs like bacteria, fungi, and parasites using a specific method of action. In the last few years, using too many antibiotics has caused a problem. It has made germs stronger and able to resist antibiotics. Bacteria that can't be killed by antibiotics are causing dangerous problems for animals and people. The bacteria are also able to survive the antibiotics, making the problem worse. Antimicrobial resistance happens naturally and can occur in different ways. Many things can cause bacteria to become resistant to antibiotics. Using antibiotics too much and not in the right way in hospitals is a big problem because it makes harmful germs stronger against different antibiotics.

When there is more antibiotic, it makes it easier for bacteria to become resistant to it. Harmful bacteria can also get antibiotics from animal food and farming, where they are added to the food. They are being used to treat people, but this is causing resistant bacteria to develop and spread easily from one person to another. Many people believe that natural or herbal products are a better choice than antibiotics. Even though it's not likely that these products will cause resistance, we still need to work hard to understand how they work, what they're made of, and how they're made. It's difficult to make natural products in labs because they are complex to make. Many marine bacteria and microorganisms have not been studied yet, and they may have important natural substances in them. Antimicrobial peptides are important for new medicines that can fight against drug-resistant bacteria. AMPs like Magainins, Cathelicidins, Lactoferrins, and Defensins are a potential source for these new medicines. Using viruses to treat bacterial infections, known as phage therapy, was first discovered in the 20th century and was thought to be a potential treatment. In 2007, it was approved as safe for use in farming and veterinary medicine to control bacteria in food products. Now it is commonly used to fight many bacterial diseases. An enzybiotic is a combination of enzymes and antibiotics. It is made up of lytic enzymes that are naturally found in viruses, bacteria, and in body fluids like tears, saliva, and mucus[8], [9]. These enzymes have properties that can kill bacteria or fungi.

They are often used to keep food fresh and add flavor. In the beginning, all the enzymes from bacteriophages that could break down bacterial cell walls and be used as antibiotics were called enzybiotics. Later, enzymes that can kill fungus were added to enzybiotics. Even before biotechnology began, enzymes and proteins were used as medicines. Enzymes connect with their targets in a very exact and strong way. They change many different molecules into the things we want using a catalyst, which makes chemical reactions happen faster. This makes them stand out from all the other drugs. Microbes have many different enzymes, and it's easy to make more of them by changing the environment or genes. This is a big advantage when using enzymes from microbes. Therapeutic enzymes are good because they are cheap and dependable. Because they produce a lot, these are easy to change and make better. Digestive and metabolic enzymes can be used by themselves or with other treatments to help with diseases like leukemia, skin ulcers, heart problems, Parkinson's disease, inflammation, digestive problems, and pancreatic issues. They are also used to diagnose, investigate, and monitor many serious diseases. When enzymes are used as medicine to treat gram-positive bacteria, they break down the cell wall. Gram-negative bacteria have a hard time because they have a tough outer layer. Enzymes only target specific things and do not harm the good bacteria in our bodies[10], [11]. The main types of enzymes used for treating medical conditions are discussed below. Peptidoglycan is a key part of the cell wall in both types of bacteria, gram-positive and gram-negative. The main parts of the bacterial structure are N acetyl muramic acid (NAM) and N acetyl glutamic acid (NAG), which are joined together by a peptide chain to make a strong mesh.

Many bacteria have a mix of enzymes called peptidoglycan hydrolases. In general, these are involved in controlling how a cell wall grows, making peptidoglycan, and separating new cells when they divide and break open. They can also break the strong bonds of the peptidoglycans. Bacteriophages break down the wall of bacteria to release new phages at the end of their life cycle, with the help of special enzymes. Special enzymes help bacteriophages break down the bacteria wall to release new phages at the end of their life cycle. Bacteriophages use special enzymes to break through the bacteria wall and release new phages as they complete their life cycle. The wall of bacteria is broken down by bacteriophages with the assistance of special enzymes, which allows them to release new phages at the end of their life cycle. Peptidoglycan hydrolase has two parts at each end. The N terminal breaks down the cell wall and the C terminal helps the protein bind to the cell wall. Attaching this domain to its cell wall base is important for cutting the bacterial cell wall effectively. The cell wall binding domain doesn't identify the peptidoglycan because its structure stays the same[12]. Breaking things down well needs the binding domain to attach to its cell wall material.

Special receptors in the peptidoglycan layer help the enzyme molecule to attach to the vulnerable bacteria. Enzymes can stick to specific bacteria because they have a special binding power. This means they work only on bacteria that they can stick to. These enzymes help make spheroplasts needed to transform cells by taking things out of bacterial cells. It kills the bacteria and releases new viruses. Peptidoglycan hydrolases are a great treatment for Enterococci infections. These infections are often hard to treat with regular antibiotics because the bacteria have become resistant to them. Peptidoglycan hydrolases can kill the bacteria and are very effective at doing so. These enzymes can be used outside in plants and animals, either in their natural form or in a modified form. They are also produced in greater amounts inside genetically modified plant or animal organisms to make them more resistant to infections. Hydrolase enzymes are helpful in making products like ointments and creams for skin and tissues in cosmetics and medicine. Peptidoglycan hydrolases are enzymes that include the following types. Lysins, which are found in bacteriophages, are capable of

destroying cell walls by enzymatic action. When the pH drops below their isoelectric point, they become positively charged due to their alkaline nature.

New bacteriophages are released by breaking the bacterial cell wall. The addition of enzymes to gram-positive bacteria results in the swift destruction of the bacteria as their cell walls are broken down. This is a powerful way to fight these bacteria. Lysins can be sorted into five main groups based on the specific tasks they do with enzymes: N-acetylmuramyl-L-alanine amidases, Endopeptidases, N-acetylmuramidases, Endo- β -N-acetylglucosaminidases, and Lytic trans glycosylases. Lysin can be used to break open both types of bacteria cells, with or without using another chemical to help. The salmonella phage lysin called SPN1S was 30 times more effective at breaking down bacteria compared to the chicken egg white lysozyme and other similar enzymes. The LysAB2 lysin from the *Acinetobacter baumannii* virus was able to kill seven different types of bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus sanguis*, *Acinetobacter baumannii*, *Escherichia coli*, *Citrobacter freundii*, and *Salmonella* bacteria. Lysin's ability to break down the cell wall of bacteria is used to preserve food and detect harmful pathogens. Phage lysins can also be used to keep food safe by killing harmful bacteria. This is important for people who are sick or old and for animals, because they are more vulnerable to getting sick from these bacteria.

The Lysin LysZ5 from the *Listeria monocytogenes* phage can make the bacteria in soy milk almost impossible to find, even when it is stored in the refrigerator. Staphylococcal lysin LysH5 kills *Staphylococcus* bacteria. Gold in milk. The enzyme and bacteriocin work well together and could be used to keep food fresh by killing harmful germs even in small amounts. Streptococcal lysin PlyC helps protect against the bacteria *Streptococcus pyogenes*, which can cause infections in the nose. During childbirth, using enzymes can help protect newborns from infections caused by *Streptococcus agalactiae* in the mother's genital area. Using lysins to control harmful bacteria inside the body. An infection called aureus endophthalmitis caused damage to the eyes of mice. But when the mice were given a single injection of the enzyme a few hours later, it greatly decreased the number of bacteria in their eyes. This also helped protect the retina at the tissue level. Singh and his colleagues wrote about this in 2014. A study injected *Enterococcus faecalis* into mice and found that a medicine called IME-EF1 can protect against this infection better. *Faecalis* compared to the virus that makes it. A cream with a special ingredient called chimeric lysin ClyS87 worked well on the bacteria on the skin of mice. The lysin ointment worked better than mupirocin

It helped lower the number of bacteria in the lungs and kept the mice from getting sick with pneumococcal bacteria in their blood. The Cpl-7s bacteria killers made the baby zebrafish live longer. Lysins can get rid of biofilms, which are a key part of many harmful bacteria and make them resistant to a lot of germ-killing drugs. The most common germs that cause infections in biofilms are staphylococci. SAL-296, CHAPk97, SAL-140, PlyGRCS57 Lysins phi11 Ply187, and the LysH5 phage lysine can remove staphylococcal biofilms. Lysins can be used to find and measure bacteria in food, and as a type of disinfectant that targets specific types of bacteria, according to Hoopes et al. In 2009 PlyC is a disinfectant that can kill *Streptococcus equi*. Lysins also help to prevent unhealthy bacteria from contaminating ethanol fuel during fermentation. Viruses have a special enzyme called endolysins that can break down the cell wall of bacteria. This causes the bacteria to burst and release new virus particles. Some viruses have another type of enzyme called virion-associated peptidoglycan hydrolase (VAPGH). Endolysin is a protein made by viruses that infect certain types of bacteria. It has two parts: one part that breaks down the bacterial cell wall, and another part that helps it attach to the cell.

These parts are connected by a small flexible region. Phage-encoded peptidoglycan hydrolases (PGH) called Endolysins break down peptidoglycan during infection to help the virus penetrate the cell wall. When cleaned, both PGH types can kill bacteria when put on cells from outside the body. Phages have enzymes that break down the carbohydrates in the bacterial cell wall. "Sugars outside the bacterium can keep it safe from drying out, harmful substances, and the body's defenses. "Bacteriocins are proteins made by certain bacteria that can kill other bacteria. They are produced by both types of bacteria, gram-positive and gram-negative. These peptides are made naturally or changed by Mitomycin C to stop other bacteria from growing. They kill bacteria by latching on to them and causing changes in their metabolism, biology, and shape. The first bacteriocin was found in *Escherichia coli* and was called colicins. Until now, these are some of the most diverse types of antibacterial peptides that can kill bacteria by stopping them from making their cell walls, making holes in their outer membrane, or stopping enzymes from working. Bacteriocins are put into three groups based on their genes and how they work in the body.

These can handle high temperatures and are small (S). Bacteria named *Staphylococcus aureus* and *Bacillus cereus*. Because of their positive charge, bacteriocins attach to the phosphate groups on cell membranes, which are negatively charged. This causes them to go into the membrane and make holes in it, which results in the cell dying. Class II bacteriocins are different from class I bacteriocins because they don't have a certain type of peptide chain, which makes their structure simpler. This class has three subcategories: II-A (pediocin, enterocin, and sakacin), II-B (lactococcin G, plantaricin, and lactacin F), and II-C (AS-48 from *E. coli*). Class III bacteriocins are types of antimicrobial peptides. Examples of this class include Helveticin J and Millericin B. Bacteriocins are useful in many ways. Lantibiotics are bacteria-killing substances that can be used in food. They are the only ones approved by the FAO/WHO and considered safe since 1969. Additionally, it is approved as a natural preservative in countries in the European Union and has the designation E234. These bacteriocins are really helpful for keeping people healthy because they are not very harmful to the body. They are great for treating blood pressure, inflammation, allergies, skin infections, mastitis, herpes infections, cavities, and peptic ulcers.

These are mostly used to treat problems with the kidneys and urinary system, as well as a type of severe stomach infection. The bacteriocins made by LAB are safe to use as food preservatives because they are not poisonous, work against a wide range of bacteria, and can survive different levels of acidity. This helps food last longer without spoiling. Autolysins are enzymes made by bacteria that help with cell growth, division, and building the cell wall. They also help with protein secretion and forming peptidoglycan in the cell. The LytA amidase from *Streptococcus pneumoniae* was the first autolysin to be tested as a potential antibacterial treatment, among many others. They tested how well it kills bacteria compared to another medicine called Cpl-1 lysin and cefotaxime. Both LytA amidase and Cpl-1 lysin were more effective than cefotaxime. LytA was the most effective at reducing the number of bacteria in the peritoneal fluid and blood compared to the other agents studied. Lothar and others published their research in 1987. The autolysins from different types of *Staphylococcus* bacteria have enzymes that can break down certain substances and also help the bacteria stick to surfaces.

AutolysinE (AtlE) helps cells stick to a surface and makes biofilm. The altered atlE strain was much less harmful than the normal type in a rat infection model using a catheter. Autolysin S (Aas) and Autolysin C (AtlC) stick to fibrinogen, and Autolysin S (Aas) also clumps together sheep red blood cells. The person is from *S. Epidermidis* kills bacteria, and helps *S.* stick to things. Gold particles attract and bind to fibrinogen on the surface. Autolysin

Aaa helps the bacteria stick to fibrinogen and fibronectin. Lysozyme is an enzyme found in mucus that breaks down certain substances. It was first found in the nose and later discovered in plants, animals, and microbes like bacteria, viruses, and fungi. These enzymes break down the bacterial cell wall and kill bacteria. Egg whites from chickens and milk from animals are the main sources of lysozyme used in making things at factories. Egg whites have 3400 mg/L of lysozyme and milk has 5840 mg/L. Glutamic acid and aspartic acid help break down the glycosidic bond between NAG and NAM. They need to be in the catalytic center for the enzyme to work properly.

Lysozyme was first tried out in pigs in the mid-2000s. The study found that a special protein from goat milk can make changes in the body's metabolism, gut bacteria, and gut structure. However, improvements in growth performance because of the lysozyme were not seen. Lysozymes, along with antibiotics, can help prevent and treat bacterial infections like sore throat, tonsillitis, diarrhea, and wounds. They were used to carry antibiotic molecules to bacterial cells. Enzymes in a gel were used to treat wounds and acne on the skin. The gel also helped prevent infections from skin piercings. Several formulations of the gel were determined to be beneficial for this application. Mouthwashes contain lysozymes to eradicate oral bacteria, while sprays for respiratory infections also use this enzyme. Lysozymes have the ability to break down viral RNA, which could help fight HIV. In the future, they might be used to treat HIV infections.

CONCLUSION

Enzymes are very important in creating new medicines because they help with finding and making drugs that can help people get better. Their ability to efficiently and accurately change biological processes makes them very important. Studying enzymes for making medicine in a natural way is good for the environment because it uses sustainable methods and is what people want. Using enzymes to treat genetic disorders and combining protein engineering with enzyme studies show how we can create personalized treatments and new ways to help people with these disorders. Enzymes and immunotherapy coming together to treat cancer in a new way.

This can help the immune system fight cancer in a better way. Enzymes help doctors test for and treat diseases in a more personalized way. They do this by using biomarker tests and diagnostic tools. Even though it's tough to make enzymes for medicine in a consistent way, it's really important to make sure they're safe and effective because the rules for how to make them keep changing.

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