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Milk Protein Hydrolysates: Improved Biotechnology from Conventional Dairy Milk

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Abstract—Milk protein hydrolysates are classified as one of the modern biotechnologies in which a much shorter peptide is used to hydrolyze the protein in milk. This biotechnology approach benefits the industry by supplying more bioactive properties to traditional milk protein, and also helps to remove the milk protein allergy that certain people experience. The most popular process used to generate bioactive peptides from a whole protein molecule can be called enzymatic hydrolysis. Another way of generating milk protein hydrolysates is through fermenting milk proteins through the use of proteolytic enzyme starter culture, and it can generate hydrolysates in huge amounts. The key concern that emerges from this industrial milk is that because of the issue of lactose intolerance, if it may lead to non-consumable goods. With the coming out of protein from hydrolysis, this biotechnology may fix this issue and help to deal with it.

Keywords—milk protein hydrolysates, bioactive properties, allergy, enzymatic hydrolysis, microbial fermentation

2.1 Introduction

Biotechnology is an application of any living organisms and biological species on developing new and useful products. It can also be expressed as the use of biological derivatives to produce a specific material for a certain use. The Biotechnology industry has recently been giving a great contribution to the community not only in innovation but also in economy. It was said that technological innovation capability will upgrade a firm's competitive capabilities. The technological innovation capability can also be enhanced by intra-organizational learning activities [1]. In addition, scientific research will positively influence technological performance and improve the rate of innovation.

In building scientific-capabilities, there are two strategies that were being suggested. First, an investment in basic research will give the opportunity for scientific findings. Second, scientific knowledge from the external environments should be absorbed and applied to the process of development. The Biotechnology industry is the most suitable industry that can be studied as it consists of a lot of innovation and is widely recognized for its scientific research [2]. This industry in Taiwan is growing at a faster rate and it has a big potential. It has been stated that the combination of many innovations with the development process and potential technology will continue improving the performance in an organization [1].

Biotechnology has been applied in many types of areas such as medical, industrial and also agriculture. In food processing, it has also been applied by the use of microbial inoculants that have the ability to enhance the chemical properties such as taste and nutritional value. The use of micro-organisms on the food selected for the purpose of preservation and variety of

production such as enzymes, flavor compounds and food ingredients. This can be concluded that the application of biotechnology on food processing consists of specified selection of microbial species in order to improve the overall performance.

The food that is being applied with biotechnology applications must be assured of its quality and safety. The safety of the final product with its physical, chemical and microbiological hazards must be absent in a public health risk. The food safety among the consumers have now become their concerns as there are many food scares that can affect their health such as mad cow disease and the melamine contamination. Therefore, pathogenic bacteria detection in food is one of the methods for food-safety monitoring. The method of monitoring will also be applying the uses of biotechnology in order to increase the speed of detection and improve the performance of the product [3].

Another product of food that applied biotechnology method is milk protein hydrolysates. The process of this product is by going through hydrolysis of milk protein with the help of enzymes and the purpose of the process is to decrease allergenicity, increase adsorption and improve functional properties of proteins for its end-use application [4]. Generally, milk can be considered as the most complete food. It provides a wide range of benefits such as basic nutritional requirements including additional health content that are contained in its bioactive components. The main problem arise from this conventional milk is that once could lead to inconsumable of the products due to lactose intolerant problem. With the emergence of hydrolysis proteins, this biotechnology could solve and help to cope with such problems. All of the advantages which milk protein hydrolysate exists help to solve problems that arise from consuming conventional milk. The advantages will be further discussed in the report. For the process, there are other processes that can produce milk protein hydrolysates such as fermentation whereby it generates similar sized peptides. The bioactive components that contain in the derived milk protein are beneficial to the gastrointestinal tract including some biological activities like suppressing the colonization of pathogenic bacteria [5].

2.2 Biotechnology in Milk Protein Hydrolysates

The product of milk protein hydrolysis is currently being processed by going through enzyme-catalyzed hydrolysis of selected protein. The product is being developed for nutritional purposes and physical functionality. It is also being used to remove intact protein molecules and maintain the nutritional value of milk in infant formula. In addition, whey protein hydrolysates also applied the same type of process and it is being used in sports nutrition. This is due to the fact that it consists of a high level of branched-chain amino acids. It will be metabolized in muscle tissue and this will increase the rate of recovery of muscle after doing any exercises. Whey protein hydrolysates are also said to have a high rate of digestion and this will also provide a fast access to nutrition for muscle [6].

The hydrolysis process usually happens in nature that exists in the gastrointestinal intact and it is stimulated in the laboratory with the use of biotechnology methods. As the milk proteins transit through the tract, it also is being exposed to enzymes such as pepsin, trypsin and chymotrypsin that are capable of breaking down into smaller peptides and it will be further digested. The method of biotechnology will be applied when the milk proteins are being treated with food grade enzymes or by the process of fermentation with bacteria and this will release milk protein hydrolysates [5].

2.2.1 Enzymatic Hydrolysis

Enzymatic hydrolysis can be considered as the most frequent method that is being used to produce bioactive peptides from a whole protein molecule. Pepsin and trypsin are examples of enzymes that can be used to achieve the purpose of the process [7]. Table 1 showed the enzymatic hydrolysis of milk proteins using proteases and the effect on the functional properties of hydrolysates [8]. Furthermore, the process requires a moderate condition where the pH value is between 6.0 and 8.0 and temperature is between 40 and 60 ºC. This is to minimize any side reactions and to maintain the composition of amino acids constantly throughout the process. One of the advantages of this process is that it can increase the solubility and heat stability of the peptides.

Table 1: Enzymatic hydrolysis of milk proteins using protease and effect on function properties of hydrolysates [8]

Protein source	Enzymes	Mechanism of action	Parameters of hydrolysis	Degree of hydrolysis	Changes in functional properties
Milk protein concentrates	Chymotrypsin	Endoproteases	pH 8.0/50.0°C/5-10 min	24.3-24.4%	At pH value between 4.6 and 7.0 the solubility increases. Hydrophobic surface and strength of gel decreases. An increased in emulsification capacity
	Trypsin		pH 8.0/37.0°C/10-60 min	14.8-15.1%	
	Pepsin		pH 2.0/37.0°C/240-720 min	5.0-5.7%	
	Papain		pH 6.8/60.0°C/30-180 min	7.2-9.8%	

Water addition and pH adjustment will be conducted in order to achieve a successful protein hydrolysis process. Raw material of protein will dissolve in a buffer at a specified pH. Another method that can be added in the reaction is by using a buffer and the addition of water will give the advantage to monitor the progress since the buffer is required to maintain the target pH value and prevent pH variations from happening. However, there is an effect of using a buffer in this process whereby the proteolysis could not be determined since the indicator for the enzymatic process to complete is when the pH decreases and the process will no longer occur.

Theoretically, this process will have an accumulation of H^+ and causes the pH of the reactant to decrease with time. Hence, hydrolysis rates must be maintained at an optimum rate by avoiding the inactive protease activity failure. This can be done by adding sodium hydroxide, NaOH, a suitable base that can neutralize H^+ and bring back the pH value to optimum value. The side effect of the NaOH addition will cause a high content of sodium in the product. Therefore, the addition of NaOH is not necessary. When the process of protein hydrolysis is complete, there are some methods that can be used to stop the reaction. First, the pH value in the reaction can be altered to a value that can make the enzyme inactive. An adjustment of pH value between 4.0 and 5.0 is recommended since the enzyme activity of enzyme will be activated out of this range. In addition, the adjustment will enhance the separation of the soluble peptides during centrifugation to produce peptide-rich supernatant. Second method is by holding the temperature above 80°C for about 5 to 20 minutes which will denature the protease and prevent the enzymatic reaction from proceeding. The reaction mixture can also be stored in a cold temperature below 20°C as it also stops the enzymatic reaction. Figure 1 showed the flow chart for the enzyme digestion to produce protein hydrolysates. When the process is complete, the reaction mixture will undergo centrifugation in order to isolate the supernatant as the peptides were placed [9].

In order to conduct this process, it requires an approval status of 'generally recognized as safe' (GRAS) and the status of 'food grade' quality is also considered as safe. Trypsin, pepsin and chymotrypsin are some of the enzymes that have the approved 'food grade' quality [5]. Trypsin is widely used in this process because it has the highest angiotensin-converting enzyme (ACE)-inhibitory peptides and calcium binding production [7]. There are also other gastrointestinal enzymes and variety enzyme combinations that can be used for enzymatic hydrolysis of whole protein molecules but are not considered as 'food grade' status. At the end of the enzymatic hydrolysis process, there is an additional treatment that will be given to the hydrolysates such as ultrafiltration, heat treatment and activated carbon treatment. The reason for this treatment is to eliminate the bitterness that comes from the hydrolysates [5].

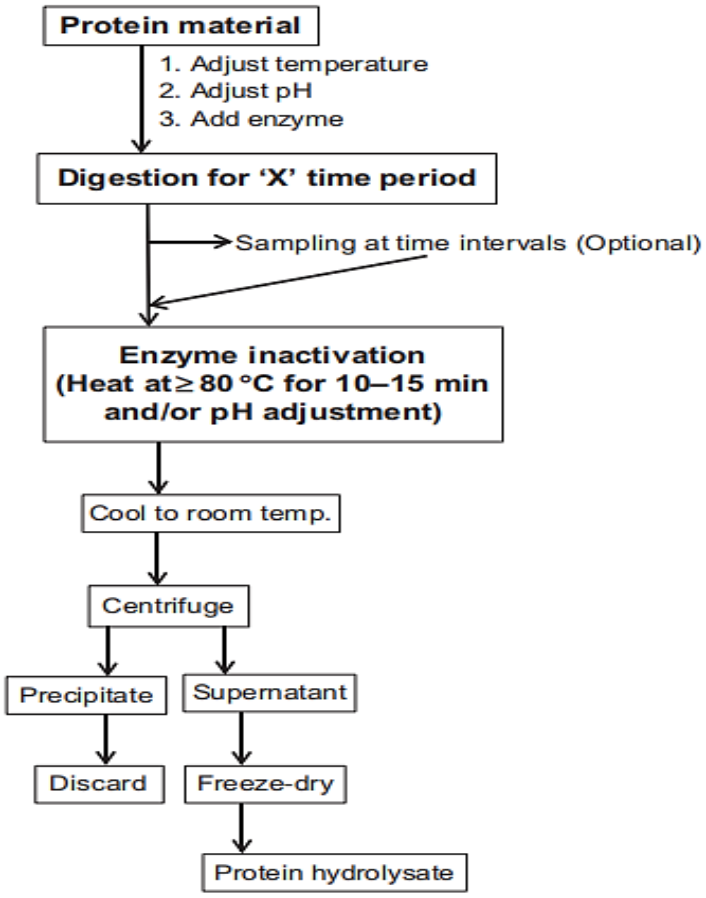

Figure 1: Flow chart for enzymatic hydrolysis process [9]

2.2.2 Microbial Fermentation

Another method of producing milk protein hydrolysates is through fermentation of milk proteins by using proteolytic enzyme starter culture and it can produce hydrolysates in a huge quantity. However, as the microorganisms used for this process have such characteristics that are toxic and pathogenic, safety measures must be taken seriously. There are two types of proteolytic enzymes and it depends on their hydrolyzing mechanisms. First is the endopeptidases that hydrolyze peptide bonds within protein molecules and second is exoproteases that hydrolyze nitrogen or carbon terminal peptide bonds [5]. Moreover, yogurt and cheese starter bacteria have given a result whereby it produces different bioactive peptides in milk during fermentation.

A past research on this process stated that the fermentation of milk protein with dairy starter culture gave an antioxidant activity. The peptides that are being generated showed an increasing value due to the activity [7]. As the fermentation is due, the proteolytic enzymes system microorganisms will break down the milk protein and bacterial culture will usually be used to produce hydrolysates. Lactic acid bacteria (LAB) is a proteolytic system that stores cell envelope-associated proteinases, endopeptidases, aminopeptidases, tripeptidases and dipeptidases which can help in producing hydrolysates. Milk protein will undergo degradation and produce free amino acids and peptides for the growth of LAB. Figure 2 represents the proteolytic system of LAB [10].

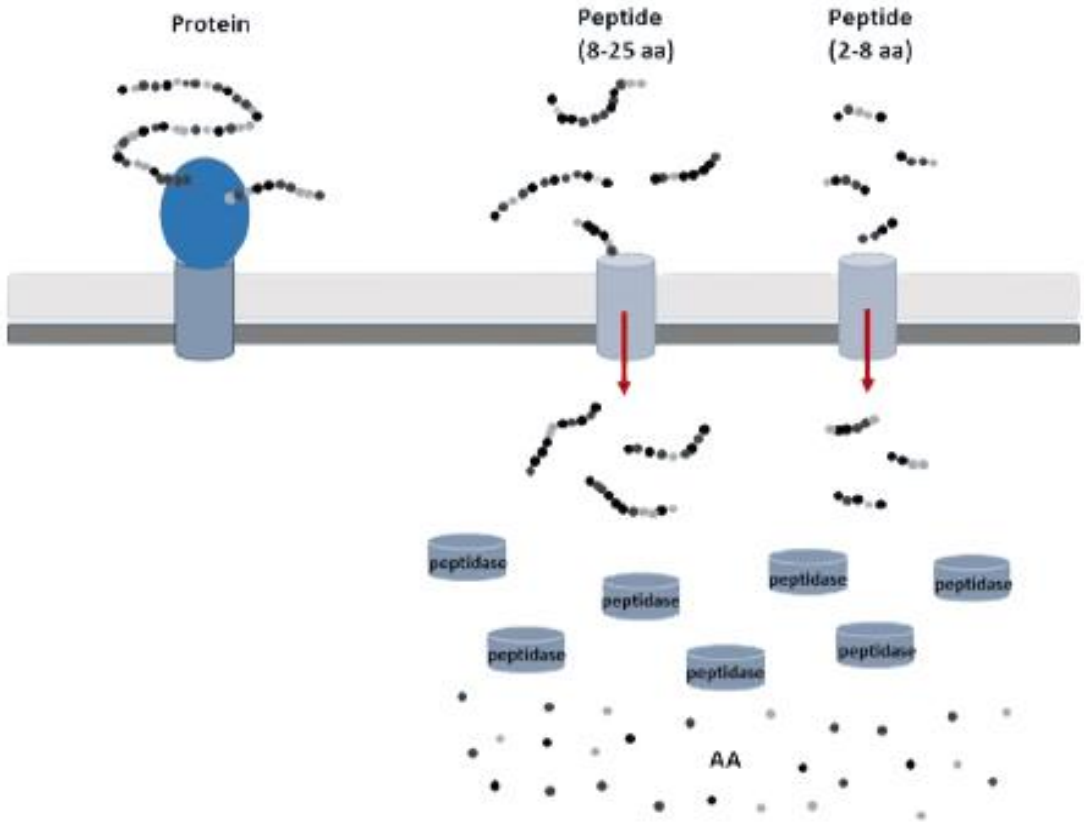

Figure 2: Proteolytic system of LAB [10]

The hydrosylates that are not being utilized by the bacteria will be promoting various bioactivities. LAB has the ability of hydrolyzing more than 40% of peptide bonds which also produces oligopeptides from 4 to 40 amino acid residues. There are some parameters that can affect the production of hydrosylates from milk protein such as enzyme-substrate ratio, composition of medium, heat treatment, temperature, pH and carbon-nitrogen ratio. The use of LAB culture with food grade enzymes is a good biotechnology method in order to hydrolyze milk proteins. This is because the method will increase the amount of peptide content of hydrolysate and create more diversity in terms of bioactivity of hydrolysates [5].

2.3 Advantages of Milk Protein Hydrolysates

2.3.1 Overview

Milk protein hydrolysates are known as one of the industrial biotechnologies which are being used to hydrolysis protein in milk into a much shorter peptide. Generally, the conventional dairy milk which is present in the industry contains the majority of water content and others contain different volumes of fats, protein and carbohydrates on different types of milk [4]. Technically, milk is considered as food with a lot of nutritional benefit and one of the nutritional benefits which caught biotechnologist attention are the nine essential amino acids contained in milk [11]. Thus, altering this protein into a shorter peptide contributes with vast advantages in such aspects. This method of biotechnology helps the industry by providing bioactive properties more to conventional milk protein and also helps to eradicate allergies to milk protein that certain people possess. There are a lot of advantages which could be discussed from milk protein hydrolysates in terms of cost, production and energy used and how it is different compared to conventional one.

2.3.2 Advantages in Term of Cost

In terms of cost, the method for milk protein hydrolysates (MPH) is now more preferable due

to its simplicity compared to the native milk. Nowadays, the increasing demand of milk protein hydrolysates (MPH) leads to the enhancing and better method of protein hydrolyse.

One of the methods discussed which cost savings are by the addition directly of the enzyme chosen to the solution of protein. The process might be a little bit extended compared to conventional milk but then it provides a better result. The addition is not considered too expensive due to the use of living microbes or enzymes, for example Lactobacillus casei. The addition then will create hydrolysis induction until the desired hydrolysis degree. Then after the reaction completes (protein hydrolysed), the reaction will stop when the solution is heated. This is ensuring inactive enzymes [12]. Even though the process production cost is slightly high compared to conventional milk, it is still preferred by industry.

2.3.3 Advantages in Term of Production and Energy Used

In terms of production, there are 3 methods well known for hydrolysates of milk protein. All those methods carried out with respective pros and cons regarding each process. One of the methods had been explained by adding selected enzymes to the milk protein solution. The method sure is cost effective but there are also several cons that might be encountered. One of the disadvantages is that hydrolytic products are difficult to regulate. Time and state of the reaction significantly affect the form and concentration of the hydrolytic products but then if the time is being controlled carefully, misled products will not occur [12].

For bioreactor membrane method, commonly used in the continuous removal including hydrolysis chosen hydrolytic materials. Reaction vessel is in a closed circuit connected to a membrane system. Within the reaction vessel, the chosen enzyme is put into the protein solution. After starting incubation, pumped the vessel contents, allows the hydrolytic product into the membrane system to be selectively separated based on its molecular mass and the cutoff of membrane molecules. This process is much more efficient compared to classical methods due to their process membrane retaining the enzyme from flow back to reaction vessels. From previous research, this process had been used by using Casein protein and the enzyme for the protein to hydrolyse was Aspergillus oryzae. The product has reduced immunologically active casein and immunologically active whey proteins by 99% and 97% respectively [13].

Enzyme immobilization is also carried out through the biotechnologies process. The advantages by using this method/ energy for production are selectively isolated by membrane filtration to the hydrolytic products easily in the enzyme problem immobilised in the membranes. Enzyme immobilisation is carried out on a membrane or solid matrix, covalently bound and used for protein hydrolysates preparation. The immobilised enzyme is either mixed for a certain amount of period in the protein solution. Next, extracted by filtration or the protein solution is going through an enzyme bed. The hydrolysate protein is collected at the end column [12].

2.4 Effect of Milk Protein Hydrolysates on Today Society

2.4.1 Overview

Generally, the general definition defines milk as any liquid that is appropriate for consumption of mankind, offering nutrition, refreshment and delicious flavor and taste. It can have a stimulating effect on people/society, or not. Additionally, to one or more of the above purposes, functional beverages have other health benefits. But certain societies need to face up to reality and problems arise from beverages. One of the problems arises from a portion of society which is lactose intolerant. Lactose intolerant society cannot consume dairy products especially milk because of the existence of lactose (a type of sugar found in dairy products). Those with hydrolysate protein will come out a pure protein with a little amount of lactose, consumable for lactose intolerant consumers [13]. Other effects might vary towards different kinds of society and those effects are important to be recognized with.

2.4.2 Protein Hydrolysate

The production of protein hydrolysates consists of a mixture of proteins and peptides that resulted from the hydrolysis process. The process will make the peptide bonds broken from the in proteins (Figure 3(a)). Figure 3 shows the process of protein hydrolysis and its product. Protein hydrolysates can also be applied in various products whereby it can be used in

clinical and sport nutrition which function as a digestion support. Moreover, the product can also be considered as a good source of bioactive peptides. The variation in the peptides will have different kinds of effects (Figure 3 (b)) [14].

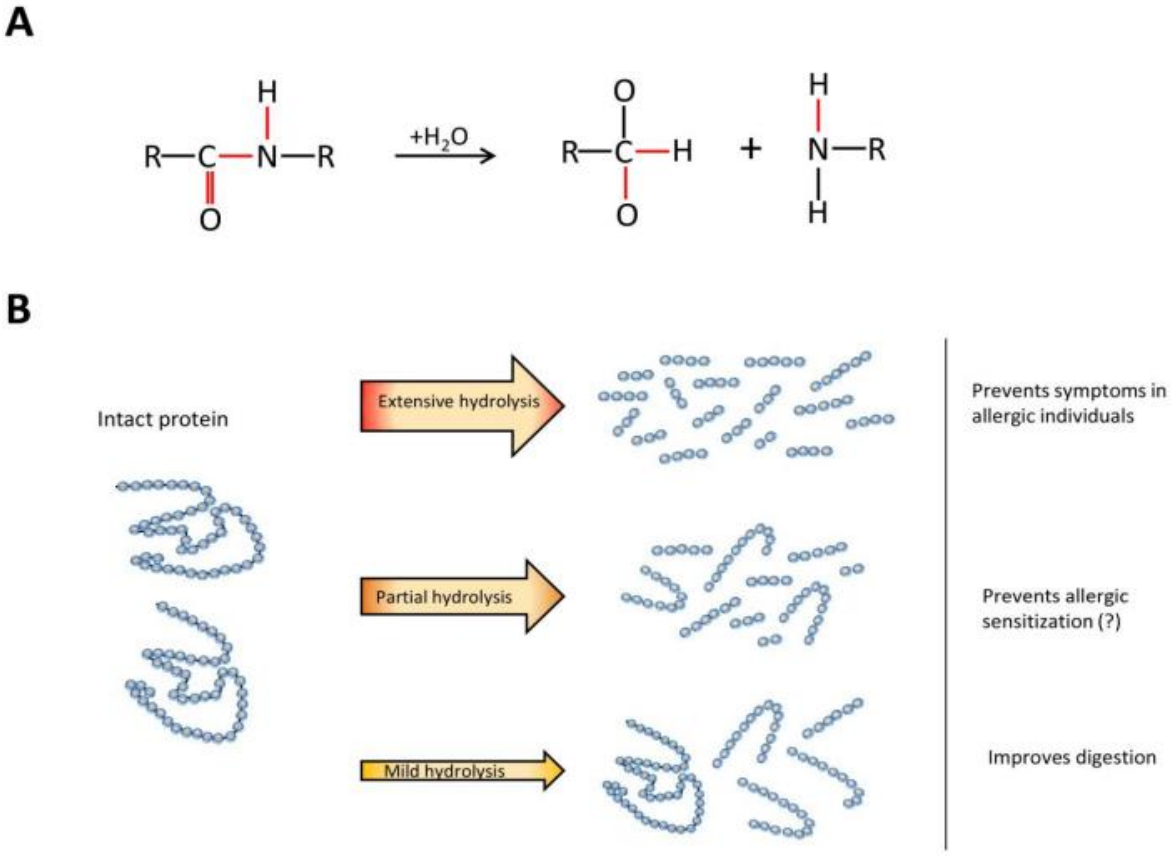

Figure 3: Protein hydrolysis process and its product. (**A**) reaction of protein hydrolysis (**B**) different peptides give different effects [14]

2.4.2.1 Protein Hydrolysate on Human Nutrition

When humans consume protein hydrolysate, it has been shown that the intake of protein hydrolysates results in a rapid absorption of amino acids contrasted to intact protein or amino acid mixtures which are free. Protein hydrolysates can also have peptides which are biologically active exhibiting a few physiological effectiveness. Furthermore, relative to the intact proteins,

They are distinguished by hypoallergenic properties. All of these amino acids, peptides and hypoallergenic characteristics generate protein hydrolysate which prior for medical food. The medical food could be stated as supplements, weight control, sport nutrition, and even for those patients who suffered with digestion [11]. On the other hand, people who are severe with phenylketonuria were also benefited with the consumption of modified protein hydrolysates.

Table 2: Protein hydrolysates in human nutrition [17]

Protein Supplementation	Clinical Use
● Energetic drinks ● Geriatrics products ● Sports nutrition ● Weight-control diets	● Phenylketonuria (PKU) ● Hypoallergenic infant formula ● Acute and chronic liver disease ● Short bowel syndrome ● Crohn's disease ● Pancreatitis ● Ulcerative colitis

2.4.2.2 Protein Hydrolysate on Biotechnology Application

Even the uses itself are considered biotechnology, protein hydrolysate are also used for growing microorganism by providing nutrition to the microorganism. A wide area of biotechnology has been covered including cell culture, recombinant culture, plant cell culture and also as a media for growing microorganisms [12].

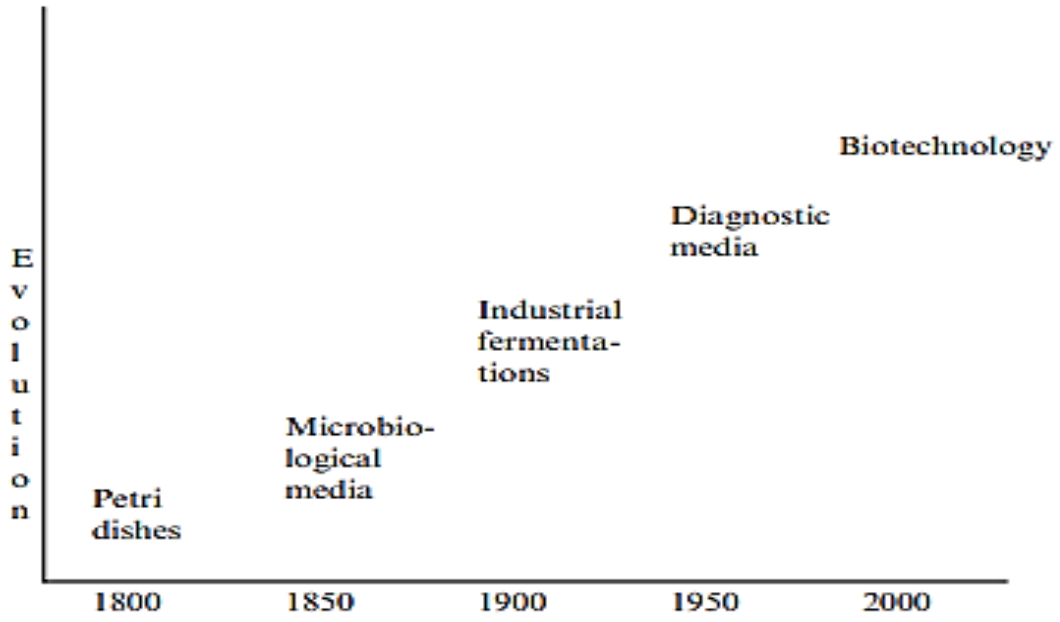

Figure 4: Protein hydrolysates evolution on biotechnology [18]

2.4.2.3 Protein Hydrolysate for Food Industry Application

For food industry society, the effect brings significant impact towards achieving a good yield of product. It is recognized to be a better substitute as it has great emulsifying properties. This is due to the limited hydrolysis of protein; it changes their functional properties. Thus in return for their emulsifying properties, it has been used to emulsify food products. One of the examples is an emulsifier for meat [12].

2.4.2.4 Protein Hydrolysate for Animal Feeding

In many concerns, when the quality of feed contains high protein, the livestock will become more productive. Animals need to be fed with high quality food. The effect from good quality food will then be helping for improved protein quality products [12].

The improved protein quality product here could be varied. One of the incorporated protein hydrolysate includes production of quality and high amount of output of milk. This is due to quality animal feeding associated with protein hydrolysate application. Others also include quality meat from animal feeding.

2.4.2.5 Protein Hydrolysate for Fermentation Industry Application

Fermentation is the most crucial in living up to sustainability and biotechnology. The method of fermentation itself helps in order to increase the lifespan of certain products. For microbial cultures, a medium is needed to act as a nutrition support for the microbes to be fermented. The use of protein hydrolysate is always on point for medium purpose for microbial cultures [12].

Thus the use of protein hydrolysate in the fermentation industry is ideally to improve microbial cultures plus to maximize fermentation product.

2.4.2.6 Protein Hydrolysate for Cosmetic Industry Application

Cosmetic industry has become an important product which is mainly useful for women. Cosmetic here could also refer to a product for treating and nourishing all body parts including skin, nose, eyes, lips and also hair. The use of protein hydrolysate is also involved with the cosmetic industry [12].

Currently, plant and animal sources also have enzymatic protein hydrolysates function mainly in personal care products as skin and hair care agents [12].

2.4.3 Milk Protein Hydrolysate

2.4.3.1 Prebiotic Activity of Milk Hydrolysates

The importance of prebiotic activity could be seen clearly from breastfeeding the infant. This process of biotechnology could become one of the substitutes for breastfeeding methods due to their effectiveness, targeting mother and newly child born society. From research, breastfeeding helps to colonise the gut by the aid of healthy microbiota [5]. Commonly, gut microbiota healthily provides metabolic, nutritive, and protective functions. Infants really need this nutritive support as it develops a balanced immune system for individuals. Other than that, the microorganism involved also helps from milk to release growth substrates and the vaccine responses effectiveness.

Surprisingly, Anindya [13] provide with a findings in which milk hydrolysates could also come out with near significance as breastfeeding could provide.To be reffered, a proteolytic casein hydrolysate and whey protein hydrolysates fermented with strains of Lactobacillus casei effectively stimulate Bifidobacteria spp. Other than that, both peptides support Bifidobacterium and Lactobacillus spp. growth is generated by proteolytic digestion of β-lactoglobulin which is bovine milk containing many whey proteins that cannot be found in human milk [5]. From the research, clearly hydrolyse milk engages with such an outcome that could provide prebiotic activity for human health. The effect brings out good value which later could help future society in concern for mother and newly born baby. From studies, the use of milk protein hydrolysate for infant nutrients is the most important compared to others. Figure 5 shows the market size of milk protein hydrolysate in some of the applications in the United States.

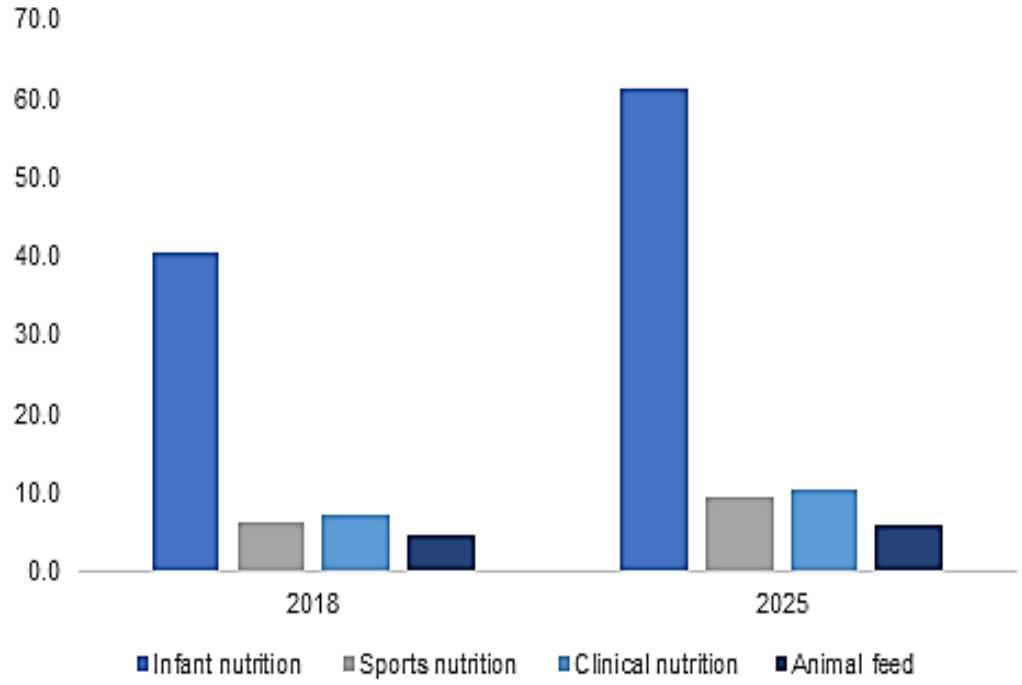

Figure 5: Market size of milk protein hydrolysate by application in the United States. Figure shown infant nutrition purpose dominate market due to its effectiveness (Adapted from 'U.S. Milk protein hydrolysate market size by application' by gminsight, 2019) (Source: www.gminsights.com)

2.4.3.2 Gastrointestinal Mucosal Integrity of Milk Hydrolysates

In between conjunction of the gut-associated lymphoid tissue (GALT) of the gastrointestinal tract exist with the intestinal epithelial cell layer. This region in our body contains the most amount of lymphocytes, used for the immune system. There is also a functioning as the protective layer (the mucus) and covering the intestinal epithelial cell layer. This protective layer helps in prevention of pathogenic microorganisms, including harmful toxins plus allergens from entering the body. Mucins is what is called mucus gel with glycoproteins composed [5].

The relation of mucins with selected biotechnology is that milk hydrolysate can affect mucins secretion. The modulation of milk hydrolysates in the production of mucin can help in the creation of dietary strategies to improve and defend the mucous layer [5]. The help to society may assist those who suffer with thin epithelial cell layers as protective mucus are extremely low to face up physical impact or microbial damage.

2.4.3.3 Gastrointestinal Immune System Modulation of Milk Hydrolysates.

Effect to society of hydrolysate milk protein could also impact on the immune system. Specifically, immunoglobulins could be increased in the body when consuming several milk protein hydrolysates. These immunoglobulins act as glycoprotein that specifically identify and assist in the removal of antigens from bacteria or viruses via a highly complex and precise immune response [5]. Thus, increasing the immune system is possible with the correct amount of milk hydrolysate protein.

Eventually, the existence of intestinal mucosa is for the battle of 'physiological inflammation'. Low inflammation is required to fight against pathogenic bacteria. Anti-inflammatory from a variety of milk hydrolysate are there for the purpose of gastrointestinal immune system. This immunisation is important as an inflammatory act and tissue repair [15]. Recent studies show that milk hydrolysates can modify the gastrointestinal tract's immune system by influencing the proliferation and maturation of localized immune cells [5].

Some of the product examples of protein hydrolysates are yogurt or milk fermented by *Lactobacillus casei* and milk fermentation products of *L. Helveticus*. Both of the products have the function of increasing cell proliferation and villous area in the intestine including enhanced the expression of calcinuerin in the small intestine. As for milk protein hydrolysates, it increases the level of hemoysin in serum and improves the phagocytosis of macrophages [14].

2.4.3.4 Food Products of Milk Protein Hydrolysates

In several areas the utilization of milk protein hydrolysates as modifying food texture was defined. Not only can milk protein hydrolysed be consumed, it is also used in food products. Casein was hydrolysed with pepsin hydrolysis while complete hydrolysate (CH) and supernatant obtained by centrifugation (S). Both CH and S have been used to substitute, in part, the phosphate salt used to produce processed cheese. The casein hydrolysate and its soluble fraction showed good emulsifying properties, meltability, microstructure and textural properties in processed cheese, except for the high use of the S fraction [12]. The application of casein hydrolysates and the fraction thereof did not adversely affect the organoleptic properties of the processed cheese [12].

Other than that, other food products involving milk protein hydrolysate include production of cheese. The introduction of milk protein hydrolysates involves beneficial outcomes on probiotic survival in yoghurt and the product consistency. The introduction of casein hydrolysate or whey protein hydrolysates increased acidification and reduced yogurt coagulation period, decreased post-acidification during storage, improved probiotic survival, improved organoleptic properties and yogurt and yogurt adhesiveness by open texture and decreased graininess were characterized [12]. From (kwok et. al) studies, the use of hydrolysate mixture mixed with traditional emulsifier, casein resulting with compromising result (Table 3).

Table 3: Different emulsifiers sensory properties of manufactured process cheese [19]

Treatment	Flavor	Texture
T[2]	6.35 ± 1.31[b]	6.05 ± 1.36[b]
H[3]	4.75 ± 1.68[a]	4.25 ± 1.52[a]
S[4]	4.40 ± 1.35[a]	4.60 ± 1.64[a]
H+T[5] 3:1	6.45 ± 1.15[b]	6.20 ± 1.44[b]
S+T[6] 3:1	6.10 ± 1.74[b]	5.50 ± 1.80[ab]

[1]Means with a common superscript in the same column are not significantly different ($p > 0.05$). Means of triplicate. As the value increases from 1 to 9, the intensity of the sensory properties increase.
[2]T = control, 3% of traditional emulsifier (mixture of sodium phosphate and sodium citrate)
[3]H = 3% hydrolysate
[4]S = 3% supernatant
[5]H+T = 3% mixture of hydrolysate and traditional emulsifier
[6]S+T = 3% mixture of supernatant and traditional emulsifier
[7]Ratio of hydrolysate (H) or supernatant (S) to traditional emulsifier (T)

As shown in Table 3, the process cheese score was 6.45 and 6.10, respectively (hydrolysate mix with traditional emulsifier casein and supertant mix with traditional emulsifier casein. From the result, it clearly showed that casein hydrolysate could replace conventional cheese production due to its improved emulsifying activity by hydrolysate.

2.4.3.5 Milk Protein Hydrolysates for Clinical Nutrition.

As explained before, some people might experience being allergic or lactose intolerant. Food allergy can be described as "an adverse immune-mediated health effect that occurs when exposed to a specific food [12]." For infants and babies, cow milk is the most allergenic food. The problem of allergy to cow milk (CMA) varies from almost 2 percent of newborn babies. When newborn babies get international protein they are sensitized either in cow's artificial milk or in substitute of commercial human milk. Its symptoms can include gastrointestinal, cutaneous, breathing or general symptoms [12].

Hydrolysates have long been used to treat these allergies and intolerance, plus recently to

avoid atopic dermatitis in infants at high risk. Hydrolysates have to be free of immunogenic and antigenic peptides and proteins for milk allergic patients [12]. Controversial findings based on immunogenicity and allergenicity of molecular-weight peptides below 3000 Dalton have been published [12]. Peptides smaller than 2500 Dalton are considered not immunogenic. It is worth noting that partially hydrolysed protein can induce specific antigen tolerance [12]. The ingestion of partially hydrolyzed whey proteins will induce particular oral tolerance to ß-lactoglobulin thus hydrolyzing the whey protein hydrolysate extensively. They concluded that in the prevention of allergies in at-risk infants, comprehensive casein hydrolysates and partially hydrolyzed whey protein methods could be noted as an effective substitute for human milk. Consumption of partial whey protein hydrolysate has been commonly prescribed to prevent allergic disease from emerging in early childhood. Nevertheless, a randomized trial did not disprove the idea for the use of partially hydrolyzed whey proteins in sewage for the prevention of allergic diseases in infants at high risk [12].

Next is for phenylketonuria (PKU). The one of phenylketonuria (PKU) is prelevant genetically inborn metabolism errorsIt is characterized by high blood levels of Phe and significant excretion of its metabolites due to the deficiency of the hepatic enzyme phenylalanine hydroxylase, which converts Phe into Tyr. PKU is estimated to predominate at a ratio of 1/15,000. Placenta protects the prenatal infants, so that the baby is born naturally. Blood Phe rise begins within a day of birth and causes brain damage if not treated. In untreated PKU subjects permanent neurological damage occurs and is clinically expressed as mental retardation [12].

Dietary restriction of consumed Phe, when done early in infancy, is the effective treatment for PKU subject. Nevertheless, food and drink intake should contain limited and regulated quantities of Phe in order to preserve normal development of PKU samples as Phe is an essential amino acid for the integrity of the neurosystem. Several low Phe preparations were produced by enzymatic hydrolysis of milk protein fractions and corresponding elimination of free Phe from the hydrolysate by adsorption with numerous matrixes or through nanofiltration [12].

As for clinical purposes, milk protein hydrolysate contains a Glycomacropeptide (GMP) [12]. This GMP is known to be used for cheese production. Glycomacropeptide (GMP), during cheese development, is an amino acid glycosylated peptide. The use of GMP as an alternate source of protein for phenylketonuria (PKU) diets was referred to with outstanding review. GMP is currently industrial-scale isolated from cheese whey and commercially exists as a food product, with outstanding safety records. GMP does not contain aromatic amino acids like Phe. It is therefore special for phenylketonuria (PKU) nutrition and other commercial GMP foods like food and beverages which are now available on the market. However, in order to produce a healthy diet, supplementation with other essential amino acids which are lost from GMP likely tryptophan is required [12].

In conclusion, for clinical purposes, milk protein hydrolysate induces with so much effect. The effect is for food allergy/intolerant and for phenylketonuria (PKU) and thus help nowadays society to bring out good deeds.

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