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Mini Review

Utilizing Coagulant Plants in the Development of Functional Dairy Foods and Beverages: A Mini Review

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Abstract

Proteases are commonly available to the dairy industry and becoming saturated in their potential for further productivity among health-conscious people, which has driven positive attention towards plant-based coagulants. This review explored the possibility of coagulant plants as an ideal choice in the development of functional dairy foods and beverages and the benefits that come along to health. Dairy products like cheese require coagulation of milk by using enzymes such as rennet either in its original state, purified or genetically modified. Animal and microbe sourced coagulants have been facing many challenges due to increasing public awareness. Plant proteases are not new and have been identified previously to have the ability to coagulate milk but plants are less explored and understood in their potentials in being a milk coagulant and fortifying the curd with useful biological activities. Currently, people are looking for functional foods that provide various health benefits when consumed rather than calorie-rich food, which causes diseases. In recent times many plants are able to coagulate milk. These particular plants have been identified as an ideal choice in the development of functional dairy foods and beverages due to their dual ability of first coagulating the milk and then fortifying the curd with biologically useful compounds.

Key words: Plant proteases, coagulant, dairy industry, plant-based coagulants, functional dairy food

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INTRODUCTION

The dairy industry is an important and lucrative industry. The global dairy market was worth USD 413.8 billion in 2017 and is expected to exhibit decent growth in the upcoming years¹. One major product of the dairy industry is cheese. Coagulants are a crucial element in cheese making and rennet, which are derived from animal origin². However, due to the various controversies surrounding animal rennet and as the potential of the proteases commonly available for the dairy industry become saturated in terms of further productivity, proteases from different sources are being investigated as substitutes. One suitable alternative is plant-based coagulants. According to Silva and Malcata³, the application of plant-based coagulants allows for targeted cheese production and hence contributes to improve the nutritional input of those populations on whom restrictions are improbable by the use of animal rennet. The benefits of using plant instead of animal proteases, such as rennet or genetically modified organism (GMO)/living modified organism (LMO)-derived enzymes are many. Some of these benefits include ethical, religious and cultural acceptability⁴. The rich source of bioactive molecules in plants makes them a source for biotechnological applications⁵. Studies have shown that useful proteins can be obtained from plants. For instance, soy proteins can be obtained from soy beans and are good for combating cardiovascular diseases because they can reduce blood lipid levels⁶.

Currently, the shift in attention among people is towards functional foods that provide various health benefits when consumed rather than calorie-rich food, which causes diseases⁷. Furthermore, issues such as the use of enzymes sourced from slaughtered young animals⁸ from religiously unacceptable sources⁹ (Fig. 1), controversies of modern biotechnological techniques¹⁰, prion-related diseases¹⁰ and many more have promoted the use of plants as coagulant. Hence, this review explored the use coagulant plants in the development of functional dairy foods, beverages and the benefits that come along to health due to their dual ability of first coagulating the milk and then fortifying the curd with biologically useful compounds.

SUBSTRATES WITHIN MILK

Milk proteins: A total of 87.4% of milk content is water, which is a polar inorganic compound and is known as a universal solvent. Due to its polarity, water can react with many molecules and assist the condensation process of lactose into



Fig. 1: Pecorino di farindola cheese made from porcine rennet

Source: Suzzi *et al.*⁹

the simple sugar galactose and glucose. From the remaining 12.6%, milk is made up of 3-4% total protein and its protein includes all 9 essential amino acids required by humans¹¹ (Fig. 2). Milk proteins are synthesized inside the mammary glands of mammals and the protein in cow's milk is one of the key reasons that milk is a crucial component of the human diet. The complete protein content of milk is composed of numerous specific proteins and 60% of the amino acids used to construct the proteins are acquired from the animal's diet¹².

Generally, two primary groups of milk protein are characterized, namely, casein and whey protein. Among them, the primary protein is casein. Caseins are distinct molecules that are comparable in structure but each protein has its own amino acid composition, genetic variations and functional properties¹³. Caseins have the appropriate amino acid composition that is crucial for the growth and development of the young ones and are fairly digestible in the intestine. Casein is one of the most abundant organic components of milk, in addition to lactose and milk fat¹⁴. Three different caseins, namely, alpha-casein, beta-casein and kappa-casein, naturally exist in milk and can be isolated using electrophoresis¹⁵.

Casein (Fig. 3) usually exists in the form of micelles (a dense protein granule). Caseins are first phosphorylated, followed by the binding of calcium ions to phosphate to initiate the formation of casein micelles. Casein micelles play an important role in the digestion of milk in the body and serve as a basis for the milk processing industry including the cheese-making industry¹⁶. As proposed by Guetouache *et al.*¹⁷, kappa-casein is the most studied casein due to its stabile micelle and its important role in dairy processing. All other proteins present in milk, such as immunoglobulins, serum albumin, enzymes and nitrogen compounds are classified together under whey proteins¹⁸.

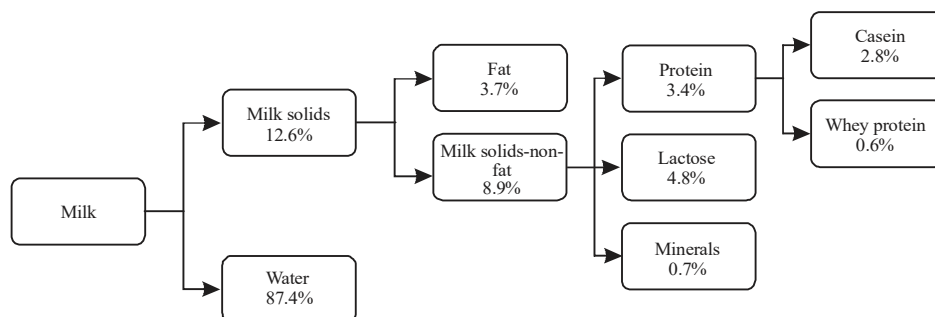


Fig. 2: Constituents of milk

Source: Guerra¹¹

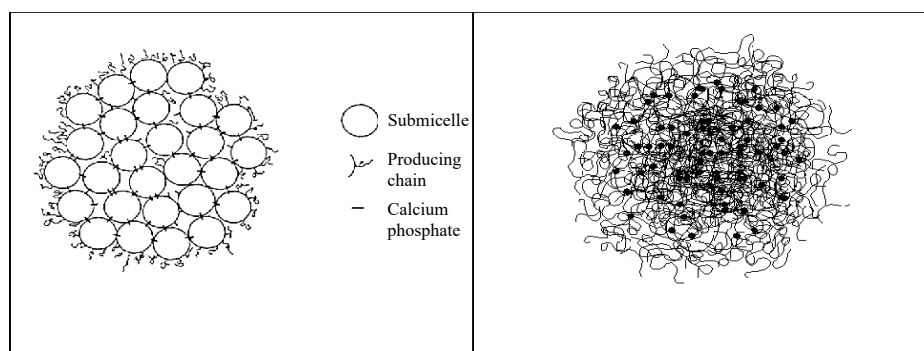


Fig. 3: Model (cross-section) of casein micelle

Source: Gaucheron¹⁶

MECHANISM OF MILK COAGULATION

Milk coagulation is essentially the process of milk (liquid) clotting to form curd (a gel-like structure). Fox *et al.*¹⁹ reported that milk coagulation is the primary step in most forms of dairy processing for the purpose of texture development in products such as; yogurt and cheese. The earliest report of milk coagulation stated that the coagulation of milk may have been accidental in the beginning, before the invention of pottery (5000 B.C.)²⁰. During that period, milk was normally kept in bags made from the stomachs of ruminants and the enzymes remaining in the stomach tissue, particularly renin, would cause the coagulation of milk during storage. In the modern dairy processing industry, at least two types of coagulation are used extensively, namely, enzyme-mediated coagulation and acid-mediated coagulation²¹.

The coagulation step has a large impact on the efficacy of the cheese production industry. Coagulation separated milk into solid parts, curd and liquid parts, whey. After a firm curd is obtained, the whey is discarded²². This is because it is the curd that will be transformed into cheese. In this process, the fats and casein are concentrated and solidified while the water that makes up 87% of the milk is removed together with the

whey protein²². Milk consists mainly of casein micelles (Fig. 3) and these micelles play the most important role in milk coagulation. Casein micelles are hydrophobic, which means they are non-polar molecules that repel the water molecules by not forming ionic or hydrogen bonds with them¹⁶.

The presence of stabilizing factors in the casein micelles prevents coagulation from occurring²³. The first factor is kappa-casein, which is present on the surface of casein micelles. Kappa-casein is responsible for the hydrophobic characteristic of casein. The three-dimensional structure of kappa-casein enhances hydrophobicity and forms hair-like layers on the surface of casein²⁴. The second factor is the negative charge of the casein micelles. Milk has a pH of 6.5-6.7, which makes it slightly acidic. At this pH, the casein micelles repel one another due to their negative charges²⁵. However, various methods, such as the addition of enzymes, acid treatment or heat-acid treatment, cause the aggregation of casein micelles and make them stick to one another. This sticking process is known as the process of coagulation²⁶.

Enzyme-mediated coagulation: The enzyme-mediated coagulation of milk is achieved through the modification of casein micelles using chymosin, also referred to as rennet, a

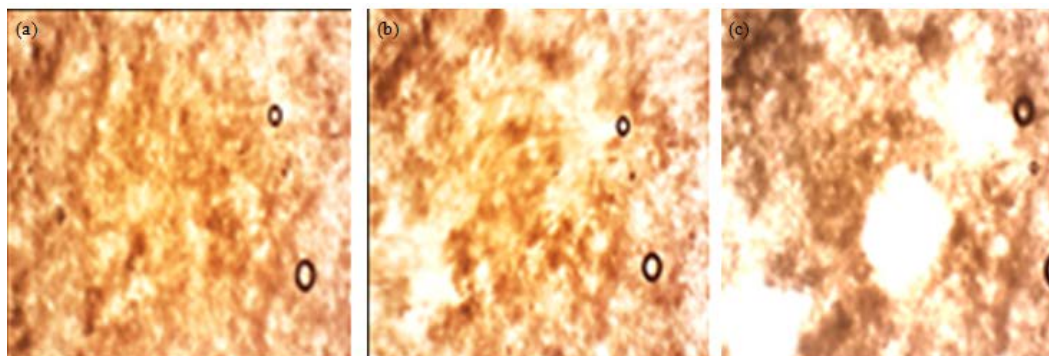


Fig. 4(a-c): A tiny drop of milk under light microscope, (a) 100× magnification, (b) Before coagulation and (c) During coagulation with rennet and after coagulation (transparent gaps visible due to curd and whey formations)

proteolytic enzyme. Chymosin is the most active and abundant component in animal rennet and it cleaves the peptide bond Phe (105)-Met (106) of kappa-casein²⁷. Chymosin is a type of protease enzyme that is closely related to pepsin. Rennet is a complex enzyme with chymosin as its main component and can be derived from animals, including cows or pigs and from plant sources²⁶.

Generally, there are three phases in chymosin-mediated milk coagulation²⁷. During the first phase, kappa-casein is enzymatically hydrolysed to produce hydrophobic para-kappa-casein and a hydrophilic macro-peptide (also called casino-macro-peptide)²⁸. The para-kappa-casein remains attached to the micelle core, while the macro-peptide diffuses into the whey. This process immediately results in the steric destabilization of kappa-casein due to the loss of the surface potential of a negatively charged group²⁸. According to Anema and Li²⁹, when approximately 70% of the kappa-casein is hydrolysed, the colloidal stability of the micelles is greatly disturbed allowing the spontaneous, secondary aggregation phase to take place. When molecular chains connect to form a three-dimensional network through hydrophobic interactions, a gel-like structure forms as casein micelles lose their solubility. The gel is then further solidified through calcium cross-linking. Lastly, in the third phase, syneresis occurred in which the trapped whey is expelled from the casein network through contraction, which is usually promoted by cutting and stirring³⁰.

The coagulation of milk is a complex process and its coagulation properties are influenced by various factors, such as; milk quality, breed of animal and season. However, the easiest and most obvious parameters that can be determined and compared are the enzyme concentration, pH, calcium chloride concentration and temperature³¹ (Fig. 4). When the enzyme concentration in milk increased, the number of

collisions between the rennet and caseins, especially kappa-casein, increases. An increase in the concentration of enzymes, such as; rennet, decreases the coagulation time due to a higher level of kappa-casein proteolysis. Hence, micelles need more kappa-casein cleavage to aggregate faster³². Normal milk has a pH of 6.5-6.7. When the pH of milk is lowered to approximately pH 6.0-6.4, the milk coagulates into curd more quickly. This occurred because lowering the pH reduces the electrostatic repulsion between micelles and increases rennet activity. These changes caused the coagulation to occur at a faster rate²⁵.

A high amount of CaCl₂ adds more calcium ions to the solution. Calcium ions help to speed up the coagulation time of the milk as they act as a cofactor and reduce the repulsive forces between casein micelles and promote the aggregation of milk proteins³³. According to Ong *et al.*³⁴, increasing the coagulation temperature also decreases the coagulation time. As documented, the effect of increasing coagulation temperature contributed to the improvement of milk coagulation in terms of velocity and texture of curd formation. The heat promotes strong bond formations between the denatured whey proteins and casein micelles and increases the rate of coagulation. In historical times, rennet obtained from the stomach of ruminants was used for milk coagulation. However, with the development of science and technology, various other proteases are also being used for cheese-making. Data in Table 1 showed some examples of coagulants and enzymes identified to have milk-coagulating capabilities.

Acid-mediated coagulation: As opposed to the enzymatic approach, the acid coagulation of milk uses acid to lower the milk pH, causing an alteration in the properties of casein micelles. The addition of acid destabilizes the colloidal stability

of the micelle compounds by breaking the calcium bonds between the micelles and resulting in the initiation of coagulation⁴⁰. For research purposes, the acidification of milk is usually performed by adding a precise volume of hydrochloric acid and gluconic acid to lower the pH of the milk⁴¹ to below 6. Under normal conditions, milk has a pH of 6.7 and this pH is lowered to the acidic pH of 4.6. This low pH alters the interactions of calcium phosphate molecules and micelles⁴⁰. Calcium phosphate is a nanocluster that traps the micelle, forming compounds of micelles. Once this interaction is damaged, the compounds break apart. Micelles are destabilized and begin to stick to each other, forming a gel-like structure⁴².

The acid used to lower the pH can be exogenous or endogenous. Examples of exogenous acids are citric acid or acetic acid. These types of acid are directly added to the milk to induce coagulation. Acids can also be produced by a carrier endogenously, such as bacteria. *Lactobacillus* bacteria release lactic acid, hence, they can be used to produce endogenous acids⁴³. Some disadvantages of this method include non-uniform flavour profiles due to the addition of acid and the high production cost required to purchase the acid. The use of acid-mediated coagulation from natural sources may be challenging in terms of controlling the pH. Such is the case with the pH environment created by *Lactobacillus* bacteria growth when the production of dairy products, such as cheese, is practised as a skill rather than a controlled industry⁴².

ISSUES WITH ANIMAL OR MICROBIAL DERIVED COAGULANTS

In animal rennet, chymosin is the active part of the enzyme used in cheese making and is extracted and purified from the stomach of calves that have not been weaned from their mother's milk⁴⁴. The production process involved many steps that make the enzyme very expensive. Calves are also killed at a young age because as they get older, the chymosin content in their body decreases, one main reason for this decrease in chymosin production is that as the calf grows older, the chymosin enzyme is replaced with pepsin⁴⁵. The un-weaned calf is separated from its mother within two days of birth⁸. Although the secretion of chymosin does not completely stop in adult cows, it is excreted in small amounts. Hence, an ethical issue arises in slaughtering very young animals for protease sources. People for the ethical treatment of animals (PETA) opposes the slaughtering of such animals⁴⁶. There are also religious constraints in using animal sources of milk coagulants. Due to this restriction, individuals with certain

religious beliefs are prohibited from consuming certain food products derived from forbidden animals. Particularly in Islam, religious individuals are restricted from consuming any porcine-related food as they are claimed to be made from unclean animals. Muslims do not consume porcine sources of rennet as it is considered 'haram' because porcine is related to pigs⁴⁷. In Italy, a unique local food product known as Pecorino cheese is made using rennet derived from porcine sources⁹. However, the small amount of porcine-derived rennet used in cheese-making, which might be removed together with the whey after cheese production, makes assuring the halal authenticity of the cheese difficult. Moreover, both in Hinduism and Buddhism, it is prohibited to consume beef or beef-related products out of respect for the animal⁴⁸.

Another issue with animal rennet is that vegetarians around the world are not able to consume dairy products produced with animal rennet⁴⁹. These people are constricted by their diet and if cheese is produced using animal-sourced coagulants, vegetarians will not consume it⁵⁰. Due to the growing number of people following vegetarian diets, several substitutes for animal rennet have been made available, allowing the supply of milk coagulants to meet the demand for cheese production and providing alternative sources for vegetarians. Plant-based coagulants are an appropriate alternative for animal rennet for the dairy-consuming vegetarian community as no controversial issues regarding animals will be raised. Due to advances in technology, microbes are also widely used as a vector to produce milk coagulants through genetic engineering. Certain microbial sources of coagulants reduced the yield of the curd, which is due to the inefficiency of the microbes to aggregate the casein micelles. This results in a softer curd with a lower yield⁵¹. Though microbial sources of coagulants are cheaper than plant and animal sources of coagulants, their milk clotting activity/proteolytic activity (MCA/PA) ratio is poor⁵². Moreover, many arguments are being raised against the use of genetically engineered foods. Some individuals believe that genetically modified calf rennet is fundamentally unnatural and unsafe for health and creates dangerous side effects as it may serve as a vector for various diseases¹⁰. The BSE or TSE infection of the abomasum can also lead to issues for the use of animal rennet⁵³.

PLANTS AS COAGULANT SOURCE

Plants are our main source of food and are very beneficial because almost all parts of the plants, such as seeds, roots, flowers, fruits and leaves can be utilized for our everyday needs. For example, people eat the leaves of some plants,

Table 1: Milk coagulants and their enzymes

| Coagulant type | Milk-clotting enzyme | References |
|------------------------------------|----------------------------|---------------------------------------|
| Calf rennet | Chymosin | Kumar <i>et al.</i> ³⁵ |
| Adult bovine rennet | Pepsin | Kumar <i>et al.</i> ³⁵ |
| Porcine rennet | Pepsin | Kumar <i>et al.</i> ³⁵ |
| Microbial (<i>Rhizomucoi</i>) | Mucorpepsin | Armstrong <i>et al.</i> ³⁶ |
| Microbial (<i>Cryphonectria</i>) | Endothia-pepsin | Cooper ³⁷ |
| <i>Cynara cardunculus</i> | Cardosin | Gomes <i>et al.</i> ³⁸ |
| Malayan pit viper venom | Metalloproteinase kistomin | Vejayan <i>et al.</i> ³⁹ |

Table 2: Types and sources of milk-clotting plant proteases

| Type of protease | Protease name | Source of protease | References |
|------------------|-------------------------|----------------------------|--------------------------------------|
| Aspartic | Cardosins and Cyprosins | <i>Cynara cardunculus</i> | Ordiales <i>et al.</i> ⁶⁰ |
| | <i>Cynara</i> | <i>Cynara scolymus</i> | Chazarra <i>et al.</i> ⁶¹ |
| Cysteine | Oryzasin | <i>Oryza sativa</i> | Feijoo-Siota and Villa ⁶² |
| | Bromelain | <i>Ananas comosus</i> | Bahmid ⁶³ |
| | Ficin | <i>Ficus racemosa</i> | Devaraj <i>et al.</i> ⁶⁴ |
| | Papain | <i>Carica papaya</i> | Bornaz <i>et al.</i> ⁶⁵ |
| Serine | Zingibain | <i>Zingiber officinale</i> | Huang <i>et al.</i> ⁶⁶ |
| | Cucumisin | <i>Cucumis melo</i> | Uchikoba and Kaneda ⁶⁷ |
| | Lettucine | <i>Lactuca sativa</i> | Lo Piero <i>et al.</i> ⁶⁸ |
| | Streblin | <i>Streblus asper</i> | Tripathi <i>et al.</i> ⁶⁹ |

such as spinach, lettuce and celery. Spinach is low in fat and high in zinc, protein, fibre, iron and calcium. Spinach helps to improve eyesight, regulate blood pressure and build stronger muscles⁵⁴. Celery has long fibrous stalks that are rich in antioxidant nutrients and roots that are nutritious⁵⁵. Cauliflower, broccoli, carrots and many other plants can provide many different nutrients to our body. These plants consist of various components and one such is the proteases present in certain plants that are capable of coagulating milk. The plants that have these proteases have the potential to be used as plant-based milk coagulants. Plant-based milk coagulants have a variety of benefits as substitutes for animal-based milk coagulants. The demand for plant coagulants is increasing rapidly due to an increase in global demand for cheese and a decreased supply of calf rennet⁵⁶. Plant coagulants also have high proteolytic activity. Ginger, muskmelon and artichoke are all known for their high proteolytic activity⁵⁷. Plant coagulants are also not associated with Halal restrictions, while animal coagulants cannot be consumed by certain groups of people due to religious constrictions. Plant proteases can also lower plasma cholesterol and blood pressure levels, unlike animal sources of protein⁵⁸. Proteases have been divided into groups, based on the catalytic mechanism used during the hydrolytic process. The main catalytic types are aspartate, serine, cysteine and metalloproteases⁵⁹, but the plant proteases used as milk coagulants have been reported to only be from the first three groups, not from the metalloproteases group⁵⁷. Table 2 showed some examples of milk-clotting plant proteases and their sources.

Cynara cardunculus, commonly known as cardoon, is a thistle-like plant in the sunflower family that is domesticated in the western and central Mediterranean region⁷⁰. *Cynara cardunculus* has a purple-violet flower and spiny green leaves. In ancient times, the flowers of cardoons have been used in artisanal cheese making, mainly in Portugal, where it is claimed to be superior for cheeses. Cardoons contain cardosin, a protease enzyme that has high milk-clotting activity². Cardosin is prepared by drying and grinding the stamen plucked from the flower into powder and then soaking it in water to extract the milk-clotting enzymes. The solution is then filtered to remove any unnecessary residues and is ready to be used in the first step of cheese making. Artichoke, *Cynara scolymus*, is another plant that has strong coagulant properties. The leaves of the artichoke have a high content of aspartic enzymes⁷¹. *Cynara* enzymes from the flower of this plant can also be used as an alternative for milk clotting. The strength of the enzyme extracted from its flower gets increased in the presence of high levels of calcium. *Cynara* has been used for years in the Spanish and Portuguese cultures for cheese production. Typically, there are three types of coagulants found in the flower of the artichoke: Cyanarases A, B and C. These rennets are capable of preserving the distinct and native flavours of traditional cheeses⁵⁷.

The latex of the fig tree, *Ficus carica* has coagulant properties, is widely found in the Gaziantep region of Turkey and is used to produce Gaziantep cheese. The process of producing milk coagulant from the latex is delicate because the latex has a viscous texture⁵⁷. The fig tree has a high proteolytic yield compared to the artichoke. The fig tree also

has high proteolytic activity, with a clotting/proteolytic ratio of 1/50. However, the fig tree gave a bitter taste and a strong odour as well as a browning effect, to the cheese, which makes it less favourable in the cheese-making industry⁷². Zingibain is a cysteine protease enzyme found in common ginger that was found to have the ability to coagulate milk⁶⁶ and that is used in China to make ginger milk curd, a famous Cantonese dish. Proteases, such as papain and bromelain, were also found to have milk coagulating properties and are famously used for meat tenderization⁷³.

FUNCTIONAL FOODS MARKET

Nutrients, herbs and dietary supplements are major constituents of nutraceuticals that make them instrumental in maintaining health, acting against various disease conditions and promoting quality of life. In early times, functional foods included food fortified with vitamins or minerals and subsequently, the focus shifted to foods fortified with various micro-nutrients. Currently, food products that offer multiple health benefits in a single food are being developed⁷⁴. The future of the functional food market looks bright: The worldwide revenue for functional food is projected to increase from approximately 300 billion U.S. dollars in 2017 to over 440 billion U.S. dollars⁷⁵ in 2022. Functional foods and supplements are now available for almost any disease. The targets of functional foods included the immune response, cancer, gastrointestinal diseases, mood, memory and alertness, energy, strength, stamina and ageing⁷⁶.

Plant-based food products as functional foods: Functional foods consist of technologically advanced ingredients that provide various health benefits⁷⁷. The definition of functional food changes from country to country and therefore, there is no commonly acknowledged definition. The term 'functional food' was born in Japan as Japanese individuals were the first to identify that food can play a role other than just supplying energy to the body⁷⁶. Functional foods are described as "food products strengthened with special constituents that have beneficial physiological effects"⁷⁸. Japan is the greatest market for functional foods and it became the first nation to approve these items through the enactment of Foods of Specified Health Use (FOSHU). According to The European Commission Concerted Action on Functional Food Science in Europe (FuFoSE), "a food item is considered functional if it has positive and essential dietary effects on at least one human body function"⁷⁸. According to the US National Academy of Sciences Food and Nutrition Board, functional food is defined as "any modified food or food ingredient that gives a positive health effect past the traditional nutrients it comprises of"⁷⁸.

Functional food is becoming increasingly popular and the main reason for the increased popularity of functional foods is due to the health benefits they provide to consumers. Globally, the life expectancy of the current population continues to rise as does the contribution made by older individuals to the total population⁷⁹. Consumers are taking more responsibility for their health and well-being and they believe that food contributes directly to their health, therefore, they are increasingly turning to their diet to enable them to do so⁷. As people age and grow old, various diseases become more prevalent. However, the medical services for the ageing population are costly, which results in an increase in the interest in functional foods. The expanding cost of healthcare, the increases in life expectancy and the desire of older people for enhanced quality of their later years are reasons for the development of functional foods⁸⁰. However, functional foods are usually more expensive than their regular counterparts and the demand for food is high, especially among the higher income population. The reason behind the price is justified as the development of the functional foods required extensive research and development⁸¹.

Plant-based food products are excellent sources of functional foods as plants are naturally found in the environment and are abundant in many parts of the world. Plants contained nutrients and added health benefits that could be used to treat various kinds of diseases and this particular trait makes them an ideal choice to be used as ingredients for functional foods⁸². Numerous plants or physiologically active ingredients derived from plants have been investigated for their role in disease prevention and health. Examples include *Mangifera indica* seed kernel⁷⁴ and essential oils of *Ocimum basilicum* L. which have antibacterial activity⁸³, rhizome of *Alpinia conchigera* has having antimicrobial activity⁸⁴, plants such as; *Tinospora crispa*⁸⁵, *Eucheuma cottonii* and *Padina* sp.⁸⁶ which have anti-diabetic activity and *Plectranthus amboinicus*⁸⁷ which showed antioxidant activity.

The micro-nutrients in plants are incorporated into different types of food to produce functional foods that can be consumed by people of all religions and with a wide-range of preferences. These functional foods include prebiotics, functional drinks, bakery products and dairy products. One example of a local Malaysian plant-based food that is considered as a functional food is 'Tempeh'⁸⁸. 'Tempeh' is a local Malaysian dish which is a fungal fermented soybean food that offers many proven health benefits, such as excellent digestibility and protection against diarrhoea and chronic degenerative diseases⁸⁸. Another example is Mangosteen peel drink, which is famous in southeast Asia. Mangosteen peel drink was found to provide antioxidant and anti-inflammatory benefits to its consumers in a study conducted by Xie *et al.*⁸⁹.

Prebiotics are another example of indigestible plant fibre that feed probiotics or good bacteria living inside the large intestine. Prebiotics selectively stimulated the bacterial species that reside in the colon, such as; Lactobacilli and Bifidobacterium, to aid in digestion⁹⁰.

FUNCTIONAL DAIRY FOOD OF THE FUTURE

Plant-based diets are increasing in popularity as increasing numbers of people are becoming health conscious, therefore, plant-based diets are becoming a trend worldwide. In 2017, it was found that in the U.S., approximately 6% of people were vegan compared to only⁹¹ 1% in 2014. Plants presented many benefits to their consumers and a plant-based diet can be a great asset to human health. For example, plant-based diets are a good source of a variety of nutrients, such as; vitamins, minerals and fibre and are naturally lower in added sugar and saturated fat than diets high in animal-derived and processed foods⁹². Diets consisting of fruits and vegetables help prevent many chronic diseases, such as type 2 diabetes, hypertension and cancer. For example, carotenoids in carrots help to neutralize the free radicals that cause cell damage, anthocyanins in berries can help to ensure good vision and flavonoids in apples help control inflammation⁹³.

The importance of functional food rather than calorie-rich food is gaining popularity in the western world and in modern society worldwide. The increasing prevalence of obesity is alarming and health-conscious people avoid the many general staple foods due to their high calorie contents. Obesity has been regarded as one of the main causes of diabetes in the Asian region⁹⁴. To counter this, various studies have been conducted and it was found that plants reduce the risk of diabetes. Turmeric, for example, is a spice that has various benefits, including lowering blood sugar levels. This effect occurred as a result of curcumin, an active ingredient in turmeric⁹⁵. Moreover, curcumin also benefits kidney health. This factor is important as diabetes is one of the leading causes of kidney disease⁹⁶. Long-term diabetes control is typically determined by measuring haemoglobin A1c, which reflects the average blood sugar level. In a previous study, cinnamon was consumed for 90 days by patients with type 2 diabetes and it was found that the patients exhibited a sharp reduction⁹⁷ in haemoglobin A1c. Studies have also shown that broccoli can lower insulin levels in the body and protect the cells from the free radicals that are produced during metabolism⁹⁸.

Another critical illness receiving a great deal of attention is cancer. Cancer is a disease that is generally caused by the

modernization of our lifestyle, although this is not necessarily always the case. Many culprits have been identified as the initiator of cancer and one is diet, i.e., processed food⁹⁹. Cancer is another leading cause of death worldwide and more than half of cancer cases are incurable. To reduce the rate of cancer, many different types of efficacious health interventions that can result in dramatic reductions in mortality and disability at relatively modest costs are being researched and discovered¹⁰⁰.

The causes of cancer can be many but one way to help reduce the development of cancer is via the diet. Other than consuming nutraceuticals, which have been identified as potential therapeutic agents for cancer¹⁰¹, the phytochemicals that are found in many plant-based foods were also found to reduce the growth of cancer cells¹⁰². An example is broccoli, which contained indole-3-carbinol, a molecule that can target and destroy breast cancer cells. Broccoli also contains sulforaphane, which is a compound that has potential anticancer properties¹⁰³. Curcumin, a principal component found in turmeric was found to decrease the growth of colon cancer by targeting a specific enzyme related to cancer growth¹⁰⁴. In addition, it was also found that certain phytochemicals, such as antioxidants, have the potential to aid in cancer treatment. A study by Mut-Salud *et al.*¹⁰⁵ found that antioxidants could promote the effectiveness of antitumor treatment and even protect healthy tissues against damage induced by oxidative stress. Another study on traditional Chinese medicine used against cancer found that there is a positive linear relationship between antioxidant activity and the anticancer effect of aqueous herbal extracts. This finding suggested that the antioxidants in the aqueous herbal extracts might contribute to their anticancer effects¹⁰⁶. Therefore, it is believed that antioxidants have the potential to function as anticancer components. Oxidative stress has also been identified as a root cause of the development and progression of several diseases¹⁰⁷. Supplying exogenous antioxidants or increasing the endogenous antioxidant defences of the body is an excellent way to counteract the undesirable effects of reactive oxygen species (ROS)-induced oxidative damage. Many plants were found to have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants that are capable of attenuating ROS-induced oxidative damage¹⁰⁷.

Although the discoveries and inventions related to food preparations have been beneficial, there are also dangers associated with them. Some people have changed their paradigm of food beliefs from being merely for calories to being preventive medicine, hence popularizing functional foods. It is therefore, expected that the trend of future dairy

functional foods will include the incorporation of plants with the dual effects of acting as milk coagulants and providing health benefits associated with their inherent medicinal biological properties.

CONCLUSION

The increasing issues related to coagulants in the dairy industry has resulted in exploring plants as source of coagulants and this is even more, so, because plants have useful medicinal benefits within them. Through this study, milk coagulating plants was identified to be the future of functional dairy food due to their dual ability of first coagulating the milk and then fortifying the curd with biologically useful compounds.

SIGNIFICANCE STATEMENT

Functional food is gaining relevancy in western and modern societies around the world. Hence, this review explored the potentials of such endeavours in area of dairy industry. It described challenges faced by this industry related to the use of animal and microbe derived natural or modified coagulants. Almost all the challenges can be overcome using plant-based coagulants and provide a future trend for functional dairy food. This study will help researchers to understand the potential of the dual effects of plants in coagulating the milk as well as enhancing the curd with biologically important micro-constituents, thus forming a functional dairy food.

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