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Microencapsulation in milk

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ABSTRACT: Functional foods can potentially play a key role in preventing health risks associated with eating habits. The development of healthy foods through the addition of bioactive compounds poses many technical challenges. These substances cannot tolerate the conditions of the stomach and in most cases are unable to reach the site of absorption. Microcapsulation has created new opportunities that could revolutionize the food and dairy industry. Microencapsulation greatly helps to improve the transport of bioactive compounds by reducing particle size and increasing surface area by volume. Microcapsulation is a useful tool for improving the transport of bioactive compounds in foods, especially probiotics, minerals, vitamins, lutein, fatty acids, lycopene and antioxidants, peptides, phytosterols, polyphenols, bioflavonoids and fibroids. Future research is likely to focus on the transfer and potential use of common encapsulation methods, in which two or more bioactive substances can be combined for a synergistic effect. In this article are discussed some aspects related to encapsulation methods, coatings, bioactive compounds and their use in food processing, especially dairy products. Future research is likely to focus on aspects of delivery and the potential use of co-encapsulation methodologies, where two or more bioactive ingredients can be combined to have a synergistic effect.

Keywords: *Bioactive compounds, Functional, Healthy foods, Ingredient, Microcapsulation*

INTRODUCTION

Consumers today are more conscious than ever about the beneficial health aspects of the foods they consume. Beyond satisfying hunger, it is expected that the foods should also have properties to prevent disease and ameliorate physical and mental well-being [1]. According to a report by the World Health Organization (WHO), diets and dietary habits are among the major risk factors that lead to the development of fatal diseases like cancer, coronary heart diseases, obesity, etc. [2]. Functional foods can potentially play a key role in preventing the health risks related to dietary habits. Several functional foods are already on the market and a lot more are to appear in the near future. Functional dairy foods represent the biggest proportion of the functional food market, comprising about 42.9% of the market share [3]. Milk and dairy products have comprised a significant part of the human diet all around the world since ancient times. Due to the presence of nearly all

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essential nutrients, milk is popularly known as a balanced food for all age groups. Recent research suggests that apart from delivering basic nutrients, milk is a significant source of bioactive components. A number of dairy products have already found their way into several food products for nutritional enhancement. Milk is enriched with a variety of nutritional elements, making it one of the important complete foods in nature, however, recently some bioactive components and a few other beneficial health components have been incorporated into dairy products. In this modern age, people expect their food, besides providing basic nutrients, should nourish their health, ensuring protection against certain diseases. As a consequence, dairy companies are continuously launching new products supplemented with specific nutrients or bioactive components. A large number of research studies are being conducted to develop commercially adoptable dairy products aimed at particular groups who are deficient in specific nutrients as well as at the general population. In addition, there has been a significant research directed to helping the large numbers of lactose-intolerant people around the world. Microencapsulation is a physicochemical process of protecting the nutritional or functional elements from environmental and gastric conditions and releasing those in the intestinal condition [4]. In this regard, Microencapsulation is a physicochemical process of protecting the nutritional or functional elements from environmental and gastric conditions and releasing those in the intestinal condition [4]. Microencapsulation (ME) has been defined as ‘the technology of packaging solid, liquid and gaseous materials in small capsules that release their contents at controlled rates over prolonged periods of time’. Microencapsulation (ME) is the envelopment of small solid particles, liquid droplets or gases in a coating (1–1000 mm). Such technologies are of significant interest to the pharmaceutical sector (e.g. for drug and vaccine delivery), but also have relevance for the food industry. The matrices in contact with food are generally natural components, but mainly they must be Generally Recognized as Safe (GRAS) for human health. The industrial production of foods often requires the addition of functional ingredients [1]. Typically, these are used to control flavor, color, texture or preservation properties, but increasingly ingredients with potential health benefits are also included. Adding bioactive ingredients to functional foods presents

Table 1. Physical processes and general consideration.

Method	Principle	Comments	Reference
Spray drying	Core is dispersed into aqueous encapsulant solution and atomized into a drying chamber	Most commonly used method, cost-effective	[17]
Spray chilling	Core is dispersed into coating solution and sprayed into a cold environment to solidify the carrier material	Used for protection of water – soluble cores, also suitable for cores that are sensitive to temperature	[14]
Extrusion	Emulsion dispersion containing the core passed through a die at high temperature and pressure unto a bath for solidification of the particle	Used primarily for encapsulation of flavors and volatile cores in glassy matrices	[11]
Fluidized bed coating	Particles are suspended in air and a coating is applied	Used for achieving finer control over release properties of the core	[6]
Inclusion complexation	An inclusion complex is formed between cyclodextrin and the core	Encapsulation of flavors and lipophilic nutrients	[10]
Coacervation	Coacervates are formed when two oppositely charged biopolymers associate and phase separate	Can entrap high loadings of cores, has been used in encapsulation of flavours and many nutrients	[18]

many challenges. Particularly with respect to the stability of the bioactive compounds during processing and storage and the need to prevent undesirable interactions with the carrier food matrix [5].

This technology is relatively new to the dairy industry and has already found several applications, such as encapsulation of omega-3 polyunsaturated fatty acids, chitin, peanut sprout, lactase, iron, vitamin C, probiotic bacteria, and many more [6-8].

Microencapsulation of functional ingredients in milk

A wide range of encapsulation procedures have been proposed for the microencapsulation of the bioactive components to be fortified in milk but no single procedure has become universally applicable [6]. The suitability of any microencapsulation technique relies primarily on the molecular structure and characteristics of an individual bioactive substance [9]. Coating materials are chosen, based upon the characteristics of the individual functional components and the type of vehicle products. For the application of microencapsulated nutraceutical materials into milk, several methods have been investigated so far [10].

Probiotic

Probiotic bacteria are ‘defined, live microorganisms which, administered in adequate amounts, confer a beneficial physiological effect on the host’. These bioactive ingredients have been at the forefront of the development of functional foods, particularly in dairy products, and thus deserve particular attention in the scope of this review. Probiotics present two sets of problems when considering ME: their size (typically between 1 and 5 mm diameter), which immediately excludes nanotechnologies, and the fact that they must be kept alive. At least five ME methods have been applied to probiotics: spray-coating, spray-drying, extrusion, emulsion and gel particle technologies (which include spray-chilling). The main purpose of probiotic encapsulation is to protect cells against an unfavorable environment, and to allow their release in a viable and metabolically active state in the intestine. Microparticles should be water-insoluble to maintain their structural integrity in the food matrix and in the upper part of the GI tract; above all, particle properties should allow progressive liberation of the cells during the intestinal phase. For ME of microorganisms, the most used polymers (all natural, inexpensive, biocompatible and GRAS) are chitosan (obtained from arthropods), alginate (a polymer extracted from seaweed), carrageenan, whey proteins, pectin, poly-l-lysine, and starch [11]. Different types of starch and modified starches have been tested as entrapping agents of probiotics. Unfortunately, in some cases, the low pH and the presence of proteases, two of the conditions commonly experienced by probiotic organisms during their passage through the stomach, diminish their adhesion to starch. Resistant starch is not degraded by the pancreatic amylase and arrives at the intestine in an indigestible form. This provides a good release of bacterial cells in the large intestine and offers them prebiotic functionality. The materials are used alone (monolayer) or in combination: in this last case, coating the microcapsules with an additional film can avoid their exposure to oxygen during storage and can enhance their stability at low pH. For example, one of the most common double (or triple) layer strategy is represented by an inner layer of alginate, containing the entrapped microorganisms, then covered by a monolayer of chitosan that might be eventually contained in a further outer layer of alginate, chitosan or other polymer. Chitosan-coated alginate beads give better protection in simulated gastric conditions than other outer coating films [2]. Different foods containing encapsulated probiotic cells are present on the market. Belgium group Barry Callebaut produces chocolate containing encapsulated probiotic cells that do not negatively affect the taste, texture or mouth feel of the final functional product, and which, at doses of 13. 5 g per day might be sufficient to positively affect the gut microbiome. In some cases, inulin or other prebiotics have been added to probiotics in the manufacturing of the bar called ‘Attune’ [8].

Conjugated linoleic acid

Conjugated linoleic acid of several bioactive lipid components in milk, conjugated linoleic acid (CLA) has attracted considerable attention for having beneficial health functions, such as being anti carcinogenic and anti-atherogenic. In order to utilize the bioactive functions of CLA, it should be protected from oxidation during food application. Recently, one study reported that whey protein concentrate (WPC) has been a very effective coating material to prevent the oxidative deterioration of CLA .This study revealed that the application of WPC-coated CLA did not cause any objectionable change in the sensorial properties of the vehicle. Later on, Choi *et al.* manifested that the Maillard reaction products (MRPs) of whey proteins and maltodextrin showed better microencapsulation efficiency and solubility as a coating material [9]. However, in vitro and in vivo studies have to be performed to confirm the efficiency of microcapsules in releasing CLA in the gastrointestinal condition.

Chitosan

Chitin is the second most abundant natural biopolymer on earth [12]. The deacylated form of chitin, called chitosan, has been of great interest for the past few years due to its broad range of health-promoting functions. Following the approval of chitosan as a feed additive by the United States Food and Drug Administration (USFDA), the food industry also is trying to utilize its nutritional effects, and consequently, some developed countries have recognized chitosan as a functional food ingredient [12]. Milk is one of the potential vehicles for chitosan supplementation, but-thecharacteristicbittertaste, alongwithitsoff-flavorand the color of chitosan or chitin-derived products, is a big constraint on its application to milk. As a result, the incorporation of microencapsulated Chito oligosaccharide in to milk has been studied using polyglycerol monostearate (PGMS) as a coating material [9]. Having 88.08% encapsulation efficiency, the chitosan microcapsules were found to be very stable and only 7.6% of Chito oligosaccharide was released from the microcapsules during 15 days of storage at 4°C. With regard to the physicochemical and sensorial properties of milk (Fig. 1.), microencapsulated Chito oligosaccharide had a very insignificant effect. Therefore, there is a huge prospect of developing microencapsulated Chito oligosaccharide-supplemented milk for the mass market.

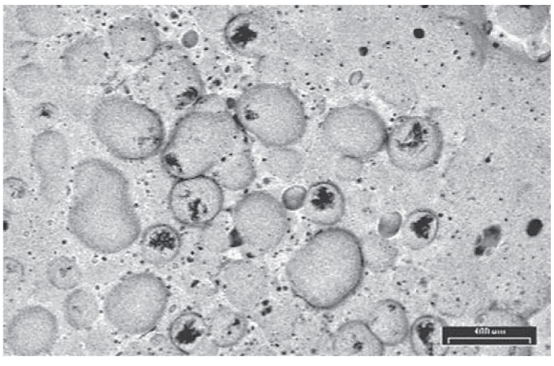

Fig. 1. Photomicrograph of microencapsulated Chito oligosaccharide with polyacylglycerol monostearate. The photograph was taken at 50× magnification. Source: [9].

Peanut sprout extract

Resveratrol, a famous anti-aging polyphenol, is also known for its anti-arthritic, anti-inflammatory, and anticarcinogenic properties [13]. A number of other health-promoting functions of resveratrol have been discovered through some recent investigations. Due to its potential beneficial health effects, incorporation of resveratrol into food products is becoming a popular practice in the food industry. Various sources of resveratrol have been reported but the greatest source is known to be peanut sprout extract [13]. From the peanut sprout (Fig. 2). The extreme sensitivity of resveratrol to environmental conditions is considered a major challenge in its food application. Water-in-oil-in-water (w/o/w) technology has been emerged recently as an advanced microencapsulation procedure which is currently being used to protect functional components. To protect the functional effects of resveratrol, a study demonstrated that peanut sprout extract could be microencapsulated with a greater efficiency of 98.74% under conditions optimized by response surface methodology [11]. To produce w/o/w emulsion, MCT was used as primary coating material and whey protein concentrate (WPC), maltodextrin, and gum Arabic were used as secondary coating material to entrap the peanut sprout extract. This invention was followed by another work which was conducted regarding the viability of w/o/w emulsion technology to preserve the functional effects of resveratrol as well as the quality of milk [11]. This study demonstrated that higher concentrations of peanut sprout extract microcapsules (PPSEM) had a significant effect on the pH and color values of milk while lower concentrations of PPSEM did not produce any noticeable change in the physicochemical properties of milk. It was determined that the concentrations of PPSEM of up to 0.1% (w/v) could be used in formulating functional milk while maintaining the physicochemical and sensory properties of milk.

Microencapsulation of vitamins

Supplementation of micronutrients into foods has become a common approach to increase the daily consumption of essential vitamins and minerals. Food fortification began long ago in the industrialized countries for the efficient control of several deficiencies, such as vitamin A and D, several B vitamins, iodine, and iron. In recent years, some developing countries have also started implementing food fortification to combat micronutrient deficiencies. Given the enormous success of iodine fortification in salt, a number of other food products are considering supplying specific micronutrients. Fortifying milk and dairy products can play a vital role in the supply of essential micronutrients. Vitamin D fortification of milk started in the United States in the 1930s on the recommendation of the American Medical Association's Council on Food and Nutrition in order to fight the prevalence of rickets in children [14]. The initiative proved successful and currently, in the United States and Canada, it is mandatory to fortify milk with vitamin D [14]. This success has led to the initiation of vitamin A fortification of milk as required by regulation in the 1940s. Even though apart from these two types of vitamins, the addition of other trace elements into milk has not become common practice, food scientists are trying to incorporate some other important trace elements into milk using the appropriate technology. The purpose of vitamin fortification in food products is mainly to meet the special nutritional needs of infants and aged people and to prevent diseases in nutrient deficient populations. This aim cannot be attained unless a proper delivery system is determined, which can supply the nutrient to the appropriate site by protecting it from both environmental and gastrointestinal conditions until it reaches the appropriate site. Water-soluble vitamins are sensitive to environmental conditions, such as temperature, pH, moisture, light, etc. and are susceptible to processing and storage. The sensitivity and the risk of degradation of vitamins can be prevented by encapsulating them with suitable coating materials. Ascorbic acid (vitamin C) is a water-soluble vitamin and is one of the essential micronutrients to be incorporated into milk. This element has very important functions in the human body. Besides its antioxidant role, ascorbic acid plays a crucial role in enhancing the absorption of iron by converting ferric iron into a ferrous state [11]. In order to overcome some of the shortcomings regarding the instability of ascorbic acid during its incorporation into milk, microencapsulation has been proposed as a possible solution. Both dry powder and liquid microcapsule technologies are applicable to encapsulate ascorbic acid. Of several factors, the choice of coating materials, the physicochemical properties of the core materials, the encapsulation process, and the ultimate properties of microcapsules all have to be considered. Lee *et al.* have determined the suitability of PGMS as a coating material for ascorbic acid during its incorporation into milk [11]. In their research, the encapsulation efficiency was 94.2% and the release of ascorbic acid during 5 days of storage was limited up to 6.7% with no significant change in the sensorial properties of the fortified milk. In an earlier study, these authors demonstrated that along with PGMS, MCT could also be a potential candidate as a coating material [11]. In vitro examination carried out in this study indicated that these two coating materials showed very high levels of efficiency in both preserving the ascorbic acid in the simulated gastric fluid and releasing it in the simulated intestinal fluid. This study has revealed that the encapsulated ascorbic acid, when consumed with milk, increased the serum iron, which implied that the microencapsulated ascorbic acid increased the bioavailability of iron. Iron is an essential microelement and has several important functions in the human body. Lack of this element leads to one of the most prevalent nutritional deficiencies around the world called iron deficiency anemia (IDA) which affects nearly 20% of the world's population. Iron deficiency usually results from the inadequate supply of iron in the diet, poor bioavailability of iron from the digested foods, or a combination of the two. Therefore, it is suggested that the intake of iron could be enhanced, especially through foods fortified with iron. Milk and dairy products are being considered as suitable iron-fortifying vehicles due to their high consumption and as an outstanding source of essential nutrients. Moreover, milk is well known for its low content of iron (0.2mg/kg), despite being abundant with other nutritional elements [11]. Therefore, fortification of milk with iron could be an important solution in the fight against iron deficiencies. For an effective iron supplementation, it is crucial to select the appropriate iron compound with a high degree of bioavailability, and more importantly, to select the suitable technique which is safe, does not change the organoleptic qualities of the food vehicle and helps provide iron with high level of stability and bioavailability. It has been suggested that microencapsulation can prevent the negative effects, such as fat oxidation, the off-taste from the iron, the off-color, and sedimentation from iron fortification, and can secure an effective supplementation of iron in milk and dairy products. For a feasible iron fortification in milk, microencapsulation of iron salts has begun using a type of phospholipid called SFE-171as a coating material and afterwards a series of studies were carried out to improve this technology [5]. Ferrous sulfate microencapsulated with lecithin (SFE-171) has been studied in animal and human subjects and it was reported that this product had the same bioavailability as ferrous sulfate and did not deteriorate the organoleptic quality of the fortified products [5]. A few investigations revealed that the constitutive ingredients of milk, such as casein, calcium, and whey protein lessen the bioavailability of ferrous ion by interacting with this ion, and this negative interaction has been shown to be avoided by microencapsulating iron with SFE-171 [13]. Kwak & et al revealed that encapsulating the iron salts with fatty acid ester (FAE) can increase both the microencapsulation efficiency and iron fortification efficiency. Using PGMS, they obtained 75% microencapsulation efficiency. The microcapsules could effectively protect the iron until it reached the intestine. Moreover, the resultant milk, after the addition of the PGMS-coated iron capsules, did not show any change in the organoleptic quality [13].

Microencapsulation of lactase

Lactose, a type of carbohydrate, is the second biggest component in milk. Generally, this component comprises about 4.8–5.2% of milk. After intake, lactose is hydrolyzed into glucose and galactose, which are easily absorbed in the small intestine. However, a large number of non-Caucasians, aged people in many Western countries, and various ethnic population groups are intolerant to lactose, as they lack adequate amount of lactase (β-D-galactosidase EC 3.2.1.23) in their gastrointestinal tract [3]. When lactase-deficient people consume milk, they have one or more of the following clinical symptoms: abdominal pain, watery diarrhea, bloating, and cramping, and these symptoms are generally known as lactose intolerance [7]. To assist the large number of lactose-intolerant people, several efforts have been undertaken so far. For the very first time, β-D-galactosidase was added to the milk which hydrolyzed lactose during processing. A technique called “lactase immobilization,” where lactose was continuously hydrolyzed, further promoted the process. In terms of lactose hydrolysis, this method was simply ingenious. But lactose-hydrolyzed milk did not attract much attention due to the high sweetness resulting from the hydrolysis of lactose. Therefore, there was a demand for hydrolyzing the lactose after consumption. A few years later, a further advanced technique known as UF/RO, was introduced whereby lactose was removed from the milk through ultra-filtration (UF). However, removal of lactose resulted in the loss of some important macro/micro nutrients from milk and the loss of the original sweetness of milk. Moreover, milk with lactose removed was inefficient in calcium absorption and translocating it into bone. Microencapsulation is the most efficient method so far to aid lactose maldigests as it is capable of withholding the activity of lactase in the stomach and releasing the entrapped lactose in the intestine. This method has been recognized as one of the most advanced and commonly used techniques in the food industry in order to protect the beneficial effects of certain food ingredients. Due to being entrapped, β-D-galactosidase cannot hydrolyze lactose until it reaches the human gastrointestinal tract. For the microencapsulation of lactase, a wide range of research has been conducted with different methods and a variety of coating materials. One of the preliminary research studies was done by Baik *et al.*; they used polymerized 1,6-diaminohexane and sebacoyl chloride as a coating material. To prevent the immediate hydrolysis of lactose, entrapment of β-galactosidase in a lipid vesicle was also tried [4]. The vesicle is generally called liposome; in this strategy β-galactosidase is entirely enclosed by a phospholipid membrane which is later destroyed in the stomach by the presence of bile salts. However, according to their claim, the entrapment efficiency was only 28%. To overcome the instability of the conventional liposome suspension, dried liposomes have been produced using the dehydration-rehydration method in some instances [15]. Kim and his fellow researchers developed dried liposomes containing β-galactosidase in the presence of trehalose and they found it had very high entrapment efficiency; in addition, trehalose could prevent the fusion of liposomes and the leakage of the entrapped enzyme [16].

In order to advance the microencapsulation technique, one of the most innovative works was revealed by Kwak *et al.* whereby lactase was microencapsulated with fatty acid ester [5,7]. To microencapsulate the lactase, the choice of coating materials is very important. In particular, it is necessary that the coating materials should be stable in the gastric ambience, while in the intestinal condition they essentially need to be hydrolyzed easily. Kwak and his group have determined MCT, PGMS or a combination of those two as suitable coating materials. In a subsequent study, it was shown that the β-galactosidase microcapsules coated with MCT or PGMS remained intact in the gastric juice and are hydrolyzed when they reached the upper intestine. The reason is attributed to the sensitivity of the microcapsules to bile salts and pancreatin present in the intestinal fluid. The maximum efficiency of microencapsulation was found when the ratio of coating to core material was 15:1. As a coating material, MCT was inexpensive, but the quality of MCT was not so satisfactory. On the other hand, PGMS is a very high quality material while it is expensive. Therefore, in order to obtain a viable coating material the research group of Kwak tried a combination of MCT and PGMS with a ratio of 5:5. The yield of microencapsulation was maximal with the combination product when the ratio of coating and core material was 15:1. Through some functional tests and clinical demonstrations, milk containing β-galactosidase coated with fatty acid esters did not show any change in sweetness during storage, meanwhile it maintained the characteristic taste and flavor, and was digestible to lactose-intolerant people [17]. During microencapsulation a small amount of β-galactosidase remained encapsulated to partially hydrolyze the lactose. This partial breakdown increased the sweetness of milk to some extent. To solve this, centrifugation of the residual enzymes has been suggested [7]. However, this process involved a high cost and was not suitable for large-scale production. Later, from the same inventors, a special technique called "ozone treatment" made it possible to inactivate the residual enzymes without affecting the sensory profile [3, 18]. Furthermore, this method could eliminate the pathogenic microorganisms from the food system. These inventions have proved a huge success in solving lactose-intolerance problems; however, one crucial aspect regarding microencapsulation of β-galactosidase still remained unaddressed. Since the water-in-oil (w/o) emulsion technique was used to encapsulating β-galactosidase, the resulting microcapsules made with MCT or PGMS were in liquid form. And these w/o microcapsules were found to be easily oxidized under normal environment conditions. Hence, the storage of microencapsulated β-galactosidase was a major concern; besides, the liquid type of microcapsules was inconvenient to transport. In order to overcome the disadvantages relating to w/o emulsion, recently an innovative work has been done by Kwak and his fellow researchers. They applied the water-in-oil-in-water (w/o/w) technique to encapsulate β-galactosidase. The w/o/w emulsion technique facilitates longer storage of microcapsules as it can be dried into a powder form; as a result, the delivery of the substance is now remarkably conve-

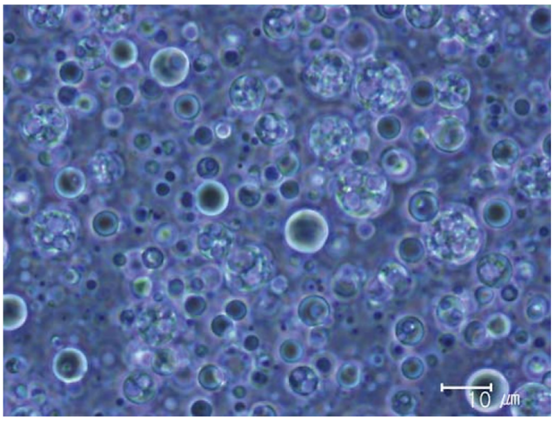

Fig. 2. Water in oil in water (w/o/w) emulsion of microencapsulated lactase. Source: [9]. See plate section for color version.

nient. In this double emulsion technique, two coating materials were used, whereby the inner phase was hydrophobic in nature and the outer phase was hydrophilic. MCT was used as the inner coating material and whey protein isolate (WPI) was chosen as the outer coating material. The hydrophilic characteristics of the WPI allow it to be dispersed in milk easily; the conversion of powder form is also associated with the hydrophilic nature of the outer phase of the w/o/w emulsion (Fig. 2). It was also shown that the yield and quality of microcapsules were further increased with this new technology.

It is obvious from the latest research results discussed that some of the inventions have great potential to aid lactose maldigesters and can make meaningful progress in the processing of dairy products. In particular, the, double emulsion technique invented by Kwak *et al.* is potentially a prime candidate for industrial application.

CONCLUSIONS

Considering the health tendencies of the modern food technology, the use of bio-based active films as packaging materials is very important. Many nutrition experts and food research institutes are looking for new ingredients with possible health benefits. Milk and dairy products comprise the major portion of the functional food market and these are essential parts of our daily diets. Hence, the application of functional ingredients to milk is worthwhile. For the efficient delivery of bioactive ingredients into milk, microencapsulation is regarded as a feasible solution. Using this technology the proper delivery of the bioactive ingredients can be ensured by protecting the functionality until the ingredients reach the target site in the intestine. Reduced dose and cost efficiency are two other potential advantages of microencapsulation. Microencapsulation has been used to deliver food ingredients and bioactive. Encapsulated ingredients have a superior performance, such as successful delivery of ingredients into foods, and a potential for enhancing bioavailability of bioactive components. Microencapsulated vitamins are used for nutritional purposes to fortify foods and as antioxidants.

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