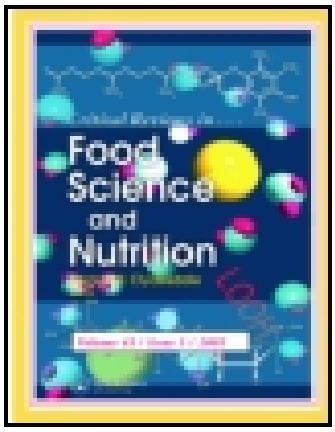

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Application of Liposomes in Some Dairy Products

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Application of Liposomes in Some Dairy Products

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The application of liposomes as potential carriers to deliver the food components is considerably innovative technology. While the application of liposome technology has been very limited to date, researches indicating the potential of liposomes for improving the flavor of ripened cheese using accelerated methods, the targeted delivery of functional food ingredients, the synergistic delivery of ascorbic acid and tocopherols for promoting antioxidant activity in foods and the stabilization of minerals (such as iron) in milk has been performed. In food industry, liposomes and nanoliposomes have been employed to encapsulate the flavoring and nutritive agents and, also, they have been suitable candidates to deliver the antimicrobials. In this paper,

the application of lipase, proteinase, nisin and flavor-containing liposomes in products during the processing (such as cheese maturity) as well as the application of liposomes-encapsulated micronutrients (such as iron) in milk are reviewed.

Keywords Dairy, Iron, lipase, liposome, nisin, proteinase

INTRODUCTION

Principally, the nanotechnology is recognized as an adverse field of scientific interests, which covers variety of steps such as apparatus and materials usages, process and production, wherein the majority of dimensions are in the nano-scale (Mozafari, 2007)

Nanometric types of liposomes, known also as Nanoliposomes, in fact are vesicles consisted of phospholipid bilayers, which have encapsulated a certain quantity of aqueous media (Zeisig and Cämmerer, 2001). The mechanism by which liposome is constructed might be attributed to unfavorable interactions existing between water and phospholipids molecules, wherein the polar proportions of phospholipids come in contact with the aqueous media present at interior and exterior sides of bilayer, and conseqently the hydrophobic hydrocarbon residues encounter each other inside a yielded bilayer (Jesorka and Orwar, 2008). In fact, the liposomes, which are tightly arranged bilayer vesicles, are result of energy input to the aggregated phospholipids molecules, wherein the occurring aqueous phases are occupied (Mozafari et al., 2008). Since lipid vesicles are composed by both aqueous and lipid proportions, the liposomes are claimed as being appropriate candidates to entrap, transfer, and release the substances soluble in lipid and water as well as amphiphilic materials (Mozafari et al., 2008; Khosravi-Darani et al., 2007).

There are variety of literature available employed liposomes to deliver therapeutic agents, as analytic tools, for modeling the biological membranes and as carriers for proteins, enzymes, vitamins, antioxidants and flavors in food products matrix (Jesorka and Orwar, 2008; Mozafari et al., 2008; Date et al., 2007; Kimball, 2008; Mozafari et al., 2006; Taylor et al., 2005a, b).

The liposomes, comparing another encapsulation methodology such as extrusion, fluidized beds and spray drying, are well-known for the stability that they present to substances soluble in aqueous media within applications with high a_w (Desai and Park, 2005). It has been demonstrated that the entrapped substances through liposome encapsulation show great stability against exterior factors such as temperature and pH changes as well as chemical and enzymatic alterations (Mozafari et al., 2008; Mozafari et al., 2008). Since the liposomes could be nature-oriented, regulatory hurdles capable to interfere their usages if foodstuff are principally decreased or removed, therefore novel formula might be created (Mozafari et al., 2008; Taylor et al., 2005; Gibbs et al., 1990; Breukink et al., 1999). The bacteriocins, when incorporated directly into food systems, are susceptible to be decomposed by proteolytic reactions or interacted by food constituents, thus the encapsulation of bacteriocins could be a fair solution to such issues (Da Silva Malheiros et al., 2010).

The liposomes have been suggested as an appropriate means to enhance the cheese ripening, due to their simplicity and easy composition modification (De Vos et al., 1993; Mulders et al., 1991; Xia and Xu, 2005). Because the ripening is a complex process and cheese making has diverse steps in every different type of cheese, no single liposome will be able to meet whole requirements for all types of cheeses. The flavor of cheese is probably composed of multiple various flavor constituents. The final flavor is formed over the ripening period as an elastic, bland curd is transferred into ripened cheese. Since a considerable portion of expenses belongs to ripening process, as a result of inventory and refrigeration costs, the acceleration of cheese maturing has been in interest focus of researchers (Kirby et al., 1991; El Soda and Pandian,

1991). One of the ways to shorten the prolonged maturation stage is to utilize the encapsulated enzymes by liposomes (El Soda and Pandian, 1991). The present article discusses the characteristics and the potential applications of liposomes for containment and encapsulation of different nano-techno-materials such as lipase, proteinase, nisin, iron, flavor and antioxidants in food products.

LIPOSOME FORMATION AND THE MAIN METHODS OF PRODUCTION

The Chemistry of Liposomes

When phospholipids are in contact with aqueous systems, due to their amphiphilic character, in order to protect their hydrophobic portions against molecules of water they create aggregated matrix with hydrophilic sections out touching aqueous environment. Then, provided that the enough energy is supplied, these aggregated complexes organize themselves to form liposomes, also known as nanoliposomes, (the well-arranged and closed bilayer vesicles). Within the process, the present hydrophobic components such as certain vitamins, nutrients and drugs in aqueous phase are encapsulated in liposomes by dissolving these substances together with the lipids. In turn, lipophilic components may interact with cyclodextrins to create complexes and consequently to be entrapped in aqueous part of nanoliposomes and liposomes (McCormack and Gregoriadis, 1994).

It should not be considered that the creation of nanoliposomes and liposomes is by accident. In fact, when phospholipids come in touch with hydration media, the lipid vesicles are created having one or a series of bilayers, with water molecules as separator. Lasic et al. (2001) proposed that the preference for symmetric membranes is being flat (spontaneous curvature ≡ Co=0) and

to curve them the sufficient energy is needed. The membrane curvature is determined by type of employed lipids and the availability of sterols. Cylindrical-shaped phospholipids, for example phosphatidylcholine (PC) able to construct bilayer sheet structures, may accept curvatures to create eventually vesicles. However, because of geometrical limitations these complexes usually are slightly unstable structures, when the stabilizing agents, such as sterols (e.g., cholesterol) are absent (Mozafari, 2005).

The fundamental to explore and produce the liposomes and nanoliposomes is to comprehend the phase transitions and fluidity of phospholipid membranes. Sine their phase behavior play essential role to present the properties including deformability, aggregation, fusion, permeability and protein binding which are influential in stability and behavior of lipid vesicles within biological media (New, 1990).

Methods of Liposome Production

There are numerous techniques for preparation of liposomes and nanoliposomes, and some books and reviews are available in this regards (Mozafari, 2005; New, 1990; Vemuri and Rhodes, 1995; Watwe and Bellare, 1995; Arshady, 2001; Mozafari, 2006). The choice of an appropriate methodology is dependent on various factors: the physicochemical properties of both targeted component for encapsulation and liposomal ingredients; the potencial toxicity and effective concentration of the encapsulated material; the nature of the solution the lipid vesicles being dispersed; the extra processes required over the period of usage and delivery of the vesicles; optimum size, polydispersity, and shelf-life of the vesicles and last but not least, batch-

to batch reproducibility and possibility of large-scale production of safe, efficient liposomal products (Mozafari, 2008; Gomez-Hens et al., 2006).

The most common technique is to let the preparation containing chloroform and/or methanol solution of phospholipid, cholesterol, and the targeted hydrophobic materials for encapsulation to be evaporated, that finally the thin film is left. The bilayer sheets of the amphiphilic/hydrophobic compounds, then, are separated off the bulk to yield multilamellar vesicles (MLVs), through supply of an aqueous phase and hydrophilic substance, along with providing enough quantity of mechanical or thermal energy (Mozafari, 2005; Lasic, 1988; Lasic, 1998; Mozafari and Mortazavi, 2005). MLVs are appropriate candidates to encapsulate the variety of lipophilic substances, since they own the large lipidic phase. Though in regard to encapsulate the hydrophilic materials, ULVs (unilamellar vesicles, consist of an internal aqueous core entrapped by bilayer phospholipid, are suitable tools. liposomes can be produced through several technophobes such as sonication and extrusion of MLV through polycarbonate filters for ULV-type, and the process of electroformation for GV-type (giant vesicles, with diameters in micrometer ranges) (Estes et al., 2005). In later technique, to prepare the liposomes firstly a film of lipids is deposited onto an electrode surface, made of indium tin oxide or platinum wire, AC electric fields are applied. The yielded GV-types are mainly unilamellar, which make them ideal models of cellullar membranes to be exploited as microscale bioreactors (Estes et al., 2005). Figure 1 shows the methods of liposome production. The main methods are discussed here:

Mechanical Methods

Some conventional methods of liposome production include the reverse-phase evaporation technique, ether injection technique, freeze-thaw method, and rapid solvent-exchange method. mentioned techniques yield ULVs or MLVs, based on the selected procedures (Szoka and Papahadjopoulos, 1978; Deamer and Bangham, 1976; Schieren, 1978; Pick, 1981; Buboltz, J.T.; Feigenson, 1999). Predominantly, the above-mentioned techniques yield heterogeneous mixtures of liposomes, if an extra processing step (e.g. sonication or filtration) is not employed. This heterogeneous population of particles, which mostly contains MLV-type liposomes within micrometric range in diameters, can be converted to a homogeneous mixture of unilamellar vesicles just by application of freeze-thaw cycles (for instance, 10 cycles of freezing in liquid N_2 and thawing at 25 ºC) (Palankar, 2008). Alternatively, nanoliposomes could be obtained, through filtration of MLVs frequently by employment of small pore filter membranes making the average diameter of the liposomes progressively smaller, provided that the right sizes for filters are chosen. Thereby, the tendency in liposomes to turn into ULVs is provoked (Mozafari et al., 2008). The Similar findings have been indicated by application of other processes such as sonication of MLVs and/or passing the MLVs through a microfluidizer (Vemuri and Rhodes, 1995).

Nanoliposomes can, also, be produced using an extruder, wherein the MLVs are extruded through nanometric filters. Regarding ULV preparation, MLVs are extruded in sequence through polycarbonate membranes under slightly low pressures by an extruder (Hope et al., 1985; MacDonald et al., 1991). Liposomes are susceptible to become contaminated by nonliposomal

aggregates, which might remained in the starting syring, therefore to avoid this phenomena it is necessary to fulfill an odd number of extrusion passes. It is worth noting that each preparation technique itself possesses an adverse mechanism of liposome formation. For example, it is suggested that the formation of liposomes is as a result of bilayered phospholipid flakes (BPFs) generation, which is followed by transmission of vesiculate into larger liposomes (Lasic, 1988).

Gould-Fogerite and Mannino (1993) suggested that the formation of LUVs as a function of agarose plug diffusion, calcium-EDTA chelation, and rotary dialysis methods is inter-mediated by structures known as cochleate cylinders. Talsma et al. (1994) proposed that the production of lipid monolayers at gas-water interfacial, due to application of bubble technique, could act as a start-up component to create further vesicle.

Principally, molecules of water and phospholipids enter the hydrophilic-hydrophobic interactions, which results in formation of liposomes and nanoliposomes. In respect to liposome formation the significant point is the undeniable role of underside interactions occurring between phospholipids and water molecules in creation of phospholipid membranes, besides importance of applied preparation technique. Therefore, to lead the membranes to create vesicles with right size, acceptable polydispersity, structure and elasticity as well as encapsulation efficiency is the main goal rather than producing the bilayer membranes in the random way (Mozafari, 2005; Mozafari and Mortazavi, 2005).

Non-Mechanical Methods

Methods Based on Replacement of Organic Solvents by Aqueous Media

In present preparation technique, instead of an aqueous solution, organic solvents such as water miscible or immiscible are employed. Alternatively, the technique based on the way of organic solution addition is classified into injection method, i.e. injection of the lipid containing organic solution into the aqueous medium, and proliposome-liposome method, i.e. stepwise incorporation of aqueous phase to the organic phase, ethanol especially. Moreover, in emulsification techniques, that is the reverse-phase evaporation and the double emulsion, a water-immiscible solvent is applied instead of an aqueous phase, therefore the liposomes highly capable to encapsulate the hydrophilic and lipophilic components are yielded.

The Ethanol Injection Method

In this method, the MLVs are obtained due to lipid solution of ethanol incorporation to a high quantity of buffer. For the present technique variety of drawbacks could be counted such as heterogeneity in particles size, high dilute liposomes, difficulty in removal of ethanol completely due to azeotrope formation between ethanol and water molecules, and possible biological inactivation of active macromolecules because of ethanol present in medium even at low quantities (Batzri and Korn, 1973). Despite the mentioned drawbacks, the considerable advantage of present technique is its capability to entrap the variety of drug components are entrapped appropriately for instance large hydrophilic proteins by passive encapsulation, small

amphiphilic drugs by a one-step remote loading technique, or membrane association of antigens for vaccines (Wagner et al., 2006, 2007).

Proliposome-Liposome Method

In present technique basically the liposome dispersion is yielded due to dilution of initial proliposome preparation with an aqueous phase (Maitani et al., 2001). It is claimed that this method is an appropriate technique to encapsulate the broad-spectrum of water and alcohol soluble drug components, and comparing the other passive entrapment-based techniques, is highly efficient in encapsulation phenomena.

Reverse-Phase Evaporation (REV)

In this technique to hydrate the lipid, same as other techniques based on injection, they firstly get soluble in an organic phase and then are subjected to an aqueous phase. Since the applied organic phase is necessary to be immiscible touching the aqueous phase, as a result an emulsion of oil in water is obtained, being followed by further dilution with aqueous phase to yield liposome (Tur´anek et al., 2003). The method is significantly popular, offering the favorable high rate in entrapment process near 50%, which can be even better promoted by application of the double emulsion preparation method (one variation of the microemulsion method), resulting in unilamellar liposomes (Szoka and Papahadjopoulos, 1978).

Methods Based on Detergent Removal

In this method, to solubilize the lipids within micellar media the detergents (such as, bile salts or alkylglycosides) with high solubility in organic and aqueous systems are employed. In fact, the molecules of detergents within aqueous phase are in equilibrium with lipid portion of the micelle. The physical properties of yielded vesicles such as shape and size are under the influence of chemical character and quantity of detergents as well as the nature of lipids applied. The cosolubilization of proteins and phospholipids occurring in membranes is the highly common procedure used to reconstruct the membrane proteins (Frokjaer, 1989; Jackson and Litman, 1982). There are multiple ways available to eliminate the detergents residue off the mixed micelles including gel chromatography, dilution, and dialysis using hollow and/or membrane filters as well as adsorption to cyclodextrins or hydrophobic resins (Driessen and Wickner, 1990; Schurtenberger et al., 1984; Brunner et al. 1976; Goldin, 1979; Milsmann et al., 1978).

LIPOSOMES APPLICATION IN DAIRY PRODUCTS

There are many potential applications for liposomes in the food industry including dairy industry, from the protection of sensitive ingredients to increasing the efficacy of food additives. There are literature available claiming that it is possible to modify the pharmacokinetics properties of drugs, herbs, vitamins and even enzymes through employment of phospholipids-derived liposomes and microscopic lipid vesicles. Moreover, liposomes have been used to present targeted delivery of the components being encapsulated in dairy industry. The main

applications, so far, have been aimed to change the texture of food components, encapsulating food components or additives, developing new tastes and sensations, controlling the release of flavors, and increasing the bioavailability of nutritional substances (Chaudhry et al., 2008). In dairy industry the first use of liposome belonged to in cheese making (Law and King, 1985). Other applications of liposomes are encapsulating antimicrobials, enzymes and minerals (like iron) in dairy products. Figure 2 indicates main role of liposomes in dairy products. Table 1 represents selected publications on using nonoliposomes in these products. Below, some important applications of liposomes in dairy products are discussed:

Application of Liposomes for Supplementation of Iron in Milk

Today, iron deficiency is highly recognized as the nutritional deficiency worldwide, which is mainly due to its insufficiency in dietary intake, lack of bioavailability, or both at the same time (Horton and Ross, 2003; Navarrete et al., 2002). This phenomenon should not be taken for granted as could cause anemia, when a blood hemoglobin level falls below standard, because of iron deficiency as one of the vital components in blood formation process (Gaucheron, 2000).

Since there is an antagonism between the iron and milk calcium, milk suffers from lack of iron (0.2–0.4 mg L^{-1}). Therefore, in order to enrich the milk with iron and to protect it against iron-calcium antagonism, fat oxidation consequences and metallic off-flavor, addition of enough iron into milk as microencapsulated by liposomes has been proposed. In this regard, iron salts highly soluble (e.g. ferrous sulfate) for their cheapness and higher bioavailability as well as preventing the negative effects of free iron are most applicable (Augustin et al., 2001).

In iron-containing nanoliposomes, using antioxidants such as ascorbic acid in the structure of liposomes is a common practice in order to protect the ferrous ion against oxidation (Xia and Xu, 2005).

Application of Liposomes in Cheese

Cheese is the milk-based nutritious product which is made mostly from cow's milk in variety of tastes, textures, and shapes, which are consequence of biochemical reactions over the ripening time. Since the cheese ripening is slow and costly, and sufficient time for activity of spoilage microorganisms is available, there are attempts to shorten this period by adding the flavoring agents, enzymes, texture improving components and to protect cheese against spoilage through incorporation of preservatives in encapsulated form by liposomes (El Soda and Pandian, 1991). Here, applications of proteinase, lipase and nisin in cheese production are expressed:

Proteinase

Most of the researches regarding the application of proteinase-containing nanoliposomes in cheeses are related to Cheddar cheese. Kheadr et al. (2000) recommended the use of encapsulated bacterial proteinase or fungal proteinase to accelerate Cheddar cheese ripening without producing flavor or texture defects. It is worth noting that in comparison with bulk-food phase, enzymes present more stability in concentrated solutions (Thompson, 2003) and also, it is possible to utilize stabilizing components such as thermostabilizers (e.g., sugars), being entrapped with the enzyme to protect the enzyme against high temperatures over processing steps (Kirby, 1991; Jackson and Lee, 1991). As a matter of the fact, if the lipid vesicles are

tailor-made the entrapped enzymes by them would not enter the interactions with the substrate, which results in having them inert and inactive within the food products. In addition, through fine tuning the composition of liposomes, the time, duration and the rate at which the enzyme is released can be managed.

It is claimed that application of enzymes encapsulated by liposome could result in enhancing the quantity of enzymes active in flavor formation concentrated in the curd, comparing the distribution of free enzymes within the whole-milk mixture (Desai et al., 2005). The application of milk fat was that of the first attempts to encapsulate the enzymes for improvement of cheese ripening duration. It is indicated that the flavor defects, caused by incorporating the free enzymes, decline as liposome-encapsulated enzymes are employed. Several advantages have been suggested as a result of using proteinases encapsulated by liposome, for instance to minimize the milk nitrogen losses from whey, to protect whey against being contaminated by proteinases, to control bitterness development as well as texture defects (Kirby and Gregoriadis; 1984). Since the circumstances at which proteinases are released, pH 5.0 and the temperature of 10 °C not much desirable by some, from the liposomes, their enzymatic activity is threatened and significantly dropped.

In addition to proteinase, starter cultures are effective in formation of desirable sensory properties of cheese types, as incorporation of Lactobacillus cultures enhanced texture changes and flavor production over ripening (El Soda et al., 2000; Oommen et al., 2002). Puchades et al. (1989) showed that the amount of free amino acids increased as a function of lactobacilli incorporation over cheese maturation time, however Abboudi et al. (1990) indicated that the cheese flavor was promoted as a result of increase in levels of the methyl ketone generated

Lactobacillus casei ssp. *casei* L2A. Tre´panier et al. (1991) as well, reported the same and observed 40% increase in flavor intensity of Cheddar cheese, when was incorporated by the similar strain; while choosing the cultures to incorporate, there are considerable criteria which should be taken on account involving the autolysis and proteolysis properties of the adjunct strain (Awad et al., 2000; Hannon et al., 2003). Though, Lactobacilli have been recognized as highly appropriate, El Soda et al.(1999) found that some of them autolyze at low rates and also, peptidase and esterase are produced at low levels in cheese slurry.

Lipase

Lipolysis of milk fat is critical in flavor changes of different cheese types, which is caused by lipases present in the rennet, rennet paste preparations and/or microbial lipases as well as natural ones (Chilliard et al., 1984; Desnouveaux et al., 1986; Catalano et al., 1985). One of the contributors to flavor of the matured cheese is free fatty acids (FFA), as a result of milk fat being hydrolyzed (Adda et al., 1982). Although, to achieve an appropriate flavor, while avoiding rancid off-flavors, researches should be conducted on finding the suitable ratio of FFAs individually (Woo and Lindsay, 1982). In addition, FFAs contribute to the aroma development by acting as precursors in the formation of methyl ketones, secondary alcohols, aliphatic as well as aromatic esters (Adda et al., 1982; Ha and Lindsay, 1991; Urbach, 1991).

There are several literatures available in which have been tried to enhance the lipolysis through incorporation of free lipolytic enzymes either at initial stages, milk or at final product, cheese curd (Law and Wigmore, 1985; Ashour et al., 1986; Omar et al., 1986, 1987; Lin and Jean, 1989; Ezzat, 1990; Kocak et al., 1996). It has been indicated that the application of free

enzymes is susceptible to present premature attack on substrate, which in turn results in an excessive lipolysis, being followed by defects in flavor as well as texture (Kocak et al., 1996). Therefore, to replace them with enzymes being microencapsulated has been suggested to overcome the mentioned issues. As a general rule, in order to avoid defects in the quality of cheese, the incorporation of liposomes intended to provide cheese with enhancement and promotion of ripening efficiency should be fulfilled under the control. Moreover, in preventing the off-flavors development besides guaranteeing the controlled release of Liposome core, the composition of Liposome is the key factor, that should be behaved carefully. Pro-lipo made Liposomes have been proved to be trustful candidate to carry the lipase in the attempt to accelerate the ripening process through faster fat breakdown, while avoiding the flavor defects formation.

Nisin

Nisin, an antimicrobial peptide (304 kDa) composed of 34 amino acids, involving unsaturated amino acids and lanthionine residues, is produced by variety of *Lactococcus lactis* strains. Nisin, an antimicrobial peptide (304 kDa) composed of 34 amino acids, involving unsaturated amino acids and lanthionine residues, is produced by variety of *Lactococcus lactis* strains. (De Vos et al., 1993; Mulders et al., 1991). Nisin is an efficient component in preventing the growth of board-spectrum of gram-positive bacteria and has been given GRAS (generally recognized as safe) status; which is why it is allowed to be employed as a natural antimicrobial in several foodstuff (Delves-Broughton, 1990a, b). Despite its efficiency to inhibit the *Listeria monocytogenes* has been reported, since in commercial mostly it is incorporated directly to

foodstuff, nisin is likely to be decomposed by enzymes and/or to interact with present components (e.g. proteins and lipids), which in turn results in activity defect (Abee et al., 1994; Crandal and Montville, 1998; Jung et al., 1992).

The most recognized mode of action by which nisin inhibits the bacteria activity is to form pores in the bacterial cytoplasmic membrane based on the barrel-stave mechanism and/or wedge model, which results in leakage of vital small cytoplasmic substances (Kirby and Gregoriadis, 1984; Driessen et al., 1995; Bennik et al., 1997; Ruhr and Sahl, 1985; Winkowski et al., 1994).

Since Dha residues are determined present in almost all products resulted from nisin decomposition, it is an evidence for their position in biological activity of the nisin peptide (Chan et al., 1989; Rollema et al., 1991). Nisin possess the great hydrophobic portion in its structure, especially the sections 1 through 19 completely hydrophobic, however Lys12 is excepted, and this portion has been identified as in charge to insert the nisin peptide into the lipid membran (El Jastimi and Lafleur, 1999). Therefore, it may be possible to provide the protection for the Dha residues by this portion being incorporated into the liposome membranes. In the meanwhile, the conformation of the peptide is affected and consequently the formation of β-turns is evoked, as a result of the association created between nisin peptides and phospholipid membranes, which in turn, contributes to the liposome membrane interlinked with nisin molecules, as comparing the unstructured nisin peptides the structured offer the higher stability (El Jastimi and Lafleur, 1997; Daeschel et al., 1992).

Today, concentrated nisin is being produced in variety of forms in commercial scale to be utilized in foodstuff involving pasteurized cheese spreads (Mazzotta et al., 1997). The nisin is mostly exploited as a free form in cheese industry, such as Nisaplin (Aplin and Barret, Ltd),

which is expensive and has drawbacks such as lower activity, stability, and bioavailability (Roberts and Zottola, 1993). In addition, cheese making process may be interfered by free nisin and even the cheese quality may be damaged as a result of growth inhibition in the starter culture or nonstarter LAB critical in ripening and flavor development (Morgan et al., 1997). The growth inhibition of pathogens and spoilage microorganisms within cheese has been reported by incorporation of microencapsulated nisin, and no interfere with the cheese-making process was seen, therefore its application as powerful antimicrobial for cheese preservation was proposed (Benech et al., 2002). Moreover, the lysis of starter was provoked as a function of nisin, which was followed by release of intracellular enzymes and consequently casein hydrolysis, thus the associated flavor development was accelerated (Pritchard and Coolbear, 1993).

Liposomes have been proved as appropriate carriers in controlled release of nisin within cheese matrix. In fact, the key characteristics of nisin such as stability, availability, and distribution are improved as being entrapped by liposomes immobilized their membrane. The antimicrobial effectiveness of nisin for a short-term and long-term preservation of cheese, by release of encapsulated nisin and desorption of membrane-immobilized nisin, respectively, is desirable (Daeschel et al., 1992; Cutter and Siragusa, 1997; Pritchard and Coolbear, 1993; Lau et al., 1991; Bower et al., 1995; Bower et al., 1995; Siragusa et al., 1999).

CONCLUSION

Multiple applications can be considered for liposomes and nanoliposomes in variety of fields such as cosmetics, diagnostics, drug and gene delivery, long-lasting immunocontraception, and food nanotechnology as food substances carriers, since they are biodegradable and

biocompatible. There are many potential applications for liposomes in the dairy industry, ranging from the protection of sensitive ingredients to increasing the bio-availability of micronutrients (such as iron in milk), promoting the efficacy (stability and controlled activity) of food additives (such as enzymes and nisin in cheese), accelerating the production of some foodstuffs (such as cheese) and nano-encapsulation of flavor compounds. Liquid milk and cheeses have been the main dairy products in which liposomes have been applied. The existence of encapsulated nisin provides both short-term (by release of encapsulated nisin) and long-term (desorption of membrane-immobilized nisin) antibacterial action, therefore, the preservation of food products is improved. At present, the use of liposome-encapsulated enzymes involves an expensive extra ingredient for cheese making. Further investigations could be in-line with industrialization of nano-liposome applications in dairy industry.

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Type of fortified product	Type of encapsulated material	Form of encapsulated material	Method of production	Functionality in product	Sources
Milk	Iron	Microencapsulated FeSO4 with lecithin	Reverse-phase evaporation , thin-film hydration, and freeze-thawing	Increased bioavailability	De Vos et al. (1993)
Cheese	Proteinase	Encapsulated *Bacillus subtilis* neutral proteinase and cyprosins	Pro-lipo H	Accelerate cheese ripening, decrease the flavor defects, reduced whey contamination	Kirby et al. (1984), El Soda and Pandian (1991) , Thompson (2003)
	Lipase	Encapsulated Palatase M or lipase 50	Pro-lipo H	Excessive lipolysis, development of flavors reduced the firmness	Chilliard et al. (1984), Kocak et al. (1996)
	Nisin	Liposome-encapsulated nisin Z	Pro-lipo H	Inhibits of gram-positive pathogens and spoilage bacteria	Delves-Broughton (1990a, b), Abee et al. (1994)

Table 1 Selected publications on using nonoliposomes in dairy products

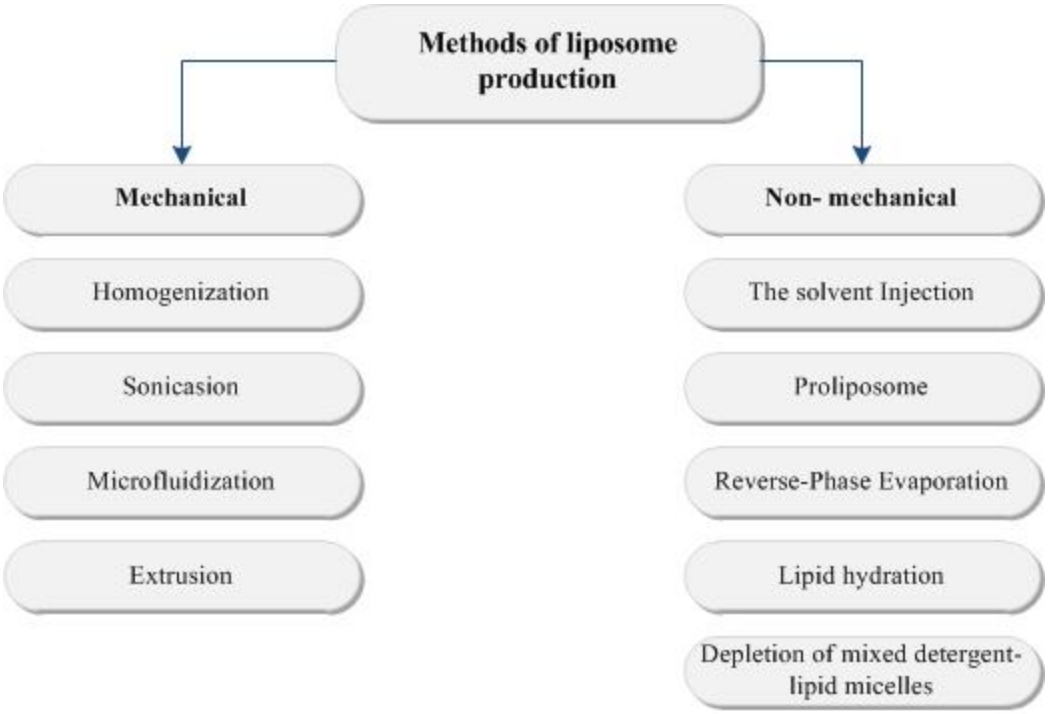

Figure2. Methods of liposome production

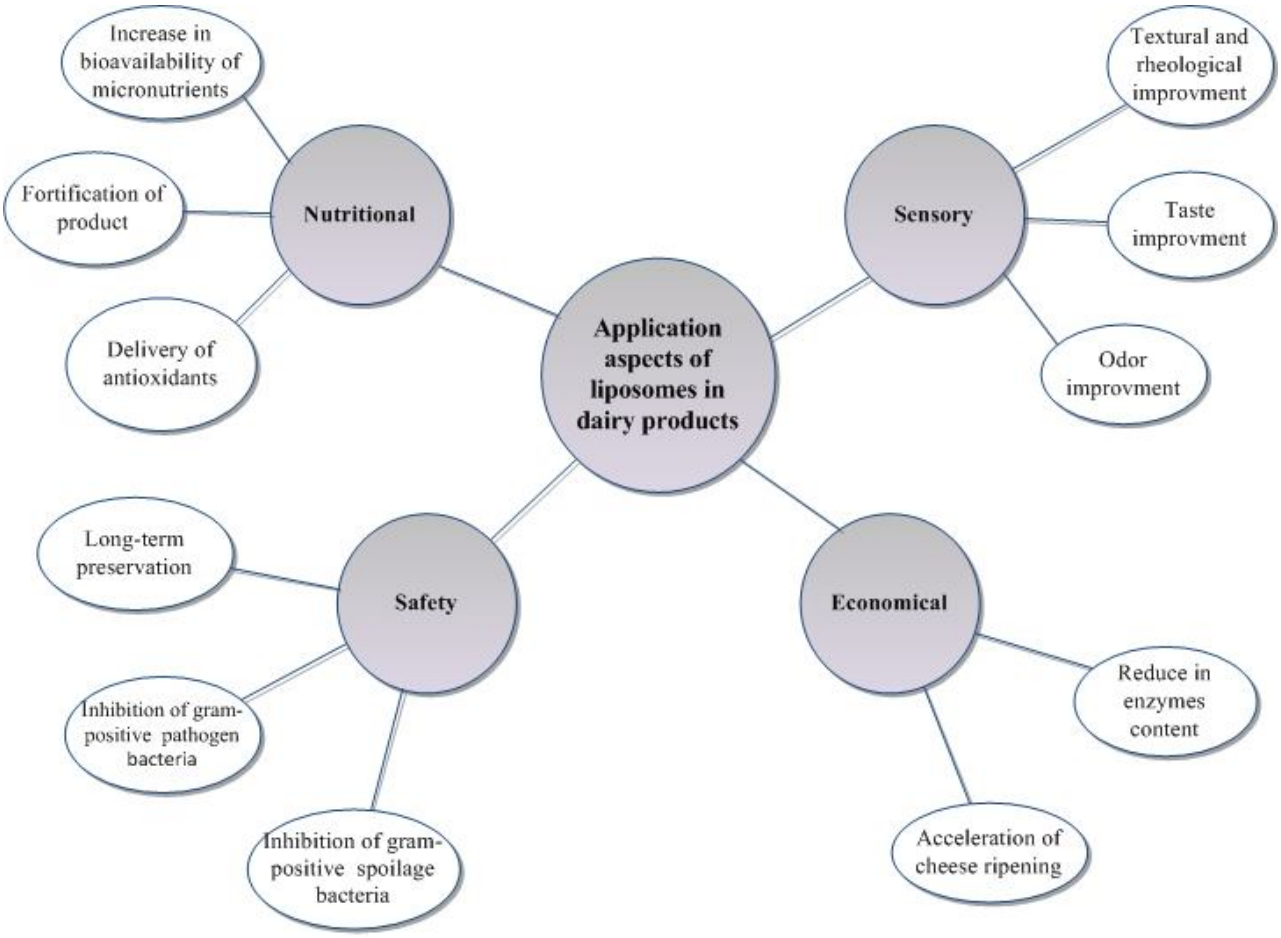

Figure 3. Main application aspects of liposomes in dairy products