ISSN: 2501 - 2568 (2016) Volume 1, Issue 1, 106 - 119
Journal of Food Bioengineering and Nanoprocessing
open access journal
www.nanobiofoods.com
Review
received: 10.12.2015
revised: 15.01.2016
accepted: 22.02.2016
published: 03.03.2016

Milk proteins as nanocarriers

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ABSTRACT

There are many applications that are used to enhance the food quality and bioavailability by nano-encapsulating the nutrients and food additives in food industry. Developing food tastes, providing hygienic food storage and extending the shelf life of the product, masking colors, flavours and odors, improving the bioavailability of nutrients and providing controlling release etc. can be taken as examples. Recent studies about nanoencapsulation include vitamins, antioxidants, colours, flavours, and preservatives. A number of nano systems became available for the delivery of nutrients with the development of nanotechnology.Milk proteins are naturally nontoxic proteins which don't require more mechanical energy to obtain. Milk proteins are natural encapsulation vehicles because of their ability to bind different bioactives and the ability to form hydrogels. There are some researches about casein micelles, reassembled casein micelles, β-lactoglobulin and α-lactalbumin. Recent researches show that natural casein micelles can be used for the production of nanogel particles. In addition to this, casein micelles and reassembled casein micelles (rCM) have been used for coating material. In other respects, β-lactoglobulin has been used for carrying bioactive compounds based on entrapment the components in protein hydrogels and α-lactalbumin nanotubes have been used as vehicles for drugs and components cause of its cavity.In this review, we focus on nanotechnology in food industry, milk proteins as nanocarriers and nutrient delivery.

Keywords: *Nanotechnology, nanoscience, milk proteins, nanocarriers, drug delivery, nanoencapsulation.*

INTRODUCTION

A nanometer is one-billionth of a meter (10^{-9} m). A human hair in a diameter approximately 60,000 times, a sheet of paper is 100,000 times, a red blood cell is 2,000 to 5,000 times bigger than one nanometer (Sekhon, 2010). The National Nanotechnology Initiative describe the nanotechnology as "Analyze, process, manipulate and develope nanoscale materials at 1-100 nm" (National Nanotechnology Initiative 2011). Nanotechnology interested with matter that ranges from 1-100 nm.

Nanotechnology is usable in other science fields include chemistry, biology, physics, material science and engineering (Kapre & Kakade, 2014). Nanofood term define the food which has been manufactured, produced, processed by using nanotechnics (Momin *et al.*, 2013). Nanotechnology has many applications such as tissue engineering, targeted delivery of drugs, biomedical engineering.

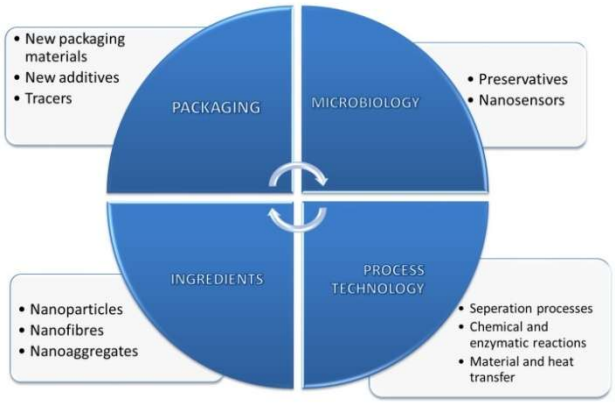

Figure 1. Summary of potential applications for nanotechnology in food science (Weiss & Gibis, 2013)

Additionally, nanotechnology can be used into the food processes such as nanosensors,

targeted delivery, safety of foods, manufacturing and producing new products and smart packaged foods (Ranjan *et al.*, 2014). Furthermore nanotechnology can enhance bioactive compounds specifications such as water solubility, thermal stability and oral bioavailability (Ezhilarasi *et al.*, 2013).

Nanotechnology applications in food industry can be summarized as follows Figure 1.

ENCAPSULATION

Encapsulation is a process that entraps a matter in a material to generate particles from few nanometres to a few millimetres (Burgain *et al.*, 2011). Encapsulation is a rapidly expanding technology that has potential applications in many areas including pharmaceutical and food industries (Ezhilarasi *et al.*, 2013). One of the first aim of encapsulation is to protect biologically active functional materials and microorganisms from negative conditions such as light, heat, moisture or oxygen through relation between core and encapsulation material. Therefore, encapsulated material is protected and the functionality of the product is increased (Tavares *et al.*, 2014). For example, a probiotic bacteria is inactivated in the stomach and gastrointestinal tract by the negative conditions. Probiotic bacteria can be protected by encapsulation until they reach where they are supposed to be active (Boom 2011).

We may review different types of encapsulates as shown in Figure 2 to understand encapsulating further. In the reservoir type, the active agent has been coated by a shell, therefore reservoir type may also be called capsule. In the matrix type, the active agent is much more dispersed over the carrier material. Active agent can be found on the carrier material surface. The coated matrix type is combination of reservoir type and matrix type. The coated matrix type is coated by a shell (Zuidam & Shimoni 2010; Burgain 2011).

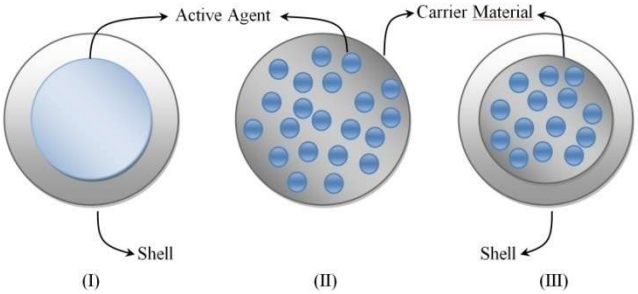

Figure 2. Encapsulation systems; (I) reservoir type, (II) matrix type, (III) coated matrix type (Zuidam&Shimoni, 2009; Burgain 2011).

Encapsulation applications of bioactive compounds in food industry include hiding flavours, colours and odours, controlling oxidative reaction, increase shelf-life etc (Burgain 2011). Some encapsulation techniques and processes for bioactives and food ingredients are summarized in Table 1.

Types of encapsulation;

1. Nanoencapsulation (1-100 nm)
2. Microencapsulation (0.1-5000 µm)
3. Macroencapsulation (>5000 µm)

Table 1. Overview of some encapsulation techniques and processes for bioactives and food ingredients (Tavares 2014; Zuidam&Shimoni, 2010)

Technique	Procedure	Examples
Spray drying	Drying of the encapsulated material dispersed in the shell material. Basically, dispersing or dissolving active material in aqueous solution then atomizing and dehydrating. Starch, polysaccharides, maltodextrins and/or proteins used for encapsulation.	Encapsulation of flavor compounds, polyphenols and vitamins
Spray cooling/ chilling	Incorporation of the core material in the warm and liquefied shell material (often vegetable oils). Dispersing or dissolving active material in heated lipid solution then atomizing and cooling. Relatively low-cost encapsulation technique. Through product atomization, and consequently cooling, micro-capsules are formed	Encapsulation of flavor compounds, minerals, vitamins and probiotics
Freeze drying	Co-lyophilisation of the core and the shell materials after a homogenization process. Dissolving or dispersing active agent and carrier material in water then freezing the	Encapsulation of flavor compounds,

Technique	Procedure	Examples
	sample and drying under low pressure. Grinding (optional). Normally, this technique produces non-uniform particles.	fatty acids and probiotics
Extrusion	Formation of core material droplets that became microparticles after immersion in a hardening bath with the shell material. Basically, melting the coating, dispersing or dissolving active in the coating then extruding with twin-screw extruder and cooling. Normally, the shell material is a glassy carbohydrate matrix. The core material may be released in a high temperature medium. The encapsulation efficiency is small, moreover the produced particles show high stability and an extended shelf-life.	Encapsulation of flavor compounds, vitamins and food ingredients (lactic acid)
Spinning disk	Passage through a spinning disk of a suspension of the core material in the shell material. During processing, the shell material forms a thin film around the core material particles.	Encapsulation of cells (yeast)
Supercritical fluid extraction	This technique is similar to spray drying, except that the shell material and the core material are solubilized/dispersed in a supercritical fluid.	Encapsulation of heat-sensible coresas vitamins and polyphenols
Fluidized bed	The shell material is atomized onto core material fluidized by an upward stream of air. First fluidizing active powder and spraying the coating then dehydrating or cooling. This technique is applied for solid particle encapsulation (100 mm to few mm).	Encapsulation of acidulates, vitamins and cells
Cocrystallization	Spontaneous crystallization of a supersaturated solution of sucrose simultaneouslywith the addition of the core material, forming a crystalline irregular network, allowing the encapsulation into the pores of the network.	Encapsulation of acids, flavor compounds, antioxidants and minerals
Coacervation	Phase separation of one or many polyelectrolytes from a solution and deposition of the colloidal particles around the active ingredient suspended or emulsified in the same reaction media. When hydrocolloids are used, they can be cross-linked using appropriate chemical or enzymatic agent. Basically preparing o/W emulsions with lipophilic active in oil phase, mixing under turbulent conditions then inducing three immiscible phases and cooling. Crosslinking (optional).	Encapsulation of fatty acids and flavonoids
Liposomes	Dispersing lipid molecules in water with active agent in lipid or water phase and reducing size by high shear or extrusion. Removing free active (Optional). Spherical particles consisting of a membranous system formed by one or more concentric bi-layers of lipids (often phospholipids). They can be used in the entrapment, delivery and released of water-soluble, lipid-soluble and amphiphilic materials.	Encapsulation of vitamins, enzymes and peptides
Inclusion	Mixing carrier, active and water together and incubating, Drying (Optional) This technique refers to the supra-molecular association through non-covalent interactions of a ligand (“encapsulated” compound) into a cavity formed by a “shell” material (e.g., cyclodextrins).	Encapsulation of vitamins, flavor compounds and essential oils

NANOENCAPSULATION

Nanoencapsulation is a technology which assembles substances in miniature size and it has been used to perform some techniques like nanocomposite, nanoemulsification, and nanoestructuration. Moreover, it helps to ensure the ultimate product performance which also includes controlled release of the core (Sekhon 2010).

There are two technics for production of nanomaterials and these are “top-down” approach and “bottom-up” approach. The top-down approach includes physically reduced materials to nanoscale by grinding, milling, lithography and etching technics. Using dry milling technology to obtain a water-bending capacity wheat flour of fine size is a good implementation of this method. The bottom-up approach technics such as self-assembly and self-organization are derived from biology implementations and include chemical synthesis for building larger structures atom by atom or molecule by molecule(Momin 2013). The “bottom-up” method depends on the self-assembling properties of molecules under specific thermodynamic conditions to assemble supramoleculer structures, microstructures to produce desired functional materials (Sanguansri 2006).

Nano-sized or nano-encapsulated food additives and supplements may help to enhance the dispersibility of fat-soluble additives in food products, enhance the flavour of the food, provide hygiene for the food storage, decrease the usage of fat, salt, sugar, and preservatives; and also develope in the uptake and bioavailability of

nutrients and supplements. Vitamins, antioxidants, colours, flavours and preservatives are the samples that exist today(Chaudhry and Castle 2011).

Level of protection and bioavailability of the trapped materials are significantly affected by the capsule size and material. By means of the developments of nanotechnology, the delivery of the active ingredients in the system can get some of the convenient nano systems. The nano delivery systems that used in the current applications are nanoemulsions, nanoliposomes, nanoparticles and nanotubes. (El-Salam & El-Shibiny 2012). Reducing the nutraceuticals partical size causes more surface area per unit volume, consequently their biological activity are increased therefore bioavailability, delivery properties and solubility of nutraceuticals may be improved. Thus, entrance of low particle sized nutraceuticals to the bloodstream from the gut become easier (Ezhilarasi 2013).

High- pressure value homogenizers or microfluidizers with a droplet diameter that are less than 100-500 nm, generate nanoemulsions. Functional food ingredients may be included in the droplet, which is the interfacial region, or the continuous phase in order to decrease the chemical degradation process (Rashidi &Darani 2011).

Basically, nanoemulsions are an emulsion at nanoscale in which the disperse phase consists of nanoparticles. Nanoemulsions are formed by shear-induced repturing. For making a repatable stable emulsion, some factors must be considered and controlled such as selecting a suitable composition, controlling of addition to formulation which components in what order and the effective shearing that can repture droplets effectively. Additionally there are some exigences for nanoemulsions. First of all, the dispersed phase molecules must not soluble in the continuous phase. The easiest method to achieve this situation is chosing insoluble liquid for dispersed phase. Secondly, choosing of components is an important stage for formation of lyotropic liquid crystalline microemulsion phases which is an undesirable situation due to short chain alkalines, water, alcohols and surfactans can be formed. Third one is the surplus of surfactant of continuous phase which allows new surface area for nanoscale droplets to be coated. Last exigences is necessity of resizing microscale droplets into nanodroplets (Mason *et al.,* 2006).

Through the novel encapsulation and delivery properties that include oil in water in oil (o/w/o) and water in oil in water (w/o/w) emulsions, numerous emulsions may be used as a delivery system. The emulsions that cover more than one layers may produce a novel delivery system including oil droplets which are confined by multilayer interfaces. (nanomer thick layers do not arise from the same polyelectrolyte.) They are more stable to the stresses caused by environment than conventional oil in-water emulsions with single layer interfaces. Functional ingredients (e.g. proteins, polysaccharides, and phospholipids) are baited in the core of a multilayer emulsion delivery system in this kind of applications. (Rashidi & Darani 2011). Nanoemulsions with small droplet sizes are specific type of collodial dispersion. Usually nanoemulsions coating range are between 10 nm to 200nm. Nanoemulsions increase transportation ability, solubility, dispersibility, availability and accessibilty of bioactive compunds, food and drugs such as alpha tocopherol, antioxidants, polyunsaturated fatty acids, hydrophobic vitamins, flavour and aroma ingredients. Additionally, nanoemulsions are better encapsulation systems than conventional emulsions (Adjonu *et al.,* 2014).

The nanoscale nutraceutical compounds can be classified as lipophilic type and hydrophilic type according to their solubility in water. Lipophilic type nanoscale nutraceutical compounds are insoluable in water but hydrophilic type nanoscale nutraceutical compounds are soluable. Lipophilic compounds are soluble in lipids and organic solvents although hydrophilic type compounds are insoluable. Some nanoencapsulated lipophilic nutraceuticals are lycopene, β-carotene, lutein, phytosterols and docohexaenoic acid (DHA). Ascorbic acid, polyphenols, etc. can be given as examples of nanoencapsulated hydrophilic nutraceuticals (Ezhilarasi 2013).

Liposomes occur one or more lipid or fosfolipid bilayers in aqueous phase which other molecules react with it and they are closed, continuous ester phospholipids. Nanoliposomes

have same physical, chemical and thermodynamic properties as liposomes however nanoliposomes could be stable against aggregation and their nanometric size remains stable during storage. Nanoliposomes can entrap, deliver and release water and lipid soluable bioactive components by their amphiphilic structure. This property is the major advantages of nanoliposomes (Kuan *et al.,* 2012). Liposomes and lipid vesicles are largely convenient systems to deliver a broad spectrum of substances in functional food, pharmacological, agricultural, biochemical, biological, etc. Liposomes and lipid vesicles are closed, continual bilayered structures made mostly of lipid and/or phospholipid molecules. They may also be produced by the heating method which does not have any harmful chemical or procedure. Liposomes may encapsulate enzymes to enhance the speed of ripening of cheese and also vitamins to enhance the nutritional standards of daily products(Rashidi & Darani 2011).

Carbon nanotubes are regular carbon allotropes of various appearance ratio along with dominant surface area lengthing from a hundred nanometers to micrometers along with diameters of 0.4–2 nm for single-walled (SWNT) and 2–100 nm for multiple-walled (MWNT) carbon nanotubes(Celiker & Mallikarjunan 2012). Nanotubes are actually magnetic metal dots which contain more residue forming from typical form of hexagon to perforated carbon tube form. The features of nanotubes contain thermal resistance and a powerful and flexible structure which might be used in medical vehicles, sport equipments, alumina, and industrial food transaction devices. (Rashidi & Darani 2011).

A variety of applications of nanoparticles within the food industries are focused on (i) sensory developments (taste/color enhancement, texture modification), (ii) enhanced absorption and pre-determined delivery of nutrients and bioactive compounds, (iii) stability of active contents like nutraceuticals in food structures, (iv) packaging and product development to enhance the shelf life, (v) sensors to evaluate the safety of food, (vi) as an antimicrobial effect against to the pathogenic bacteria (Ranjan *et al.,* 2014).

Food Safety and Risks

Protection of products from biological, chemical, physical and radiation contamination have to come to mind when using the term "food safety". Using nanotechnology as a novel food and dairy processing caused new health and environmental concerns (Qureshi *et al.,* 2012).

Nanoparticles can be absorbed by skin contact, respiration and intestines and they enter to the bloodstream when added to the food, water and drugs (Maynard 2006). Generally the effect of nanoparticles on human body such as nanotoxicity varies by surface properties, particle size, chemical composition and how single nanoparticles gathered. Possible risks of nanomaterials can also be determined by the size of the nanoparticles that enter the body, penetration areas, potential accumulation and translocation of nanoparticles in the body. Some factors can be considered such as contact duration of nanoparticles, toxicology of nanoparticles, determining of nanoparticle toxicity by toxicological databases, biological nanoparticle transformation for evaluation of nanotoxicity (Chau *et al.,* 2007).

Nanomaterials have increased contact surface area. This property makes nanomaterials more reactive and mobile, and this may cause DNA mutation, carcinogenic effects by forming free radicals in body (Quareshi *et al.,* 2012).

On the other hand, researches revealed methods to produce harmless nanomaterials (Zhang *et al.,* 2003). In this research minimizing particle size to nanoscale diminished the toxic effect of the substance. Another research paper revealed that nitrogen enriched carbon nanotubes established to the trachea of mice are less toxic than normal carbon nanotubes, which can be used in food packaging technology (Chau *et al.,* 2007).

MILK PROTEINS AS NANODELIVERY

Biologically active functional components are protected and associated in a targeted delivery systems at begining from the time of preparation, storage, digestion and absorption into human body. For the good targeted delivery systems functional components have to be released at

desired times and in desired areas (Kuan *et al.*, 2012). Synthetic polymers such as polyacrylamide, polyamides, polyphenylesters and polyurethanes can be used in the biomedical and pharmaceutical delivery systems. Nevertheless, synthetic polymers are not allowed to use in food processing due to requires GRAS ingredients. For this reason, polysaccharides such as alginate, carrageenan, pectin, dextran and chitosan, proteins such as zein, casein and whey proteins and lipids such as medium chain triglycerides, tristearin and corn oil are preferable for food processing because of their biodegradability and non-toxicity. However, using these polymers can be revealed some problems such as price due to their performance and processing like other biodegradable food-grade polymers (Ramos *et al.*, 2014).

Milk proteins can bind various bioactives or entrap them by forming supra-molecular structures, emulsions or hydrogels, thus they are interesting encapsulation agents(Tavares *et al.*, 2014). Milk proteins such as β-lactoglobulin can be denatured by pressure, heat and pH etc. and the denatured components reassemble to form larger structures (Momin *et al.*, 2013).

Through their serine-phosphate residues, Caseins α-s1, α-s2, and β bind calcium in a natural way, moreover they do the same to calcium phosphate nanoparticles. As it is mentioned before, it can be seen as the main historic mission of these proteins. Lactoferrin binds Fe^{+3}mainly, on the other hand, it may bind other metal ions such as copper, manganese, chromium, and aluminium in living organisms, too.

Milk proteins can bind hydrophobic molecules with the help of some mechanisms, mostly hydrophobic interactions, van der Walls appeal, and also hydrogen bonds. Caseinate, and isolated β-CN, have been demonstrated to bind vitamin D, as it seems via hydrophobic interactions, and β-CN has been demonstrated to bind a chemotherapeutic medicine, mitoxantrone, with the combination of hydrophobic and ionic interactions.

The amphiphilic structure of many milk proteins offers splendid surface properties. They can absorb at oil-water interfaces and stabilize emulsions and this ability they have can be influenced by their litheness, structure, position of aggregation, pH, ionic power (especially calcium ions) and temperature. The line of surface activity which was reported for separate milk proteins was: β-CN > monodispersed casein micelles > BSA > α-la > α_s-CNs = κ-CN > β-lg.

Certain main milk proteins are self and co-assemblers in a natural way. Caseins are organized in micelles in nature. These micelles are spherical clusters of 50-500 nm and they are held all together mostly by hydrophobic interactions and calcium-phosohate nanoclusters, bridging between their serine-phosphate residues.

Milk proteins own perfect gelation properties, for example, acid or rennet curd formation of caseins and heat caused gelation of whey proteins or of the whole milk proteins. Acid gelation of caseins is originated from isoelectric precipitation and rennet-caused gelation is originated from the proteolytic cleavage of κ-CN's hydrophilic "hairy layer" that results in micelle aggregation(Livney 2010).

Milk protein concentrates are preffered choice of ingredient in many products such as clinical and nutritional beverages, cheese, yogurt and ice cream due to their high protein and low lactose content, existence of caseins in micellar form and whey proteins (Gazi & Huppertz 2015). The advantages of milk and diary products as ingredients are colorless, tasteless, more stable for processing, non-toxic and they have specific physicochemical properties (Ramos *et al.*, 2014).

The main milk proteins are caseins and the whey proteins.

Caseins and casein micelles

The major protein of milk is casein. The average diameter of casein is 150 nm and it present in the form of large colloid units called casein micelles (Celiker & Mallikarjunan 2012). Caseins exist in milk (94%) are generally formed as assembled micelles of sizes which range from 50 to 500nm. Caseins are the fragments of proteins that sediment at pH 4.6. They are mostly thought intrinsically unstructured proteins. (Tavares *et al.*, 2014). Caseins are belonged to a family of open-structured, proline-rich phosphoproteins. The major four caseins in bovine milk are α-s1-CN, α-s2-CN, β-CN and κ-CN. They are different in these

situations; the number and sequence of amino acids, number of phosphorus atoms, and proline and carbohydrate ingredients. (El-Salam & El-Shibiny, 2012). The αs1-CNs generate %40 of the total casein fraction, αs2-CNs generate %10 of the total casein fraction and β-CNs generate %35 of the total casein fraction in bovine milk (Huppertz 2013). α-s1-CN, α-s2-CN and β-CN can bind to the amorphous micellar calcium phosphate cluster based on their phosphoserine residues and then a stabilized protein shell is formed. κ-CN cover the surface of the micelles and provides a hydrophilic, charged and diffuse surface layer. Additionally, κ-CN strengthens the micelles through intermicellar electrostatic and steric repulsion such as polyelectrolyte brush (Sahu *et al.,* 2008). αs1-CN, αs2-CN and β-CN are sensitive to calcium however κ-CN is insensitive to calcium (Huppertz 2013).

Casein micelles have porous structure and may be subjected to physical applications such as heating, boiling, cooling, drying (Ramos *et al.,* 2014). Casein micelles may be considered as a designed nano device in a natural way for the delivery of minerals, especially Ca to neonate but their structures are not fixed. Temperature changes, ionic strength, pH, water activity, and high hydrostatic pressure treatment cause some changes in size distribution of casein micelles due to the lack of a rigid three dimensional tertiary structure (El-Salam & El-Shibiny, 2012).pH dependent dissociation of casein from the casein micelles may occur by heating milk. When the milk is heated, low rate casein dissociates from the casein micelles at low pH without consideration of heating temperature. Increasing the pH and the temperature up to about 70 ℃ causes gradationally more casein dissociates from the casein micelles. The dissociated casein are composed of large amount of casein proteins include α_s-casein, β-casein and κ-casein under these conditions. The dissociation of κ-casein increases with increasing temperature and pH at temperatures above about 70℃. The amount of α_s-casein and β-casein decreases with increasing temperature and pH. Therefore the low level of α_s-casein and β-casein are dissociated at about 100 ℃. Usually casein micelles surrounded by κ-casein at low pH. κ-casein passes through from casein micelle to the serum when the pH is increased (Anema & Li 2015). Casein micelles are consist of several units which are linked together such as calcium ions, phosphates and citrates. The casein micelles are seperated into two parts as defined in the submicelle model and Holt model. First part is consists of α-caseins and β-caseins and this part located inside the micelles. This part of casein micelles has hydrophobic behavior. Second part consists of κ-casein and this part located outer surface. This part of casein micelles has hidrophilic behavior (Pomastowski *et al.,* 2014).

Caseins, in milk, form surplusly hydrated colloidal corpuscles named casein micelles on a dry-weight basis, casein micelles include about 94% caseins and also 6% minerals, mostly calcium phosphate. The caseins are synthesized more particularly in the mammary glands, guessing that one of their functions is to ensure amino acids which are necessary for the improvement of the neonate. Caseins are conjugated proteins because they frequently esterified to serine residues with phosphate groups (Gutiérrez*et al.,* 2013). Along with that mission, caseins provide the milk to be supersaturated in calcium phosphate due to caseins capacity to bind divalent and multivalent ions. As a result of this property, casein micelles are the ones that are natural devices for calcium and phosphate delivery to neonates (Tavares, 2014). Casein is responsible for the synthesis of hemoglobin and plasma proteins therefore casein is the main protein and most useful structure in milk. On the other hand caseins trigger the proliferation of lymphocytes and phagocytic activities of macrophages due to their phosphoprotein groups (Pomastowski *et al.,* 2014).

Caseins have great emulsification, gelation, water binding properties, biological activity and digestibility(Sahu *et al.,* 2008; Qureshi *et al.,* 2012). Therefore, casein micelles are natural nano-capsular carrier for nutraceuticals (Qureshi *et al.,* 2012). Casein is essential protein for many bioactive peptides. Peptides that derived from casein are occur during enzymatic digestion and these peptides take part of regulatory function of an organism. These functions include antihypertensive activity, mineral binding, opioid agonist function, hormone like-activity and

immunomodulatory processes. Casein can be designed and used as a diatery supplement for athletes and caseins antimicrobial peptides have inhibatory effect on pathogenic microorganisms (Pomastowski *et al.*, 2014).

Lately, enzymatic cross-linking plus subsequent removal of the calcium and calcium phosphate from the modified micelles have used the natural casein micelles preparation of nanogel particles. Caseins are perfect substrates about cross-linking by transglutaminase owing to their low level of minor and tertiary structures. Also, they can link glutamine residues in the peptide chain with the help of lysine residue in a covalent way. It has been reported that treatment of casein micelles with TGase can induce intra-micelle cross-linking in minimum cross-linking through micelles (El-Salam and El-Shibiny, 2012).

Reassembled casein micelles (rCM) may be prepared either with acid casein or with sodium caseinate but using these methods to prepare a rCM successfully needs to be controlled by the important parameters (e.g. pH, temperature, ionic strength). The table sums up the steps that are followed for the preparation of rCM from acid casein and/or sodium caseinate. The size of native casein micelles was a bit smaller than the size of rCM. Moreover, the properties of the native and reformed casein micelles were not the same (El-Salam and El-Shibiny, 2012).

Table 2. The preparation of the reassembled casein micelles from the acid casein and sodium casein (El Salam & El-Shibiny, 2012).

Mounsey et al, 2005	*Semo et al, 2007*
Acid Casein (88 g) Mixed 5 min in 912 gr deionised water	200 mL Sodium Caseinate (5g/kg) Mixed with 4mL K_3citrate (1M), 24 mL K_2HPO_4 (0.2 M) and 20 mL $CaCl_2$ (0.2 M)
Casein Dispersion (88 g) pH adjustment to 7.1 in 15 min with $Ca(OH)_2$ (100g/kg)	Caseinate Solution (88 g/kg) Adding 8 times 2.5 mL K_2HPO_4 (0.2 M) with 15 min intervals, 5 mL $CaCl_2$ (0.2 M) and mixed at 37 ^oC
Calcium Caseinate Micelles (18 mg Ca/g protein) Titration with $CaCl_2$ $2(H_2O)$(113 g/kg) + Na_2HPO_4 (116g/kg)	Reassembled Casein Micelles (400 mL) pH adjustment to 6.7 and ultracentrifuged (1 hour)
Reassembled Ca Caseinate Phosphate Heating at 60 ^oC and 20 min	Precipitate (185 MPa)
Reassembled Casein Micelles	Reassembled Casein Micelles

The re-assembly of caseinate was applied by Semo *et al.*,(2007) to encapsulate vitamin D_2.

Consecutive additions of mixture of potassium hydrogen phosphate and calcium chloride which was dropped into a solution of sodium caseinate and tri-potassium phosphate induced the re-assembly procedure. Semo *et al.*, 2007, achieved casein micelle-like structures with a diameter of nearly 156 nm. The structures also let the influential encapsulation of vitamin D_2 of roughly 27%. Vitamin D_2 was effectively protected from photochemical degradation by this way. All these casein supra-molecular structures were influential in the protection of thermolabile β-carotene facing thermal degradation throughout industrial processes like sterilization, pasteurization, and baking (Tavares *et al.*, 2014).

Sahlan and Pramadewi (2012) carried out an experiment to test the capacity of caseins to encapsulate flavonoids with the help of isolating a casein fraction from cow milk by combining a rennet hydrolysis and a little reducement of pH. Casein nanoparticles that have a mean diameter close to the diameter of native casein micelles, which was 109 nm, were achieved. They possessed an encapsulation proficiency of about 42%, answering to an encapsulation of nearly 1.0 mg flavonoid for each gram of casein(Tavares *et al.*, 2014).

Roach *et al.*, (2009), investigated that the association of triclosan (TCS) with the bovine casein micelles. They reported that caseins were able to improve the solubility of TCS up to %80 without pressure and association increased by %30 with pressure (at 300MPa). Reformed micelles exhibit better viscosity than skim milk at 100 MPa, however viscosity has decreased after more pressure due to change of particle morphology. The enhanced solubility of TCS provides better casein micelles as a potential delivery system for triclosan (Roach *et al.*, 2009).

Zimet *et al.*,(2011) used reassembled casein micelles and casein nanoparticles as nanovehicles for u-3 polyunsaturated fatty acids. In the study, they demonstrated that casein can be bound to docosahexaenoic acid (DHA), an important u-3 polyunsaturated fatty acid. The results suggested that DHA had a high tendency towards self-

assembly into casein and there were an average of 3-4 DHA binding areas in each protein molecules. Reassembled casein micelles with DHA sized 50-60nm were prepared and no significant effect of heat treatment (20s pasteurisation at 74) on the particle size was found. It was also observed that Reassembled casein micelles with DHA and casein nanoparticle systems with DHA have a protective effect against DHA oxidation (Zimet *et al.*, 2011).

Saiz Abajo *et al.*, (2012) encapsulated casein micelles and β-carotene. In this study, in the first stage, the liquid micelle solutions and control samples (non-encapsulated β-carotene samples) were heated at 80 ^{o}C for 8 h and in the second stage, the sterilization step, samples were heated for 14 min at pH 7, incubated at 121 ^{o}C for 9 min and finally cooled to a final temperature of 26.5 ^{o}C for 23 min. In the third stage, pasteurization, samples were heated for 7 min at pH 6.7, incubated at 100 ^{o}C for 14 min and finally cooled to the final temperature, 26 ^{o}C, for 21 min. In the fourth stage, the thermal resistance of free β-carotene in the encapsulated β-carotene and cookies were checked. 400MP hydrostatic pressure was applied for 5 min for the fifth, final, stage. It was demonstrated that β-carotene samples coated with casein micelles were protected against degradation during industrial processes such as heating, sterilization, pasteurization and high hydrostatic pressure were applied (Sáiz-Abajo *et al.*, 2013).

β-casein nanoparticles can serve as effective oral nano carriers to stabilize and solubilize hydrophobic drugs. Mandelbaum and Danino (2009) focused on encapsulation of celecoxib and budesonide, which are known with their low bioavailability, and they tried to nanoprocess these molecules in β-casein. Light microscopy studies showed that the concentration and the size of the drug crystals were significantly reduced in the presence of casein and that β-casein in solution has the ability to stabilize and interact with drugs (Elzoghby *et al.*, 2011).

Zhu and Li (2003) prepared polymethylmethacrylate (PMMA)/casein core/shell nanoparticles by copolymerization of methyl methacrylate with casein micelles excited with a small amount of copper ions. The presence of casein micelles supported the emulsion polymerization of the monomers and provided particle stabilization (Elzoghby *et al.*, 2011).

Pan *et al.*, (2007) increased the hydrophilicity of dextran-linked casein during the Maillard reaction, a naturally occurring and non-toxic reaction that occurs during cooking and storage.

Nanoparticles encapsulated with insoluble β-carotene were produced by spontaneous formation of dextran-linked casein copolymer. Spherical nanoparticles with a hydrodynamic diameter of 200 nm were produced with β-carotene as core material and dextran as the shell. The hydrophilic dextran shell brought the particles into a stable dispersed state across a pH range of 2-12. The encapsulated β-carotene was in a state such that it could be released by trypsin or pepsin hydrolysis. The amphiphilic character of the particles allows them to deliver unstable and hydrophobic food and drugs (Pan *et al.*, 2011).

Sahu *et al.*, (2008) investigated the interaction between curcumin and casein molecules by using steady-state fluorescence spectroscopy and casein molecules' potential as a carrier of curcumin (diferuloylmethane) which is isolated from rhizome of turmeric (*Curcuma longa*). Curcumin is a natural polyphenolic compound and studies about curcumin has shown that the ability to inhibit carcinogenesis in a some kind of cell lines such as breast, cervical, colon, gastric, hepatic, leukemia, oral epithelial, ovarian, pancreatic and prostate cancer. However, because of the curcumins low solubility in aqueous solution (2.99×10^{-8} M) and its poor bioavailability limits curcumins clinical effect. They reported that, encapsulating with polymeric micelles, liposomes, polymeric nanoparticles, lipid based nanoparticles and hydrogels has increased curcumins aqueous solubility and bioavailability. Also it was reported that curcumin molecules interacted with casein molecules. Curcumin molecules bound the low-polarity regions of casein molecules. Under physiological buffer conditions, curcumin-casein micelles nanoformulation exhibited similar cytotoxic effects on human cervical cancer cell line Hela compared to an equal dose of free curcumin (Sahu *et al.*, 2008).

Whey proteins

Whey proteins are usually globular proteins and offer many possible biological functions. Whey

proteins consist of β-lactoglobulin (β-Lg), α-lactalbumin (α-La), immunoglobulins (IG), serum albumin (BSA), proteose peptones (PP), lactoferrin (LF) and lactoperoxidase (LP) (Ramos *et al.,* 2014). The main whey proteins in bovine milk are β-lactoglobulin and α-lactalbumin, which are synthesized only by mammary glands. These proteins are considered responsible for providing amino acids to new offspring. In addition, α-lactalbumin acts as a coenzyme during milk generation, adjusting the synthesis of lactose. Moreover, β-lactoglobulin has many binding sites for hydrophobic ligands such as fatty acids and vitamins (Tavares *et al.,* 2014).α-lactalbumin has a high affinity Ca^{+2} binding site and this affinity increases α-lactalbumins stability against higher temperatures. However α-lactalbumins stability is decreased by binding of Zn^{+2} ions onto Ca^{+2}-loaded α-lactalbumin and this makes the protein biased to protease hydrolysis (Celiker & Mallikarjunan 2012).

Protein foams and protein emulsions can enhanced by nanosize particle clusters. On the other hand, the whey protein clustering can be induced due to some factors such as the existence of chemicals or electrolytes, changing the net charge, increasing hydrostatic pressure, partial enzymatic hydrolysis, electrical fields and ecpecially temperature (Ramos *et al.,* 2014). The whey proteins can denature with heating at temperatures above 70℃. κ-casein or other denatured whey proteins can interact with denatured whey proteins by heating due to thiol disulphide exchange reactions. The dissociation of α_s-caseins and β-caseins are inhibited by this interaciton (Anema & Li 2015). Whey proteins are generally globular structures. Structure of whey proteins consist of polypeptide chains and these polypeptide chains have acidic or alkali and hydrophobic or hydrophilic amino acids in a balanced way (Ramos *et al.,* 2014). Therefore, whey proteins are special emulsifiers in food because of their amphiphilic properties. Whey proteins amphiphilic features provide whey proteins to adsorb onto the surfaces of oil droplets. In addition to this whey proteins stabilize emulsion droplets against destabilization when droplets are formed (Adjonu *et al.,* 2014).

In order to use the whey proteins as bioactive molecules, in particular β-lactoglobulin as a carrier, they must be incorporated into a hydrogel structure. Hydrogels are water absorbing networks that hold high amounts of water while maintaining the network infrastructure (El-Salam & El-Shibiny 2012). Whey protein fractions have great coating properties when compared with other proteins, they pretend as a barrier against moisture, lipids, oxygen, flavors or aromas and food ingredients carriers such as antioxidants, antimicrobials, flavors and nutraceuticals. Whey proteins improve mechanical stability when used as a coating material.They have been tested for coating material on salmon, peanuts, cereals or fruits and whey protein coating exhibited oxygen and moisture barriers and fine aroma (Ramos *et al.,* 2014).

In the production of nanotubes from whey protein, α–lactalbumin, molecules are configured automatically by collection of partially hydrolyzed molecules. Partially hydrolyzed α–lactalbumin has a tendency for spontaneous aggregation. At neutral pH and in the presence of a suitable cation, this structure creates automatically generated blocks and tubes with several micrometers in length and only 20 nm in diameter (Graveland-Bikkera & Kruif 2006).

Whey protein concentrates (WPC), whey protein isolates (WPI), whey protein fractions such as α-lactalbumin, β-lactoglobulin, casein glycomacropeptide, lactoperoxidase and protein hydrolysates are obtainable in the market. Whey protein concentrates and whey protein isolates are largely used as gelling, surface active agents and water binding agents. These whey protein products have specific physicocehmical properties and functional properties with the nature of amphiphilic, electrostatic charges and nutritional benefits. Additionally, their ability to form foams, hydrogels, emulsions, self assembling structures, nanoemulsions and nanostructures for delivery of bioactive compounds are proper for food and non food applications. Specially, β-lactoglobulin is responsible for gelation and emulsification

properties of whey protein concentrates and whey protein isolates (Ramos *et al.*, 2014).

Lactoferrin, a simple glycoprotein from the iron-binding protein family, has been reported to increase the bioavailability of iron and has bacteriostatic, antioxidant, anti-inflammatory, and immune system properties (Tavares *et al.*, 2014).

One of the most important features of α-lactalbumin is the presence of a cavity. Through this cavity, α-lactalbumin nanotubes can be used as a tool for masking and preserving encapsulated molecules such as drugs, vitamins, and enzymes or encapsulated compounds (Graveland-Bikkera & Kruif 2006).

Protein-coated nanoparticles sensitive to nutraceutical compounds provide additional protection in the gastrointestinal tract. In a study by Chen and Subirade (2005), chitosan nanoparticles (around 100 nm) were coated with β-lactoglobulin. The coating capability of the natural globular and heat denatured forms of β-lactoglobulin was studied and a high sensitivity to pH was identified. The protein attraction between β-lactoglobulin and chitosan appeared to be due to electrostatic, hydrophobic, and hydrogen bonding interactions. When the gastrointestinal release of these natural β-lactoglobulin-coated nanoparticles was simulated, certain features were noted that appeared likely to slow the release of molecules (Chen & Subirade, 2005).

Zimet and Livney (2009) used β-lactoglobulin and β-lactoglobulin with pectin nanomaterials as a carrier for ω-3 polyunsaturated fatty acids. The β-lactoglobulin DHA complex and its pectin-containing electrostatic nano-compound showed very good colloidal stability. A transparent dispersion with 100 nm average particle size was obtained. These compounds are thought to be useful for the enrichment of clear, lean acidic beverages (Zimet &Livney 2009).

In their study on the use of whey proteins for encapsulation and controlled targeted delivery, Gunesakaran *et al.*, (2007) reported that hydrogel and/or nanoparticle systems of whey proteins can be used for controlled delivery of bioactive compounds. They stated that the hydrogels, especially at a pH above the isoelectric point, showed pH-sensitive swelling behavior which was directly related with the release kinetics. They also demonstrated that the release kinetics of the hydrogels coated with sodium alginate could be modified. Moreover, they showed that the average size of the nanoparticles prepared with β-lactoglobulin could be reduced by pre-heating at 60 ^{o}C, a process that improved the uniformity of the particle sizes (Gunasekaran *et al.*, 2007).

Li *et al.*, (2012) studied the epigallocatechin-3-gallate (EGCG) encapsulation ability of β-lactoglobulin at a wide pH range (2.5 to 7.0) with intensive heat treatment (30-85 ^{o}C/20 min) and 1-10 mg/ml β-lactoglobulin and 1:2 - 1:32 EGCG content. Nanoparticles and the encapsulation of EGCG were formed by heat simultaneously. As a result, β-lactoglobulin-EGCG nanoparticles were successfully prepared to protect the antioxidant activity of the EGCG. These results suggested that β-lactoglobulin can be used as a preservative for EGCG and other polyphenols in foods and beverages (Li *et al.*, 2012).

Zhang and Zhong (2009) developed a new method for preparation of whey proteins based on the use of microemulsions as nanoreactors. This study showed that the heat stability of whey proteins was further improved by enzymatic crosslinking prior to the pre-heating process. They stated that this improvement is a function of Tgaz concentration and crosslinking process. The obtained nanoparticles with >100nm particle diameter were heat stable. Moreover, they reported that this study will lead to attractive prospects for fundamental studies involving interfacial events of dissolved protein solutions with microemulsions, detailed structures in different enzymatic crosslinking states (process, kinetics, and possible substrate concentrations), structural differences in the enzymatic crosslinking during over-heating, and physicochemical properties of possible protein nanoparticles related with pre-heating conditions (El-Salam & El-Shibiny, 2012).

Giroux *et al.*, (2010) prepared whey protein nanoparticles by thermal denaturation, pH cycle and Ca^{2+} crosslinking. Depending upon the conditions, the diameter of the nanoparticles ranged from 100-300 nm; the particle volumes decreased with increased Ca concentration at pH cycle. The prepared nanoparticles had good

stability in the presence of disintegrating agent (El-Salam & El-Shibiny, 2012).

The generation of core/shell nanoparticles is a current approach used to avoid the difficulties faced in the use of whey protein nano hydrogels for nutraceuticals delivery. Core/shell nanoparticles are nano structures in which the core is coated with a material coated with another material. The selected shell material should prevent the aggregation of the particles. Core/shell structures improve the thermal and chemical stability of the nanoparticles and enhance their dissolution. The shell also prevents the core material from oxidation (El-Salam & El-Shibiny, 2012).

Jones *et al.*, (2010), used two methods to prepare nanoparticles from a thermally denatured β-lactoglobulin-pectin complex. The first method included the preparation of β-lactoglobulin nanoparticles and then coating them with pectin. The second method included the heating of both β-lactoglobulin and pectin at the same time. These two methods were compared and it was reported that in the second method, surface coating was higher with pectin, and thus tended to reduce agglomeration (El-Salam & El-Shibiny, 2012).

CONCLUSIONS

Milk proteins are important for neonates, middle aged and aged people. They have great emulsification, gelation, water binding properties, biological activity and digestibility. They are natural devices to deliver calcium and phosphate besides that, they provide amino acids to the neonate. Therewithal milk proteins are remarkable due to their biodegradability and nontoxicity, binding metal ions and entrapment some components such as antioxidants, antimicrobials, flavors, nutracuticals, vitamins, bioactive agents, drugs etc. in a natural way, having a perfect jelation properties, delivering properties and coating properties in food processes. The description of milk proteins' GRAS (Generally Recognized As Safe) is the major advantage of them that may increase their usage as a nanomaterial in food industry. Although all the risks of some new products that are achieved by using nanotechnology have not profoundly researched yet, nanomaterials dont have to cause any health risks for workers, producer and consumers, and dont have to cause any damage to the environment. Also determining of convenient coating materials for bioactive compounds, optimal conditions (such as pH, temperature etc.) and amount of bioactive compounds should be considered to achieve a succesful nanoencapsulated products. Notwithstanding the milk proteins have excellent properties, there are few studies which report the use of nanomaterials in the milk industry as nanocarriers. Increasing of the researches in this area and the integrating of this emerging technology to the food industry will provide convenience for both producers and consumers by preventing damages to the useful bioactive components during the production, stocking and consumption stages.

Milk proteins as nanocarriers are more advantageous than other coating materials. Since they are natural products, no mechanical power is required to achieve them and produce nanocarriers from them, and their nontoxic structure.

REFERENCES

Adjonu R., Doran G., Torley P., Agboola S., 2014, Whey protein peptides as components of nanoemulsions: A review of emulsifying and biological functionalities, Journal of Food Engineering, 122, p.15–27.

Anema S.G., Li Y., 2015, Reassociation of dissociated caseins upon acidification of heatedpH-adjusted skim milk, Food Chemistry, 174, p.339–347.

Boom R. M., 2011, Nanotechnology in food production, in: Frewer L. J., Norde W., Fischer A., Kampers F. (Eds), Nanotechnology in the Agri-Food Sector. Wiley-VCH Verlag GmbH & Co. KGaA, Boschstr, pp 39-57.

Burgain J., Gaiani C., Linder M., Scher J., 2011, Encapsulation of probiotic living cells: From laboratory scale to industrial applications, Journal of Food Engineering 104, p.467–483.

Celiker H. K., Mallikarjunan K., 2012, Better Nutrients and Therapeutics Delivery in Food Through

Nanotechnology, Food Engineering Reviews, 4, p.114-123.

Chau C. F., Wu S. H., Yen G. C., 2007, The development of regulations for food nanotechnology, Trends in Food Science & Technology, 18, p.269-280.

Chaudhry Q., Castle L., 2011, Food applications of nanotechnologies: An overview of opportunities and challenges for developing countries, Trends in Food Science & Technology 22, p.595-603.

Chen L., Subirade M., 2005, Chitosan/β-lactoglobulin core–shell nanoparticles as nutraceutical carriers, Biomaterials, 26, p.6041–6053.

El-Salam M. H. A., El-Shibiny S., 2012, Formation and potential uses of milk proteins as nano delivery vehicles for nutraceuticals: A review, International Journal of Dairy Technology, 65, p.13-21.

Elzoghby A. O., Abo El-Fotoh W. S., Elgindy A. N., 2011, Casein-based formulations as promising controlled release drug delivery systems, Journal of Controlled Release 153, p.206–216.

Ezhilarasi P. N., Karthik P., Chhanwal N., Anandharamakrishnan C., 2013, Nanoencapsulation techniques for food bioactive components: A Review, Food Bioprocess Technology, 6, p.628-647.

Gazi I., Huppertz T., 2015, Influence of protein content and storage conditions on the solubility of caseins and whey proteins in milk protein concentrates, International Dairy Journal, 46, p.22-30.

Graveland-Bikkera J.F., Kruif C.G., 2006, Unique milk protein based nanotubes: Food and nanotechnology meet, Trends in Food Science & Technology 17, p.196–203.

Gunasekaran S., Ko S., Xiao L., 2007, Use of whey proteins for encapsulation and controlled delivery applications, Journal of Food Engineering 83, p.31–40.

Gutiérrez F. J., Albillos S. M., Cassas-Sanz E., Cruz Z., Garcia-Estrada C., Guerra-Garcia A., Garcia-Reverter J., Garcia-Suarez M., Gaton P., Gonzalez-Ferrero C., Olabarrieta I., Olasagasti M., Rainieri S., Rivera-Patino D., Rojo R., Romo-Hualde A., Saiz-Abajo M-J., Mussons M-L., 2013, Methods for the nanoencapsulation of beta-carotene in the food sector, Trends in Food Science & Technology 32, p.73-83.

Huppertz T., 2013, Chemistry of the caseins, in: P. L. H. McSweeney, P. F. Fox (Eds), Advanced Dairy Chemistry: Volume 1A-Proteins: Basic Aspects, 4th Edition, Springer Science + Business Media, New York, pp 135-161.

Kapre S., Kakade G., 2014, Nanobasics and nanotechnology, Journal of Advances in Applied Sciences and Technology, 1(2), p.130-132.

Kuan C. Y., Fung W. Y., Yuen K. H., Liong M. T., Nanotech: Propensity in foods and bioactives, Critical Reviews in Food Science and Nutrition, 52, p.55-71.Li B., Du W., Jin J., Du Q., 2012, Preservation of (−)-Epigallocatechin-3-gallate Antioxidant Properties Loaded in Heat Treated β-Lactoglobulin Nanoparticles, Journal of Agricultural and Food Chemistry, 60, p.3477−3484.

Livney Y. D., 2010, Milk proteins as vehicles for bioactives, Current Opinion in Colloid & Interface Science, 15, p.73–83.

Mason T. G., Wilking J. N., Meleson K., Chang C. B., Graves S. M., 2006, Nanoemulsions: formation, structure, and physical properties, Journal of Physics: Condensed Matter, 18, p.635-666.

Maynard A. D., 2006, Nanotechnology: Assessing the risks, Nano Today, 1(2), p.22-23.

Momin J. K., Jayakumar C., Prajapati J. B., 2013, Potential of nanotechnology in functional foods, Journal of Food Agriculture, 25(1), p.10-19

Mounsey J. S., O'Kennedy B. T., Kelly P. M., 2005, Influence of transglutaminase treatment on properties of micellar casein and products made therefrom. Dairy Science and Technology, 85, p.405–418.

National Nanotechnology Initiative-NNI, 2011, What is Nanotechnology, Available from: http://www.nano.gov/nanotech-101/what/definition. [5.12.2014].

Pan X., Yao P., Jiang M., 2007, Simultaneous nanoparticle formation and encapsulation driven by hydrophobic interaction of casein-graft-dextran and β-carotene,Journal of Colloid Interface Science, 315, p.456–463.

Pomastowski P., Sprynskyy M., Buszewski B., 2014, The study of zinc ions binding to casein, Colloids and Surfaces B: Biointerfaces, 120, p.21–27.

Qureshi, M. A., Karthikeyan, S., Punita K., Khan, P. A., Uprit, S., Mishra, U. K., 2012, Application of nanotechnology in food and dairy processing: An overview, Pakistan Journal of Food Sciences, 22(1), p.23-31.

Ramos O. L., Pereira R. N., Rodrigues R., Teixeira J. A., Vicente A. A., Malcata F. X., 2014, Physical effects upon whey protein aggregation for nano-coating production, Food Research International, 66, p.344–355.

Ranjan S., Dasgupta N., Chakraborty A. R., Samuel S. M., Ramalingam C., Shanker R., Kumar A., 2014, Nanoscience and nanotechnologies in food industries: opportunities and research trends, Journal of Nanoparticle Research, 16, p.24-64.

Rashidi L., Darani K. K., 2011, The Applications of Nanotechnology in Food Industry, Critical Reviews in Food Science and Nutrition, 51, p.723-730.

Roach A., Dunlap J., Harte F., 2009, Association of triclosan to casein proteins through solvent-mediated high-pressure homogenization, Nanoscale Food Science, Engineering, and Technology, 74(2), p.23-29.

Sahu A., Kasoju N., Bora U., 2008, Fluorescence study of the curcumin-casein miçelle complexation and its application as a drug nanocarrier to cancer cells, Biomacromolecules, 9, p.2905-2912.

Sáiz-Abajo M., González-Ferrero C., Moreno-Ruiz A., Romo-Hualde A., González-Navarro C. Z., 2013, Thermal protection of b-carotene in re-assembled casein micelles during different processing technologies applied in food industry, Food Chemistry 138, p.1581–1587.

Sanguansri P., Augustin M. A., 2006, Nanoscale materials development - a food industry perspective, Trends in Food Science & Technology 17, p.547-556

Sekhon B. S., 2010, Food nanotechnology – an overview, Nanotechnology, Science and Applications, 3, p.1–15

Semo E., Kesselman E., Danino D., Livney Y. D., 2007, Casein micelle as a natural nano-capsular vehicle for nutraceuticals. Food Hydrocolloids, 21, p.936–942.

Shew A., 2008, An Interesting, Interdisciplinary, Ideological Split, Bulletin of Science, Technology & Society, 28 (5), p.390-399.

Tavares G. M., Croguenneca T., Carvalhoc A. F., Bouhallab S., 2014, Milk proteins as encapsulation devices and delivery vehicles: Applications and trends, Trends in Food Science & Technology, 37, p.5-20.

Weiss J., Gibis M., 2013, Nanotechnology in the food industry, Ernaehrungs Umschau International 60(4), p.44–51.

Zhang Q., Kusaka Y., Zhu X., Sato K., Mo Y., Kluz T., Donaldson K., 2003, Comparative toxicity of standart nickel and ultrafine nickel in lung after intratracheal instillation, Journal of Occupational Health, 45, p.23-30.

Zimet P., Livney Y. D., 2009, Beta-lactoglobulin and its nanocomplexes with pectin as vehicles for -3 polyunsaturated fatty acids, Food Hydrocolloids 23, p.1120–1126.

Zimet P., Rosenberg D., Livney Y. D., 2011, Re-assembled casein micelles and casein nanoparticles as nano-vehicles for -3 polyunsaturated fatty acids, Food Hydrocolloids, 25, p.1270-1276.

Zuidam N. J., Shimoni E., 2010, Overview of microencapsulates for use in food products or processes and methods to make them, in: Zuidam N. J., Nedovic V. (Eds), Encapsulation Technologies for Active Food Ingredients and Food Processing, pp 3-29

Conflicts of Interest

The authors declare no conflict of interest.