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Current trends in Nano encapsulation of flavours and aromas

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ABSTRACT

Nano-encapsulation of flavour and aroma represents an efficient alternative in increasing their stabilities, prolong sensory perception, bio availability, and improve their efficiency. Presently, the applications of nanotechnology in the food industries are in the areas of nanoparticle coatings for packaging applications, health-promoting products, and beverages. Apart from the advantages, nanotechnology has raised a number of safeties, ethical and regulatory issues as a result of little knowledge regarding the impact of nano-sized materials on human health. While there are some reported studies on nanocapsule-containing fragrances or perfumes, very few studies have focused on nano-encapsulation of flavor and aroma. Currently, various techniques such as emulsification, complex coacervation, and supercritical fluid are being employed in nano-encapsulation of flavor and aroma. This review attempts to examine the current state of knowledge and limitations on the technology of nano-encapsulation of flavour and aroma.

Keywords: *Nano-encapsulation, flavor, safety.*

INTRODUCTION

Nano-encapsulation is an aspect of nanotechnology which involves the entrapment of bioactive agents within carrier materials in Nano-scale dimension. This technique is employed to enhance stability of volatile compounds, reduce evaporation and degradation of these compounds in terms of aroma and flavor (Khayata, et al. 2012; Sáiz-Abajo, 2013). Nano-encapsulation is referred to as the technology used for encapsulating bioactive substances or active food ingredients in miniature or at the Nano-scale range of 10 to 1,000 nm (Lopez et al. 2006). The carrier or materials used in the entrapment of these active compounds are called Nano-particles, Nano-materials or Nano-carriers. The word 'Nano' refers to a particle size or shape to a magnitude of 10^{-9} or less than 1,000 nm (Quintanilla-Carvajal et al. 2010; Sanguansri and Augustin, 2006).

Nano-materials or nano-carriers for encapsulation must be made of biodegradable food –grade components which are stable during processing, storage and consumption. Generally, Nano-encapsulation techniques use either the top-down or bottom-up approaches for Nano-material development. In the top-down approach, application of specific tools in which size reduction and structure shaping for desired application of the Nano-particles is developed. Emulsification and emulsification-solvent evaporation are Nano-encapsulation techniques found in this approach. In bottom-up approach on the other hand, Nano-particles are designed by self-assembling and self-organization of molecules. Other Nano-encapsulation techniques found in this approach include, coacervation, Nano-precipitation, inclusion complexation and supercritical fluid techniques (Sanguansri and Augustin, 2006; Mishra et al. 2010).

Nano-encapsulation of compounds/ ingredients has been well developed in the pharmaceutical and cosmetic industries. There is now growing research on Nano-encapsulation in the food sectors. Bioactive compounds are being encapsulated effectively in order to increase their physical stability, concentration and protecting them from the interactions with the food ingredients, while inhibiting microbial growth in

the food areas where microorganisms are preferably located (Weiss et al. 2009).

The main function of encapsulation is to shelter the active ingredients from adverse environmental conditions such as light, moisture and oxidation and also promote its shelf life and control the release of encapsulates (Shahidi and Han, 1993). Microencapsulation is an already established technology employed in food system for stabilization of flavor and aroma in food ingredients. The technique in microencapsulation involves the entrapping of tiny particles or droplets of volatile flavor and aroma in active compounds in micro-particles or microcapsules with diameter ranging between 3 and 800 μm (Meena et al. 2011). One of the major advantages of nanoparticles over micro-particles in the food system include improved sensory properties, extended flavor perception and better mouth feel (Moraru et al. 2003).

Particle size is the main factor that affects the delivery of any bioactive compound to various sites within the body (Kawashima, 2001; Hughes, 2005). Therefore, Nano-encapsulation has gained greater interest as it has high potential to enhance bioavailability, improved controlled release, and enable precision targeting of bioactive compounds in a greater extent than microencapsulation (Mozafari et. al., 2006). Nano-encapsulation technologies have the potential to meet food industry challenges concerning the effective delivery of health functional ingredients and controlled release of flavor compounds (Sekhon, 2010).

TYPES OF NANO-ENCAPSULATION

Nano-emulsion

Nano-emulsion is produced by the use of high-pressure valve homogenizers or micro-fluidizers into droplet diameters of less than 100 to 500 nm (McClement, 2004). The small droplet size produced has unique rheological and textural properties which is desirable in the food industry (Chaudhry et. al., 2008). Applying Nano-emulsion in food products can promote the use of less fat without compromising creaminess. Therefore, this gives consumer a healthier option (Shegokar et.al. 2010). Encapsulating functional components within the droplets is able to retard chemical degradation processes by engineering the properties of the interfacial layer surrounding them (McClements and Decker, 2000).

Nanoparticles

Nano-particles are solid, submicron particles ranging from 1 to 100 nanometres (Couvreur et. al., 1995; Couvreur, 1988). Nanoparticles can be developed from a variety of materials such as synthetic polymers, proteins and polysaccharides. Biopolymeric nanoparticles are nanometer- sized nanoparticles produced using food-grade biopolymers such as proteins or polysaccharides (Chang & Chen, 2005; Gupta & Gupta, 2005; Ritzoulis et. al., 2005). The formations of these particles are by aggregative (net attraction) or segregative (net repulsion) interaction of single biopolymers separated into smaller nanoparticles. Polylactic acid (PLA) is one of the most common components of biodegradable bio-polymeric nanoparticles that are used to encapsulate and deliver drugs, vaccines, and proteins. However, it is rapidly removed from the bloodstream, remaining isolated in liver and kidneys. Therefore, PLA requires an associative compound such as polyethylene glycol to deliver the active components to other parts of the body (Riley et. al., 1999).

Nanodispersion and Nanocapsules

Functional ingredients such as vitamins, antimicrobials, antioxidants, and preservatives come in various molecular and physical forms which include polarities, molecular weights, and physical states. They are rarely used in pure form; normally they are being produced in the form of dispersions or capsules using nanotechnology. Nanocapsules are vesicular system in which the bioactive compound is confined in a cavity surrounded by a unique polymer membrane (Couvreur et. al., 2002). Nanodispersions and nanocapsules are ideal mechanisms used to deliver the functional ingredients. An effective delivery system must be able to transport the functional ingredients to its desirable site as well as

preventing the ingredients from chemical or biological degradation, and controlling the rate of release of ingredients under certain environment conditions (Ozimek et. al., 2010).

Nanospheres

Nanospheres are polymer matrices where the bioactive compounds are dissolved, entrapped, encapsulated, chemically bound or adsorbed (Couvreur et. al., 2002; Chen et. al., 2006). They are nanoscale porous particles formed by crosslinking the polymer chains. However, depending on the copolymer composition, the central core can become more or less solid- like making it hard to have clear separation between micelles and nanospheres (Chen et. al., 2006).

Nanocochleates

Nanocochleates are purified soy based phospholipid consisting about 75% by weight of lipid which is cigar-shaped multilayered structure with spiral solid lipid bilayer (Thangavel & Thiruvengadam, 2014; Momin & Joshi, 2015). They are nanocoils which are wrapped around the micronutrients to stabilize and protect them as well as increase the nutritional value of processed food (Sinha et. al., 2008; Mannino, 2003). Nanocochleates may be used in Nanoencapsulation because of their properties which promotes cross membrane diffusion for charged and impermeable molecules (Ramasamy et. al., 2009). They have been used to deliver proteins, peptides and DNA for vaccine and gene therapy applications. In addition, the advantage of nanocochleates is that they are resistant to degradation in gastrointestinal tract and this make them suitable for oral delivery (Zarif et. al., 2003).

Nanolaminates

A Nanolaminate consists of 2 or more layers of nanometer dimensions materials that are physically and chemically bonded to each other. It can be used in the preparation of edible coatings and films (Ozimek et. al., 2010). It is most likely to be used as coating material which is attached to food surfaces, instead of as self-standing films, due to their extremely thin nature which makes them very fragile (Kotov, 2003). Nanolaminates serve as moisture, lipid, and gas barriers; they also improve the textural properties of food; and serve as carrier for functional ingredients (Ozimek et. al., 2010).

Nanofibers and nanotubes

Nanofiber is produced by a manufacturing technique using electrostatics force which has diameters ranging from 10 nm to 1000 nm. It has only a few applications in food industry as nonofiber is not composed of food- grade substances (Ozimek et. al., 2010). Biopolymer nanofibers that are produced through electrospinning technology can have good potential in food and nutraceutical formulation, coatings and bioactive food packaging (Fernandez, 2009). Nanotubes are being widely used in non-food applications. Carbon nanotubes with low-resistance conductors or catalytic reaction vessels are examples of nanotubes used in non-food applications. It has been reported that under certain environmental conditions, certain globular milk proteins (α- lactalbumin) can self-assemble into similar structured nanotubes (Graveland-Bikker et. al., 2006; Graveland-Bikker & De Kruif, 2006; Gutiérrez, 2013). It is a potential new carrier for nanoencapsulation of nutrients, supplements, and pharmaceuticals (Bugusu et. al., 2009).

Nanomicelles

Nanomicelles are sub-100 nm spherical particles formed spontaneously upon surfactant addition after critical micelle concentration has been reached (Momin & Joshi, 2015). A study has been carried out on the possibility of loading vitamin D2 into casein micelle using the natural self-assembly tendency of bovine caseins. The result shows that the vitamin was about 5.5 times more concentrated within the micelles than in the serum. Therefore, it may be useful as nanovehicles for entrapment, protection, and delivery of sensitive hydrophobic nutraceuticals within other food products (Semo et. al., 2007).

Nanoliposomes

Nanoliposome, or submicron bilayer lipid vesicles (<30 or 30- 100 nm vesicles) is a new technology for the encapsulation and delivery of bioactive ingredients. It possesses and maintains nanometric size range during storage and application (Mozafari et. al., 2006). Due to their biocompatibility and biodegradability, nanoliposomes have wide application not only in food technology, as well as other fields such as nanotherapy, cosmetics and agriculture.

Incorporation of bioactive components into nanoliposomes is able to enhance its performance by improving their solubility and bioavailability, in vitro and in vivo stability, as well as preventing their unwanted interactions with other molecules. Besides, nanoliposomes is cell-specific targeting, which is a prerequisite to attaining drug concentrations required for optimum therapeutic efficacy in the target site while minimising adverse effects on healthy cells and tissues (Mozafari, 2010).

NANOENCAPSULATION TECHNIQUES

Nanoencapsulation techniques are rather more complex than microencapsulation techniques. The purposes of Nanoencapsulations are to prolong the functionality of food during production and storage, to prevent formation of off-flavor compound, to protect the textural and structural properties of food and to reduce enzyme activities of food (Fogliano & Vitaglione, 2005). There are two approaches in Nanoencapsulation techniques which are top-down and bottom-up approaches. A top-down approach includes emulsification and emulsification-solvent evaporation where the Nano products are formed through size reduction. On the other hand, coacervation, Nanoprecipitation, inclusion complexation and supercritical fluid technologies are classified as bottom-up approaches formed by self-assembly and self-organization of molecules (Sanguansri & Augustin, 2006; Mishra et al., 2010). Among the techniques, they can be divided into three classifications which are physical, chemical and physicochemical processes. Physical processes include spray drying and freeze drying; chemical processes include molecular inclusion and physicochemical processes which include simple or complex coacervation (Yanez et al., 2003). The main idea of Nanoencapsulation is to produce Nanosuspensions by coating wall materials in liquid or dried form.

Coacervation

Coacervation is a technique involving phase separation of colloid particles and the forming of new liquid phase called coacervate (Korus, 2001). Coacervation process is an encapsulation technology based on the theory of electrostatic attractions between oppositely charged biopolymers, hydrogen bonding and hydrophobic interactions. There are two types of coacervations which are simple coacervation and complex coacervation. Simple coacervation only involves one type of polymer whereby a charged bioactive component (normally hydrophilic agents) is attached to an oppositely charged biopolymer forming coacervate. On the other hand, complex coacervate involves many types of biopolymers (two or more). In complex cases, two oppositely charges biopolymers (positive and negative) are used to form electrostatic force so that the bioactive component is trapped within a particle (Madene et al., 2006). Jincheng et al. (2010) had encapsulated capsaicin using the complex coacervation technique in gelatine by cross-linking with gluteraldehyde to obtain a 100nm nanoparticle to increase its stability and mask its undesirable odor. Glutaraldehyde is added to stabilize the coacervates because the complex coacervates are highly unstable.

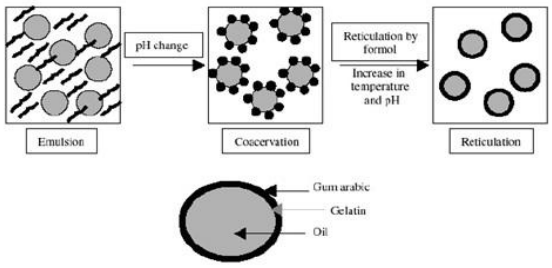

Figure 1. Principle of the complex coacervation method.

Besides gelatine, previous studies showed that maltodextrin (Wang et al., 2008), acacia (Xing, 2005) and chitosan (Gan & Wang, 2007) have been used as the wall materials to improve the colloidal stability so that the degraded compounds and the release of undesirable odor can be controlled. The complex formed and types of the interactions between biopolymer depend on the biopolymer type, pH and concentration of the biopolymer used. Besides that, bovine serum albumin was

encapsulated by chitosan and poly (ethyleneglycolran-propyleneglycol) to control the release of encapsulated protein (Gan & Wang, 2007). However, coacervation process has many disadvantages; it is complicated and expensive (Flores et al., 1992).

Emulsifications

Emulsification is dispersions of two immiscible liquids where one is being dispersed in the other. There are two types of emulsions: oil-in-water emulsion (o/w) and water in oil emulsion (w/o). Oil in water emulsion involves lipophilic active agents like β-carotene, plant sterols, carotenoids and dietary fats whereas water in oil emulsion involves water-soluble food active agent like polyphenols (Zuidam & Shimoni. 2010).

The emulsification process normally needs high energy input. The process is combined with others methods such as: emulsion-diffusion method, precipitation of pressurized emulsions, emulsion-droplet coalescence and emulsification-solvent. Firstly, emulsion-diffusion method is the emulsification process followed by the removal of organic phase (diffusion). The organic phase normally consists of biopolymer and oil in organic solvent. The emulsion is achieved using the mechanical shear stirring method (Moinard-Chécot et al., 2008). Choi et al. (2010) has used this method to produce fish oil. The objective of this method is to encapsulate the bioactive compound using less physical stress and easier step.

Secondly, the precipitation of pressurized emulsion is carried out by exposing the high pressurized solution containing active compounds with a cold aqueous solution containing stabilizers or emulsifiers. The cold solution is to allow for an intermediate saturation and give encapsulant thermal shock forming nanoparticles (Fathi et al., 2014). For example, Ribeiro et al.(2008) had used β-carotene as the bioactive compound. Using similar theory, Jafari et al. (2007) produced 700-800nm nanoparticles with good stability by encapsulating limonene using maltodextrin in combination with modified starch and whey protein concentrate (WPC). This method is called microfludization where the emulsion was forced through a collision chamber by very high pressure to form Nanoemulsion droplets (Fathi et al., 2012).

Thirdly, emulsion-droplet coalescence method involves the collision and coalescing of the emulsion containing aqueous acetic acid together with the addition of chitosan (Agnihotri et al., 2004; Grenha, 2012). Under high-speed stirring, precipitation of chitosan droplets occurred leading to nanoparticles formation. Moreover, emulsification-solvent evaporation technique involves two stages. First is the emulsification of the polymer, encapsulant and organic solvent into an aqueous phase followed by the second stage whereby the solvent is evaporated leading to polymer precipitation as nanospheres (Kumari et al., 2011; Reis et al., 2006). For instance, curcumin (Dandekar, 2010), quercetin (Kumari, 2010), α-tocopherol (Cheong et al., 2008) and β-carotene (Silva et al., 2011) have been encapsulated to improve the controlled release, stability and also the entrapment efficiency.

Kumari et al. (2010) had encapsulated quercetin in poly-D, L-lactase and polyvinyl alcohol by using emulsification-solvent technique along with freeze drying technique. Cheong et al. (2008) and Anarjan et al. (2011) have also used this method to produce Nanodispersion of α-tocopherol and astaxanthin, respectively. The Nanodispersions formed were in the range of 90-163nm. Through this process, re-coalescence can be minimized and the stability, solubility and the bioavailability can be improved. Temperature, stirring rate, type and amount of dispersing agent can significantly affect the size of nanocapsules formed. Salting-out effect method is also based on the water-miscible solvent separation from aqueous solution through the salting-out (electrolytes) or non-electrolytes such as sucrose (Mendoza-Munoz et al., 2012).

Apart from the methods discussed above, high energy emulsification methods also include high-pressure homogenization and ultrasonication. In ultrasonication, ultrasound energy is transferred to the particles to break up the hydrogen bonds within particles to reduce the size of nanoparticles (Fathi et al., 2014). For example, Kentish et al. (2008) optimized the operating conditions on flax seed oil using ultrasonication method producing 135nm nanoparticles. For high pressure homogenization, the coarse emulsion of biopolymer resulted from dispersion is forced

through an orifice at high pressure (100-2000 bar) breaking the particles to nanoscale size. For an example, Yuan et al. (2008a, b) encapsulated β-carotene using the emulsifiers Tween-20, Tween-40, Tween-60 and Tween-80 to increase the stability of nanoparticles formed using high-pressure homogenization technique. They reported that Tween-20 produced the smallest droplet size compared to others.

Molecular inclusion

The inclusion complexes involve the fitting of a molecule into the biopolymer compound forming ligand or lattice through hydrogen bonding, Van De Waals forces and hydrophobic interactions. In food industry, many studies (Loftsson & Kristmundsdottir, 1993; Reineccius et al., 2002) used cyclodextrins to encapsulate flavors. Hadaruga et al. (2006) encapsulated linoleic acid in α- and β-cyclodextrin to produce 236nm nanocapsules to improve thermal stability. There are several ways for complexing β-cyclodextrin with flavor compounds. First way is to stir the cyclodextrin with flavors and it allows precipitating and then filtering off. Second way is by blending the solid cyclodextrin and bubbling in the flavors as vapours. Lastly is by kneading the flavor substance with the cyclodextrin-water paste (Pagington, 1986; Bhandari et al., 1999). Molecular weight and shape, stearic hindrance, polarity and volatility of the core material can affect the retention of aroma compounds (Goubet et al., 1998).

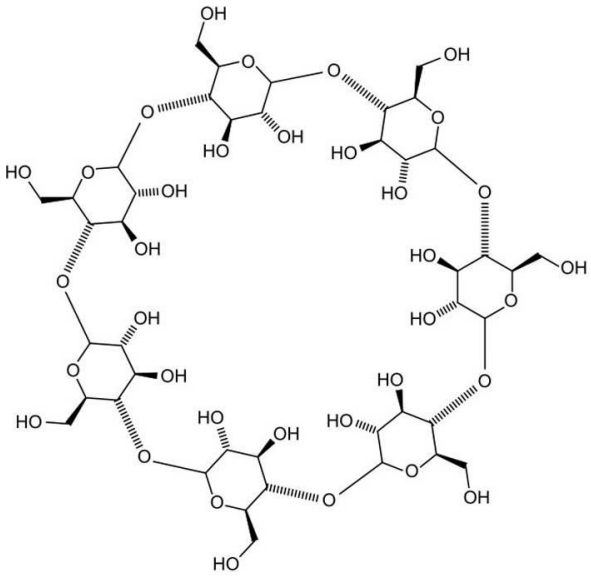

Figure 2. Structure of β-cyclodextrin molecules.

Supercritical Fluid Technique (SCF)

Supercritical fluid (either liquid or gas) is used due to its thermodynamic behaviour. Carbon dioxide is the most widely used fluid in encapsulating heat sensitive bioactive compounds. Theoretically, the bioactive compound and the polymer are solubilized in a supercritical fluid (SF) and expanded until it reaches the critical temperature. After that, the SF is removed by evaporation process and the solute particles are eventually precipitated (Reis et al., 2006).

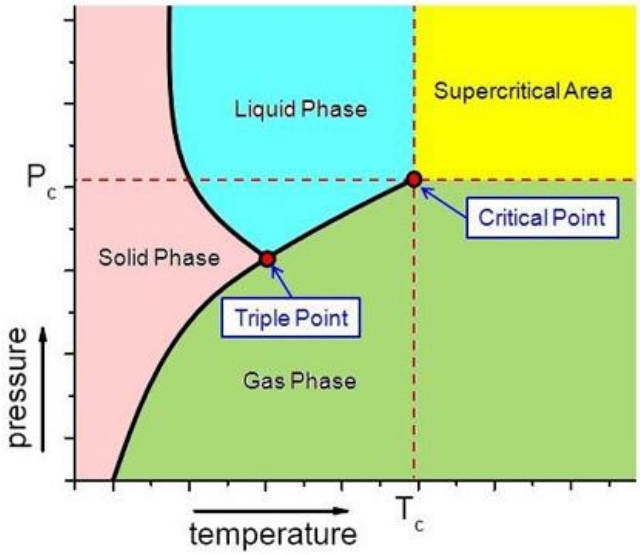

Figure 3. Supercritical fluid diagram.

There are two types of SCF techniques: Rapid Expansion of Supercritical Solutions (RESS) and Supercritical Anti Solvent (SAS). In RESS, the biopolymer and encapsulant are saturated in SCF fluid at high pressure and precipitated into Nanoparticles. In SAS, organic solvent is extracted by SC-CO_2 at the critical pressure and temperature and the biopolymers precipitated on encapsulant's surface (Wang et al., 2004). For example, Turk and Lietzow (2004) synthesized phytosterol Nanoparticles with a good stability using rapid expansion. The advantages include the formation of particles with controllable size and lesser solvent is required in the process (Cocero et al., 2009).

Spray Drying

In order to obtain higher chemical stability, the Nanosuspensions are dried into powder form to improve higher stability. One of the drying techniques is spray drying. Spray drying involves the dispersions of encapsulant in a carrier material, atomization into hot air chamber and the recovery

of Nanocapsules into cyclone separator (Watanabe et al., 2002). It is used to encapsulate many food ingredients especially the production of flavors and volatiles (Deis, 1997). Recently, De Paz et al. (2012) encapsulated β-carotene using modified n-octenyl succinate-starch using spray drying technique to produce 12µm particles.

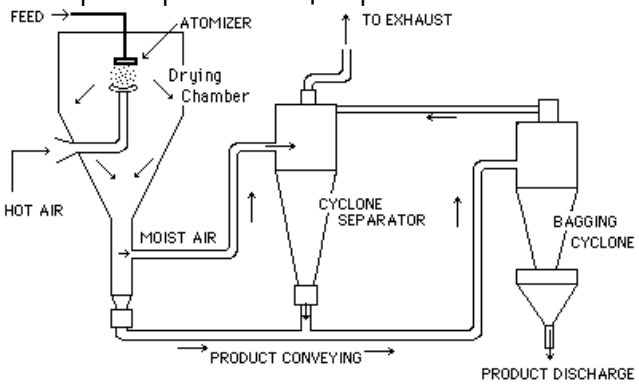

Figure 4. Spray drying process.

Besides that, Jafari et al. (2008) had encapsulated fish oil in maltodextrin to maximize encapsulation efficiency. Spray drying is widely used because it provides many advantages. The benefits include low operating cost, fast process, good stability of Nanocapsules, rapid solubility of capsules, wide choice of carrier materials, and large-scale of uniform spherical-nanocapsules (Reineccius, 1989). Processing temperature and humidity, spray dryer features, core and wall properties and particle size will significantly affect the retention of volatile material (Goubet et al., 1998). However, spray drying might cause heat-sensitive encapsulated product to oxidize producing off-flavor during the process. This problem can be solved by using fluid bed spray coating whereby the fluidized particles under hot atmosphere are coated by coating materials that are sprayed through a nozzle and adhered onto the particles (Jacquot & Pernetti, 2003).

Freeze Drying

Freeze drying technique (also refer to as lyophilisation) is the dehydration of heat sensitive materials through four main stages: freezing, sublimation (primary drying), desorption stage (secondary drying) and storage (Ezhilarasi et al., 2013). Minemoto et al. (1997) compared hot air drying and freeze drying methods on the stability of encapsulated methyl linoleate using Arabic gum. The study showed that the encapsulated final product from freeze drying gave better stability during storage compared to hot air drying method. However, unlike spray drying, freeze drying is not commercially used due to its very high operating costs and long processing time.

Recently, Nakagawa et al. (2011) and Bejrapha et al. (2011) produced capsicum oleoresin in poly-ε-caprolactone using emulsion-diffusion method combined with freeze drying. Their study showed that the Nanocapsules produced by freeze drying showed excellent dispersion characteristics through the gel network formed during the process.

DELIVERY SYSTEM

Delivery system is defined as a system that controls the rate of release of bioactive materials that are entrapped in a carrier. There are basically two common release mechanisms; delayed release and sustained release (Lakkis, 2008). This delayed release is applied in situation of flavor release in food, color release in beverages, nutritional compounds release in target site of the intestine. Meanwhile, in sustained released mechanism consistent concentration of bioactive compounds are released in the target site. This sustained release mechanism is designed to extend flavor or drug release.

In the delivery system of Nanoencapsulation, the Nanocarriers, which is defined as a Nanometer scale carrier with less than 100nm diameter has a main role of protecting the encapsulant from any unfavorable environmental condition such as pH and enzyme degradation, oxidation, etc (Fang & Bhandari, 2010; Ghosh et. al., 2009; Zimet & Livney, 2009). Nano delivery system can be made up of different macromolecules such as lipid, carbohydrate or protein based. In addition, they must be of food grade, biodegradable, stable during production, storage or consumption stage.

Lipid-based Nano Delivery System

Nanoemulsions, nanoliposomes, solid lipid nanoparticles (SLNs), and nanostructure lipid carriers (NLCs) are the recent popular lipid based delivery systems. For nanoemulsions, it is nanoscale droplets of multiphase colloidal dispersions formed by dispersing one liquid in another immiscible liquid by physical share-induced rupturing (Liu et. al., 2006; Mason et. al., 2006; Meleson et. al., 2004; Russel et.al., 1989). Nanoemulsions are usually transparent (McClements & Li, 2010; Shakeel & Ramadan, 2010). Nanoemulsions allows for large scale production using high pressure homogenization. They are toxicologically safe, and gravity stable due to the Brownian motion caused by entropic driving forces (Mason et. al., 2006). They are kinetically stable (Henry et. al., 2010; Tadros et. al., 2004), metastable and their droplets size distribution is not altered in diluted water (Gutieirrez et. al., 2008). These features make them suitable as nutrient carriers in beverage. The droplets are dispersed into small droplets and distributed widely in the gastro-intestinal tract after digestion (Talegaonkar et. al., 2010). Wulff-Perezm et. al. (2009) reported that when there is certain surfactant concentration, destabilization will happen, as a result of non-adsorbed micelles. This can be explained as depletion-flocculation effect. Stability, release and bioavailability of the nanocarrier information are much influenced by localization. A bioactive compound might have different localization in the emulsion, and this depends on the number of homogenization cycles and production temperature. Low stability in acidic conditions is a limiting factor in nanoemulsion. However, it has been reported that it can be stabilized by chitosan (Klinkesom and McClements, 2009). The chitosan coated lipid droplets are currently applied in oral delivery system (Klinkesorn and McClements, 2009).

Nanoliposomes are widely used in food research and food industry. Liposomes are formed as a spherical shell during the interaction of water molecules with phospholipid of amphiphilic compounds (Goyal et. al., 2005; Jesorka & Orwar, 2008). Liposomes are kinetically stable, and large scale production can be achieved by using natural ingredients (Huwiler et. al., 2000; Mozafari et. al., 2008; Thompson et. al., 2006; Mozafari & Khosravi-Darani, 2007; Yurdugul & Mozafari, 2004; Mozafari, 2006). Liposomes are categorized according to the numbers of bilayer and size. Their vesicles can be categorized into small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), multilamellar vesicles (MLV), and multivesicular vesicles (MVV). MLV is made up of one or more concentric lipid bilayers while MVV is made up of single lipid bilayer entrapping some small non-concentric vehicles. Nano-sized liposomes have the ability to entrap hydrophilic molecules in their interior volume, and hydrophobic compounds in the lipid bilayer parts (Acosta & Garti, 2008). Temperature sensitive liposomes, which are produced by modifying the lipid bilayers with specific polymers, help to change its solubility in water according to the critical temperature of the specific polymers used. When the condition is above critical temperature, the polymer-coated liposomes are destabilized due to interaction between liposome membrane and the hydrophobic polymer chain, and consequently release the load (Hayashi et. al., 1996; Kitano et. al., 1994; Kono et. al., 1994; Kono et. al., 1999). This is a very suitable application for flavor release in ready-to-eat meal. Meanwhile, pH-sensitive liposomes, which can be produced by amphiphilic lipid molecules such as unsaturated phosphatidylethanolamine (PE) and oleic acid, destabilize and release the load at acidic environment (Cho et al., 2009). Nanoliposomes have shorter release time. However, liposomes can be coated with different polymer to improve the limitation and achieve different requirements.

Solid lipid nanoparticles (SLN) are particles consists of solid matrix made up of lipid shell. Solid matrix provides more protection against chemical reaction, gives high flexibility in the control of bioactive compounds release. The rate of bioactive compounds release is slower when the solid matrix degradation time is longer (Muller et. al., 2000). SLN produces high encapsulation efficiency, excluding organic solvent usage and large scale production and sterilization (Mader & Mehnert, 2005; Mehnert and Mader, 2001). There are basically three types of methods for incorporating bioactive components into SLN. The first method is

homogeneous matrix model, followed by; bioactive-enriched shell and bioactive enriched core respectively. SLN is thus able to perform dissolution mechanism, burst release mechanism and membrane control release mechanism respectively (Muller et al., 2002b). The stability of colloidal dispersion can be affected by surface charge (Bunjes, 2005; Cavalli, 1997; Lim & Kim, 2002). Gastrointestinal environment may destabilize the particles and lead to aggregation and size growth. On the other hand, the bioactive capacity of the carrier system is affected by bioactive solubility in melted lipid, physical and chemical structure of solid lipid matrix and polymeric state of lipid. Space is less available when the lipid used has higher purity. Addition of solubilizer into the lipid is able to enhance solubility and after that increase the capacity. Lipid that forms crystal especially those with perfect lattice structure can easily end up expelling bioactive compounds out of the structure (Westesen et. al., 1997). In addition, there is also the risk of explosion. This problem can be overcome by delaying the recrystallize transition from α to β polymorphic by using surfactant with hydrocarbon tails that crystallizes prior to the lipid phase or preventing particle aggregation (Awad et. al., 2008; Helgason et. al., 2009; Helgason et. al., 2008).

Nanostructure lipid carrier (NLC) is a novel carrier that was developed to improve the limitation in SLN and is reported to have smaller particles than SLN (Fang et. al., 2008). It uses the same production method as SLN but different mixing lipid molecules. The output gives a depressed melting point compared to the original matrix. Moreover, the capacity of encapsulation load is increased as the lipid matrix is given a certain nanostructure. At the same time, the formation of perfect crystal is inhibited and thus the risk of explosion is neglected (Chen et. al., 2010; Muller et. al., 2002a; Muller et. al., 2002b).

Carbohydrate-based Delivery System

Generally, carbohydrate based delivery system can interact with varieties of bioactive compounds. They are thermally stable during high temperature production, unlike lipid or protein-based which can be melted or denatured. Different carbohydrates give different massive molecular structure and consequently different bioactive entrapment abilities. Carbohydrate based delivery system can be divided into plant, animal, algal or microbial origin. Starch, cellulose, pectin, guar gum, chitosan, alginate, cyclodextrins are regularly used.

Starch is of low cost and relatively pure. It is sensitive to acid and amylase. Derivatives of starch are widely developed for delivery system. Di-aldehyde starch, (Yu et. al., 2007), propyl starch (Jain et. al., 2011; Santander-Ortega et. al., 2010), octenyl succinic anhydride (OSA) modified starch, acetylated starch, PEGylated starch acetate are examples of modified starch that are designed to overcome limitation of the natural hydrophilic starch. OSA-starches are used for food and flavor ingredients (Qi & Xu, 1999). Its characteristics include ability to absorb to oil-water interface, assist in oil droplets formation, and provide long term stability during homogenization process and storage. Other than OSA-starches, acetylated starch is used for food and flavor ingredients. Acetylated starch is reported to increase hydrophobicity and resistant to enzyme hydrolysis, and also reduce swelling (Pu et. al., 2011). Linear amylose molecule is another starch form that is able to co-crystallize with lipophilic aromatic compounds. The helices formed have a hydrophobic cavity that can trap particular food bioactive components (Zabar et. al., 2009).

Similar to starch, cellulose can also be modified to become suitable encapsulating block. Some examples of modified celluloses are; Nanocrystalline cellulose (NCC), non-enteric cellulose esters and enteric cellulose esters. NCC is a rod-shaped nanoscale material with exceptional strength. It is simple in preparation and able to bind with hydrophobic or non-ionized groups (Chen et. al., 2012; Dufreene, 2010; Eichhorn et. al., 2010; Jackson et. al., 2011). Non-enteric cellulose ester such as acetate, acetate butyrate and cellulose acetate propionate has low water solubility and they may be suitable for viscous food dispersion to provide sustained release. Meanwhile, enteric cellulose esters such as cellulose acetate phthalate (CAP) or hydroxypropymethyl cellulose phthalate (HPMCP)

are insoluble in acidic condition and soluble in moderate acidic to slightly alkaline conditions. It is reported that HPMCP together with lutein was able to retain bioactive compounds with approximately 88% encapsulation efficiency, along with minimal thermal and light degradation (Heyang et. al., 2009). In spite of this, temperature-responsive hydroxypropyl cellulose carriers have been developed and they are reported to capture flavor and delay flavor release during high temperature (Heitfeld et. al., 2008).

Pectin is suitable for delivering food bioactive compounds that are sensitive to acid. This is related to its natural resistant characteristic to enzymatic digestion and its degradability by microflora in the colon (Sinha & Kumria, 2001). Low methoxyl pectin is able to form gels in the presence of divalent calcium ions and high methoxyl pectin can also form gels under acidic condition. Application of high Intensity ultrasound is able to increase rate of dissolution of the encapsulant in pectin. Besides, smaller particles can be produced if the pectin is combined with other polymer.

Guar gum is a type of carbohydrate used to thicken, emulsify and retrograde retarding agent in food products (Funami et. al., 2005; Kostyra & Barylko-Pikielna, 2007; Ribotta et. al., 2004). Guar gum is soluble in cold water, forming gels in hot water, resistant to enzyme hydrolysis in mouth, stomach, and small intestine, but susceptible to microbial degradation in colon. Native guar gum always forms highly viscous solution and thus requires modification before application. Depolymerized guar gum, which has low molecular mass and water soluble characteristic, has been used in the encapsulation of flavor oils (Sarkar et. al., 2012). Depolymerized guar gum can replace Arabic gums. Depolymerized gum together with Arabic gums is more effective in flavor retention than Arabic gum alone. Grafting of guar gum with polyacrylamide has been shown to improve the low hydration and low thermal stability of the gum (Soppirnath & Aminabhavi, 2002).

Protein-based Delivery System

Protein based delivery system shows characteristic high nutritional value, non-antigenicity, binding capacity, emulsification and gelation ability, and abundant renewable sources. Moreover, protein is less likely to be opsonized by the reticuloendothelial system (RES). The protein can be divided into animal protein and vegetal protein. Examples of animal protein that can be used as delivery system is gelatine, collagen, albumin, casein, whey protein, silk protein, and elastin. Animal protein has the advantages of synthetic polymer and absorbability, and less toxic to the degraded final product. In comparison with animal protein, vegetal protein such as zein and gliadin has the capability of yielding sustained release. In addition, high hydrophobicity of vegetal protein allows for the exclusion of the hardening treatment which may give toxic chemical crosslinks. Moreover, vegetal protein is less expensive and is readily modified for different targeting properties.

Protein based nano-carriers are potential drug carrier systems. Elzoghby et. al. (2012) has reviewed the different types of protein based nanocarriers in term of advantages, limitation, formulation, etc. The unique protein structure itself makes it a high possibility in site-specific drug conjugation and targeting by various ligands modifying the surface of the protein nanocarrier.

CHARACTERIZATION OF NANOPARTICLES

Nanoparticles are particles having a nano-scale diameter with either a micro porous structure or nano-size core compounds. Characterization has to be complete and accurate. It is important to understand the possible benefits and potential toxicity of nanoparticles in biological systems (Royal Society 2004). Parameters used in the characterization of nanoparticles include: size, structure, shape, texture, appearance evaluation, dispersion state of aggregation and sorption.

Various technologies are currently being used in the characterization of nanoparticles. Optic, confocal (Goya et. al., 2008), scanning electron microscopy (SEM), transmission electron

microscopy (TEM) (Jafari et. al., 2008), high-resolution transmission electron microscopy (HRETM), and atomic force microscopy (AFM) (Oliva et. al., 2003) are among the imaging techniques used in the characterization. Spectroscopic techniques such as static light scattering, dynamic light scattering (DLS), neutron scattering, small angle neutron scattering, nuclear magnetic resonance, and X-ray diffraction (XRD) are also useful for nanoparticles characterization. DLS is one of the most commonly reported. It measures the Brownian motion and the state of aggregation determination (Kaszuba et. al., 2008; Tiede et. al., 2008). XRD is useful in the identification of crystalline solids based on the atomic structure (Luykx, et. al., 2008). Instead of spectroscopic techniques alone, spectroscopic techniques coupled with SEM gives good analysis of chemical composition by using energy dispersive X-ray spectroscopy (EDS) and high angle annular dark field imaging (HAADF). X-ray photoelectron spectroscopy (XPS) can give a course but sensitive image of the particle surface.

Chromatography relevant techniques are able to perform separation of nanoparticles, including size exclusion chromatography (SEC), capillary electrophoresis and hydrodynamic chromatography (HDC). SEC separates particles in different solution according to the charge and distribution of the components while HDC separates particles according to their hydrodynamic radius (Tiede et. al., 2008). On the other hand, some well-established centrifugation and filtration techniques such as ultracentrifugation, ultrafiltration and nanofiltration are tools used for preparative size fractionation of samples.

Nanoencapsulation of Flavor Compounds

Flavor is the most important attributes in determining food quality. Therefore, it is necessary to preserve the flavor compound so that the food manufacturer can retain the aroma and volatile of food product during storage. Nanoencapsulation has be applied to protect the food from being oxidated, evaporated and migrated. Through the nanoencapsulation process, the coated bioactive compound with protective wall material helps to control flavor stability and its release (Madene et al., 2006).

Recently, Li and Chiang (2012) optimized the ultrasonic emulsification conditions for producing D-limonene using response surface methodology (RSM). They found out that the emulsification of D-limonene nanoemulsions work best when the power of ultrasonic was applied at 18W, for time 100-140s. Besides that, Lv et al. (2014) tested the thermal resistant behavior of nanoencapsulated jasmine essential oil. Through the complex coacervation using gelatine, there were a total of 51 compounds identified. Among them, linalool, methyl anthranilate, cis-jasmine, dihydromyrcenol, cis-jasmone and benzyl acetate were the main contributor flavor compounds for jasmine essential oil. After encapsulation, the nanocapsules can resist high temperature better. However, study showed that the intensity of the majority of the compounds decreased more than 50% when the heat processing was at (80°C) after 5 hours. After 5 hours, the flavor stability of the nanocapsulates was affected and unfavourable flavor compounds were formed such as 1-ocatanol and 2-decenal. The antioxidative characteristic of the nanocapsules is influenced by several factors like the type of wall material used, encapsulation method, physicochemical properties of the food and the storage conditions (Minemoto et al., 1999). Gunning et al. (1999) revealed that the release of flavor components was the largest when the sucrose-maltodextrin matrix was in a supercooled liquid state.

In terms of controlled flavor release, flavor can be released either by diffusion, degradation, swelling or melting (Madene et al., 2006). If the flavor can be released at a desired time at a desired rate, the loss of ingredients during processing can be reduced. Boland et al. (2004) had investigated the release of 11 flavor compounds from gelatine, starch and pectin gels. The gelatine gel showed more flavor release in the presence of saliva when compared with starch and pectin gels. Otherwise, Shiga et al. (2007) showed longer flavor retention by mixing gum Arabic and β-cyclodextrin in the feed liquid during spray drying.

SAFETY ISSUES

The lack of knowledge about the side effect of nanoparticles/nanomaterials on human health and the environment has raised a number of safety issues in environmental, ethical and regulatory aspects (EFSA 2009). The safety issues or risk assessment is associated with the type of material/particles/carrier used rather than the technology involved in nanoencapsulation. One of the most key issues regarding nanoencapsulation was the biotransformation of nanoparticles/ materials after ingestion, contact or inhalation. It is worthy of note that novel materials have different properties and react differently with food components. In order to fully understand the possible benefits and the potential toxicity of individual nanoparticles/materials in biological system, it is essential to completely and accurately characterize them (Royal Society, 2004). This issue however has not been well explored. Nanotoxicity of these particles/materials are influenced by the particle size, mass, chemical component, physical properties, and the aggregation of individual nanoparticles (Nel et al. 2006; Oberdorster at al. 2005).

There is no conclusive report regarding the undesirable effect of nanotechnology in the food sector on health. However, necessary regulatory controls and measures should be put in place as a proactive approach for protecting the consumer from potential adverse effects of this technology until it is proven otherwise. Further scientific updates will be a welcoming development to checkmate nanotoxicity of novel particles/materials being developed.

TRIVIAL

Nanoencapsulation have been widely applied not only in food, but also in other areas such as medicine, textile, cosmetics and fragrance. They have been widely studied as drug carrier, because they can improve drug sustained release, drug selectivity and effectiveness, drug bioavailability and decrease drug toxicity (Miao et.al., 2006; Palumbo et.al., 2002; da Fonseca et.al., 2008; Couvreur et.al., 1979 & Florence et.al., 1979). Xiao (2009) reported that a series of nanoencapsulate fragrances have been prepared successfully via the ionic gelation method and interfacial polymerization. Rose, sandalwood, orange and jasmine fragrances have been successfully nanoencapsulated using ionic gelation method while Polybutylcyanoacrylate (PBCA) encapsulated fragrance nanocapsule is produced using interfacial polymerization method. Nanoencapsulate fragrance is a core-wall structure with less than 100 nm. The protection of shell materials enable it to preserve the aroma when heated to 90°C and kept for 10 minutes. When the fragrances are applied into textiles, the aroma retention time is 6 months and wash resistance is more than 50 times. Another study carried out by Somasundaran et. al. (2006) showed that modified nanoparticles synthesized using inverse microemulsion polymerization technique by introducing functional groups into it produced enhancement in fragrance binding compared to the unmodified nanogels in methanol. Another study has been focus on the application of nanoencapsulation of herbal extracts *Ricinus communis, Senna auriculata and Euphorbia hirta* on 100% cotton denim fabrics. The results showed good resistance to microbial attack. After 30 industrial washes the fabrics were able to resist attack from the test bacterial strains and durability of finished fabrics was enhanced (Sumithra & Raaja, 2012).

FUTURE TRENDS

Nanoencapsulation is well applied in various food-related fields. Trends in nanocapsule construction are highlighted to manufacture, observation and measurement of the capsule

together with the focus on distribution of the size of the particles, interaction of wall and core materials, and control of the coalescence. The multiplicity of interaction among compounds at molecular and atomic levels is in the developing stages. Diffusion of bioactive components through the capsules is now a major subject of interest. On the other hand, different disciplines and techniques such as advanced non-linear dynamics, computer vision system, solid state physics, biotechnology, and novel interpretation of traditional concepts such as interfaces and product architecture are having increasing roles in nanoencapsulation. The complex phenomenon in nanotechnology and nanoengineering, and the difficulties in solving the laminar-turbulent, turbulent-turbulent multiphase interaction are the key points that need to be resolved in developing the right equipment, process and products (Quintanilla-Carvajal et. al., 2010). It is expected that more functional and stable nonoencapsulation would be produced in the nearest future.

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Conflicts of Interest

The authors declare no conflict of interest.