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REVIEW ARTICLE

Nano- and Microemulsions for Delivery of Nutraceuticals Through Livestock Products - A Review

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Abstract

Nano- and Microemulsions are based on the relationship between the discontinuous and continuous phases. These are liquid-liquid system of encapsulation which are particularly suited for delivery of hydrophobic nutraceuticals. It is likely to fabricate nano- and microemulsions from same constituents (oil, water and surfactant) but methods of preparation are different. Microemulsions form spontaneously by self assembly system. Nanoemulsions does not form spontaneously need high energy input to obtain. Therefore, prepare in devices e.g. Homogenizer, Microfluidizer, Ultrasonicators *etc*. Major benefits of nutraceuticals fortification in livestock products are it offer one of the simplest and most practical method to combat micronutrient deficiency for both poor and wealthy societies. Milk drinks, milk products, meat products are widely consumed staple foods. Delivery of hydrophobic nutraceuticals like oil soluble vit A, D, E, K, antioxidants, herbs extract, long chain fatty acids and carotenoids through food always have been challenge to food industry. Therefore, nano- and microemulsions constitute most capable systems to improve solubility, bioavailability and functionality of hydrophobic compounds. In dairy, creation of oxidatively stable transparent bioavailable beverages containing ω-3-fatty acids, phospholipids and minerals is major outcome of this technology. The addition of nutraceuticals in emulsified form in meat products is well studied and revealed that it is promising delivery system for long chain fatty acids fortification in red meat. There have been very less publications available on nano-and micro-emulsions in livestock products, therefore cited techniques needs more attention by research and development, regulations and food scientist.

Keywords: Nanoemulsions, Microemulsions, Casein Micelles, Nutraceuticals.

1. Introduction

Nutraceuticals which commonly are referred to as functional foods are substances that can be considered as food or part of a food which provide beneficial health effects of medical importance (Singh and Sachan, 2011; Vidanarachchi *et al.*, 2012). Reportedly, it was coined in 1989 by Dr Stephen L De Felice, founder and chairman of the "Foundation for Innovation in Medicine" New Jersey, USA. The emergence of nutraceuticals with health benefits provides an excellent opportunity to improve public health. Consuming healthy food has become a major trend in the last decade leading to the development of novel food processing techniques for the encapsulation and delivery of nutraceuticals. In Indian market lot of products sold in name of nutraceuticals. On the internet close to around 100 products are even listed (Patel and Singh, 2012). Emulsions are widely used within the food industry. Emulsions are dispersions of two or more nearly immiscible fluids, e.g. oil and water. Usually one liquid is dispersed in the other as fine droplets (Examples oil spreads, butter). For micro-emulsions, the mean diameter size is between 0.1 and 50 μm. Such emulsions are thermodynamically stable (Flanagan and Singh, 2006). They have relatively small particle sizes (r<50nm) compared to the wavelength of light (λ = 390-750 nm), which means they only scatter light weakly and so appear either transparent or only slightly turbid (Lesmes and McClements, 2009). However, nano-emulsions are thermodynamically unstable systems that typically have small particle sizes (r<100 nm) and they also tend to appear either turbid or opaque. The natural forms of nano- and microemulsions are casein micelles in milk, which is organized natural nano-delivery system designed to stabilize and transport essential nutrients, mainly calcium and protein, for the neonate (Livney *et al.,* 2006). More correctly, nano- and microemulsions are liquid-liquid types of encapsulation system. Both types of emulsions consist of oil, water and surfactant/co-surfactant but methods of preparation different which are discussed in next section of review.

2. Nutraceuticals

The most commonly applied nutraceuticals in industrial applications are lipids (lipid family includes fatty acids, phospholipids, carotenoids and oil-soluble vitamins), proteins (proteins family includes many food derived peptides, anti-hypertensive factor, antioxidants and immunity regulatory factor), carbohydrates (dietary fibers) and living bioactive components (probiotic bacteria, yeast) (Paul de Vos *e. al.,* 2010). They can also be classified on the basis of solubility i.e. hydrophilic (water soluble) and hydrophobic (water insoluble, Table 1). According Salminen *et al.*(2013) and Livney (2014) the fortification of hydrophobic nutraceuticals especially in clear drinks like water, soft drink, milk drinks and in meat products is very challenging comprises multiple elements like poor aqueous solubility of the hydrophobic nutraceuticals, high sensitivity to deterioration by oxidation and other chemical and physical factors, adverse sensory properties of certain nutraceuticals, high costs of the nutraceuticals and of the solubilization/encapsulation material used, processing, storage and shipment condition limitations, regulatory hurdles including strict limitations on health claims and on novel ingredients and technologies, religious constraints (kosher, halal), allergy of certain encapsulated materials, consumer demand for label friendly ingredients, poor bioavailability of certain hydrophobic nutraceuticals and more.

3. Major Benefits Nano- and Micro-emulsions

Rao and McClements (2011) suggested most important applications of nano- and micro-emulsions are therefore to incorporate hydrophobic ingredients into beverages that need to be transparent. The poor water solubility of hydrophobic nutraceuticals compounds causes enormous difficulties in delivering them in food. Garti and Yuli-Amar (2008) and Couedelo *et al.* (2011) explained delivery of oil-based nutraceuticals as emulsions. These emulsions are acts as encapsulation to resist the high acidity and enzyme activity of the stomach and duodenum, increase bioavailability of nutraceuticals, mask the bitter taste and odor, prevent it from oxidation. It ensures the product offers the finest sensory experience. It is an approach of protecting nutraceuticals in arduous journey from plant to palate, since many nutraceuticals get deteriorate along the various manufacturing and packaging processes. Their small droplet size allows for efficient delivery, accelerated release and rapid absorption of hydrophobic nutraceuticals such as vit E, Omega-3-fatty acids, flavonoids and various phyto-polyphenolic compounds (Lee and McClements, 2010).

4. Techniques for Preparation of Nano- and Micro-emulsions

There are various techniques available to produce and characterize emulsions some of them have shown be more suitable than others. Nanoemulsions can be produced using a variety of methods, which are classified as either high energy or low energy approaches (Acosta, 2009; Leong *et al.,* 2009). High energy approach uses mechanical devices such as high pressure homogenizers, microfluidizer and ultrasonic homogenizers. Low energy emulsification techniques take advantages of the physicochemical properties of these systems based on the phase transition that take place during the emulsification process. Different characterization parameters for nanoemulsions include transmission electron microscopy, nanoemulsion droplet size analysis, viscosity determination, refractive index, thermodynamic stability studies and surface characteristics. Quantification of functional compounds is done by HPLC, Dynamic Light Scattering (DLS) may quickly determine the hydrodynamic diameter of nanoparticles in a nanoemulsions, Zeta potential can indicate the stability of the nanoemulsions (Bhatt and Madhav, 2011). Microemulsions are easier to prepare than nanoemulsions and emulsions, but requires much higher surfactant concentration. More expressively basic difference between nanoemulsions and micro-emulsions are not particle size but principle of formation. Microemulsions are formed by spontaneous self assembly system. Which means under appropriate environmental conditions, certain types of food components spontaneously assemble into well-define structures E.g. Micelles, Vesicles, Fibers, tubes, liquid crystals *etc* are examples of microemulsions (McClements, 2009). Here concentration, quantity and selection of emulsifiers, surfactants and co-surfactant play prime role in bringing appropriate environment conditions.

Table 1: Major Hydrophobic Nutraceuticals Components that Need to be Delivered into Foods

Name	Types	Nutritional Benefits	References
Fatty Acids (PUFA) (MUFA)	Salmon, Flaxseed, CLA Tree nuts	Potent controllers of the imflammatory processes, Maintenance of brain function, Reduce cholesterol disposition, Reduce risk of coronary heart disease	Sarin Rajat *et al.,* 2012
Carotenoids	β-Carotene Lycopene, Lutein, Zeaxanthine	Antioxidant, vit A Precursor, Natural Food Color Antioxidant, Reduce risk of Cancer, Particularly Prostate Cancer, Natural Color Coronary Heart Disease	Yin *et al.,* 2009 Garti *et al.,* 2005 Ron *et al*., 2010
Antioxidants	Tocopherols, Flavonoids Polyphenols	Cancer, Coronary Heart Disease, Urinary Tract Disease	Sarin Rajat *et al.,* 2012
Phytosterols	Stigmasterol, β-sitosterol	Coronary heart Disease	Ron *et al*., 2010
Vitamins	Oil soluble (A, D, E, K)	Prevents Rickets, Osteomalacia	Ron *et al.,* 2010
Curcumin		Antioxidant, Anti-inflammatory, Anticarcinogenic, Natural food color,	Huang *et al.,* 2010

5. Rewards of Fortification of Nutraceuticals in Livestock Products

In several countries liquid milk fortification with vit A and vit D is mandatory. In 1930s vit D was added to milk in US to prevent rickets in children. Various steps and process have measurable impact on some specific nutrients. The fortification of livestock products such as milk, meat with nutraceuticals is a promising strategy for promoting health of wide populations. Fat soluble vitamins like vit D hardly found in skim milk and low fat dairy products consumed largely in modern societies being an important sources of Calcium and Phosphate. There are plus points of fortification of livestock products are wider consumption by all age groups (large demographic coverage), cost adorable by target population, higher stability and bioavailability of added micronutrients. It is best way to alleviate ***Hidden Hunger*** and combat micronutrient deficiency (Arora *et al.,* 2012). Hayes *et al.* (2011) suggested meat is one of the most important and commonly consumed foods and it is an excellent way to promote intake of functional ingredients without any radical changes in eating habits.

6. Nano- and Micro-emulsions in Dairy Products

The new technology presented herein may serve to enhance the health promoting properties of beverages and other dairy products. Some of the giants of the food industry such as ***Unilever*** and ***Nestle*** are also applying nanotechnology to their food products. ***Unilever*** has made ice cream healthier without compromising on taste through the application of nanoemulsions. The objective is to produce ice cream with lower fat content, achieving a fat reduction from the actual 16% to 1%. ***Nestle*** has a patent in oil-in-water emulsions (10-500nm), aiming at achieving quicker and simpler thawing through the addition of polysorbates and other micelle-forming substances (Cross ref: Silva *et al.*, 2012). Recently Huimin *et al.* (2014) demonstrated a technology for producing UHT milk enriched with Docosahexaenoic acid. Octenyl succinic anhydride (OSA starch) a hydrophobically modified starch used as emulsifier to make algae-oil emulsion in UHT milk. Algae-oil enriched drinking milk is a stable product during 11 weeks of storage. Application of high storage temperature (40˚C) does not significantly increase the oxidation process. Also, study suggested that stable algae oil emulsion can be formed by OSA starches with corn syrup. Antimicrobial micro-emulsions have also been demonstrated to effective against various food borne pathogens (Gaysinsky *et al.*, 2005; Gaysinsky *et al.*, 2007). Micro-emulsions containing Eugenol oil (Eugenol oil encapsulated in surfactant micelles) in UHT milk was effective against *Listeria monocytogenes* and *E. coli O157:H7*. Gaysinsky *et al*. (2007) study revealed that surfactant-encapsulated antimicrobials efficiency of targeting food borne pathogens affected by food composition especially fat level in milk. Study found antimicrobial activity is more in skim milk (2% fat) when compared to full fat milk (4% fat). Vit D is fat soluble is hardly found in skim milk and low fat dairy products. A Patent (US20090311329 A1) by Livney and Dalgleish (2009) invented novel approach for the nanoemulsions / nanoencapsulation and stabilization of hydrophobic biologically active compounds, particularly in non-fat or low fat edible products. A hydrophobic domain of casein micelles stabilize the nutraceuticals in aqueous systems like milk and facilitates the enrichment of low fat and fat free dairy and other food products with these bioactive molecules e.g. vit D2, A, K, E. Tippetts *et al.* (2012) reported the increased in retention of vit D3 in cheddar cheese by incorporating it as a part of an oil-in-water emulsion using milk protein as emulsifier to obtain a fortification level of 280 IU/serving. Significant retention of vit D3 observed in cheese curd emulsified oil with non fat dry milk than control. Full fat cheese curd has less retention compare to whole milk cheese curd.

7. Nano- and Microemulsions in Meat Products

Meat is considered a vital component of a healthy diet, an excellent source of proteins, essential minerals, trace elements and vitamins although it is deficient in Calcium, Fiber, Iron and vit C. Negative concern regarding meat consumption and its impact on human health have promoted research into the development of novel functional meat products (Hayes *et al.*, 2011). Meat products enriched with omega-3 polyunsaturated fatty acid is recent trend in meat industry. The problem with addition of PUFA in meat products is they are highly susceptible to chemical degradation under processing and storage conditions. Caceres *et al*. (2008) and Pelser *et al*. (2007) study shown oil-water (O/W) emulsions is most to be suitable systems to incorporate n-3 fatty acids into food products like ham, sausages and meat patties. In addition Caceres *et al.* (2008) suggested even after encapsulation of PUFA in emulsion, various antioxidants are usually needed to be control oxidation. Using various antioxidants such as tocopherol, Ethylene diamine tetra-acetic acid (EDTA), Catechins, sodium citrate and erythorbate within base emulsions have helped overcome many problems related to their low oxidative stability. According to Salminen *et al.* (2013) the problem with incorporating emulsion delivery systems into meat products is that most food matrices differ in respect to their interactions with incorporated emulsion. The high salt and mineral concentration in the meat products may cause electrostatic screening of the protein charges in the emulsion, thus introducing flocculation or coalescence. Furthermore, processing conditions (mixing, heating, freezing, freeze-thawing) of meat products may likely influence the stability of emulsions delivery systems. Another important result of the work of Joe *et al.*(2012) was that sunflower oil based nanoemulsions preservation technique is able to extent the shelf life and maintain the quality of mackerel steaks during storage. Study was conducted to determine influence of nanoemulsions on the microbiological, proximal, chemical and sensory qualities of Indo-pacific king mackerel steak stored at 20˚C for a period of 72h. Sunflower oil based nanoemulsion treatment showed initial reduction in heterotrophic, H_2S and lactic acid bacterial populations in 12h, followed by gradual increase in their respective populations. Organoleptic evaluation revealed treated steaks showed an extension of shelf life of 48hrs when compared with control and antibiotic treated samples.

8. Shortcomings of Techniques

The application of these techniques in many food and beverage products is currently limited because of number of technical and practical reasons. First, there is only limited number of food grade surfactants currently available for preparing and stabilizing these systems. Second, there is poor understanding of the influence of sample composition and environmental conditions on the formulation and stability of specific kinds of colloidal delivery systems. Third, it is difficult to prepare nano-and microemulsions from many commonly used edible oils, e.g. fish oil, corn oil or soyabean oil etc. (Warisnoicharroen *et al*., 2000; Salager *et al.*, 2005). Fourth, irreversible aggregations and leakage of functional compounds are the major problems of the nano- and microemulsions (Hae-Soo Kwak, 2014). Fifth, in microemulsions, it is often difficult to incorporate large hydrophobic molecules within the particle structure while maintaining the optimum curvature of surfactant minolayer (Coupland *et al.*, 1996). Sixth, in nanoemulsions, it is difficult to produce small droplets during high pressure homogenization due to high oil viscosity and interfacial tension (Woodster *et al*., 2008).

9. Conclusions

In the view of above citation we can conclude that instead of using traditional techniques as delivery medium for hydrophobic nutraceuticals, nano- and microemulsions can be use as better alternative. Protection is needed for many sensitive hydrophobic nutraceuticals as they are generally unstable and interacts with complex food matrices. In case of dairy products creation of oxidatively stable transparent bioavailable beverages containing ω-3-fatty acids, phospholipids and minerals is major outcome of this technology. It is also evident through a number of reports, analysis and patent applications that, in meat products addition of nutraceuticals in emulsified form is well studied. In red meat it is promising colloidal delivery system for long chain fatty acids. Since the feed regimens in ruminants are cumbersome, time consuming process. On the other side, from many studies it found that direct addition leads to oxidation during processing and storage. Therefore, nano- and microemulsions constitutes most capable systems to improve solubility, bioavailability and functionality of hydrophobic compounds. Food industry always seeks to use these systems for the incorporation of hydrophobic nutraceuticals in food matrices.

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