Encapsulation Technologies: A Tool for Functional Foods Development

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Abstract: ***There is a growing demand for functional foods in the market with the increasing of world's population. The main targets for this trend in consumption are foods containing plant extracts with antioxidant properties, polyunsaturated fatty acids, probiotics, vitamins and minerals. Although many of these components are unstable under normal conditions or have a residual taste, their application is limited. It is therefore necessary to use techniques which can protect the stability of these functional components, enable their application in various food matrices and enable them to be better absorbed in our gastrointestinal tract. Various sectors of the food industry have a demand for the enrichment of foods with functional compounds. This review aims at highlighting the importance and application of various encapsulating techniques of probiotics, unsaturated oils, flavours, and fruit juice. The methods and wall materials used in different encapsulation techniques would discuss in this review. Encapsulation technology is an emerging technology that can guarantee the stability of these functional ingredients and allow their application in variety of food matrices.***

I. INTRODUCTION

Foods that contain natural antioxidants, unsaturated fatty acids, probiotics, bioactive compounds and vitamins are the main functional food trend[1]. These functional ingredients can be used to improve the nutritional value and functionality of food products. Thus, food industry has pay attention for their research in products of this nature that incorporated functional ingredients. Nevertheless, many of these compounds are unstable or possess a residual taste, as well as the lipophilic nature of the compounds results in poor absorption and palatability [1].

Revised Manuscript Received on March 08, 2019.

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These would limit their application in functional foods. Thus, the application of these functional ingredients to obtain stable products and maintain their active characteristics during processing and storage until consumption represents a major challenge to food industry. Encapsulation is a process in which the active agent is trapped by wall material producing nanometer (nano-encapsulation), micrometer (micro-encapsulation) or millimeter scale[2]Micro and nano-encapsulation are rapidly expanding to solve potential bioavailability problems, offering new solutions to the food industry 's challenges. Microparticles are particles between 3 and 800 μm in diameter, and nanoparticles are colloidal particles between 10 and 1000 nm in diameter[3].

Microencapsulation is a process for the coating of active ingredients in the form of a solid, liquid or gaseous substance (the core) with a polymeric material layer (the shell) producing microparticles from the micrometer to the millimeter range[4]. The active ingredient is thus protected against oxygen, heat, moisture, light and lipid oxidation and improves the shelf- life of oil and flavours[5]. This technology is also commonly being used in food industry to cover off-flavour and color [6]. Compatibility of the core material with the shell is an important determinant for a successful encapsulation process, thus pretreatment of the core material is often needed [7]. In addition, microencapsulation can modify the physical characteristics by converting liquid into solid state to enhance its handling property. Microencapsulation is able to transform the liquid state into free flowing powder form, which is readily incorporated into food products without changing the sensory quality of the food product [8]. Reservoir, matrix and the combination of reservoir and matrix systems are the main types of encapsulates. Reservoir encapsulates have an outer shell which surrounds the active agent and can be produced by one or multiple reservoir chambers. However, the active agent exists as droplets or dispersed in the wall material in the matrix system [9].

Recently, there is more research focused on the nanotechnology to enhance the bioavailability of the active agents in pharmaceutical and nutraceutical fields Large surface area is available in nanoemulsions for intestinal enzymatic reactions, improving gastrointestinal epithelial diffusion across the mucus layer and thus improving bioavailability[10]. The integration of the functional ingredient into the micro and nano delivery system helps to protect against adverse conditions(e.g. heat, light, oxygen

and moisture) in the food chain.

It can also help improve solubility, bioavailability and enable its controlled release and delivery due to its reduced size in micro and nano systems[11].

II. ENCAPSULATION OF PROBIOTICS

According to the World Health Organization (WHO), probiotic is' live organism, which gives health benefits to the host when administered in adequate amounts '[12,13]. Probiotic is helpful in managing bowel disorders such as infectious diarrhea, antibiotic-associated diarrhea, lactose intolerance, allergy as well as modulating systemic immune response [14] and inflammatory disease [15]. Both Lactobacillus and Bifidobacteria strains [16] are commonly used in food product. In addition to that, Bacillus and some yeast, mainly *Saccharomyces cerevisiae* are also used in food products [2]. Incorporating some of these species into food product enhances its nutritional value and converts it into functional food.

To acquire the health benefit of probiotic, a viable probiotic is required so that it could reach the intestines in adequate number. The final probiotic products should therefore have a minimum concentration of 106 CFU/mL and 108 to 109 probiotic microorganisms per day are required for the probiotic to take effect in the human body. Besides, the probiotic must be able to live under manufacture and commercial conditions and still remain viable under ordinary storage condition. The challenge is that there is a low probiotic survival rate in the food product and gastrointestinal tract. Covering the live probiotic inside a physical wall will help the probiotic to survive in hostile conditions. Such method is currently gaining much acceptance to keep the probiotic and to transport them into the gastrointestinal tract [17]. One of the most effective method is microencapsulation which can protect the bacteria during the production process, food matrix incorporation process, storage and increases its applicability in food industry [18].

Main components used in probiotic micro-encapsulation are including red algae, κ-carrageenan from red algae [19] starch from maize [20] chitosan from Crustacean shell, alginate from brown algae, gellan gum from sphingomonas elodea [21] gelatin form collagen [22] Xantha gum from xanthomonascampestris [21], milk protein from milk [23], polyacrylamide from chemical synthesis [24] and polyvinyl alcohol composed of vinyl alcohol subunits [25].

There are few methods for probiotic microencapsulation, including spray drying [26] extrusion [27] and emulsion [28]. The most popular method is extrusion technique because of its gentle formulation conditions, simplicity, and cost effective for higher cell viability [29]. This method requires preparation of the hydrocolloid solution, probiotic addition and extrusion of the cell suspension through a syringe. The droplets are then transferred to a harding solution[27]. Extrusion technique can be carry out by prilling, which is performed using vibration of the nozzle or pulsation of the jet. Another technique to produce small dewdrop is through electrostatic forces. When the electrostatic field is switched on, the electrostatic force interrupts the liquid surface at the tip of the needle, forming a charged dewdrop stream. This method is not using any organic solvent. The size of the beads can be regulated easily. Mass production of beads is possible by using multi-nozzle system [30].

Calcium alginate is usually used to immobilize and encapsulate probiotic especially lactic acid bacteria, which usually in the concentration range of 0.5-5% [17] . Because alginate is low cost and safe to use in food, it forms gentle calcium chloride matrices in order to trap sensitive materials particularly in living microbial cells[31]. Microparticles alginate may be achieved through internal or external gelation. First of all, the emulsion of water in oil produces microparticles. By adding the calcium chloride solution, the alginate will be gelled to the emulsion. The microcapsule may also be formed by internal gelatin. To form an emulsion of water in oil, alginate is added to the calcium carbonate solution and acetic acid is added. Calcium carbonate discharges calcium ion and carbonic acid during the water phase. The calcium ions bind to the alginate to form the structure of the egg box[17]. For example, previous studies showed that Lactobacillus casei (Lc-01) and Bifidobacteriumlactis (Bb-12) were significantly better encapsulated in the freezing of calcium alginate beads than free cells when compared within the same strain. Compared with free cells, the probiotics encapsulated with calcium alginate had a 30 percent higher rate of survival both in the ice cream freezer and during frozen storage[32].

Alginate microparticles are, however, vulnerable to acidic conditions with pH inferior to 2. Microparticle alginate also deteriorates when monovalent ions or chelating agents such as lactates, phosphates and citrates are present. Alginate microparticle is highly permeable which lead to fast dispersion of moisture through the beads, and thus, it is hardly applicable to large industrial scale. However, this problem can be solved by combining other polymer compounds with alginate, coating the alginate with various substances or modifying the alginate structure[17]. For example, microencapsulated Lactobacillus acidophilus and Bifidobacterium spp with calcium alginate and Hi-Maze starch (a prebiotic) increased the viability of bacteria compared to bacteria encapsulated without starch. Inclusion of glycerol, a cryoprotective agent with alginate mix, increased the bacteria's survival when frozen at -20 oC[33].

Besides that, covering a semi-permeable layer of chitosan around the alginate microparticle is effective to increase the stability because this structure is sustainable against calcium chelating and anti-gelling agents and the beads are much denser and solid [29].In order to produce beads that are dense and strong, low molecular weight chitosan is preferred as it penetrates faster into the alginate matric [17]. Previous study show that in the simulated acidic conditions of gastro-intestinal tract, *B. bifidum* and, *L. gasseri* that alginate microencapsulated with chitosan-coating have higher survival rate [34]. Recent studies have shown that coating the alginate beads with chitosan increases the size of the bead and changes their load (from negative to positive). The optical microscopy showed that the probiotic has been

immobilized inside the hydrogel beads[35]. It is therefore evident that microencapsulation with alginate-gelatinized starch coated with chitosan can effectively sustain the viability of probiotics against adverse conditions of simulated human gastrointestinal disease. However, [35] it has been demonstrated that bifidobacterial encapsulation in chitosan-coated alginate beads has a lower storage or gastrointestinal stability than cells in alginate beads. This could be due to the usage of alginate hydrogel that had a relatively larger pore, thus, small molecules such like oxygen, acids, bile salts or digestive enzymes could still enter the microgels and damage the encapsulated probiotic.

Recent studies by[36] showed that Lactobacillus acidophilus and Lactobacillus casei were encapsulated using calcium alginate-gelatin and prebiotic (i.e. inulin and lactulose) by means of extrusion technique. L. and acidophilus. Casei effectively delivered viable bacterial cells to the colon against the adverse condition of simulated human gastrointestinal condition. Despite numerous researches had been done to investigate different method to microencapsulate probiotic, there is still wide variation of probiotic viability between the different strain of probiotic which it is very much related to the phenotypic and genotypic factors contributing to stress responses toward adverse condition. Further genomic functional analysis of the encapsulated probiotic organisms can help to match strains with the compatible encapsulation process to increase cell viability during storage[35] Likewise, it is helpful in selecting suitable delivery vehicles to protect probiotic during gastrointestinal transit and reach distal colon intact, so that it is still metabolically active to produce health benefiting effect. All in all, probiotic microencapsulation remains a challenge in the food science industry, research should focus on formulations that can improve the delivery of vehicles, possibly via antioxidants and cryoprotectants, to further preserve probiotic viability in the encapsulation gel matrix [37].

III. ENCAPSULATION OF HIGHLY UNSATURATED OILS

Oil containing high amounts of monounsaturated and polyunsaturated fatty acids, such as fish oil, olive oil, chia seed oil, flaxseed oil, sunflower oil and others, offers a good source of essential fatty acids that human beings need for health benefits. The increase of usage of these fatty acids in food formulation has been shown in the past years to help to decrease the risk of getting coronary heart disease, diabetes and immune response disorders. Furthermore, highly unsaturated oil provides a desired source of lipid-soluble bioactives such as tocopherols, phytosterols, carotenoids and polyphenols with significant antioxidant activity[38]. However, these oils are chemically unstable and easily oxidised when it meets oxygen, moisture, light, and high temperature because of its high contents of unsaturated fatty acids. Shelf stability, nutritional value, and sensory properties of the oils would be affected if it undergoes oxidation [39]. Encapsulation is an effective technique to protect the oil against adverse environment and oxidation of the unsaturated fatty acids, as well as increase the application of encapsulated products in food [40].

Minimum surface oil microcapsules and maximum retention of the active agent are regarded as a successful encapsulation technology. Spray drying is a promising technology that can produce more than 90 percent of powders with microencapsulation efficiency (MEE)[41,42,43,44]. Previous studies have reported that maltodextrin, sodium caseinate and soy lecithin microencapsulated kenaf seed oil with a total solid content of 40 percent and spray dried at an inlet air temperature of 160 oC compared to 180 and 200 oC would produce microcapsules with higher MEE and better lipid oxidation protection[45,46]. It was reported that spray drying of kenaf seed oil was able to protect the bioactive compounds (tocopherols, phytosteols, phenolic acids) and fatty acid profile during accelerated storage [45]. Goyal[47] reported that microencapsulated spray dried flaxseed oil showed excellent peroxide storage stability at the end of six months at 35 ± 1 ºC. Besides, the milk product that fortified with 1% level of microencapsulated flaxseed oil, showed sensory characteristics comparable with the control for up to 5 days of storage. Spray dried salmon oil had been applied in the yogurt product in the study by Estrada [48]. Oil high in unsaturated fatty acids proposed to be used as a fortifying agent in food applications to meet the nutritional requirements of omega-3 fatty acids.

Combination of wall materials can improve the quality of powders, as no single wall material has all the qualities required to be an ideal encapsulator. Calvo[49] showed that the addition of carboxymethyl cellulose and lecithin to extra-virgin olive oil based on maltodextrin improved the encapsulation yield and MEE as well as the oxidative and thermal stability of the product. This is because maltodextrin contributed to the maltodetrin-based model with emulsion stability, poor emulsification capacity and low oil retention. However, microencapsulated oil with sodium caseinate and maltodextrin showed better oil stability compared to microencapsulated oil with maltodextrin, carboxymethyl cellulose and lecithin with higher values of oxidative stability index, tocopherols and monounsaturated fatty acids found in the microencapsulated oil with sodium caseinate and maltodextrin. The main disadvantages of spray drying are the use of high temperatures in the spray drying process and the access to air. After the process, it can cause lipid oxidation on the microcapsule surface[50].

Complex coacervation is widely used for encapsulating bioactive compounds, especially lipophilic compounds in the food and pharmaceutical industries. Tuna oil that encapsulated by gelatine-sodium hexametaphosphate by complex coacervation yields high MEE (99.8 %) [51]. After the complex coacervates are formed, additional drying steps such as spray drying or freeze drying are required. Kaushik[52] reported that spraying dried complex coacervates produced higher MEE and lower surface oil than freezing dried complex coacervates due to the porous structure of freezing dried complex coacervates. However, lyophilisation showed better drying process for complex coacervates as it produced higher yield and lower

carotenoids lossess in encapsulated palm oil by complex coacervation compared to atomization [53]. Previous studies reported that complex coacervation offered protection against lipid oxidation during storage in encapsulated tuna oil, poppy-seed oil [54], flaxseed oil [52]. Protein-polysaccharide complex coacervates have been shown to be effective in delivering sensitive active material to the intestinal digestive stage with minimal damage to the gastric environment[55]. It showed the microparticles by the wall materials of chitosan/xanthan gum possess high potential for food application, especially yogurt [53].

Ionic gelation is formed by hydrophobic functional oil incorporated in an alginate solution to form an emulsion and the emulsion is dripped into a CaCl2 solution via extrusion by a needle where gelation takes place (external gelation). However, the porous structure of the alginate beads allowing the spread of acid provides limited protection for the core substances[40]. Thus, incorporation of different wall materials helped to form rigid wall and enhance the protection on core materials. High encapsulation efficiency can be produced by encapsulating the oil by ionic gelation. Morales [56] and Piornos [57] reported the use of alginate with shellac beads to encapsulate sunflower oil and alginate with lupin protein to encapsulate linseed oil yield the beads with encapsulation efficiency of 98.7 and 98.3%, respectively.

It also offered a possibility for intestinal digestion. Beads are an effective system for encapsulating oil against oxidation[57]. This technique has been proved as effective encapsulating system for easily degradable oils to be used as food additive in food industry and pharmaceutical products. Co-extrusion that combined the techniques of vibration nozzle technology and ionic gelation is used to encapsulate oil recently. Alginate solution (1-2% w/w) is widely used as the shell formulation in co-extrusion. Pectin can be added to the alginate-based shell formulations to improve core material protection, improve encapsulation efficiency, increase dietary fiber consumption and increase microcapsules' nutritional value. Strong calcium binding mechanism can be formed between Ca2+ and guluronate, and galacturonate blocks in alginate and pectin, respectively [58]. Chew[40] has shown that micro-encapsulated kenaf seed oil in high methoxyl pectin-alginate blends has a higher micro-encapsulation efficiency (76.6 percent) than alginate-only microcapsules (69.6%).

Chitosan has been intensively used to increase the mechanical stability of alginate beads by coating a chitosan layer on the alginate beads through formation of polyelectrolyte complex with alginate. The micro encapsulated kenaf seed oil with high methoxy pectin-alginate and a chitoan coating with high protection on the kenaf seed oil in an adverse gastric environment[58] offered an effective controlled release system. Alginate alone was, however, better than the combination of alginate and hydroxypropyl methylcellulose (HPMC), since the Sun-Waterhouse study showed wall materials for encapsulating avocado oil[59]. Previous studies showed that co-extrusion technology offered protection against lipid oxidation during storage on olive oil [60], avocado oil [59], canola oil [61], and kenaf seed oil [62,63]. Co-extrusion produced microcapsules with microencapsulation efficiency in a range of 50 to 77 % by previous studies [40,60].

The previous in vivo study showed that the nanoemulsion-based delivery system promises encapsulation techniques to improve the bioavailability of lipophilic bioactive compounds[10]. Kenaf seed oil has been encapsulated in oil-in-water nanoemulsions using the formulations of sodium caseinate, Tween 20 and gum arabic, and the formulation of sodium caseinate, β-cyclodextrin and Tween 20, produced by high-pressure homogenizers[64, 65], showed that nanoemulsions of digested kenaf seed oil showed good lipid digestion (85.3 percent), good bioaccessibility of antioxidants (phenolics and tocopherols) and slower phytosterol degradation compared to unencapsulated kenaf seed oil. The increased release of antioxidants from digested nanoemulsions has shown better bioavailability as increased chances of absorption by small intestine epithelial cells of antioxidants[66]. This demonstrated a high application potential in the food and nutraceutical industries.

IV. ENCAPSULATION OF FLAVOURS

Flavor and aromatic compounds play a key role in the satisfaction of consumers and influence further food consumption[67]. However, the long residual action and the thermostability of flavor and fragrance[68] are key factors which limit the progress of the flavor industry. The encapsulation method is one of the most used to enhance the stability of flavours. Microencapsulation is capable of producing unwanted odors and tastes in specific flavors and aromas. Material properties, formulation parameters and operating conditions are parameters that can influence microencapsulated products and their final properties[69]. The final properties of microparticles can affect the release rate of the active compound[70]. Several factors such as technological concerns (manufacturing and storage properties), economic feasibility and consumer satisfaction must be considered for the micro-encapsulation of bioactive molecules in the food industry[71].

Complex cooking is one of the most common chemical processes for capturing flavor compounds. Lv[72] reported that the formation of heat-resistant flavor nanocapsules with trapped essential jasmine oil was achieved by complex gum arabic and gelatin-based coacervation. Nanocapsules encapsulating jasmine essential oil (cross-linked by transglutaminase) possess good heat-resistance capabilities against humid heat at 80 °C. It was concluded that the electrostatic complexes could be used as a flavor and fragrance delivery vehicle in the food, pharmaceutical and textile industries[72]. In sweeteners, the microcapsulation process can increase the resistance to high temperatures, fluidity and prolonged sweetness sensation. Rocha-Selmi[73] microencapsulated sucralose (hydrophilic) with double emulsion, with primary W / O emulsion prior to complex coacervation, followed by dual W / O / W emulsion. The microcapsules presented low hygroscopicity and solubility, as well as neutral charge, indicating complete coacervation.

Aspartame microencapsulation was performed using the same technique, i.e. double emulsion followed bycomplex co-cervation. Sunflower oil was used to prepare primary emulsion while the wall materials used were gum Arabic and swine gelatin [73].

The microcapsules were structurally examined for water release and sorption isotherms at 36oC and 80oC. It was found that a temperature increase did not lead to an increase in the release rate, demonstrating that the microcapsules were relatively high temperature resistant (80oC).

Spray drying is a commercial process that is widely used in the production of encapsulated volatiles and flavors on a large scale[74]. Teixeira[75] indicated that a high retention of aroma compounds is provided by the spray drying technique. Spray drying can be used in many heat-labile materials, as the core material reaches only the lower temperature[76]. Well, Sultan et al.[77] The flavor encapsulation was studied by spraying the dried cells of Saccharomyces cerevisiae. For the encapsulation of d-limonene and ethyl hexanoate, yeast cells, Saccharomyces cerevisiae, which were by-products in the production of β-glucan, were used. The time and temperature of incubation showed significant effects on the encapsulation of flavor in the yeast cells. The flavor content for d-limonene was 37 wt percent and for ethyl hexanoate was 49 wt percent at 200 ° C inlet air temperature. Thus, by spraying drying, yeast can be used as a successful flavor capsulant[77].

Rubiano [78] evaluated the addition of four emulsifiers (sodium caseinate, tween 20, tween 60, and low methoxyl pectin) for the stability and formulation of d-limonene emulsions. Subsequently, the effects of spray drying variables on the product quality as well as on the overall performance of the process were determined. It was reported that the optimum conditions for the drying process were attained with an atomizer disc speed of 30000 rpm, and inlet and outlet air temperature of 156.7 ºC and 90 ºC, respectively. The corresponding solids recovery, solubility and encapsulation efficiency were more than 90% with low moisture content (2.22% w.b) and water activity (0.112), which resulted in a powder with suitable characteristics for industrialization [78]. Sosa [79] assessed the performance of the formulations for citral encapsulation using a spray dryer. Formulations contained trehalose or sucrose with or without maltodextrin and a modified starch as an emulsifier. The mixture of maltodextrin and trehalose was the best formulation because of higher glass transition temperature values which allow to maintain the glassy state of the powder in a broader temperature and relative humidity conditions. Trehalose could be used as an ingredient in the carrier formulation to encapsulate the citric flavor. Borrmann, Pierucci [80] successfully encapsulated passion fruit juice with n-octenylsuccinate-derivatised starch by spray drying. The drying process did not alter the taste and aroma of the passion fruit, and the spray dried passion fruit powder can be easily diluted to reconstitute the passion fruit juice for human consumption.

Getachew and Chun [81] extracted coffee oil flavour using supercritical carbon dioxide (CO_2) and the extracted oil was then encapsulated using polyethyleneglycol (PEG) by PGSS process. The optimized processing conditions were temperature (40 ^oC), pressure (260.1 bar), and polymer-oil ratios (6.57:1 g/g) with a maximum encapsulation efficiency of 79.78%. Analysis of fatty acid and flavor compounds confirmed that very good retention of fatty acids and flavor compounds can be obtained from the optimized process conditions. Microencapsulation through PGSS process could be employed for the production of freely flowing powdered particles that can be used in food processing industries [81]. Machado [82] produced dispersions that stabilized the limonene with modified starch in order to ensure that limonene was not solubilized during impregnation or encapsulation via PGSS. Encapsulation by PGSS retained 86% of the limonene although limonene is highly soluble in supercritical CO_2 because the depressurization of the PGSS process was fast enough so that the limonene did not solubilize and trapped inside the modified starch [82].

V. ENCAPSULATION OF FRUIT JUICE

Drying is defined as the use of heat to evaporate most of the water in a food[83]. Spray-drying is commonly used in the commercial production of fruit powders amongst many drying techniques used in food products[84]. Most of the starting materials were in either juices or pulps. The physico-chemical properties of the final products are dependent on the spray-drying parameters such as inlet temperature, feed flow rate, air flow rate, atomiser speed, types of carrier agent and their concentrations [85]. These parameters influenced the moisture content, yield, hygroscopicity, particle size, bulk density and colour pigment in spray-dried of foods [86]

Inlet temperature is the temperature of heated drying air that enters into the drying chamber. From the table, it is observed that inlet temperatures used for spray-drying of fruit powder ranged from 120 to 185 °C. Product yield of tamarind powder decreases with the increases of inlet temperature [87]. With an increase in drying temperatures, the moisture content of juice powders decreases[88]. The faster heat transfer between the product and the drying air reduces the humidity content at higher temperatures in the inlet. Powders produced at higher inlet temperatures were more hygroscopic[86] High drying temperature reduces the moisture content of the powder, increasing its hygroscopicity and thus the low moisture content of the powder tends to absorb moisture from the surrounding area.

VI. CONCLUSION

There are various techniques available that could be used to encapsulate the functional ingredients. Different functional ingredients might require different encapsulation techniques, drying techniques and wall materials to meet the specific physicochemical and molecular requirements, as well as its desirability. Encapsulation is an effective protection method by providing a protective shell barrier on the functional ingredient with many advantages. Encapsulation of functional ingredients achieves excellent characteristics of protection, stabilization, solubility and controlled release of the active agent. Encapsulation can

enhance the application in food industry by fortify the food products with specific healthy benefits and desired functionality. It can resolve the deficiencies of micronutrients worldwide. Transform the lab scale encapsulation technique into industrial scale represents the major challenge and need to further explore and overcome in the future study.

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